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Early detection of colorectal anastomotic leakage: Usefulness of clinical criteria and serum biomarkers

Nuno José Rama

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

Nuno José Gomes Rama

EARLY DETECTION OF COLORECTAL ANASTOMOTIC LEAKAGE: USEFULNESS OF CLINICAL CRITERIA AND SERUM BIOMARKERS

A Thesis applying for the Doctoral Degree in Medical Sciences submitted to School of Medicine and Biomedical Sciences (ICBAS) - Oporto University.

Supervisor - João Pimentel, MD, PhD;
Professor of Surgery at the University of Coimbra
Faculty of Medicine, University of Coimbra
Head of the Department of Surgery, Montes Claros Clinic, Coimbra.

Co-supervisor - Fernando Castro-Poças, MD, PhD;
Invited Full Professor with Habilitation, at the ICBAS-UP
Senior Consultant of Gastroenterology, Oporto Hospital Center (CHUPorto)
Outpatient Services Director, Oporto Hospital Center (CHUPorto)

Co-supervisor - Anabela Rocha, MD;
Invited Auxiliar Professor at the ICBAS-UP
Consultant Surgeon, Colorectal Unit, Oporto Hospital Center (CHUPorto)

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DOCTORAL DEGREE IN MEDICINE

SPECIALTY IN MEDICAL SCIENCES

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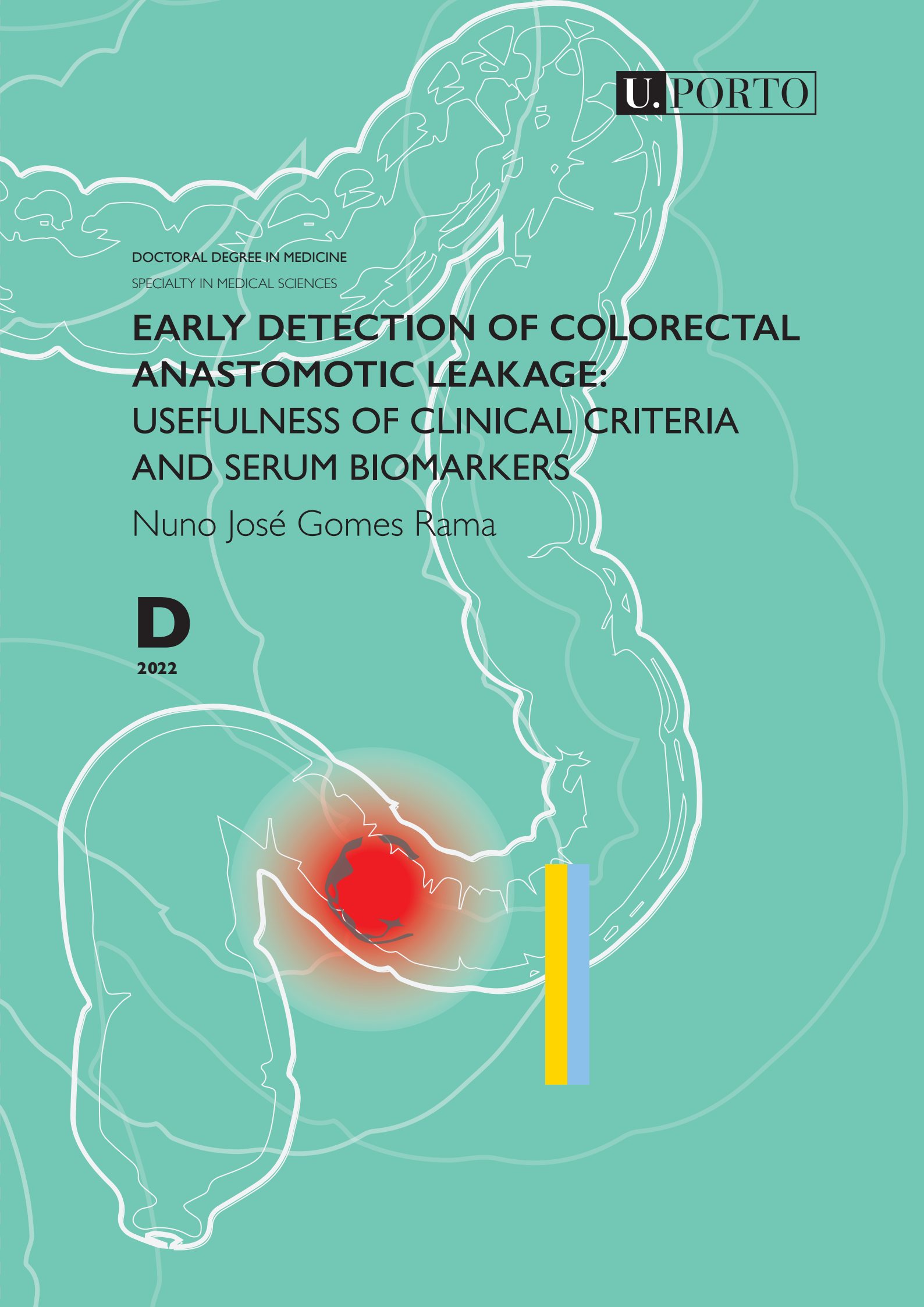

To my father, always there...

To my late mother, wherever she is...

THANKS!

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ACRONYMS AND ABBREVIATIONS

ANN - Artificial Neural Network

AR - Anterior Resection

ASA - American Society of Anaesthesiologists' classification

AUC - Area Under Curve - Receiver Operating Characteristics (ROC)

BMI - Body Mass Index

CAL - Colorectal Anastomotic Leakage

CAR - CRP / Albumin Ratio

CCI - Charlson Comorbidity Index

CHL - Leiria Hospitalar Center

CI - Confidence Intervals

CLP - Calprotectin

CMA - Cost Minimization Analysis

CRP - C-Reactive Protein

CT - Computed Tomography

CCT - Contrast CT scan

DFS - Disease-Free Survival

DULK - DUtch LeaKage score

DM - Distant Metastasis

DRG - Diagnosis-Related Group

DIACOLE - DIagnostic sCOre LEakage

EAL - Early Anastomotic Leakage

ECC - Eosinophils Cell Count

E-CRALL - Early ColoRectAL Leakage

EDTA - EthyleneDiamine Tetracetic Acid

EQ-5D-5L - Five level version of the EuroQoL 5 dimensions (EQ-5D) instrument

ERAS - Enhanced Recovery After Surgery

ESCP - European Society of Coloproctology

FTR - Failure-to-Rescue

HR - Heart Rate

IASGO - International Association of Surgeons, Gastroenterologists and Oncologists

IL - Interleukin

ISREC - International Study Group of Rectal Cancer

LAL - Late Anastomotic Leakage

LASSO - Least squAres Shrinkage and Selection Operator

LOHS - Lenght Of Hospital Stay

LR - Local Recurrence

MD - Mean Differences

MMP - Matrix MetalloProteinases

NLR - Negative Likelihood Ratio

NPV - Negative Predictive Value

OR - Odds Ratio

OS - Overall Survival

PCT - Procalcitonin

PICOS - Population, Intervention, Comparison, Outcomes and Study

ACRONYMS AND ABBREVIATIONS (cont.)

PLR - Positive Likelihood Ratio

POD - Post-operative Day

PPV - Positive Predictive Value

QUADAS - Quality Assessment of Diagnostic Accuracy Studies

RCT - Randomized Control Trial

RR - Respiratory Rate

SD - Standard Deviation

SIRS - Systemic Inflammatory Response Syndrome

SMD - Standardised Mean Differences

SOFA - Sequential Organ Failure Assessment

SP - Specificity

SR - Systematic Review

SS - Sensitivity

SSI - Surgical Site Infections

TNF - Tumor Necrosis Factor

VAS - Visual Analogue Scale

WBC - White Blood Cells

WSCE - Water Soluble Contrast Enema

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OUTLINE

This Thesis comprises a Summary, six Chapters and an Addendum as outlined below.

Summary - a brief description of the Thesis is included.

Chapter I - the rational and motivation underlying this Thesis are presented. Then, in the background, the main drivers for this work and the diagnostic approaches for colorectal anastomotic leakage (CAL) are reviewed. Finally, the research questions, objectives, and specific hypothesis within the scope of this research, are described.

Chapter II - a retrospective review of CAL incidence, diagnostic criteria, morbidity, and mortality, of the local Colorectal Division are presented. A monocentric retrospective study was designed, including all consecutive patients who underwent surgery with a colorectal anastomosis for colorectal cancer, over a 4-year period, before starting the prospective study.

Chapter III - one original article, a systematic review with meta-analysis, to evaluate the usefulness of inflammatory biomarkers to predict CAL after surgery, is included.

Chapter IV - based on two original papers, the methods, and results of the monocentric prospective study, including the E-CRALL score development and an integrated discussion of early diagnosis of CAL, are described.

Chapter V - a critical appraisal of all papers included in this Thesis, is presented, highlighting the implications of the results in daily clinical practice.

Chapter VI - potential new lines for additional research, including the new plan for a multicentric prospective study, are discussed.

Addendum - original published papers, supporting this Thesis, are gathered.

SUMMARY

BACKGROUND

Anastomotic leakage is one of the most dreaded complications following colorectal surgery, with an incidence that can be as high as 27%. It represents a significant burden for both patients and surgeons. This event is associated with increased morbidity, mortality, and healthcare costs. Moreover, it is worth mentioning the negative impact on the patient's quality of life. Nonspecific signs and symptoms often precede the acute and rapid clinical deterioration of a patient with colorectal anastomotic leakage (CAL). Late diagnosis and management increase the likelihood of an undesirable outcome. Therefore, its early diagnosis is crucial to reduce clinical consequences and costs.

Currently, there is a modest predictive ability of surgeons to identify patients at risk of CAL development. At a pre-operative stage, certain factors are associated with increased risk of CAL, such as male gender, age, smoking, neoadjuvant radiotherapy, obesity, location of tumour and immunosuppression. The surgeon should not only optimize patients before surgery, but also select those who would benefit from a diverting or a definitive stoma. Furthermore, some intra-operative factors were associated with higher CAL rates, namely surgical technical aspects,

RESUMO

INTRODUÇÃO

A deiscência anastomótica é uma das complicações mais temidas após a cirurgia colorectal, com uma incidência que pode atingir os 27%. Representa uma sobrecarga significativa para os doentes e cirurgiões. Este evento associa-se ao aumento da morbilidade, mortalidade e dos custos com os cuidados de saúde. Além disso, importa mencionar o seu impacto negativo sobre a qualidade de vida. Frequentemente, sinais e sintomas inespecíficos precedem a deterioração clínica aguda e rápida do doente que desenvolve deiscência anastomótica colorectal (CAL). O diagnóstico e a terapêutica tardios aumentam a probabilidade de um resultado indesejável. Por conseguinte, o seu diagnóstico precoce é crucial para reduzir as consequências e custos clínicos.

Atualmente, existe uma modesta capacidade de prever e identificar doentes em risco de desenvolvimento de CAL. Numa fase pré-operatória, certos fatores, como o sexo masculino, idade, tabagismo, radioterapia neoadjuvante, obesidade, localização do tumor e estado de imunossupressão, estão associados ao aumento do risco de CAL. O cirurgião deve, não só, otimizar os doentes preoperatoriamente, mas também selecionar aqueles que beneficiem de um

location of anastomosis, peritoneal contamination, lengthy procedures, significant blood losses and transfusion, among others.

Several biomarkers were proposed for early detection of post-operative septic complications, including colorectal anastomotic failure. Eosinopenia, widely available and at low cost, has been proposed as a useful biomarker to identify several sepsis-related conditions, and to distinguish that from other causes of systemic inflammatory response syndrome (SIRS). Plasmatic C-reactive protein (CRP), an acute phase liver protein, has shown to have a strong correlation with intrabdominal post-operative complications. This biomarker is reliable for SIRS secondary to surgery, increasing subsequently to surgical injury for up to 72 hours, and decreasing afterwards. In patients with post-operative complications, CRP levels remain high. Serum CRP is the most widely studied biomarker for CAL diagnosis. Some authors highlighted the usefulness of plasmatic procalcitonin (PCT) as an earlier, more sensitive, and reliable marker of CAL, even before clinical symptoms appear. Additionally, serum PCT and CRP demonstrated a good negative predictive value for CAL, enabling a safe and early discharge after colorectal surgery. Plasmatic calprotectin (CLP) has been suggested

estoma de proteção ou definitivo. Além disso, alguns fatores intraoperatórios foram associados a taxas de CAL mais elevadas, nomeadamente aspetos técnicos, localização da anastomose, existência de contaminação peritoneal, procedimentos demorados, perdas significativas de sangue e necessidade de transfusão, entre outros.

Vários biomarcadores foram propostos para a deteção precoce de complicações sépticas pós-operatórias, incluindo a CAL. A eosinopenia, amplamente disponível e de baixo custo, foi proposta como um biomarcador útil para identificar várias condições relacionadas com a sepsis, e para a distinção de outras causas da síndroma de resposta inflamatória sistémica (SIRS). A proteína C Reativa (CRP), uma proteína hepática de fase aguda, mostrou ter uma forte correlação com o desenvolvimento de complicações intrabdominais pós-operatórias. Este biomarcador é fiável para sinalizar a SIRS secundária à cirurgia, aumentando subsequentemente à agressão cirúrgica até às 72 horas, diminuindo posteriormente. Em doentes que desenvolvem complicações pós-operatórias, os níveis séricos de CRP permanecem elevados. O valor sérico da CRP é o biomarcador mais estudado para o diagnóstico de CAL. Alguns autores salientaram a utilidade da procalcitonina (PCT) plasmática como biomarcador

as an interesting early biomarker for amplified inflammatory response, in major abdominal catastrophes. Few studies investigated this biomarker as predictor for CAL, and Reisinger *et al.* have shown that CLP was even superior to CRP in CAL detection, with a high predictive effect. The best diagnostic accuracy was obtained when CRP and CLP plasma levels were combined on the third post-operative day (POD). In daily clinical practice some scores were developed, as the Dutch leakage (DULK) and DIACOLE score, as warning tools for CAL, and high scores trigger additional imaging or re-operation of patients. These scores aimed to reduce the delay in diagnosis and consequently mortality correlated with CAL.

precoce, mais sensível e fiável de CAL, mesmo antes do aparecimento dos sintomas clínicos. Além disso, as PCT e CRP séricas demonstraram um bom valor preditivo negativo para CAL, permitindo uma alta segura e precoce após a cirurgia colorectal. Em quadros sépticos abdominais, a calprotectina (CLP) plasmática pode ser um biomarcador precoce promissor da resposta inflamatória amplificada. À data, poucos estudos investigaram este biomarcador como preditor de CAL, tendo Reisinger *et al.* demostrado a superioridade da CLP, em relação à CRP, na deteção de CAL, com um elevado valor preditivo. A melhor acuidade de diagnóstico foi obtida quando os níveis de CRP e CLP no plasma foram combinados no terceiro dia pós-operatório (POD). Na prática clínica diária foram desenvolvidos alguns sistemas de pontuação, como sejam o DULK e o DIACOLE, com o objetivo de identificar doentes com CAL. Pontuações elevadas determinam investigação imagiológica complementar ou re-intervenção dos doentes. Estes sistemas de pontuação visam reduzir o atraso no diagnóstico e a mortalidade correlacionada com a CAL.

AIM

The assumption for this research project is the usefulness of post-operative biomarkers monitoring to early detect

OBJETIVO

O objetivo desta investigação é determinar a utilidade da monitorização dos biomarcadores pós-operatórios

a CAL, and therefore reduce the time to its diagnostic.

This Thesis aims to determine specific indicators for timely identification of patients who develop CAL. First, accuracy and predictive values of clinical criteria are presented. Second, predictive effect of plasma biomarkers (WBC, Eosinophils Cell Count - ECC, CRP, PCT and CLP) are determined. Third, optimized cut-off levels of CRP, PCT and CLP for patients discharging are defined. Fourth, a decision model, warning score for early CAL detection, is developed. Finally, a cost-minimization analysis to assess the economic impact of CAL is performed.

Before the prospective observational study, which represents the core work of this Thesis, a retrospective study including patients who underwent colorectal resection, over four years (2013-2016), in Colorectal Division of the local Surgical Department will be presented. The three main objectives of the retrospective study were estimation of CAL rate, assessment of diagnostic criteria of CAL, and evaluation of short-term and long-term outcomes. Afterwards, a systematic review and meta-analysis following PRISMA guidelines will be displayed, aiming to assess the added value of the serum biomarkers CRP, PCT, CLP and white blood cells (WBC) for the early detection of anastomotic leakage after colorectal surgery.

para detetar precocemente uma CAL, e deste modo reduzir o tempo para o seu diagnóstico.

Em primeiro lugar, são estimadas acuidade e capacidade preditiva dos critérios clínicos. Segundo, é estimada a capacidade preditiva dos biomarcadores plasmáticos (Contagem de leucócitos - WBC, contagem de eosinófilos - ECC, CRP, PCT e CLP). Terceiro, são definidos níveis séricos otimizados de CRP, PCT e CLP para a alta clínica. Quarto, é desenvolvido um modelo de decisão, sob a forma de sistema de pontuação de alarme, para a deteção precoce de CAL. Finalmente, é realizada uma análise de minimização de custos para avaliar o impacto económico da CAL.

Antes de desenvolver o estudo prospetivo observacional, tema central desta tese, será apresentado um estudo retrospetivo que inclui doentes submetidos a ressecção colorectal, durante um período de quatro anos (2013-2016), na Unidade de Cirurgia Colorectal local. Os três objetivos principais foram a estimativa da taxa de deiscência anastomótica, a avaliação dos critérios de diagnóstico utilizados e a avaliação dos resultados a curto e longo prazo. Posteriormente, será apresentada uma revisão sistemática com meta-análise, segundo as diretrizes PRISMA, com o objetivo de avaliar a mais-valia dos biomarcadores séricos CRP, PCT, CLP e leucócitos (WBC) para a deteção

METHODS

A prospective monocentric observational study was conducted including patients who underwent colorectal resection with anastomosis, from March 2017 to August 2019. Patients were divided into three groups: G1 - no complications; G2 - complications not related to CAL; and G3 - CAL. Five biomarkers were measured and analysed during the first five PODs: WBC, ECC, CRP, CLP, and PCT. Clinical criteria such as abdominal pain and clinical condition were also assessed. The correlation between biomarkers and CAL was evaluated. Receiver operating characteristic (ROC) curve analysis was used to compare the accuracy of these biomarkers as predictors of CAL, and the area under the receiver operating characteristic curve (AUC), specificity (SP), sensitivity (SS), positive predictive value (PPV), and negative predictive value (NPV) during this period were estimated.

Early ColoRectAL Leakage (E-CRALL) score, a warning tool for CAL was designed, based on variables from the prospective study dataset. All the potential selected variables were weighted using shrinkage methods and a lasso-LOGIT technique was used to build the score. Sensitivity, SP, NPV and PPV, AUC and discrimination

precoce de deiscência anastomótica após cirurgia colorectal.

MÉTODOS

Foi realizado um estudo de observação monocêntrico prospetivo, incluindo os doentes submetidos a ressecção colorectal com anastomose, de Março de 2017 a Agosto de 2019. Os doentes foram divididos em três grupos: G1 - sem complicações; G2 - complicações não relacionadas com CAL; e G3 – que desenvolveram CAL. Durante os primeiros cinco dias do período pós-operatório foram medidos e analisados cinco biomarcadores: WBC, ECC, CRP, CLP e PCT. Foram também avaliados critérios clínicos como a dor abdominal e o estado clínico. De igual modo, foi avaliada a correlação entre os biomarcadores e o desenvolvimento de CAL. A análise da curva ROC (Receiver Operating Characteristic) foi utilizada para comparar a acuidade destes biomarcadores como preditores de CAL, e a área sob a curva ROC (AUC), especificidade (SP), sensibilidade (SS), valor preditivo positivo (PPV), e valor preditivo negativo (NPV) durante este período foram estimados.

O sistema de pontuação E-CRALL (Early ColoRectAL Leakage), uma ferramenta de alarme para CAL, foi concebido com base em variáveis do conjunto de dados do estudo prospetivo. Todas as

threshold of E-CRALL score were estimated. A Decision Tree model to simulate effects of E-CRALL score adoption was developed, selecting values for transition probabilities and other parameters of the data from the prospective cohort population. A cost minimization analysis (CMA) was performed to compare the standard clinical practice with the test setting (with E-CRALL score adoption).

variáveis potenciais selecionadas foram ponderadas utilizando métodos de contração, sendo utilizada uma técnica de lasso-LOGIT para construir o sistema de pontuação. A sensibilidade, SP, NPV e PPV, AUC e o limiar de discriminação do sistema de pontuação E-CRALL foram estimados. Para simular os efeitos da adoção do sistema de pontuação E-CRALL, foi desenvolvido um modelo de árvore de decisão, selecionando valores para as probabilidades de transição e outros parâmetros, a partir dos dados da população da coorte prospetiva. Foi realizada uma análise de minimização de custos para comparar a prática clínica corrente com a resultante da aplicação do sistema E-CRALL (teste).

RESULTS

Twenty-five out of 396 patients developed CAL (6.3%), and the mean time for diagnosis was 9.0±6.8 days. Some operative characteristics such as surgical approach, blood loss, intra-operative complications, and duration of the procedure were notably related to the development of CAL. The length of hospital stay was markedly higher in the group that developed CAL (median of 21 vs. 13 and 7 days). For abdominal pain, the best predictive performance was achieved on POD4 and 5, with the largest AUC of 0.84 on POD4. A worsening of

RESULTADOS

Vinte e cinco dos 396 pacientes desenvolveram CAL (6.3%), sendo o tempo médio para o diagnóstico de 9.0±6.8 dias. Algumas características operatórias tais como abordagem cirúrgica, perda de sangue, complicações intraoperatórias, e duração do procedimento, estiveram relacionadas com o desenvolvimento da CAL. A duração do internamento hospitalar foi significativamente mais elevada no grupo que desenvolveu CAL (mediana de 21 vs. 13 e 7 dias). Para a dor abdominal, a melhor capacidade preditiva foi alcançada ao POD4 e 5, com o maior AUC de 0.84

clinical condition was associated with the diagnosis of CAL, presenting a higher predictive effect on POD5, with an AUC of 0.9. WBC and ECC showed a better predictive effect on POD5 (AUC=0.62 and 0.7, respectively). Those markers also presented a high NPV (94%-98%). PCT had the best predictive effect on POD5 (AUC=0.61), although presenting low accuracy. However, this biomarker revealed a high NPV on POD3, 4, and 5 (96%, 95%, and 96%, respectively). The mean CRP value on POD5 was significantly higher in the group that developed CAL compared with the group without complications (195.5±139.9 mg/L vs. 59.5±43.4 mg/L, P<0.00001). On POD5, CRP had a NPV of 98%. The mean CLP value on POD3 was significantly higher in G3 compared with G1 (5.26±3.58 µg/mL vs. 11.52±6.81 µg/mL, P<0.00005).

On POD3, E-CRALL score with a discriminant threshold of 5.51, had a SS, SP, NPV and PPV of 85.7, 66.1, 98.7 and 13.8%, respectively. On POD5, if a threshold of 8.29 was chosen, 87.4% of anastomotic failures were identified. The predictive ability of E-CRALL warning score was estimated, with an AUC from POD2 to POD5 of 0.75, 0.82, 0.84 and 0.95, respectively. Time to CAL diagnosis increased over time, being higher on POD5 (6.4 days). The best time saving was obtained on POD3, with a 5.2-day reduction compared with the baseline

em POD4. Um agravamento do estado clínico foi associado ao diagnóstico de CAL, apresentando um maior efeito preditivo ao POD5, com uma AUC de 0.9. O WBC e o ECC mostraram um melhor efeito preditivo no POD5 (AUC=0.62 e 0.7, respetivamente). Estes marcadores também apresentaram um elevado NPV (94%-98%). A PCT obteve o melhor efeito preditivo ao POD5 (AUC=0,61), embora apresentando comparativamente, uma baixa acuidade. Contudo, este biomarcador revelou um elevado NPV aos POD3, 4, e 5 (96%, 95%, e 96%, respetivamente). O valor médio da CRP em POD5 foi significativamente mais elevado no grupo que desenvolveu CAL em comparação com o grupo sem complicações (195.5±139.9 mg/L vs. 59.5±43.4 mg/L, P<0,00001). Ao POD5, a CRP apresentou um NPV de 98%. O valor médio da CLP ao POD3 foi significativamente mais elevado no G3 em comparação com G1 (5.26±3.58 µg/mL vs. 11.52±6.81 µg/mL, P<0,00005).

Ao POD3, os sistema de pontuação E-CRALL, para um limiar discriminante de 5.51, teve um SS, SP, NPV e PPV de 85.7, 66.1, 98.7 e 13.8%, respetivamente. Ao POD5, para um limiar de 8.29, foram identificados 87.4% das deiscências anastomóticas. A capacidade de previsão do sistema de alarme E-CRALL foi estimada, com uma AUC de POD2 a POD5 de 0.75, 0.82, 0.84 e 0.95, respetivamente. O tempo para o diagnóstico de CAL aumentou ao longo

results. In the prospective study, index admission comprehensive costs were markedly higher (286.3%) for CAL patients, in comparison with those without CAL [€ 9,096 vs. € 3,177 (p < 0.0001)]. After applying the E-CRALL score, episode comprehensive costs were markedly higher (425.2%) for CAL patients, in comparison with those without CAL [€ 7,876.36 vs. € 1,852.57 (p < 0.0001)]. In an overall perspective, E-CRALL score use was associated with cost savings of € 508,505.44, most of them (93.8%) at expense of non-CAL patient's savings.

do tempo, sendo mais elevado ao POD5 (6.4 dias). A economia de tempo para o diagnóstico foi otimizada com a aplicação do sistema ECRALL ao POD3, com uma redução de 5.2 dias, em comparação com os resultados da coorte prospetiva. Nesta coorte, os custos compreensivos estimados foram marcadamente mais elevados (286.3%) nos doentes que desenvolveram CAL, em comparação com os que não desenvolveram esta complicação [9,096 euros vs. 3,177 euros (p < 0.0001)]. Após a aplicação do sistema de pontuação E-CRALL, os custos compreensivos por episódio foram significativamente mais elevados (425.2%) para os doentes com CAL, em comparação com os doente sem CAL [7,876.36 euros vs. 1,852.57 euros (p < 0.0001)]. Numa perspetiva global, a utilização da pontuação E-CRALL foi associada à redução de custos de 508,505.44 euros, a maioria deles (93.8%) resultante da poupança de recursos nos pacientes que não desenvolveram CAL.

CONCLUSION

This Thesis corroborates the added value of clinical criteria as a warning sign of CAL. Plasmatic levels of CLP and CRP have potential as best early CAL predictors, while the systemic levels of WBC, ECC and PCT have limited additional value in this regard. For early discharging, optimized cut-off values of CRP, PCT and CLP were

CONCLUSÃO

Esta Tese corrobora o valor acrescentado dos critérios clínicos no alerta para o desenvolvimento de CAL. Os níveis plasmáticos de CLP e CRP têm potencial como melhores preditores iniciais de CAL, enquanto os níveis sistémicos de WBC, ECC e PCT têm um valor adicional limitado. Para a alta precoce, foram definidos valores otimizados para os

defined.

E-CRALL score was built and showed a high predictive ability, with SS and NPV of 100% after the POD4 and a significant SP (86.6%) on POD5. This study validates, internally, the E-CRALL score for early diagnosis of CAL. E-CRALL score should be included in a standard post-operative surveillance programme of CAL, proposing an early operation in case of dubious or negative imaging, to reduce the time to CAL detection and enabling its prompt management.

In terms of economic burden, this study confirms the negative impact of CAL. Overall costs of colorectal resection increased significantly, almost three times, in patients who developed anastomotic failure. Overall costs decreased in the scenario of E-CRALL adoption, revealing a noteworthy reduction of in-hospital costs, in patients with or without CAL, as compared with standard clinical practice.

biomarcadores séricos CRP, PCT e CLP.

O sistema de pontuação E-CRALL construído mostrou uma elevada capacidade preditiva, com SS e NPV de 100% após o POD4, e uma SP significativa (86.6%) no POD5. Este estudo valida, internamente, a pontuação E-CRALL para o diagnóstico precoce da CAL. A pontuação E-CRALL deve ser incluída num programa sistemático de vigilância pós-operatória da CAL, propondo a re-intervenção precoce em caso de investigação imagiológica inconclusiva, para reduzir o tempo de deteção da CAL, e permitir um tratamento rápido e mais precoce.

Em termos de impacto económico, este estudo confirma o efeito negativo do desenvolvimento da CAL. Os custos globais da ressecção colorectal aumentaram significativamente, cerca de três vezes, em doentes que desenvolveram falência anastomótica. Os custos globais diminuíram após a utilização do sistema de pontuação E-CRALL, revelando uma redução significativa dos custos hospitalares, em doentes com ou sem CAL, em comparação com a prática clínica corrente.

LISTS OF PUBLICATIONS/PRESENTATIONS

The present Thesis includes multiple research that were not only presented in national and international meetings, but also published in peer-reviewed journals. The comprehensive list of publications and presentations is displayed below.

Originated the following publications:

- **Nuno Rama**, Óscar Lourenço, Patrícia Motta Lima, Marlene Lages, Maria Pedro Guarino, Diana Parente, Ricardo Castro, Ana Bento, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

"Development of a warning score for early detection of colorectal anastomotic leakage: Hype or Hope?".

Accepted in *World Journal of Gastrointestinal Surgery* · October 2022.

Author; Chapter IV.

- **Nuno Rama**, Marlene Lages, Maria Pedro Guarino, Óscar Lourenço, Patrícia Motta Lima, Diana Parente, Cândida G. Silva, Ricardo Castro, Ana Bento, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

"Usefulness of serum c-reactive protein and calprotectin for the early detection of colorectal anastomotic leakage: A prospective observational study".

in *World Journal of Gastroenterology* · June 2022. DOI: 10.3748/wjg.v28.i24.2758.

Author; Chapter IV; Appendix 1.

- **Nuno Rama**, Marlene Lages, Cândida G. Silva, Patrícia Mota Lima, Inês Campos Gil, Óscar Lourenço, Maria Pedro Guarino, Pedro Oliveira, Maria Dixe, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

"The usefulness of inflammatory biomarkers to predict anastomotic leakage after colorectal surgery: systematic review and meta-analysis".

in *Surgery, Gastroenterology and Oncology* · September 2022 ; DOI: 10.21614/ sgo-488.

Author; Chapter III; Appendix 2.

- **Nuno Rama**, Diana Parente, Cândida G. Silva, Miguel Neves, Nuno Figueiredo, Paulo Alves, Paulo Clara, Sandra Amado, Óscar Lourenço, Maria Pedro Guarino, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

"Anastomotic Leak in Colorectal Cancer Surgery: From Diagnosis to Management or Failure - A Retrospective Cohort Study".

in *Surgery, Gastroenterology and Oncology* · September 2021; DOI: 10.21614/sgo-26-3-336.

Author; Chapter II; Appendix 3.

- Inês Gil, **Nuno Rama**, Diana Parente, Inês Sales, Paulo Alves, Paulo Clara, Sandra Amado, Miguel Coelho and Vitor Faria.

"Intracorporeal versus Extracorporeal Anastomosis in Laparoscopic Right Colectomy: Short-Term Outcomes".

in *Surgery, Gastroenterology and Oncology* · May 2018; DOI: 10.21614/sgo-23-1-33.

Co-author; Chapter I; Appendix 4.

- Kristensen HØ, Thyø A, Jøssing Emmertsen K, Smart NJ, Pinkney T, Warwick AM, Pang D, Furnée EJB, Verkuijl SJ, **Nuno Rama**, Domingos H, Maciel J, Solis-Peña A, Espín Basany E, Hidalgo-Pujol M, Biondo S, Sjövall A, Christensen P.

"Translation and international validation of the Colostomy Impact score.".

in *Colorectal Disease* · July 2021; DOI: 10.1111/codi.15635.

Co-author; Chapter I; Appendix 5.

- **Nuno Rama**, P. Ferreira, T. Jull and J. Pimentel.

"Validation of Portuguese version of the Low Anterior Resection Syndrome Score".

in *Journal of Coloproctology* · September 2018; DOI: 10.1016/j.jcol.2018.09.004.

Author; Chapter I; Appendix 6.

- The 2017 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** from Portugal).

"Safety of primary anastomosis following emergency left sided colorectal resection: an international, multi-Center prospective audit".

in *Colorectal Disease* · September 2018; DOI: 10.1111/codi.14373.

Co-author (integrating ESCP collaborating group); Chapter I; Appendix 7.

- The 2017 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** from Portugal).

"**An international multiCenter prospective audit of elective rectal cancer surgery; operative approach versus outcome, including transanal total mesorectal excision (TaTME)"**.

in *Colorectal Disease* · September 2018; DOI: 10.1111/codi.14376.

Co-author (integrating ESCP collaborating group); Chapter I; Appendix 8.

- The 2015 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** from Portugal).

"Predictors for Anastomotic Leak, Post-operative Complications, and Mortality After Right Colectomy for Cancer: Results from an International Snapshot Audit."

in *Diseases of Colon and Rectum* · May 2020; DOI: 10.1097/DCR.0000000000001590.

Co-author (integrating ESCP collaborating group); Chapter I; Appendix 9.

- The 2015 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** from Portugal).

"The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit."

in Colorectal Disease · March 2017; DOI: 10.1111/codi.13646.

Co-author (integrating ESCP collaborating group); Chapter I; Appendix 10.

Originated the following conference presentations:

"Usefulness of serum c-reactive protein and calprotectin for the early detection of colorectal anastomotic leakage: A prospective observational study".

Oral presentation at AECP (Spanish Society of Coloproctology) Annual Meeting - 2019. Valladolid, May 2019.

Poster presentation at EAES (European Association of Endoscopic Surgery) Annual Meeting - 2019. Sevilla, June 2019.

"The usefulness of inflammatory biomarkers to predict anastomotic leakage after colorectal surgery: systematic review and meta-analysis".

Oral presentation at Portuguese Surgical Annual Meeting - 2021, June 2021.

"Anastomotic Leak in Colorectal Cancer Surgery: From Diagnosis to Management or Failure - A Retrospective Cohort Study".

Poster presentation at AECP (Spanish Society of Coloproctology) Annual Meeting - 2019. Valladolid, May 2019.

Oral presentation at EAES (European Association of Endoscopic Surgery) Annual Meeting – 2019. Sevilla, June 2019.

"Intracorporeal versus Extracorporeal Anastomosis in Laparoscopic Right Colectomy: Short-Term Outcomes".

Poster presentation at 25th EAES (European Association of Endoscopic Surgery) Congress; Frankfurt. June 2017.

Oral presentation at 30th anniversary IASGO World Congress - 2018; Moscow. September 2018.

Oral presentation at Advanced Colorectal Course - 2018; Porto. November 2018.

Poster presentation at EFR (European Federation for Colorectal Cancer) Annual Meeting - 2019; Vienna. April 2019.

“Validation of Portuguese version of the Low Anterior Resection Syndrome Score”.

Oral presentation at Champalimaud Foundation Meeting “MIARC - Minimally Invasive Approach to Rectal Cancer 2017”; Lisbon. May 2017.

Oral presentation at EAES (European Association of Endoscopic Surgery) Annual Meeting – 2019. Sevilla, June 2019.

CHAPTER I

INTRODUCTION

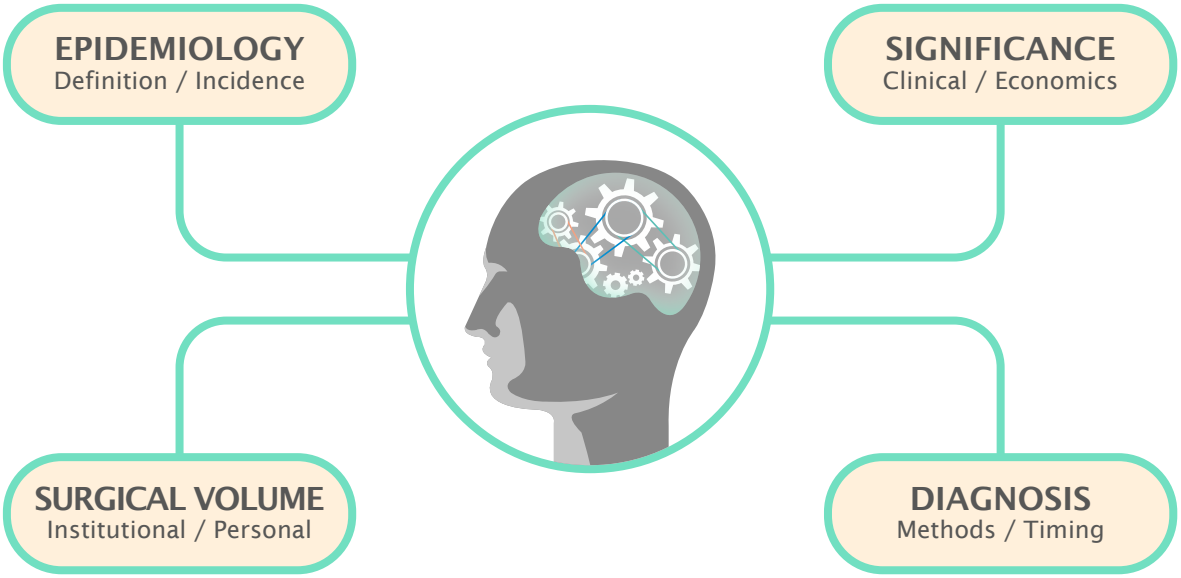
A – RATIONALE

In coloproctology there are many diseases that requires colorectal resection for proper treatment. It is important to restore bowel continuity, performing an anastomosis, whenever possible. The anastomosis can be made using many different methods, determined by several pre- and intra-operative factors. It can be handsewn or mechanic (stapled), by open or minimally invasive (laparoscopic or assisted by computer - robotics) approach. Bowel anastomosis have been reported since the mid-19th century. Jean-François Reybard, from Lion, reported the first bowel suture in 1827, carrying out and reporting, six years later, the first successful primary anastomosis after a sigmoidectomy due to colonic cancer (Hyman 2012). The first CAL-related death was described in the end of the 19th century (1899), and, since then, it is a serious and clinically relevant complication. Many surgical techniques and methods of prevention have been developed in the last decades to reduce the rates of CAL, but, unfortunately, their efficacy has been limited (Ho and Ashour 2010).

The relevance of CAL, which is the main topic of this thesis, emerges, at first, from epidemiology, especially from its significant incidence in surgical wards. Additionally, this fearsome complication is associated with a negative impact in clinical and economic outcomes. Its clinical consequences, which include mortality, morbidities, length of hospital stay (LOHS), readmissions, and long-term results (oncological and functional) are well documented in medical literature. Negative economic impact results from increasing costs with medical resources, thus compromising both reimbursement and financing, and constituting an additional financial burden for institutions. One of the ways to minimize CAL related complications is the early diagnosis, allowing a timely and appropriate management, and consequently, reducing the negative impact on morbidity, mortality, and quality of life. Diagnostic methods are paramount since nonspecific signs and symptoms frequently precede the sudden and quick clinical deterioration of CAL patients. Recently, several methods have been available or are currently under investigation for early CAL diagnosis. However, their effectiveness, usefulness and advantages are yet to be established, despite the growing number of papers recently published in the field. Finally, institutional, and personal surgical volume allowed us to undertake a prospective observational study, with the aim to timely detect CAL. *Centro Hospitalar de Leiria* (CHL - Leiria Hospital Center) serves a population of approximately 400,000 inhabitants, has a colorectal division with dedicated and skilled surgeons, as well as a significant surgical volume which allows for clinical research of this scope. **Figure 1** shows the main reasons that support

the rationale of this thesis, which are briefly developed ahead.

Figure 1. Rationale of this Research Thesis



B – BACKGROUND

In this section, a general introduction, will be reviewed the key drivers of this Thesis, and a short update of most relevant CAL diagnostic methods will be undertaken.

1 - DEFINITION

Anastomotic leakage is one of the most serious and feared complications in coloproctology. Its precise definition is still the subject of some controversy. In purely semantic terms, it is the "natural separation or opening" of a "surgical communication between two bowel segments" (Editora 2021; Porto Editora 2021). For decades, many CAL definitions have been proposed (Adams and Papagrigoriadis 2013), but consensus has been proven hard to find (Bruce *et al.* 2001; Dindo *et al.* 2004; Rahbari *et al.* 2010).

In 2004, in the first publication that reviewed the different definitions of CAL, Bruce *et al.* selected 49 studies, 29 of which presented specific definitions. This systematic review showed the need for a consensual definition to improve the investigation in this regard, proposing a set of clinical and imaging criteria correlated with the

type of CAL management. Six years later, a seminal publication by Rahbari *et al.* proposed a definition and classification of CAL after proctectomy developed by the International Study Group of Rectal Cancer (ISREC) (Rahbari, *et al.* 2010).The authors proposed a consensual definition, in which, CAL was a communication between intra- and extra-luminal spaces due to a defect in the integrity of the bowel. Presence of pelvic abscesses near the anastomosis was also considered to be a CAL (Rahbari, *et al.* 2010). A three-degree (A, B, and C) clinical classification, with impact on management, was also proposed. An anastomotic failure type "A" was considered generally sub-clinical, and would not require any active therapeutic intervention; type "B" required active management (antibiotic therapy, image-guided drainage, or transanal drainage), with no need for further re-operations; type "C" required further re-operations, by definition (Rahbari, *et al.* 2010).

At the beginning of the last decade, there was still no consensus in the definition of CAL. A proof of that was a manuscript of Adams *et al.* which aimed to evaluate the degree of agreement about the definition of CAL among colorectal surgeons who, in 2011, were members of the Association of Coloproctology of Great Britain and Ireland (ACPGBI) (Adams and Papagrigoriadis 2013). This survey showed a significant level of disagreement between colorectal surgeons, especially regarding type B of the ISREC definition. The main limitation of this research was the moderate response rate (26.4%) and its potential interpretation bias. In 2017, a publication by van Rooijen *et al.* attempted to establish the level of consensus between Dutch and Chinese colorectal surgeons (van Rooijen *et al.* 2017). In the general CAL definition category, the authors could only find consensus (rate of agreement higher than 80%) in the item " extravasation of contrast on enema". Among Dutch surgeons, there was also agreement on the item "radiological collection requiring percutaneous drainage" and "necrosis of the anastomosis visible upon re-intervention", with rates of agreement of 85 and 86%, respectively (van Rooijen, *et al.* 2017). The authors suggested setting up a Delphi panel, including colorectal surgeons worldwide, to establish a widely accepted consensual definition of CAL. This challenge was accepted by six Italian scientific societies focused on coloproctology, which conducted a modified Delphi panel in three stages, from April to May 2019, in order to establish an Italian consensual definition of CAL (Spinelli *et al.* 2020). Those consensuses were in line with the previous definition proposed by Rahbari *et al.*, but a more comprehensive and precise definition, including imaging findings, were not unanimous.

Finally, a panel of international experts recently published a similar study, aiming

to reach consensus on the definition of CAL using a modified Delphi method (van Helsdingen *et al.* 2020). This standardized the definition of CAL, considering four different categories: clinical, laboratory, imaging, and operative findings. Consensual clinical parameters included tachycardia, clinical deterioration, abdominal pain, discharge from abdominal drain, discharge from rectum, rectovaginal fistula and anastomotic defect detected by digital rectal examination. Additionally, an increase in plasma CRP or its combination with leukocytosis should raise the suspicion of CAL. Likewise, extravasation of endoluminal water soluble contrast, collection around the anastomosis, presacral abscess near anastomosis, perianastomotic air and intra-abdominal free air were clear signs of CAL on CT-scan imaging. Furthermore, indicative re-operative findings of CAL were evidence of necrosis of anastomosis or of blind loop, signs of peritonitis and dehiscence of anastomosis (van Helsdingen, *et al.* 2020).

To summarize, currently, the definition of CAL seems standardized, due to the research of the last two decades. The prospective study mentioned below integrated the consensus-based recommendation for the definition of CAL published by van Helsdingen *et al.* in 2020.

2 - INCIDENCE

The reported incidence of CAL is significant, but extremely variable, depending on several factors, such as the type of study, level of anastomosis, disease nature (benign or malignant), type of procedure (elective or urgent), among others (McDermott *et al.* 2015; Phitayakorn *et al.* 2008). In **Table 1**, adapted from a manuscript published by Phitayakorn *et al.*, the incidence of CAL is shown, as reported in the twenty most relevant studies published in the last century (Phitayakorn, *et al.* 2008). Regarding colonic anastomosis, CAL rates ranged from 0.2 to 4.0%. Concerning rectal anastomosis, the reported rates ranged from 1 to 14%. Two publications did not clearly specify the anatomic segment (colon or rectum), presenting incidences from 8 to 19%.

Table1. Colorectal anastomotic leakage incidence depicted according to anatomic location (studies published before 2000).

Study	Year	Type of study	Nº (Patients)	Colon (%)	Rectum(%)	Colorectal (%)
(Schrock et al. 1973)	1973	Retrospective	1932	2.0	2.0	NA
(Heald and Leicester 1981)	1981	Retrospective	52	NA	NA	19.0
(Brennan et al. 1982)	1982	Prospective	100	NA	NA	8.0
(McGinn et al. 1985)	1985	Prospective	118	NA	8.0	NA
(Beard et al. 1990)	1990	Prospective	143	NA	9.0	NA
(Mealy et al. 1992)	1992	Retrospective	114	NA	5.0	NA
Kracht et al. 1993)	1993	Prospective	454	4.0	NA	NA
(Redmond et al. 1993)	1993	Retrospective	111	NA	3.0	NA
(Karanjia et al. 1994)	1994	Retrospective	276	NA	9.0	NA
(Santos et al. 1994)	1994	Prospective	149	NA	7.0	NA
(Fingerhut et al. 1995)	1995	Prospective	113	NA	7.0	NA
(Sagar et al. 1995)	1995	Prospective	100	NA	7.0	NA
(Hansen et al. 1996)	1996	Retrospective	615	NA	2.0	NA
(Mann et al. 1996)	1996	Prospective	370	NA	3.0	NA
(Golub et al. 1997)	1997	Retrospective	813	1.0	1.0	NA
(Vignali et al. 1997)	1997	Retrospective	1014	NA	3.0	NA
(Dehni et al. 1998)	1998	Retrospective	258	NA	10.0	NA
(Petersen et al. 1998)	1998	Retrospective	467	NA	9.0	NA
(Rullier et al. 1998)	1998	Retrospective	413	NA	14.0	NA
(Watson et al. 1999)	1999	Retrospective	477	0.2	2.0	NA

The incidence of CAL, in the most important studies published after 2000, is presented in Table 2. It gathers 12 prospective and 18 retrospective studies, including a total of 30,562 patients. Only one retrospective study analyzed exclusively colonic anastomotic failure, reporting CAL in 6.4% of 9,333 participants (Krarup *et al.* 2012). Fourteen studies (10 retrospective and 4 prospective) reported colonic anastomotic failure rates ranging from 1.3 to 13.2%. Rectal anastomotic failure rates varied from 1.6 to 27.2%. Eight studies focused exclusively on rectal anastomotic leakage (5 retrospective and 3 prospective), presenting incidence that ranged from 3.5 to 27.2% (Akiyoshi *et al.* 2011; Bell et al. 2003; Marijnen et al. 2002; Marusch *et al.* 2002; Matthiessen, *et al.* 2008; Merkel *et al.* 2001; Welsch *et al.* 2007; Wong and Eu 2005). Eight publications (3 retrospective and 5 prospective), including 5,301 participants did not clearly detail the anatomic level of CAL, with rates ranging from 3.8 to 18% (Alves *et al.* 2002; Buchs, et al. 2008; Garcia-Granero *et al.* 2013; Italian ColoRectal Anastomotic Leakage Study 2020; Lagoutte *et al.* 2012; Petersen, *et al.* 1998; Trencheva *et al.* 2013; Warschkow *et al.* 2011a).

Table 2. Colorectal anastomotic leakage incidence depicted according to anatomic location (studies published after 2000).

Study	Year	Type of study	Nº (Patients)	Colon (%)	Rectum(%)	Colorectal (%)
(Merkel et al. 2001)	2001	Retrospective	814	NA	10.9	NA
(Alves et al. 2002)	2002	Retrospective	707	NA	NA	6.0
(Marijnen et al. 2002)	2002	Prospective	1414	NA	4.0	NA
(Marusch et al. 2002)	2002	Prospective	482	NA	11.0	NA
(Bell et al. 2003)	2003	Retrospective	403	NA	13.0	NA
(Branagan and Finnis 2005)	2005	Retrospective	1834	3.6	6.3	NA
(Wong and Eu 2005)	2005	Retrospective	1066	NA	4.0	NA
(Lipska et al. 2006)	2006	Retrospective	541	2.7	7.4	NA
(Konishi et al. 2006)	2006	Retrospective	391	2.1	4.0	NA
(Hyman et al. 2007)	2007	Retrospective	1223	2.1	4	NA
(Platell et al. 2007)	2007	Retrospective	1562	1.4	4.3	NA
(Matthiessen et al. 2008)	2007	Prospective	33	NA	27.2	NA
(Welsch et al. 2007)	2007	Retrospective	383	NA	5.7	NA
(Buchs et al. 2008)	2008	Prospective	811	NA	NA	3.8
(Frye et al. 2009)	2009	Retrospective	1228	1.7	7.5	NA
(Bellows et al. 2009)	2009	Retrospective	311	9.1	6.2	NA
(Ortega-Deballon et al. 2010)	2010	Prospective	133	12.3	17.9	NA
(Boccola et al. 2011)	2011	Retrospective	1576	5.0	8.8	NA
(Warschkow et al. 2011)	2011	Retrospective	1187	NA	NA	8.0
(Akiyoshi et al. 2011)	2011	Retrospective	363	NA	3.6	NA
(Almeida et al. 2012)	2012	Retrospective	149	13.2	16.2	NA
(Trencheva et al. 2013)	2012	Prospective	616	3.4	7.4	NA
(Krarup et al. 2012)	2012	Retrospective	9333	6.4	NA	NA
(Lagoutte et al. 2012)	2012	Prospective	100	NA	NA	13.0
(Pedersen et al. 2012)	2012	Retrospective	129	NA	NA	18.0
(Garcia-Granero et al. 2013)	2013	Prospective	205	NA	NA	8.3
(Zoran Kostić† and Slavković* 2015)*	2015	Prospective	150	4.2	12.6	NA
(Giaccaglia et al. 2016)	2016	Prospective	504	3.9	8.1	NA
(Pantel et al. 2019)	2019	Retrospective	752	1.9	1.6	NA
(Italian ColoRectal Anastomotic Leakage Study 2020)	2020	Prospective	1546	NA	NA	4.9

3 – SURGICAL VOLUME

Surgical volume and surgeon's skills seem to have impact on the rate of CAL, which has been observed in several studies but remains controversial (2018; Biondo *et al.* 2005; Damen *et al.* 2014; Frasson *et al.* 2015; Gani *et al.* 2017; Hyman *et al.* 2009; Manilich *et al.* 2013; Tang *et al.* 2001). A retrospective study by Hyman *et al.* included 556 patients submitted to colorectal resection with anastomosis and found a CAL rate of 4.9%. The incidence in high-volume surgeons varied from 1.6 to 9.9% ($p<0.01$) and the overall rate of complications ranged from 30.5% to 44% ($p=0.04$). However, authors have concluded that the variability in the rate of CAL in the surgeons is not only a result of their experience, but also from other potentially preventable factors (Hyman, *et al.* 2009). Kelly *et al.* published a meta-analysis to compare short-term and oncologic results after a colorectal resection between residents (under supervision) and specialist surgeons. They included 19 non-randomized observational studies, involving a total of 14,344 colorectal resections (61.7% performed by surgeons). The authors concluded that, in the selected cases, the procedure carried out by residents under supervision was equally adequate and safe (Kelly *et al.* 2014). A similar conclusion was found in three recent studies: the multicentric, prospective, and international snapshot audit, supported by the 2015 European Society of Coloproctology Collaborating Group; the prospective study with 800 patients conducted by García-Granero *et al.*; and the retrospective study using mega-data (N=21,827 patients), published by Gani *et al.* (2018; Gani, *et al.* 2017; García-Granero *et al.* 2017). Association between institutional or individual surgical volume and post-operative results was reviewed and analyzed by Huo et al (Huo *et al.* 2017). Forty-seven studies were included in their systematic review, with a total of 1,122,303 patients and 9,649 surgeons, in 9,877 institutions. The meta-analysis showed a positive relation between the high institutional/individual surgical volume and the short-term (morbidity, namely CAL, and intra-operative and 30-day post-operative mortality) or long-term outcomes (recurrence and overall survival). Best results were noted in the high-volume hospitals with high-volume surgeons, followed by low-volume hospitals with high-volume surgeons. The authors concluded that high-volume institutions and surgeons were associated with better outcomes for colorectal cancer surgery. However, this relationship was non-linear with no clear threshold of effect (Huo, *et al.* 2017).

The Colorectal Division of Centro Hospitalar de Leiria (CHL) performs approximately 250 colorectal resections with anastomosis every year for benign and malignant

diseases. Since 2015, a team of five surgeons is mainly dedicated to colorectal procedures. Institutional outcomes related to colorectal cancer surgery, from 2013 to 2016 are presented in Chapter II (retrospective study). My personal experience is summarized in **Appendix 11**.

4 - CLINICAL SIGNIFICANCE

The clinical impact of CAL is paramount. This complication is associated with an increase of morbidity and mortality, having a harmful effect on oncologic and functional outcomes. In terms of morbidity, there is a high rate of complications, namely infectious, that determine higher LOHS, re-operations and re-admission rates. Several studies confirmed these associations, as the ESCP collaborative studies, in which I collaborated as co-author (**Appendix 7 to 10**). (Gessler *et al.* 2017; group 2017; Hammond *et al.* 2014; Inês Campos Gil and Sandra Amado 2018; Lee *et al.* 2020; Ribeiro Jr *et al.* 2019). In CAL patients, Gessler *et al.* also found superior rates of post-operative complications and need for further re-operations. The difference was not significant for wound infection and pneumonia, but patients that developed CAL, had more severe complications, according to the Clavien-Dindo classification (Dindo, *et al.* 2004). Moreover, LOHS increases significantly in patients with severe comorbidities, as shown by Krarup *et al.* Overall LOHS was 9.7 days (95% CI, 9.5–9.9 days), but for patients with a Charlson Comorbidity Index ≥2, the adjusted increase in LOHS was markedly higher [15.0 days; (95% CI, 13.6–16.4 days); p = 0.001] (Krarup *et al.* 2015).

Mortality rate is superior in patients that developed CAL, regardless of comorbidities. The concept of "*Failure-to-Rescue*" (FTR), introduced by Silber *et al.* in 1992, represents the mortality as consequence of failure in the management of a specific post-operative complication, such as CAL (Almoudaris *et al.* 2011; Silber *et al.* 1992). Gessler *et al.* demonstrated that the occurrence of CAL was associated with a significant increment in the 30 and 90-days mortality [5 vs. 0.6% (p=0.015) and 8.3 vs. 2% (p=0.004), respectively]. In addition, all deaths occurred in patients with malignancy, severe comorbidities, and CAL type C (Gessler, *et al.* 2017). Similar findings were obtained by Krarup *et al.* In this study, 30-days mortality was significantly higher in patients who developed CAL (20.4% vs. 3.9%, p < 0.001), and the presence of CAL was associated with further increases in mortality: Hazard Ratio (HR) of 1.58 (95% CI, 1.00–2.51; p = 0.047). More recently, Ribeiro Jr. *et al.* analyzed the clinical impact

of CAL after proctectomy and observed a negative impact on adjusted mortality for age, gender, diagnosis, and surgical timing. Similar results were also found in the retrospective study conducted by the Colorectal Division of CHL, after-mentioned on Chapter II (**Appendix 3**) (Rama *et al.* 2021).

Several research have investigated the impact of CAL on the oncological outcomes, particularly in local recurrence (LR), distant metastasis (DM), overall survival (OS), and disease-free survival (DFS). There are, at time of writing, at least five systematic reviews with meta-analysis that evaluate the impact of CAL on long-term oncological results (Bashir Mohamed *et al.* 2020; Ha *et al.* 2017; Mirnezami *et al.* 2011; Wang *et al.* 2017; Yang *et al.* 2020). The work by Won Ha *et al.* includes 34 non-randomized studies regarding colorectal resections for cancer. Sixteen were retrospective, while 18 evaluated only patients who underwent proctectomy, adding up to a total of 78,434 patients. Twenty six studies carried out an LR analysis (39,745 patients), finding a higher risk of recurrence in patients who developed CAL [Relative Risk (RR)=1.90; 95% CI 1.48–2.44; I^2 = 78%] (Ha, *et al.* 2017). This association was not found in the study of Mohamed *et al.*, which included 24,446 patients submitted to curative resection for colon cancer. In the cohort of patients with CAL, 7.5% had LR, versus 6% of those without (RR=1.16; 95% CI 0.84-1.59; I^2= 16%; p= 0.36 (Bashir Mohamed, *et al.* 2020). When patients who underwent proctectomy by cancer are analyzed, the CAL increases the risk of LR, as concluded by Yang *et al.* This meta-analysis evaluated 28 studies and a total of 21,883 patients. The cohort with CAL had a higher risk of LR, despite its moderate heterogeneity (OR= 1.93; 95% CI, 1.57-2.38; I^2 = 39%; p < 0.0001) (Yang, *et al.* 2020).

Distant metastatic dissemination showed no statistically significant association with CAL in the meta-analysis carried out by Won Ha *et al.*, that included 11 studies and 10,392 patients (RR = 1.20; 95% CI 0.94–1.53; I^2 = 61%; p=0.15). However, studies that considered colon and rectum cancer separately had different results (Bashir Mohamed, *et al.* 2020; Ha, *et al.* 2017; Yang, *et al.* 2020). Regarding curative colonic resections, adjusted by time of follow-up, four studies were selected, showing a higher risk of DM in the CAL patients (HR=1.45; 95% CI 1.18 – 1.8; I^2= 0%; p=0.0003) (Bashir Mohamed, *et al.* 2020). Regarding curative proctectomy, there was no significant increase in DM in the cohort with CAL, as the meta-analysis by Yang *et al.* have showed. After excluding one heterogeneous study, 9 studies and 7,837 patients remained. They showed no increase of DM in the group of patients that developed CAL (OR= 1.11; 95% CI, 0.92-1.33; I^2= 0%; p = 0.28) (Yang, *et al.* 2020).

Finally, the influence of this complication on the OS and DFS were analyzed. The three meta-analyses above-mentioned concluded that both OS and DFS are higher in patients with colorectal cancer that did not develop CAL after curative resection. The meta-analysis by Won Ha *et al.* showed a negative impact of CAL in both OS (RR=1.36; 95% CI 1.24–1.50; I^2 = 74%; p<0.00001) and DFS (RR=1.40; 95% CI 1.20–1.63; I^2 = 86%; p<0.0001). The strength of the results was confirmed after conducting a sensitivity analysis.

The presence of complications after colorectal surgery is a determinant of gastrointestinal and genital-urinary disfunctions, which may affect quality of life in different ways. It is important to develop tools that can clearly evaluate these disorders. In this regard, the Low Anterior Resection Syndrome (LARS) Score was evaluated in the Portuguese population (Rama *et al.* 2019). It is presented here as **Appendix 6**. I also contributed, as co-author (integrating the ESCP collaborative group) to the validation of a scale to evaluate the impact of a colostomy on the quality of life (Kristensen *et al.* 2021) (**Appendix 5**). Ostomies may result from a decision of covering one anastomosis or an option of CAL management, among others. Often it becomes a "permanent" stoma, for clinical and/or technical reasons (Güenaga *et al.* 2007; Phan *et al.* 2019). Brown *et al.* recently investigated the impact of post-operative complications in long-term quality of life, in patients who underwent curative colorectal procedures. The authors concluded that post-operative complications have a negative influence on long-term quality of life. These patients have a worse score in physical and social function, body image, mobility, self-care, and discomfort due to pain, with a subtle negative effect on mental health and financial condition (Brown *et al.* 2014). In a similar study, Di Cristofaro *et al.* found that the cohort of patients with post-operative complications showed impaired emotional and physical functions after six months, demonstrating its negative psychological impact (Di Cristofaro *et al.* 2014). Similar results were found by other authors. A study by Marinatou *et al.* included 75 patients who underwent low anterior resection for rectal cancer, 25 of whom developed major CAL. The authors also evaluated the impact of CAL on quality of life in the first year, using several validated scales, and demonstrated a negative impact on physical, social, and emotional strength, and a significantly higher rate of stoma-related problems, especially skin complications (Marinatou *et al.* 2014). Recently, Hultberg *et al.* confirmed this tendency. Their study included all patients who underwent anterior resection for rectal malignancy from April 2011 to June 2013, available in the Swedish Colorectal Cancer Registry. Gastrointestinal, urinary, and

sexual functions were investigated, by postal survey two years later. The response rate was 82% (1,180 answers) and 7.5% of patients reported CAL. In CAL group, the rate of permanent stoma, as well as anal incontinence and impaired sexual function, were significantly higher, in opposition to the rate of urinary incontinence, which was reduced (Kverneng Hultberg *et al.* 2020).

5 - ECONOMICAL BURDEN

Besides its serious negative clinical outcomes, CAL is linked with a significant economic and healthcare burden. While it is easy-to-understand that post-operative complications have an expressive impact on in-hospital costs, the economic repercussions of these clinical sequelae remain unclear. Ashraf *et al.* established the costs associated with CAL following anterior resection in colorectal cancer patients. They conducted a prospective monocentric observational cohort study at a colorectal surgery department in Oxford (England). The mean total hospital in-patient cost was £6,233 ± £965 for non-CAL patients and £9,605 ± £6,908 for the 20 patients with leakage ($p = 0.0007$) (Ashraf *et al.* 2013). Similar results were obtained in an American retrospective study, including more than 100,000 colorectal surgical patients. Hammond *et al.* found a significant difference ($p < 0.01$) between mean costs in patients with ($72,905 ± 94,723) and without CAL ($25,005 ± 29,256) (Hammond, *et al.* 2014). In a recent Swiss retrospective study, La Regina *et al.* showed that the cost of patients with CAL was substantially higher (308%) and there was a remarkable difference between mean overall costs: €71,978 (± 41,114) vs. €17,647 (± 6,289), in patients with and without CAL, respectively ($p < 0.01$) (La Regina *et al.* 2019). Coming from a private institution in a middle-income country, Ribeiro Jr. *et al.* showed that the total hospital costs were six times higher ($p=0.002$) for patients with CAL (R$210,105 ± 238,091) when compared to patients without this complication (R$34,270 ± 37,613) (Ribeiro Jr, *et al.* 2019). Recently, on the same subject, Capolupo *et al.* determined that the mean adjusted in-patient cost was significantly higher (108%; $p < 0.001$) for patients with (€14,711) than for those without CAL (€7,089) (Capolupo *et al.* 2021).

All over the world, there is a large discrepancy in the remuneration of healthcare providers, whether public or private. A diagnosis-related group (DRG) is a mixed-case complexity system implemented to categorize patients with similar clinical diagnoses, increasing efficiency in inpatient care and improving transparency in hospital activities. DRG-based payment systems were gradually introduced in many

countries all over the world. In 1984, a project was initiated by the Portuguese Ministry of Health to study the feasibility of this system in Portugal. Five years later, the Portuguese government approved the new hospital financing law which includes a DRG-based structure to be introduced nationwide (Bentes *et al.* 1995). Surgical complications, such as CAL, increase the LOHS, changing both the level of healthcare and DRG selection. A specific DRG for a given patient is produced based on their severity level. Some studies showed the significant increase of overall healthcare costs in patients with CAL and the unsatisfactory DRG-based reimbursement. Ashraf *et al.* found a deficit in remuneration in a significant proportion of CAL patients, because local remuneration methods failed to identify "additional" costs. This inaccuracy was recognized as a major problem that may lead to a healthcare provider budget deficit (Ashraf, *et al.* 2013).

A retrospective data analysis published by La Regina *et al.*, observed a cost spike in both direct and indirect costs in CAL patients. They estimated an average profit per patient of €542 in the group with no complications, and an average loss per patient of €12,181, in the CAL cohort (La Regina, *et al.* 2019). Thus, the increased resource consumption was not adequately offset by the complication related DRG reimbursement, resulting in institutional net financial losses. In line with this, Capolupo *et al.* estimated that mean losses per patient with CAL (reimbursement minus costs) were €2,041, indicating that hospital reimbursement rates do not cover treatment costs (Capolupo, *et al.* 2021).

In conclusion, CAL leads to a heavy economic burden in colorectal procedures, resulting in a significant utilization of resources and increasing overall cost of both public and private healthcare providers. Remuneration tariffs seem to underestimate the real cost of a 'CAL-complicated' hospital episodes, resulting in a likely hospital financial deficit.

6 - DIAGNOSTIC METHODS

Timely diagnosis of CAL is paramount to limit related morbidity and mortality. Thus, it is important to invest in methods for early identification of a colorectal anastomotic failure. Clinical findings are the key point for diagnosis, albeit many signs and symptoms associated with CAL are hard to distinguish from those of other post-operative septic complications. However, surgeons must be aware for

early subtle clinical findings, which must be complemented by imaging and/or early re-operation. Diagnosis is often delayed due to inadequate or irregular clinical evaluation, or inconclusive imaging (Doeksen *et al.* 2007; Marres *et al.* 2017). As a result, there must be a high level of suspicion for early CAL detection, which influence the therapeutic approach. Next, the different methods available to diagnose CAL are briefly reviewed.

6.1 – CLINICAL FINDINGS

The clinical presentation of CAL is diverse, from the sudden and aggressive sepsis with multisystemic organ failure to an insidious progression, as an extended post-operative ileus. Nonspecific signs and symptoms may anticipate a fast and often abrupt clinical deterioration. However, diagnosis may remain unrecognized, which defines a sub-clinical CAL, and can represent up to 50% of cases, if only the first admission is considered (Nesbakken *et al.* 2005). Under these circumstances, additional imaging, or endoscopy, were necessary to establish the diagnosis, that is often delayed after discharge (Daams *et al.* 2014; Hirst *et al.* 2014; McDermott, *et al.* 2015). In this scenario, a standardized diagnostic approach is challenging.

6.1.1 - Abdominal pain

Abdominal pain may suggest a bothersome post-operative progression after colorectal resection, as an iatrogenic injury or ischemia, among others. (Boström *et al.* 2021; Regenbogen *et al.* 2016; van Boekel *et al.* 2019). This may also suggest that a colorectal anastomosis is not healing properly, resulting from the pathophysiological process of a secondary peritonitis. In this setting, it must be seen as a significant warning sign. Moreover, abdominal pain, may result from an insufficient analgesic policy. As a result, some analgesics are potential risk factors for CAL (Burton *et al.* 2013; Modasi *et al.* 2019; Smith *et al.* 2016). The use of opioids to control acute post-operative pain is frequently associated with ileus, which is a possible subtle clinical manifestation of CAL (Bakker *et al.* 2014; Boström, *et al.* 2021; Frasson *et al.* 2016). Abdominal pain may also be a symptom of other unrelated complications, such as urinary tract infections, pneumonia, or acute urinary retention, among others (Daams, *et al.* 2014; Hayami *et al.* 2019; Tamini *et al.* 2021). This Thesis does not aim to expand the pathophysiological mechanism of CAL, but it is possible to

speculate that even pain itself can contribute for its development. Pain can activate the sympathetic nervous system and the stress-related hormones, thus disturbing the ideal environment for colorectal healing process (Guyton *et al.* 2016; Tennant 2013). Many studies have shown increased survival in patients who underwent ß-blocker therapy after non-cardiac procedures, which is expected due to its cardioprotective properties. This protective effect is probably wider, thus reducing not only the proinflammatory events, but also the risk of post-operative complications (Ahl *et al.* 2020; Boström, *et al.* 2021).

Several studies aimed to describe the clinical picture of CAL (Boström, *et al.* 2021; Nesbakken, *et al.* 2005; Regenbogen, *et al.* 2016; Sutton *et al.* 2004; van Boekel, *et al.* 2019). Sutton *et al.* published a retrospective study including 379 patients who underwent curative colorectal cancer resection. Clinical CAL rate was 6.0% (n=22), and seven patients (32%) showed unequivocal signs of peritonitis, with abdominal pain, hyperthermia, and leukocytosis. These clinical suspicions were confirmed by water-soluble contrast enema (WSCE) and re-operation, if appropriate (Sutton, *et al.* 2004). Further ahead, will be discussed other non-abdominal symptoms that may suggest CAL. In 2005, Nesbakken *et al.* published a prospective study that analyzed the incidence of CAL, comparing the diagnostic accuracy of clinical symptoms, WSCE, and CT imaging. From the 56 consecutive patients included, five (9%) had a clinical CAL and other five had a subclinical CAL, during the first admission. The authors have estimated SS, SP, PPV, NPV and accuracy of clinical signs, that was 20, 100, 100, 85, and 86%, respectively (Nesbakken, *et al.* 2005).

Pain characteristics, particularly its intensity, were evaluated by some authors and correlated with clinical results. Regenbogen *et al.* published a recent study including 7,221 patients from 52 hospitals in the Michigan Surgical Quality Collaborative network, who underwent colorectal resections. Post-operative complications (20.3% vs. 26.4%; $p<0.001$) and re-admissions (11.3% vs. 16.2%; $p=0,01$) were less common in hospitals with lower levels of pain. As previously mentioned, this may result from the activation of sympathetic nervous system and stress-related hormones, as physiological response to surgical injury and pain (Regenbogen, *et al.* 2016). Van Boekel *et al.*, in 2017, published another study with 1,014 patients, achieving similar conclusions - that is, patients who felt more pain in the first POD had a higher rate of post-operative complications (van Boekel, *et al.* 2019).

A retrospective and observational study by Boström *et al.* aimed to determine whether early post-operative pain is an independent symptom for CAL. The authors included 3,084 patients who underwent colorectal resection with anastomosis from 2014 to 2017. A total of 189 patients developed CAL, and 121 required re-interventions for this reason. In a multivariate analysis, moderate or severe post-operative pain [Visual Analogue Scale (VAS) ≥ 4] was associated with a higher risk of CAL [OR de 1.73 (1.22–2.46), $p < 0.01$] or re-intervention [OR de 2.13 (1.37–3.30), $p < 0.01$]. Pain worsening (1 point higher in the VAS score) was also related with CAL diagnoses [OR de 1.11 (1.05–1.17), $p < 0.01$]. The authors found that increased abdominal pain is an independent marker of CAL, suggesting the need for additional diagnostic methods to confirm this diagnosis (Boström, *et al.* 2021).

Abdominal pain is one of the symptoms included on the DULK score and its modified version (see below), a scoring system developed from clinical predictors of CAL (den Dulk *et al.* 2009; den Dulk *et al.* 2013; Martin *et al.* 2015). Depending on the clinical picture, a score of four or more recommend a careful clinical re-assessment, laboratory monitoring in the next 12 hours, and a CT scan with WSCE (den Dulk, *et al.* 2009). The "new" modified DULK score only includes four criteria, one of them was abdominal pain in a non-incision location, with a score of 1 out of 4 (den Dulk, *et al.* 2013). The modified DULK score is positive if equal to one point or higher, with an SS, SP, NPV and accuracy of 97.0%, 56.8%, 99.5%, and 97%, respectively (den Dulk, *et al.* 2013). Further on, this scoring system will be discussed with more detail; here, it is important to highlight the relevance of abdominal pain as a predictor for CAL.

6.1.2 - Systemic Inflammatory Response Syndrome (SIRS)

The American College of Chest Physicians and the Society of Critical Care Medicine organized, in 1991, a conference to reach consensus about inflammatory response to infection, sepsis and related concepts (Bone *et al.* 1992). Roger Bone was the responsible for this initiative. He published the consensus document in 1992, establishing new definitions that have been used internationally ever since - specifically, the definitions of SIRS, sepsis, severe sepsis, and septic shock. SIRS was defined as the set of systemic manifestations that represent the systemic response of the body to inflammation, expressing the concept of serial reactions to injury, depending on the individual characteristics, regardless of the original cause. It is defined as the presence of two or more of the following signs: body temperature above 38°C or

below 36°, heart rate above 90/min, hyperventilation [respiratory rate (RR) above 20/minute or PaCO2 below 32 mmHg), with a serum WBC higher than 12,000 cells/μL or lower than 4,000/μL (Bone, *et al.* 1992). The usefulness of a concept of SIRS lies in its sensitivity to identify early responses, timely warning clinicians for the possibility of sepsis (in this context, secondary to CAL), with risky progression. Therefore, it promotes early evaluation and adequate monitoring of the patient. The main disadvantage is the lack of specificity, since most patients with SIRS criteria do not develop severe sepsis (Bone, *et al.* 1992).

Ten years later, in December 2001, there was a second conference, where the participants recognized the limitations of SIRS definition (high SS and low SP). They also agreed in extending the criteria that were part of the concept of SIRS/sepsis. Alterations on consciousness, presence of significant edema (or a positive fluid balance above 20mL/Kg), and hyperglycemia (higher than 120mg/dl, without previous diabetes) were included in the nonspecific variables (Levy *et al.* 2003). In a third consensus, the limitations of previous definitions were recognized, a new concept of sepsis was presented and a fast-scoring system, called quick SOFA (Sequential [Sepsis-related] Organ Failure Assessment) was introduced. This score would allow prompt identification of suspected cases of infection that had the risk of adversely evolving to sepsis. Patients are at risk if they presented at least two out of the following criteria: RR ≥ 22/min, alterations on consciousness (Glasgow Coma Scale ≤ 13), and systolic arterial pressure ≤ 100mmHg (Singer *et al.* 2016). To summarize, the recent revision of definitions and clinical criteria allows for more consistency on the epidemiological studies and clinical research, facilitating early recognition and diligent management of patients at risk of sepsis. Some of the criteria that were evaluated in patients with CAL are presented below.

6.1.3 – Heart Rate (HR)

Heart rate was studied and correlated with the development of CAL in some studies (den Dulk, *et al.* 2009; den Dulk, *et al.* 2013; Luo *et al.* 2021; Stearns *et al.* 2019; Sutton, *et al.* 2004). Sutton *et al.*, in a retrospective study including 379 patients who underwent a curative colorectal cancer resection, found that 22 of them developed clinical CAL (6.0%). Fifteen out of these 22 patients (68.0%) had a preliminary misdiagnosis, with a prevalence (59.0%) of cardiovascular disease symptoms, as tachycardia, chest pain, dyspnea, and/or edema. The diagnosis was delayed for an average of four days (from 0 to 11 days) (Sutton, *et al.* 2004). Stearns *et al.*, published a retrospective case-control study, to compare the physiological parameters. From

the 554 eligible patients (case group - left colorectal resections), 49 developed CAL and were compared with 98 patients without CAL (control group). Regarding the HR, there was a significant increase in the POD1 among CAL patients (82.8±14.2/min vs. 75.1±12.7/min; p = 0.008), maintained until POD5 (Stearns, *et al.* 2019). Recently Luo *et al.* published a retrospective study about CAL predictive value of some SIRS criteria, especially HR, RR, and body temperature. The tendency of HR progression in the post-operative period was established. It was higher in patients with CAL until the POD7, when compared with the group without CAL, and was significant from the POD1 to 5. The predictive HR effect had an AUC, from POD1 to 5, of 0.73, 0.81, 0.81, 0.75, and 0.78, respectively. On POD 2 and 3, with a HR threshold higher than 89/min, the SS and SP were 62.5 and 89.2%, respectively (Luo, *et al.* 2021). As above mentioned, HR higher than 100/min is a clinical criteria included in the DULK score (den Dulk, *et al.* 2009).

6.1.4 - Respiratory rate (RR)

In some studies, RR was also correlated with the CAL development (den Dulk, *et al.* 2009; den Dulk, *et al.* 2013; Luo, *et al.* 2021; Stearns, *et al.* 2019). Stearns *et al.* found a significant increase in RR (18.0±4.2/min vs. 16.5±1.3/min; p=0.007) after the POD3, in CAL patients (Stearns, *et al.* 2019), as well as in the study by Luo *et al.*, where the RR of patients with CAL was significantly higher from POD1 to 7. The predictive effect of RR, from POD1 to 5, had an AUC of 0.78, 0.78, 0.79, 0.78, and 0.59, respectively, and the RR threshold higher than 20/min, had a SS and SP of 62.5 and 76.9%, respectively (Luo, *et al.* 2021). Respiratory rate above 30/min or 20/min are included in the DULK score and its modified version, respectively (den Dulk, *et al.* 2009; den Dulk, *et al.* 2013).

6.1.5 - Body Temperature

Body temperature increase was associated with the development of CAL in several studies (den Dulk, *et al.* 2009; den Dulk, *et al.* 2013; Luo, *et al.* 2021; Stearns, *et al.* 2019). Stearns *et al.* registered a significant increase in body temperature (37.0±0.4ºC vs. 36.5±0.3ºC; p=0.006), although there were no cases of hyperthermia (Stearns, *et*

al. 2019). Luo *et al.* found an increase in body temperature up to the POD7, which was significant from POD2 to POD7. In this period, AUC was 0.71, 0.72, 0.78, 0.77, and 0.79, respectively. The predictive effect was better from POD4 to 6. When body temperature was higher than 37.0˚C, SS and SP were 62.5 and 85.3%, respectively (Luo, *et al.* 2021). Hyperthermia, defined as a body temperature above 38.0˚C is enclosed in the original version of DULK score (den Dulk, *et al.* 2009).

6.1.6 - Other Clinical Findings

As previously mentioned, clinical manifestations of CAL can be subtle or atypical, and this knowledge may help for timely diagnosis and treatment. In this regard, early clinical findings may be respiratory and/or neurological. Changes in the general clinical condition or post-operative ileus are, sometimes, the earliest and most frequent signs of symptomatic CAL (Bellows, *et al.* 2009). Some studies found a strong correlation between ileus and CAL, identifying a set of inflammatory cytokines involved in this process, hypothesizing that an early inflammatory response causes the ileus and, simultaneously, compromises the healing of the anastomosis (Bellows, *et al.* 2009; Boelens *et al.* 2014; Nesbakken, *et al.* 2005; Peters *et al.* 2017).

In the post-operative period, enteric drainage through abdominal tube or wound, make CAL diagnosis highly probable. Clinical examination may provide additional elements to aid in this diagnosis. Rectal examination, for example, can not only confirm suspicious drainage (bloody or purulent, for example), but also enables the confirmation of anastomotic defects in rectal walls or in the rectovaginal septum (rectovaginal fistula) (Bellows, *et al.* 2009; Nesbakken, *et al.* 2005; Tang and Seow-Choen 2005; van Helsdingen, *et al.* 2020). Tang *et al.* concluded that, when rectal examination is carried out by an experienced surgeon, it may provide trustworthy additional information when compared with WSCE, to evaluate anastomotic healing before stoma closure (Tang and Seow-Choen 2005). This highlights the usefulness of physical examination not only in the acute stage, but also for the delayed diagnosis of CAL.

To sum up, a significant rate of CAL is asymptomatic (subclinical), and diagnosis are often delayed. Tachycardia, deterioration in clinical conditions, abdominal pain, abdominal or anal discharge, as well as anastomotic defects under rectal examination are suggestive findings of CAL.

6.2 - BIOMARKERS

Considering the difficulties around the diagnosis of CAL, as well as the need for its prompt detection, additional methods are necessary to increase its diagnostic accuracy. A biomarker is defined as an indicator or clearly measurable attribute that suggests the severity or presence of a pathogenic process or physiological state (FitzGerald 2016). In 2016, the Food and Drug Administration (FDA) and the National Institutes of Health (NIH) put forward a simplified definition, which considers the biomarker as a "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention" (Califf 2018).

The ideal biomarker for CAL, in daily clinical practice, will be significantly present in a state of CAL, reliable, with persistent stable concentrations, without correlation with the primary disease, highly discriminatory, accessible, and cheap (Komen *et al.* 2008). It is easily noticeable that this demanding set of requirements is difficult to achieve. Therefore, in this field, several biomarkers are proposed. They are shown in **Table 3**, considering their sampling location (systemic or intraperitoneal) and their pathophysiological category (ischemia, inflammatory, or microbiological).

Considering the diversity of available biomarkers (systemic or intraperitoneal), their availability for daily clinical use, and the specific scope of this Thesis, will only be considered for review, the most frequently used plasma biomarkers. Serum CLP was also included in this review, despite the scarce research available on this topic, so far (Reisinger *et al.* 2014).

Table 3. Summary of biomarkers evaluated in the context of colorectal anastomotic leakage.

	ISCHAEMIA	INFLAMMATORY	MICROBIOLOGICAL
SYSTEMIC	LBP	IL-1, IL-2, IL-5, IL-6, IL-8, IL-10 TNF-α, Receptor 1 TNF # GM-CSF Interferon-γ ¥ MAC; § MBL; VEGF Presepsin Calprotectin ð WBC / Neutrophils C Reactive Protein (CRP) Procalcitonin Sodium (Na+) Prothrombin fragment 1+2 Thrombin antithrombin complex Soluble fibrin; ± TPA; ¤ PAI 1 Platelets Albumin Haemoglobin Proteins	
PERITONEAL	¶ MMP (1-3;7-9;13) Glucose Lactate Pyruvate Glycerol Lysozyme ‡ LBP ¢ TIMP (1 e 2) pH levels	IL-1, IL-6, IL-10 TNF-α Þ VEGF C Reactive Protein (CRP) Procalcitonin	*Escherichia coli* *Enterococcus faecalis* *Pseudomonas spp.* *Klebsiella spp.*

(# GM-CSF - Granulocyte–macrophage colony-stimulating factor; ‡ LBP, lipopolysaccharide-binding proteins;¥ MAC - Membrane attack complex ; § MBL - Mannin-binding lectin; ¶ MMP - Matrix Metalloproteinase; ¤ PAI 1 - Plasminogen activator inhibitor 1; ¢ TIMP- Tissue Inhibitor of Metalloproteinase; ± TPA - Tissue plasminogen activator; Þ VEGF - Vascular endothelial growth factor; ð WBC – White blood Cells count)

6.2.1 – White Blood Cells Count

In an early scenario, the first line of nonspecific host defense is endorsed by phagocytic cells, such as macrophages, monocytes, and neutrophils, enhanced by alternative complement pathway. Additionally, immunocompetent cells and immunoglobulin start a specific immune response (Bone 1991; Caille *et al.* 2004). In the presence of abdominal or pelvic infection, the release of endotoxins and cytokine production, triggered by the coagulation cascade, lead to white cell's mobilization. Then, chemokines attract leukocytes towards the infective site. Their absolute numbers increase, specifically in the postcapillary venules. Through diapedesis, they reach the infective focus and amplify the inflammatory response (Bone 1991). As previously shown, SIRS criteria are part of this exacerbated leukocyte response resulting in a WBC above 12,000 cells/μL (Bone, *et al.* 1992).

The role for the early diagnosis of CAL is still controversial, due to its nonspecific nature and wide variability. White blood cells may increase after the surgical injury or signaling a post-operative complication, regardless of whether they are medical (pneumonia or urinary infection) or surgical (surgical site infection or organ/space infection), among others. In a retrospective study by Warschkow *et al.* with 1,187 patients, WBC gave little contribute for the early detection of inflammatory complications, with an accuracy significantly lower than plasma CRP. In this study, there were differences depending on the POD and the presence of related septic complications. From POD3 to 5, SS, SP PPV and NPV values ranged from 44% to 52%, 52% to 69%, 24% to 42% and 64% to 81%, respectively (Warschkow *et al.* 2011b). Several studies have similar results, with SS and AUC ranging from 58% to 74%, and 0.63 to 0.77, from POD5 to 7, respectively (Giaccaglia, *et al.* 2016; Käser *et al.* 2014; Kørner *et al.* 2009). Usually, WBC is peaked at the time of CAL diagnosis. In the study by Garcia-Granero *et al.*, WBC at a cutoff of 5,910 cells/mm^3, showed SS of 91%, SP of 77%, PPV of 19%, NPV of 99% and an AUC of 0.82 (Garcia-Granero, *et al.* 2013). More recently, Smith *et al.* analyzed the trajectory of some biomarkers in the post-operative period, and WBC had an overall AUC of 0.76 (0.69 – 0.82) (Smith *et al.* 2018).

6.2.2 - Eosinophil Cells Count

Eosinopenia is a common inflammatory response in acute infections. First described by Zappert *et al.* (1893), it results from the production of chemotactic factors related with the stress response, determining a sudden local sequestration of eosinophils (Bass 1975; Bass *et al.* 1980; Karakonstantis *et al.* 2019). Other mechanisms have been proposed for the depletion of eosinophiles, including the effect of stress hormones (corticosteroids and catecholamines) and myelosuppression (Bass 1975; Best *et al.* 1952). The relation of eosinopenia with the inflammatory syndrome, has been well documented in several studies. Commonly, sepsis is associated with leukocytosis (WBC > 10,000 cells/mm^3 and serum ECC lower than 40 cells/mm^3 (Gil *et al.* 2003; Shaaban *et al.* 2010). Eosinopenia has been proposed as a useful biomarker to identify severe sepsis and to distinguish from other causes of SIRS. On the other hand, eosinophilia is rare in sepsis, and its presence suggests a different diagnosis (Karakonstantis, *et al.* 2019; Mitre 2013). Some studies analyzed the relevance of ECC in the diagnosis of sepsis. Shaaban *et al.* estimated a SS, SP, PPV and NPV of 81%, 65%, 66% and 80%, considering an ECC cutoff point of 50 cells/mm^3 (Shaaban, *et al.* 2010). Its usefulness as prognostic marker, for mortality in critical ill patients, has been well-established. Moreover, ECC seems to be interesting as biomarker, due to its low cost and wide availability (Garnacho-Montero *et al.* 2014; Mitre 2013). At intensive care unit admission, ECC under 40 cells/mm^3 and maintained over the first week is an independent prognostic factor for mortality. Normalization of ECC in 2 or 3 days is frequently seen in septic shock survivors (Abidi *et al.* 2011; Terradas *et al.* 2012). Recently, ECC monitoring was proposed as a marker for positive evolution in septic patients under antibiotic therapy (Davido *et al.* 2017). In a recent meta-analysis by Lin *et al.*, the incidence of eosinopenia in septic patients ranged from 23.2 to 92.7%, presenting a pooled SS and SP, positive and negative likelihood ratios (PLR and NLR), and pooled odd ratio (OR) of 0.66 (0.53-0.77), 0.68 (0.56-0.79), 2.09 (1.44-3.02), 0.49 (0.34-0.71), and 4.23 (2.15-8.31), respectively. The overall AUC was 0.73 (0.68-0.76), with the ECC cutoff points of 40 cells/mm^3 and 25 cells/mm^3 showing the highest SS (79%) and SP (83%), respectively. For the diagnosis of sepsis, ECC was not superior then conventional biomarkers. However, due to its availability, fastness, simplicity, and low cost, it can be used in the daily clinical diagnosis of sepsis (Lin *et al.* 2021). Its specific usefulness in the early diagnosis of CAL is yet to be established.

6.2.3 - Serum C-Reactive Protein

Plasma C-reactive protein (CRP) is an acute phase reactant with liver synthesis and plasma levels under 0.8 mg/L, in healthy individuals. It has shown a strong correlation with post-operative complications, namely after abdominal surgery. Due to its short half-life (19h), plasma CRP is a reliable marker of SIRS secondary to surgery, since it increases in response to surgical injury for up to 72 hours, decreasing afterwards. Serum CRP significantly increases in response to the proinflammatory cytokines release, such as interleukin (IL)-6 (IL-6), tumor necrosis factor α (TNF-α), and IL-1β. This may occur in the inflammatory acute response to infection, tissular injury, or in neoplasms, (Gray *et al.* 2021; Lagoutte, *et al.* 2012; Welsch, *et al.* 2007). In patients with post-operative complications, CRP levels remain high. Plasma CRP higher than 140 mg/L, on POD3, is a strong predictor of major abdominal septic complications, showing a SS and SP of 81.7% and 61.6%, respectively (Almeida, *et al.* 2012; Straatman *et al.* 2015; Su'a *et al.* 2017). Serum CRP is the most widely studied biomarker for CAL diagnosis. The most recent scientific evidence is supported by a meta-analysis from Yeung *et al.*, including the highest number of studies (n=23) and patients (n=6,647), so far. Mean time until the CAL diagnosis was 7.70 days, and CRP levels was markedly higher in patients who developed CAL ($p<0.001$). Means difference ranged from a minimum of 15.19 (5.88-24.50) and a maximum of 112.10 (89.74-134.45) on POD1 and 5, respectively. The AUC analysis found a cutoff point of 148, 123, 115, 105, and 96 mg/l, from POD3 to 7, respectively, and a SS and SP of 95% (POD3) and 100% (POD4 to 7) (Yeung *et al.* 2021). These results prove the strength of this meta-analysis, due to its significant sample size (Almeida, *et al.* 2012; Singh *et al.* 2014; Warschkow *et al.* 2012). One of the main limitations of this meta-analysis is its significant heterogeneity (I^2), ranging from 91% (maximum, on POD2) to 54% (minimum, on POD5). Another limitation was the lack of randomized control trials included on the analysis. In conclusion, CAL is associated with significantly higher CRP plasma levels, and estimated cutoff values are sufficiently robust to evoke CAL suspicion after POD3, if clinically appropriate. Recently Rama *et al.* carried out a meta-analysis (cf. **Chapter III**) about the usefulness of serum biomarker in this scope, and found a pooled AUC of 81.8 and 86.8%, on POD 3 and 5, respectively. The highest pooled combination of SS and SP was found on POD4 (80.5%) and POD5 (82.4%), respectively. Cutoff points derived from the POD3 and 5 were 147.3 ± 14.30 and 112.8 ± 25.3 mg/dl, respectively. It is also noteworthy the high NPV of serum CRP in this setting, which may be useful as a predictive indicator to exclude CAL, thus allowing safer earlier discharge.

6.2.4 - Serum Procalcitonin

Some authors have highlighted the usefulness of PCT as an early, more sensitive, and reliable subclinical marker of CAL (Facy *et al.* 2016; Giaccaglia, *et al.* 2016; Giaccaglia *et al.* 2014; Lagoutte, *et al.* 2012). Procalcitonin is a prohormone of calcitonin, a protein with 116 amino acids, produced by thyroid C-cells, neuroendocrine cells from the gastrointestinal tract, and lung K-cells, and with baseline levels under 0.1 ng/ml. PCT release is induced directly by the lipopolysaccharide of circulating bacteria, or indirectly by several inflammatory cytokines, such as IL-6 and TNF-α (Vijayan *et al.* 2017). Levels of PCT increase significantly (up to 5,000 times), from 2 to 4 hours, in patients with severe sepsis, persisting until recovery (Gilbert 2010; Pfäfflin and Schleicher 2009). Procalcitonin half-life ranges from 22 to 26 hours, an advantage when compared to other acute phase reactants (Lee 2013; Limper *et al.* 2010). In opposition with CRP and other acute phase reactants, plasma PCT levels barely increase due to viral infections, making them useful to distinguish bacterial from viral infections (Lee 2013). Serum PCT is also useful to distinguish the diagnose of SIRS from that of sepsis/septic shock (Harbarth *et al.* 2001; Lee 2013). Procalcitonin depuration is preferably renal, thus, patients with a compromised renal function may have spurious elevation in the serum PCT levels (Giaccaglia, *et al.* 2016; Giaccaglia, *et al.* 2014).

In a recent meta-analysis published by Su'a *et al.*, eight studies and a total of 1,693 patients were analyzed, with a CAL rate ranging from 5.4% to 13%, with an average of 8.5% (6.1%-11.8%; I^2 - 0.68%). Regarding its diagnostic accuracy, the level on POD5 was the highest, with optimal cutoff points varying from 0.25 to 680 ng/ml from the POD3 to 5, with NPV and PPV varying from 95 to 100%, and 34%, respectively. As the PCR, PCT demonstrated a good NPV for CAL, promoting the early and safe discharge of patients selected after colorectal surgery (Cousin *et al.* 2016; Giaccaglia, *et al.* 2016; Giaccaglia, *et al.* 2014). The cutoff values estimated for SP ranging from 95 to 100% were 23.5 and 3.0 ng/mL, respectively. The estimated AUC was 0.88 on the POD5 day. In patients without complications, the PCT tended to normalize in the POD3. The authors found that PCT was useful as a test to exclude the possibility of CAL after elective procedures. However, as an isolated test, it has limited usefulness to diagnose CAL (Su'a *et al.* 2020). In this regard, Giaccaglia *et al.* estimated a better AUC of PCT on POD5 compared with CRP (0.86 vs. 0.81); both are higher than the WBC in both PODs (0.601 and 0.611, respectively). Moreover, on POD5 it is important to highlight that the combination of PCT and CRP improved the diagnosis of CAL (AUC 0.901) (Giaccaglia, *et al.* 2016).

To sum up, based on the recent evidence from the meta-analysis by Rama *et al.*, it was found that the diagnostic accuracy of CRP and PCT was similar in all days of the POD (from the first to the seventh), showing the highest values on the POD5, and higher values for PCT (92.8%). However, this biomarker is modest as CAL predictor when evaluated individually. A combination of biomarkers could improve their predictive ability, but a data meta-regression was not possible due to the small number of studies in the meta-analysis.

6.2.5 - Serum Calprotectin

Calprotectin is a heterodimeric peptide (36 kDa) that connects to calcium, representing nearly 60% of the proteins in the cytoplasm of neutrophils and monocytes. As a protective protein, it is distributed not only in myelomonocyte cells, but also in epithelial cells, keratinocytes, among others. It has regulatory functions in the inflammatory process, and an antimicrobial and anti-proliferation action. In acute phase, plasma CLP levels are increased and correlated with high neutrophils and CRP levels (Aadland and Fagerhol 2002; Johne *et al.* 1997). However, this relation is not always linear. Sander *et al.* found a weak correlation between CLP, PCR, and WBC in severe sepsis, suggesting that these parameters may reflect different aspects of inflammatory response (Sander *et al.* 1984). Plasma CLP levels monitoring is relevant in several clinical conditions, such as inflammatory and infectious diseases, and some neoplasms. Hence, increased serum CLP can be found in cystic fibrosis, rheumatoid arthritis, Crohn's disease, ulcerative colitis, and several bacterial infections. Additionally, different medications, as immunosuppressors, aspirin, anti-TNF agents, and statins, can alter plasma CLP levels (Agilli and Aydin 2015). As a marker of neutrophil activation, CLP may be an interesting early marker of systemic inflammatory response, such as major abdominal catastrophes (e.g., CAL). To date, few studies investigated the predictive value of this biomarker in CAL (Cikot *et al.* 2016; Reisinger, *et al.* 2014).

The prospective observational study published by Reisinger *et al.* included 84 patients who underwent elective colorectal resection with primary anastomosis. Eight patients developed CAL, with a median clinical diagnosis on the POD6. This research demonstrated that serum CLP is better than CRP in the detection of CAL, with the highest diagnostic accuracy on the POD4 (AUC 0.96). For a cutoff value of 541 ng/mL, SS, SP, positive and negative LR, NPV and PPV were of 100%, 91%, 11,

0, 100%, and 55%, respectively. The best diagnostic accuracy was found with the combination CRP and CLP plasma levels on the POD3, with SS, SP, and PLR and NLR of 100%, 89%, 9.09 (4.34 - 16), and 0 (0.00 – 0.89) (Reisinger, *et al.* 2014).

6.2.6 - Other Serum Biomarkers

As previously mentioned, diverse publications demonstrated the increase of serum CRP levels after the POD3, in patients who developed post-operative complications, as CAL. Moreover, Shimura *et al.* showed that CAL patients had markedly low levels of albumins, when compared with non-CAL population (Shimura *et al.* 2018). Thereby, recent studies suggested the relationship CRP/albumin (CAR) as a clinically useful inflammatory composite biomarker, to predict post-operative complications, in different surgical fields (Ge *et al.* 2017). Paliogiannis *et al.* recently published a retrospective multicentric study involving 1,183 patients who underwent elective curative colorectal cancer resection. On the POD4, CAR was significantly higher in the CAL group, vs. no-CAL group [67.2 (51.5–88.6) vs. 25.9 (1.4–36.1); $p< 0.0001$]. After ROC analysis, CAR presented a good predictive accuracy for CAL [AUC of 0.83, (0.79-0.86)], higher than single levels of CRP and albumin. The CAR also showed a high ability to predict post-operative mortality [AUC of 0.98, (0.96-0.99)]. The authors concluded that the CAR is a cheap and widely available biomarker with suitable predictive abilities for post-operative morbidity and mortality. However, further studies will be needed to confirm these data.

Kaser *et al.* proposed hyponatremia as an inflammation marker, defined by a sodium plasma level under 136 mmol/l, and comprising the most common electrolytic clinical disorder. Hyponatremia results from the release of vasopressin or anti-diuretic hormones driven by the intravascular depletion or significant third space losses. However, vasopressin release may directly result from IL-6 effect, by neuroimmunoendocrinological pathways (Hoorn and Zietse 2008; Käser, *et al.* 2014; Swart *et al.* 2011). This relationship between systemic inflammation and hyponatremia is well known in pneumonia and spontaneous bacterial peritonitis associated with hepatic cirrhosis (Käser, *et al.* 2014). Other authors also proved this association in urinary infection, infectious colitis complicated with perforation, among other (Käser *et al.* 2013; Käser, *et al.* 2014). Two studies showed the correlation between hyponatremia and CAL. Kaser *et al.*, published a retrospective study with 1,106 patients and an overall rate of CAL of 7.3% (9.0% in the rectum and 5.4% in

the colon). On CAL diagnosis, hyponatremia showed a SS and SP of 23% and 93%, respectively. Combination of WBC and hyponatremia presented a SS, SP, NPV and PPV of 68%, 75%, 97%, and 18%. On the POD4, this combination had an AUC of 0.51. In a slow progress post-operative period, hyponatremia should raise the suspicion of CAL, in face of its high SP. The simultaneous presence of leukocytosis increases the suspicion, and further imaging investigation should be considered (Käser, *et al.* 2014). Zhang *et al.* conducted a retrospective study including 498 consecutive patients with colorectal cancer, with an overall CAL incidence of 5.4%. For a cutoff point under 139.5 mmol/L, an AUC of 0.65 was estimated, corresponding to a NPV of 97.2%. If combined with leukocytosis, the NPV increased to 99.1%, being worth and useful to exclude CAL diagnosis. After a multivariate analysis, this combination had independent predictive ability for CAL (Zhang *et al.* 2020).

Table 4 summarizes the diagnostic accuracy of the abovementioned plasma biomarkers, based on the best evidence currently available, including recent meta-analysis, randomized trials, and prospective or retrospective studies.

Table 4. Diagnostic accuracy of main plasmatic biomarkers, regularly used in daily practice (4th and 5th post-operative days)

	SS	SP	PNV	PPV	AUC
WBC	91% (74% – 100%)	77% (71% – 83%)	99%	19%	82%
EOS	66% (53% - 77%)	68% (56% - 79%)	NA	NA	73% (68% - 76%)
CRP	100% (63% – 100%)	100% (63% – 100%)	NA	NA	100% (79% – 100%)
PCT	NA	NA	100%	34%	86% (79% - 94%)
CLP	100% (NA)	91% (NA)	NA	NA	96% (90% - 100%)
CAR	80% (67% – 89%)	87% (84% – 90%)	NA	NA	83% (79% – 86%)
HYPONATREMIA	17% (3% – 48%)	90% (87% – 93%)	97%	5%	NA

[(CAR – CRP- Albumin Ratio; CLP – Calprotectin; CRP – C reactive Protein; EOS – Eosinophils Count; PCT - Procalcitonin; WBC – White blood Cells count; NA – Non-Available]

6.2.7 - Score Systems

Another strategy to anticipate CAL diagnosis includes pooling clinical and laboratory variables in a weighted scoring system, to improve the different diagnostic accuracy measures of these variable, if used separately. The design complexity, the need for external validation, and the difficulties for implementation in daily clinical practice, are some of the challenges of score systems. So far, some scores have been developed for early CAL diagnosis, such as the Dutch leakage (DULK) score (den Dulk, *et al.* 2009), its modified version (den Dulk, *et al.* 2013), the Diagnostic Score Leakage (DIACOLE) score (Rojas-Machado *et al.* 2016), and those based on artificial intelligence methods (Adams and Papagrigoriadis 2014). They aimed to early identify patients

with suggestive CAL finding, based on a cutoff point (discriminant threshold), to establish a management plan that includes additional examinations or re-operation (den Dulk, *et al.* 2013; Rojas-Machado, *et al.* 2016).

The first score was published by den Dulk *et al.* and resulted from a study that aimed to test the usefulness of a standardized protocol of post-operative surveillance in early CAL diagnosis and its impact in mortality reduction. A control group with 1,066 patients was defined and followed under a standard post-operative surveillance protocol, consisting of routine clinical criteria. The score included general signs and symptoms, physical findings, as well as laboratory and nutritional data (see **Table 5**). Patients scoring from 4 to 7 had close clinical surveillance, with biomarkers (CRP and WBC) each 12 hours and imaging case-by-case (abdominal CT scan). Patients scoring 8 or higher required abdominal and pelvic CT scan with WSCE. Later, the protocol was applied prospectively in 223 patients undergoing colorectal resection with anastomosis. An overall CAL rate of 7.0% and 9.4% were estimated, in the control and standard-monitoring groups, respectively. The delay in diagnosis was significantly reduced (from a median of 4 days to 1.5, p=0.01), and maintained after multivariate analysis. The CAL mortality rate reduced from 39% (29 of 75 patients with CAL), in the control group, to 24% (5 out of 21 patients with CAL) in the standard-monitoring group, although it did not show statistical significance (p=0.21). The authors concluded that the introduction of this standard-monitoring protocol was useful and significantly reduced delays in diagnosis from the first symptoms to the confirmation and CAL management. The impact on mortality was not statistically significant, but this reduction in the prospective cohort was worth mentioning (den Dulk, *et al.* 2009). Several limitations were identified, namely the comparison between a multicentric retrospective and a monocentric prospective cohort, or the high number of scoring criteria (thirteen), that makes it difficult to use, in a regular basis.

Table 5. Original DULK score (adapted from Den Dulk, *et al.* 2009).

Item	DULK *Score*	Score (points)
General		
Fever	> 38ºC	1
Heart Rate	> 100/min	1
Respiratoty Rate	>30/min	1
Urinary Output	< 30 ml/h or 700 ml/day	1
Mental Status	Agitation or lethargic	2
Clinical Condition	Deterioration	2
Local physical examination		
Signs of ileus	Present	2
Abdominal pain, other than wound pain	Present	2
Gastric retention	Present	2
Fascial dehiscence	Present	2
Laboratory investigation		
Signs of infection	Increase in WBC or CRP≥ 5%	1
Kidney function	Increase in Urea/Creat. ≥ 5%	1
Diet (Nutritional status)		
Tube feeding	Present	1
Total parenteral nutrition	Present	2

(CRP – C reactive Protein; Creat. – Creatinine; WBC – White blood Cells count);

More recently, a modified version of the DULK score was developed aiming the reduction of the number of parameters (simplification) and its easier routine employment. Den Dulk *et al.* simplified the original version, from a prospective cohort with 782 patients, adopting multivariate logistic regression modeling. The new parameters selected were clinical condition, location of abdominal pain, serum CRP levels, and RR (including a new cutoff point, higher than 20 cycles/min) - **Table 6**. The new score showed and overall SS, SP, PPV, and NPV of 97%, 53%, 16%, and 99%, respectively. This performance was obtained with only one positive score item, and the comparison with the original version did not show significant differences. The authors found that both scores were useful for early clinical CAL diagnosis, and the new version had the advantage of user-friendliness in daily clinical practice (Den Dulk, *et al.* 2013).

Table 6. Modified DULK score (adapted from Den Dulk *et al.* 2013).

Item	DULK *Score*	Score (points)
Respiratoty Rate	> 20/min	1
Clinical Condition	Deterioration	1
Abdominal pain, other than wound pain	Present	1
Signs of infection	Increase in CRP ≥ 250 mg/l	1

(CRP – C reactive Protein);

After the simplified DULK score was published in 2013, Martin *et al.* validated the original version of the score using a prospective cohort of 100 patients, with a similar post-operative follow-up protocol. The DULK score proved to be useful for CAL diagnosis, with a SS, SP, VPN and AUC of 91.7%, 55.7%, 98.%, and 0.83, respectively (Martin, *et al.* 2015). The routine use of this scoring system allowed a CAL diagnosis 3.5 days earlier. Therefore, the authors reinforced the adoption of this tool for the early diagnosis of CAL (Martin, *et al.* 2015).

One year after the publication by Martin *et al.*, Rojas-Machado *et al.* proposed a new score system, known as DIACOLE, with the same goal, but with a different design method. From a systematic review of literature with meta-analysis the authors identified and weighted potential clinical and laboratory CAL findings. The score was constructed using 13 significant parameters, weighted using the Napier's logarithm of the OR, and was validated in a longitudinal, observational, and retrospective case control study (including 41 patients with CAL and 82 patients without CAL) – see Table 7. The AUC was estimated for both DIACOLE and original DULK scores, from POD5 to 9, and ranged from 0.84 to 0.91 and 0.64 to 0.89, respectively. The authors recommended a laboratorial reassessment with intensive clinical surveillance whenever the score was higher than 3.065, or imaging (abdominal and pelvic CT scan with WCSE, if higher than 5.436 (corresponding to SS and SP of 82.9%). For simplification purpose, the authors developed an online calculator (Rojas-Machado, *et al.* 2016).

Table 7. DIACOLE score (adapted and modified from Rojas-Machado *et al.* 2016).

DIACOLE parameters	Weight
Post-operative clinical signs and symptoms	
Fever (>38ºC)	1.517
Prolonged ileus (>3 PODs)	1.497
Diarrhea	1.366
Abdominal pain	1.632
Post-operative complications	
Cardiac	1.879
Respiratory	1.742
Urinary	1.209
Neurological	2.318
Wound infection	1.500
Post-operative laboratory findings	
Leukocytosis (>12G/L)	1.788
Blood urea levels > 48 mg/dl	2.206
CRP plasma levels > 20 mg/dl (from POD3)	3.602
Others	
Post-operative blood transfusion	1.635

PODs – post-operative days; CRP - C-reactive protein

In 2013, Adams *et al.* developed a predictive model for CAL based on artificial intelligence methods including 19 input variables, signaling as risk factors for colorectal anastomotic failure (Table 8). The internal validation of this model was carried out in a case-control study with 76 patients with similar clinical features (20 with CAL, and 56 patients without CAL). The estimated SS, SP, and AUC were 85%, 82.1%, and 0.89, respectively. The external validation was conducted prospectively in 12 consecutive pilot patients and was obtained a SP of 83.3%. The authors suggested that artificial intelligence methods can help in the early detection of CAL on daily clinical routine (Adams and Papagrigoriadis 2014).

Table 8. Leak-detection ANN model Variables (adapted and modified from Adams and Papagrigoriadis 2014)

Variable	Weight	Variable	Weight	Variable	Weight
Day 5 CRP	0.127	Stoma	0.063	Day 1 CRP	0.014
Day 4 CRP	0.124	Day 2 PLT	0.034	Location	0.009
Day 1 PLT	0.119	Day 4 Hb	0.034	Day 3 PLT	0.006
Pre-op Hb	0.116	Pre-op PLT	0.027	Day 3 Hb	0.004
Day 5 PLT	0.109	Day 5 Hb	0.022	Day 2 Hb	0.002
Day 1 Hb	0.078	Day 3 CRP	0.021		
Day 4 PLT	0.072	Day 2 CRP	0.021		

ANN - Artificial Neural Network; CRP - C reactive protein; PLT - platelet count; Hb - hemoglobin; Location - level of anastomosis (small bowel, left, right, or pelvic); Stoma - defunctioning stoma present.

6.3 - IMAGING

Diagnosis of CAL is challenging, and imaging plays a significant role, especially in insidious subclinical cases, preventing not only the consequences of diagnostic delay, but also unnecessary re-operations with potential morbidity and mortality. Moreover, imaging may guide drainage of abdominal or pelvic collections, avoiding further interventions (Daams, *et al.* 2014; Hirst, *et al.* 2014; Hyman 2009; Vallance *et al.* 2017). The main imaging modalities used to diagnose CAL are simple abdominal x-ray, WSCE and CT scan, but data on their accuracy are limited by the timing of the study and the expertise of the radiologist, among others. Below is presented a brief review of this modalities.

6.3.1 - Simple Abdominal X-ray

The usefulness of simple abdominal x-ray in CAL diagnosis is limited, and available evidence of its accuracy are scarce and outdated. Williams *et al.* reviewed radiological studies from 31 patients who underwent mechanical colorectal anastomosis. Clinical anastomotic failure was identified in 10 patients, and 9 displayed stapling lines disruptions in the abdominal x-ray. The authors proposed this sign as suggestive of CAL, and helpful in some dubious or subclinical cases (Williams *et al.* 1991). Another potentially useful sign is the long-lasting and sustained presence of intraperitoneal air, better perceived in a chest or standing abdominal x-ray. It is a fast, simple, and noninvasive method, useful in other acute abdominal conditions. Most clinical

CAL occurred from the POD5 to 7, and in this period, less than 30% of patients had subphrenic air sign on the x-ray. Sequential and comparative studies can be useful, and increasing subphrenic air sign should raise CAL suspicion (Tang *et al.* 2000). In short, this imaging method has limited clinical usefulness, but can complement some suspicious and doubtful post-operative clinical pictures, suggesting the need for further accurate imaging modalities, such as the CT scan.

6.3.2 – Water-Soluble Contrast Enema

Current evidence is not consensual about the best imaging method for CAL diagnosis, but WSCE and contrasted CT scan are the most used techniques (Daams, *et al.* 2014; Doeksen *et al.* 2008; Hirst, *et al.* 2014). The accuracy of these methods is extremely variable, depending on the radiologist expertise, the inter-observer variability, the location of the anastomosis, and the diagnostic context itself (Doeksen, *et al.* 2008; Haynes *et al.* 1986). Water-soluble contrast enema has been safely used for decades to evaluate the integrity and tightness of the anastomosis, with a variable overall SS and SP that is around 85 and 20%, respectively (Nicksa *et al.* 2007). However, most patients (about 90%) never developed clinical CAL, which makes its routine usage questionable and not recommended, according to some experts, (Akyol *et al.* 1992; Daams, *et al.* 2014). A classic study by Goligher *et al.* concluded that WSCE would be unnecessary when compared to digital rectal examination or lower gastrointestinal endoscopy (Goligher *et al.* 1970). Similarly, Williams *et al.* did not recommend the systematic use of WSCE to exclude CAL, unless there were an adverse clinical progress (Williams, *et al.* 1991). The usefulness of WSCE in left colorectal anastomotic failure was also questioned by Akyol *et al.* who established its SS and SP as 52.2% and 86.7%, respectively (Akyol, *et al.* 1992), and Tang *et al.* compared the digital rectal examination with WSCE to evaluate the anastomotic integrity before stoma closure. They concluded that WSCE was less precise, with a significant higher rate of false positives then digital rectal examination (6.4% vs. 3.8%, respectively) (Tang and Seow-Choen 2005). Other authors defended the systematic use of WSCE, emphasizing its advantages. Nicksa *et al.*, compared WSCE with contrast CT scan in a retrospective study including 36 patients with CAL. Water-soluble contrast enema showed better accuracy than CT scan, even due to the high prevalence of lower colorectal anastomosis (Nicksa, *et al.* 2007). Eighteen patients were evaluated with a WSCE and 15 (83.3%) demonstrated extravasation of contrast material. In the 26

patients with a distal CAL, 17 WSCE were performed, with 15 (88%) demonstrating a leak. In contrast, only 2 of 17 (12%) CT scans were positive in this group of patients ($P < 0.001$).

More recently, Habib *et al.* published a meta-analysis including 1,142 patients from 11 studies, and evaluated the usefulness of WSCE and clarified the natural history of radiological CAL. This study established a SP, SS, NPV, and PPV of 95.4%, 79.9%, 98.4%, and 64.6%, respectively. Considering these results, the authors concluded that WSCE is effective to exclude clinical CAL, providing relevant information in lower rectal anastomoses (Habib *et al.* 2015).

6.3.3 - Contrast CT Scan

Contrast CT scan (CCT) is frequently used to detect post-operative complications in colorectal procedures. Despite the higher surgical volume, there is scarce evidence about its accuracy in the diagnosis of CAL. In many centers the preferred modality was the abdominal and pelvic CCT, providing a more detailed image of anastomosis and neighboring structures or findings, such as abscess or hematoma (Daams, *et al.* 2014; Eckmann *et al.* 2004; Hirst, *et al.* 2014; Holl *et al.* 2017; Hyman, *et al.* 2007; Khan *et al.* 2008). Combination of CCT with WSCE can identify a set of highly suggestive findings of CAL, such as the endoluminal contrast extravasation, presence of pneumoperitoneum or perianastomotic collection. Clinical significance of intraperitoneal air depends on surgical approach and POD, among others (van Helsdingen, *et al.* 2020). However, inconclusive, or doubtful CCT findings may lead to false-positive or false-negative results and causing unnecessary further re-operations or diagnostic delays. False-negative results can be affected by the timing of CCT and may require serial examinations. However, logistic, financial, and safety (radiation exposure) are worth limiting factors (Komen, *et al.* 2008). Combination of CCT and WSCE may reduce these false-negatives rate, mainly in lower colorectal anastomosis (Hirst, *et al.* 2014).

Power *et al.* included, in a case-control study, 99 patients (76 with clinical suspected cases of CAL, and 26 control patients being investigated for post-operative sepsis), to identify CCT predictive findings for CAL. The incidence of CAL was 31.5% (23/73), and CCT were performed, on average, on the POD5. The only significant CCT finding associated with CAL was the perianastomotic collection (containing liquid and air)

(p=0.04). In control group, free or localized intrabdominal air were identified for up to 9 and 26 days, respectively (Power *et al.* 2007). Nesbakken *et al.* published a prospective study comparing the diagnostic accuracy of clinical symptoms, WSCE, and CCT, that showed a SS and SP of 50% and 89%, 60% and 100%, and 57% and 100%, respectively (Nesbakken, *et al.* 2005).

Marres *et al.* studied the diagnostic accuracy and the delay of CCT, after reviewing the clinical records of 628 patients and selecting 127 who had undergone CCT. The CAL rate was 7.8% (n=49), and CCT had a SS, SP, NPV, and PPV of 73%, 91%, 88%, and 78%, respectively. Regarding the CAL diagnosis, it was significantly higher in patients with false-negative CCT (1 day; $p<0.05$). This delay was associated with a significant increase in mortality (4.2% vs. 45.5%; p=0.005) and LOHS (median of 28 days vs. 54 days; $p<0.05$) (Marres, *et al.* 2017).

Kornmann *et al.* published a systematic review to estimate the CCT diagnostic accuracy and its usefulness in the decision-making process. Eight studies and 221 abdominal CTC were included, but the overall quality of the studies was poor. Overall SS was 68% (59% - 75%) and SP, estimated in only two studies be calculated in 2 studies, ranged from 78% and 100%. Regarding the combination of CCT and WSCE for lower colorectal anastomosis, SS of the 3 studies selected was 92% (80% - 97%). The authors highlighted the low quality of the included studies and its impact on the quality of evidence and limitation of conclusions. They recommended that the limited SS of CCT should be considered, to avoid diagnostic and management CAL delays (Kornmann *et al.* 2013).

A study by Gervaz *et al.* aimed to build a predictive model for CAL diagnosis improvement, including plasma biomarkers and CCT findings. In the score proposed, WBC > 9.9 G/l [OR = 14.8; (2.3–194.7), p=0.001], presence of intra-abdominal fluid ≥ 500 cm^3 [OR = 13.4; (2.0–179.5), p=0.003] and air located near the anastomosis [OR = 9.9; (1.7–106.5), p=0.006] were strongly associated with the presence of CAL. The risk of CAL was 0, 6, 31, and 100% in patients scoring 0, 1, 2, and 3 points, respectively. An overall AUC of 0.83 (0.72-0.94) was estimated for this model. This score includes inflammatory and imaging parameters and was not yet validated but seems to be promising to quantify the risk of CAL (Gervaz *et al.* 2013).

6.4 - SURGERY

As before mentioned, CAL detection is paramount and challenging. Diagnosis is often delayed and confirmed in re-operation, despite the availability and innovation of diagnostic modalities (Chang *et al.* 2016; Kirshtein *et al.* 2008). In face of clinical CAL picture and after initial optimization, re-operation -frequently a laparotomy -, is needed. However, in selected cases re-laparoscopy is feasible and safe, and its usage has been increased (Chang, *et al.* 2016). Unclear and subtle clinical findings were associated with diagnostic delay and growing morbidity and mortality. In these cases, further re-operations, especially laparoscopic, can also be appropriate methods for CAL diagnosis and management, with acceptable morbidity (Cuccurullo *et al.* 2015; Vennix *et al.* 2013). Kirshtein *et al.* compared the results of early re-laparoscopy (<48h) vs. late re-laparoscopy (> 48h) due to suspected post-operative complications. From the 7,426 patients submitted to laparoscopy in the study period, 57 (0.7%) underwent re-laparoscopies. In the "early" group (n=37) the most frequent indication was intense abdominal pain (46%), followed by signs of peritonitis (35%), while in the "late" group (n=20), signs of SIRS (30%) and peritonitis (25%) were the most common indications. In 16 patients (28%), re-laparoscopies were negative, and in 37 the treatment was performed by laparoscopic approach (65% of all patients and 90% of those who presented findings in their re-laparoscopy). Length of hospital stay, and complication rates were significantly higher in the "late" group ($p<0.003$ and $p<0.05$, respectively). Mortality rates were also higher but not significant (10% vs. 2.7%) (Kirshtein, *et al.* 2008). Recently, Fransvea *et al.* investigated the outcomes of redo-laparoscopy for the management of early post-operative complications following laparoscopic colorectal surgery. In this systematic review, 19 studies and 1,394 patients requiring re-operation after laparoscopic colorectal resection were included, and 9 studies were selected for pooled analysis. The laparoscopy was adopted in 38.2% (n=539) of these patients, and CAL was the most common recommendation (64.4% of all redo-surgeries). The mean LOHS was significantly shorter in the redo-laparoscopy than in the redo-open group ($p < 0.001$). Additionally, a significantly lower risk of mortality was observed in the redo-laparoscopy cohort ($p = 0.009$). The authors concluded that laparoscopy is a valid and effective approach for the treatment of complications following primary laparoscopic colorectal surgery (Fransvea *et al.* 2021). Similarly, Rotholtz *et al.* concluded that early redo-laparoscopy (within 48 hours after the suspicion of a complication) provides higher chances for the use of a laparoscopic approach for management, providing a higher probability for good

post-operative outcomes, despite a greater risk of negative findings in the re-operation (Rotholtz *et al.* 2021). Consensual definition of CAL includes typical intra-operative findings, such as necrosis of the anastomosis, necrosis of the blind loop, as well as dehiscence of the anastomosis and signs of peritonitis. These signs of CAL found during re-operation should be described in the operation report, highlighting its relevance in daily clinical practice as well as for research purposes (van Helsdingen, *et al.* 2020).

7 - TIMING

The timing of CAL detection is an important issue for clarifying the real incidence, especially in low or ultralow colorectal anastomosis (Hyman, *et al.* 2007). The CAL diagnostic gap is highly variable and can be as long as one-year. Colorectal anastomotic leak may be differentiated on early (EAL) and late (LAL). This distinction is based on specific risk factors and different pathophysiological mechanisms (Floodeen *et al.* 2013; van Helsdingen, *et al.* 2020).

Early anastomotic leakages are failures with clinical and/or imaging manifestations in the first admission or until the POD 21 or 30, depending on the author (Lim *et al.* 2016; Morks *et al.* 2013; Shin *et al.* 2010). This type of CAL is more frequent in younger male patients with high BMI, after laparoscopic approach or urgent setting, and without covering stoma. Its development is usually related with technical difficulties and longer procedures with intra-operative complications such as bleeding or narrow pelvis (Floodeen, *et al.* 2013; Shin, *et al.* 2010; van Helsdingen, *et al.* 2020). Generally, they are located more posteriorly in the circular stapler line (Floodeen, *et al.* 2013). From the pathophysiological point of view, patients with EAL have higher levels of MMP (*Matrix metalloproteinases*), in the peritoneal fluid drainage, especially MMP-8 and MMP-9 (Pasternak *et al.* 2010). They comprise up to two thirds of CAL and their onset is more or less early (first post-operative days), in a clinical picture of sudden abdominal discomfort or pain (Morks, *et al.* 2013). It frequently requires further re-operation and culminates with the need for a stoma, usually permanent (Lim, *et al.* 2016; Morks, *et al.* 2013).

On the other hand, LALs are typically identified after discharge, mostly after POD 21 or 30, depending on the author (Floodeen, *et al.* 2013; Morks, *et al.* 2013; Shin, *et al.* 2010). Significant risk factors for LAL are the female gender, ageing and

their related comorbidities (high ASA score and Charlson's comorbidity index), low BMI and neoadjuvant radiotherapy. Its location is frequently anterior, in the linear stapling section of a J-pouch or lateral-terminal anastomosis (Floodeen, *et al.* 2013). Regarding its pathophysiological mechanism, LAL seems to be related with insufficient microcirculation in the anastomosis or in the linear stapling section. This features probably justify a more insidious biological behavior (Floodeen, *et al.* 2013; Karliczek *et al.* 2010). This type of CAL comprises up to one third of patients, with an uneventful early post-operative recovery. Afterwards, patients developed symptoms such as sacral pain, and perianal or vaginal discharge (Lim, *et al.* 2016). In conclusion, both subtypes of CAL, EAL and LAL, have significant differences regarding their anatomic location and timing, depending on different pathophysiological mechanisms, and justifying the assumption that they are two distinct entities.

C - OBJECTIVES / HYPOTHESIS

1 - THESIS OBJECTIVES

The following main research question was defined: **"Where should we look to timely identify a patient with anastomotic leakage after colorectal surgery?"**. This research question enclosed five additional objectives:

First, to **determine the accuracy and predictive value of clinical criteria** (post-operative progression pattern of abdominal pain, and clinical condition evolution) **for the early diagnosis of colorectal anastomotic failure;**

Second, to **establish the predictive effect of plasma biomarkers (WCC, ECC, CRP, PCT and CLP) in CAL-patients**;

Third, to **define the optimized cut-off values of CRP, PCT and CLP for a early discharge of patients**, according to the enhanced recovery after surgery protocols;

Fourth, to **develop a decision model (score), using fewer parameters, that might early predict an anastomotic failure after colorectal surgery**;

Finally, to perform a cost-minimization analysis for examining the economic impact of potential false positives (i.e., excessive investigations) and negatives (i.e., missed diagnoses).

The general hypothesis to be tested herein is: ***Post-operative monitoring of biomarkers improves the early diagnosis of colorectal anastomotic leakage, shortening the time to CAL detection***.

2 - WHAT IS NEW IN THIS RESEARCH?

One of the innovation of this study was the degree of statistical analysis of clinical data, with the adoption of classification methods, cluster, and linear discriminant analysis. For early prediction of colorectal anastomotic failure, regression models were applied to build a simple decision model (warning score). The score was experimented in a test scenario and a cost minimization analysis was conducted, to estimate the impact of delayed, wrong, or missed diagnoses.

CHAPTER II

RETROSPECTIVE STUDY

A - BACKGROUND

In this chapter, the Thesis conceptual framework was characterized, based on the candidate´s significant institutional and personal experience on colorectal disease management, which allows and justifies the relevance of this research, as before mentioned. Thus, a retrospective study including patients who underwent open or laparoscopic colorectal resection from January 1, 2013, to December 31, 2016, performed in Colorectal Division of Surgical Department at CHL was designed. Main reasons for this time study period election were institutional contextualization of the pre-study phase, sufficient study sample size and inclusion of suitable period of colorectal division activity. Thereafter, were assessed the group of patients who underwent colorectal resection with anastomosis for colorectal cancer and finally the subgroup of patients who developed anastomotic leakage. Data collection was made from individual clinical reports, as summarized in **Table 9**.

The aims of the study were to estimate the incidence of CAL, to assess the criteria used to define CAL (clinical, radiological, and surgical findings), and to evaluate short-term and long-term results (morbidity, mortality, and oncological outcomes related to CAL).

This study, entitled *Anastomotic Leak in Colorectal Cancer Surgery*: From *Diagnosis to Management or Failure - A Retrospective Cohort Study*, was published as an original article in the Surgery, Gastroenterology and Oncology, the official scientific journal of International Association of Surgeons, Gastroenterologists and Oncologists (IASGO). It is available in the **Appendix 1** (Rama, *et al.* 2021). The study was supported by the Ministry of Health – Incentive Program for the Integration of Care and Valuation of Patients' Pathways in the National Health Service of Portugal after applying for the "PAIRAR" project. The study was approved by the Ethics Committee of the CHL.

B - SUMMARY OF MAIN RESULTS

From January 1, 2013, to December 31, 2016, 480 out of 915 patients met the inclusion criteria (**Appendix 1 - Figure 1**), all of them with colorectal cancer and operated in the Colorectal Division at CHL. Procedures for benign disease (n=243; 26.6%), without anastomosis (n=72; 7.9%) and for stoma closure (n=65; 7.1%) were excluded. Patients with pouch surgery, re-intervention or small bowel resection were also not included.

Table 9. Variables selected for collection in the retrospective study

Variables	
Demographic	Age Gender
Pre-operative	Comorbidities Smoking and alcohol habits Allergies Previous abdominal surgery Steroids or immunosuppression in the last 6 months Pre-operative diagnosis Pre-operative staging Bowel preparation American Society of Anesthesiologists grade
Intra-operative	Type of anesthesia Anastomosis technique Blood loss Blood transfusion Surgical complications Level of surgical contamination Duration of surgical procedure Surgical specimen Surgical approach
Post-operative	Morbidity Mortality Time of follow-up Intensive care unit stay

This cohort (N = 480) is composed mostly by men (n= 287; 59.8%), with colon cancer (n=353; 73,5%) and a mean age of 70.4 ± 12.57 years. Thirty-seven patients developed CAL (7.7%) and the rate decreased gradually each year, from 9.1% in 2013 to 5% in 2016 (Appendix 3 - Figure 2). Anastomotic leak was more frequent in men (n=26; 70.3%), left colectomy and proctectomy (n=25; 67.5%) and in the laparotomic approach (n=13; 35.1%) or conversion (n=5; 13.5%). Clinical characteristics and different surgical approaches are summarized in (Appendix 3 - Tables 1 and 2).

Thirty-two patients (86.5%) had CAL diagnosis at the first hospital admission and five had the diagnosis deferred. Mean time for CAL detection was 6.8 days (day 2 to 17) and was most common on day 5. Twenty-five patients were diagnosed based on clinical criteria, including biomarkers (leukocyte and CRP), and in these sub-group, the diagnosis was made earlier (5.6 ± 2.1 days). These patients had a shorter LOHS

(26.1 vs. 40.9 days), which is not statistically significant (p=0.073). The remaining twelve required additional exams, such as abdominal and pelvic CCT scan and/or lower gastrointestinal endoscopy. Three out of 12 CAL patients scanned did not show unequivocal signs in CT scan. In this subgroup, diagnosis was reached later, with statistical significance [8.5 ± 4.2 days, (0.7 to 4.8), p=0.004].

Six patients were managed non-operatively and four needed an image-guided drainage of intraabdominal collections (one by transrectal access). Twenty-four out of 31 patients (64.8%) were submitted to anastomotic takedown and Hartmann's procedure, and six (16.2%) underwent refashion of the anastomosis with covering stoma. Twelve (32.4%) out of the 37 patients required Intensive Care Unit admission and fifteen (40.5%) received parenteral nutrition. Over 34.9 months of follow up, 20 out of 37 patients (54.1%) maintained bowel continuity, including preserved primary or refashioned anastomosis (n=10; 27%) and Hartmann reversal status (n=10; 27%). The main causes for not closing the stoma were patient refusal and morbidity (n=10) and cancer dissemination (n=4). The causes for secondary anastomotic failure were stenosis (n=2) and local recurrence (n=1) – **Appendix 3 - Figure 3**.

Concerning morbidity, the rate of complications was significantly higher in the CAL-patient group. Based on the Clavien-Dindo classification, 26 out of the 37 patients (70.2%) had grade III and IV complications, vs. 34 patients in the group who had no CAL (7.7%) (table 5). Mean LOHS was significantly higher in the CAL cohort [(10.5 vs. 31.3 days - < 0.0005 (14.9 to 21.9) p< 0.0005, and 83.8% vs. 6.1%, (6.0 – 89.4), p< 0.0005, respectively].

Overall and specific 30-day mortality were higher in CAL-patient group [21.6% vs. 4.7%, (8.1 – 32.9), p< 0.0005, and 13.5% vs. 1.8%, (1.1 – 14.7), p<0.0005, respectively]. Comparing both two-year periods, 30-day mortality was lower in the second one (2015-16) in both groups (with and without CAL) [27.2% vs. 15.5% (7.6 to 34.9, p=0.417), and 6.1% vs. 2.3% (0.1 to 7.8, p=0.049), respectively].

This study aimed to assess the impact of CAL on the OS, with an average follow-up of 47.4 ± 23.2 meses. Patients without CAL had a 5-year OS (in all stages) higher than CAL-patients group (63.3%, vs. 52.9%). Comparing Kaplan-Meier's survival curves, the Gehan-Breslow-Wilcoxon test shown statistical significance in OS between the groups (62.4 ± 1.5 vs. 50 ± 6.6 months; p=0.009) – **Appendix 3 - Figure 4**. Statistical analysis by subgroups (at different stages) shown the same trend of higher OS in the group of patients without CAL - **Appendix 3 - Figure 5**. On the other hand,

colon cancer patients who developed CAL had a significant lower 5-year OS, 50%, vs. 66.3% (p=0.002). This significant difference was not observed in the CAL rectal cancer cohort, as the 5-year OS was 55.6% vs. 65%, in the no-CAL cohort (p>0.05) - **Appendix 3 - Figure 6**.

C - SUMMARY OF MAIN CONCLUSIONS

This is an original study in the Portuguese population, presenting overall results similar to other studies with the same purpose, where two thirds of CAL patients were diagnosed earlier based exclusively on clinical criteria, and CAL cohort had longer LOHS, higher morbidity and mortality (78.3% and 21.6%, respectively), and rate of re-operations. With appropriate caution, the results of this study will be compared with the prospective observational study further presented. It was emphasized that CAL rate decreased in the second two-year period, probably reflecting the creation of the local Colorectal Division, with a surgical team dedicated to colorectal disorders management. Mortality following a complication as CAL (FTR) is a useful metric to evaluate different management options, to determine their impact on survival, and to perform institutional benchmarking. Early detection of CAL can be a strategy to reduce this specific mortality. Further prospective studies will be useful to obtain added-value evidence in this topic.

CHAPTER III

SYSTEMATIC REVIEW AND META-ANALYSIS

THE USEFULNESS OF INFLAMMATORY BIOMARKERS TO PREDICT ANASTOMOTIC LEAKAGE AFTER COLORECTAL SURGERY: SYSTEMATIC REVIEW AND META-ANALYSIS

Nuno Rama[1-5], Marlene Lages[2,3], Cândida G. Silva[2,3], Patrícia Motta Lima[1], Inês Campos Gil[1], Maria Guarino[2,3], Pedro Oliveira[4], Maria Dixe[2,3], Anabela Rocha[4], Fernando Castro-Poças[4] and João Pimentel[5]

1. Colorectal Division, Leiria Hospital Center, Leiria, Portugal

2. School of Health Sciences, Polytechnic of Leiria, Leiria, Portugal

3. Center for Innovative Care and Health Technology (ciTechCare), Polytechnic of Leiria, Leiria, Portugal

4. School of Medicine and Biomedical Sciences, University of Oporto, Porto, Portugal

5. Montes Claros Clinic, Coimbra, Portugal

A - INTRODUCTION

Minimal access surgery and standardised recovery protocols have improved patient recovery after colorectal surgery. Regardless of these developments, anastomotic leakage remains a major complication after colorectal surgery, with a reported incidence ranging from 2 to 7 per cent when surgery is performed by experienced surgeons (Iancu *et al.* 2008; Matthiessen, *et al.* 2008; McDermott, *et al.* 2015), increasing up to 8 to 14 per cent in low colorectal resections (Kang *et al.* 2013; Platell, *et al.* 2007; Trencheva, *et al.* 2013). Early diagnosis of CAL is crucial to limit the clinical consequences of this complication, allowing its prompt treatment (Kang, *et al.* 2013; Trencheva, *et al.* 2013). Colorectal anastomotic leakage contributes to possible patient morbidities, hospital re-admissions and overall healthcare costs. Furthermore, complications such as CAL and re-operations are considered a quality indicator in colorectal surgery (Kang, *et al.* 2013).

Although some risk factors have been identified and reported, it remains difficult to predict the development of CAL in individual patients (Singh, *et al.* 2014). Intraabdominal sepsis can be similar to physiological systemic inflammatory response syndrome (SIRS) to surgery, especially in the immediate post-operative period (Sammour *et al.* 2012). This leads to a delay in clinical diagnosis, increasing the risk of patients being discharged before diagnosis and then readmitted with CAL (Sammour, *et al.* 2012; Singh, *et al.* 2014). Late detection

of CAL may lead to the development of sepsis, multiple organ dysfunction or death. Thus, early diagnosis of CAL, at the asymptomatic stage, is of paramount importance.

Several studies have suggested the use of serum biomarkers to ease the early detection of post-operative septic complications. In colorectal surgery, some biomarkers have been identified for detecting various stages of early ischaemia, inflammation and necrosis (Chuang *et al.* 2006). Eosinopenia has been proposed as a biomarker that might help to identify several sepsis-related conditions, distinguished from other causes of SIRS (Garnacho-Montero, *et al.* 2014). Serum C-reactive protein (CRP) has been shown to have a strong correlation with post-operative complications, including abdominal surgery (Almeida, *et al.* 2012; Straatman, *et al.* 2015). The usefulness of procalcitonin (PCT) has been highlighted as an earlier, more sensitive, and more reliable biomarker of CAL, even before symptoms appear. Moreover, PCT and CRP have been demonstrated to have a good negative predictive value for CAL (Giaccaglia, *et al.* 2016; Giaccaglia, *et al.* 2014). Calprotectin (CLP) can be a biomarker for amplified inflammation early in major abdominal complications. There are currently few studies that have investigated CLP as a predictor for CAL. Reisinger *et al.* showed that CLP is a better biomarker for detecting CAL than CRP (Reisinger, *et al.* 2014). However, data regarding the diagnostic accuracy of the combination of clinical and laboratory markers for the diagnosis of CAL is still scarce. Further studies are needed to ascertain whether the addition of serum biomarkers can improve the early diagnosis of CAL. This systematic review and meta-analysis aimed to assess the added value of the serum biomarkers CRP, PCT, CLP and white blood cells (WBC) for the early detection of anastomotic leakage after colorectal surgery.

This research, entitled *Usefulness of Inflammatory Biomarkers to Predict Anastomotic Leakage after Colorectal Surgery: Systematic Review and Meta-Analysis*, was published as an original article in the Surgery, Gastroenterology and Oncology. It is available in the **Appendix 2** (Nuno Rama 2022).

B - METHODS

The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Transparent Reporting of Systematic Reviews and Meta-Analysis guideline (Moher *et al.* 2009), with PROSPERO registration number 161692.

1 - Literature search

A comprehensive search was performed in MEDLINE, Embase, PubMed, Web of Science, Scopus, and Cochrane databases, including the following controlled terms from MeSH: Eosinophils OR C-reactive protein OR Procalcitonin OR Calprotectin AND Colon OR Rectum OR Surgery OR Morbidity. Research articles published until 31st of August 2021, restricted to humans and written in English were considered and included in this study. Review articles were excluded. Additionally, references from the published literature that met the inclusion criteria were identified by searching relevant papers, systematic reviews, and meta-analyses manually. The results of all searches were combined to eliminate duplicate articles. The abstracts obtained by the search were used by two reviewers (N.R. and I.G.) independently to select suitable articles, after which the full-text versions were retrieved and independently reviewed for inclusion by the two reviewers.

2 - Study selection

Studies were assessed for inclusion independently by two authors, and any disagreements over inclusion and exclusion were resolved by consensus. Studies were included if they met the following Population, Intervention, Comparison, Outcomes and Study (PICOS) criteria: (1) patients over the age of 18 years; (2) intervention included colorectal surgical procedure with resection and anastomosis, with or without a protective stoma, regardless of the pathology that motivated the procedure, as well as the elective or urgent character; (3) the comparison group was patients without CAL; (4) outcomes assessed were CAL rate, area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV); (5) studies with different designs as presented in Table 10 .

Table 10. Design of the included studies.

Randomised Controlled Trials
Cluster-Randomised Controlled Trials
Non-Randomised Cluster Controlled Trials
Controlled Before and After Studies
Interrupted Time Series
Before-After Study without a Control Group
Comparative Studies with Historical Controls

3 - Data extraction

Data were extracted by three authors (N.R., M.G., M.L.) and entered predefined tables. The primary outcome of interest was CAL, defined as reported in the studies included. The measure of diagnostic accuracy, namely, ROC curve, AUC, sensitivity, specificity, PPV and NPV, were recorded to perform a diagnostic meta-analysis. Data reported in the text, graphs or figures of the studies were used to obtain the median or mean biomarker values on each post-operative day (POD) for the following patient groups: those with CAL, any infectious complication, and no complications. Corresponding authors were contacted to obtain the necessary data when it was not made available from the article or supplementary material.

4 - Quality assessment

Quality assessment of the studies was performed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) 2 tool (Whiting *et al.* 2011). The QUADAS 2 tool assessed the risk of bias and concerns about applicability in four key domains: patient selection, index test, reference standard, and flow of patients through the study and timing of tests, classifying them as low risk, unclear risk, and high risk. The tool was tailored to suit the content of studies and the purpose of this review and applied independently by three authors (N.R., M.G., M.L.).

5 - Data analysis and synthesis

To summarise and compare studies, where available, mean and standard deviation (SD) values for each biomarker in two groups of patients (with or without CAL) were directly pooled and analysed with standardised mean differences (SMDs), mean differences (MDs) and 95% confidence intervals (CIs) (Faraone 2008). Measures of diagnostic accuracy, including area under ROC, AUC, sensitivity, specificity, PPV and NPV, were recorded to enable a diagnostic meta-analysis to be performed. Study-specific estimates were pooled using random-effect models. Two sets of meta-analyses were performed based on the biomarker, and POD.

The statistical heterogeneity among studies was assessed using the I^2 index (Higgins *et al.* 2003), thus reporting the percentage of variation in the global estimate that was attributable to heterogeneity (I^2 = 25%: low; I^2 = 50%: moderate; I^2 = 75%: high).

Forest plots were created to illustrate the effects in the meta-analysis of the different studies and the global estimation. R (R Core Team, 2020) and RStudio (RStudio Team, 2020) were used to perform all analyses. The R package meta was used to conduct standard meta-analysis (Balduzzi *et al.* 2019), and the *R package mada* was used for meta-analysis of diagnostic accuracy. Statistical significance was defined as a p value <0.05.

Qualitative methods were used to analyse the degree of conceptual agreement of the different CAL definitions used in the included studies, based on a recently established consensus definition (van Helsdingen, *et al.* 2020). Different conceptual categories of the consensus were considered, and each individual definition was split and whether each category was mentioned was recorded.

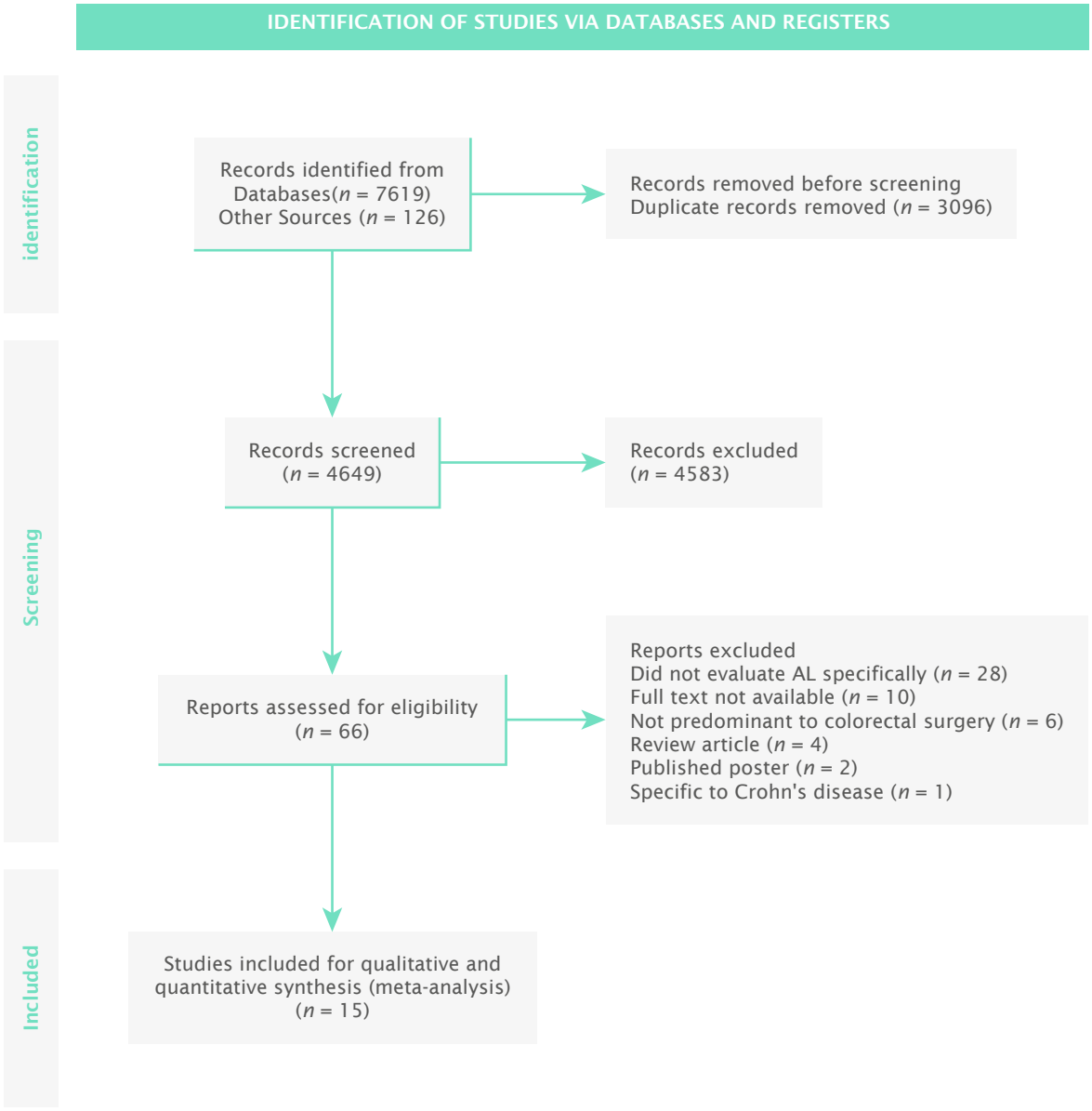
C - RESULTS

A PRISMA flowchart illustrating the selection of articles included in this systematic review is presented in **Figure 2**. Fifteen studies (Almeida, *et al.* 2012; Baeza-Murcia *et al.* 2021; Garcia-Granero, *et al.* 2013; Giaccaglia, *et al.* 2016; Giaccaglia, *et al.* 2014; Italian ColoRectal Anastomotic Leakage Study 2020; Jin and Chen 2021; Lagoutte, *et al.* 2012; Messias *et al.* 2020; Ortega-Deballon, *et al.* 2010; Pantel, *et al.* 2019; Pantoja Pachajoa *et al.* 2021; Scepanovic *et al.* 2013; Stephensen *et al.* 2020; Zoran Kostić*† and Slavković* 2015) met the defined inclusion criteria and had adequate data to be included in the meta-analysis.

1 - Study characteristics

The characteristics of the fifteen included studies are summarised in **Table 11**. All studies included patients undergoing both colonic and rectal surgery. Ten of the fifteen studies were prospective studies.

Figure 2. **PRISMA flow diagram of the study selection process.**



2 - Risk of bias

The results from the QUADAS-2 assessment are shown in Table 12. Eight studies (Almeida, *et al.* 2012; Garcia-Granero, *et al.* 2013; Jin and Chen 2021; Lagoutte, *et al.* 2012; Messias, *et al.* 2020; Pantoja Pachajoa, *et al.* 2021; Scepanovic, *et al.* 2013; Stephensen, *et al.* 2020) reported measuring CRP routinely during the post-operative period, whereas the other seven (Baeza-Murcia, *et al.* 2021; Giaccaglia, *et al.* 2016; Giaccaglia, *et al.* 2014; Italian ColoRectal Anastomotic Leakage Study 2020; Ortega-Deballon, *et al.* 2010; Pantel, *et al.* 2019; Zoran Kostić*† and Slavković* 2015) did not have CRP data available for all patients on each day. Only two studies (Garcia-Granero, *et al.* 2013; Lagoutte, *et al.* 2012)

Table 11. Summary of the characteristics of included studies evaluating biomarkers.

Reference	Study design	Study interval	Elective, *n* (%)	Approach, *n* (%)	Colonic/rectal surgery, *n* (%)	Operation for cancer, *n* (%)	*n*	CAL rate, *n* (%)	Biomarkers assessed
Ortega-Deballon *et al.* 2010	Prospective	11 months	133 (100)	Open 117 (88) Min inv 16 (12)	57/78 (42/58)*	82 (61.7)	133	21 (15.5)	CRP WBC
Almeida *et al.* 2012	Retrospective	22 months	164 (95)	Open 142 (82) Min inv 31 (18)	138/35 (80/20)	129 (75)	173	24 (13.9)	CRP WBC
Lagoutte *et al.* 2012	Prospective	13 months	100 (100)	Open 65 (65) Min inv 35 (35)	68/32 68/32)	52 (52)	100	13 (13.0)	CRP PCT
Garcia Granero *et al.* 2013	Prospective	17 months	205 (100)	Open 162 (79) Min inv 43 (21)	144/61 (70/30)	150 (73.2)	205	11 (5.4)	PCT CRP WBC
Scepanovic *et al.* 2013	Prospective	18 months	156 (100)	Open 156 (100) Min inv 0 (0)	85/38 (69/31)**	151 (96.8)	156	15 (9.6)	CRP WBC
Giaccaglia *et al.* 2014	Prospective	12 months	101 (100)	Open 89 (88) Min inv 12 (12)	77/24 (76/24)	93 (92.1)	101	9 (8.9)	PCT CRP WBC
Kostić *et al.* 2015	Prospective	20 months	150 (100)	n.s.	85/65 57/43)	150 (100)	150	15 (10.0)	CRP
Giaccaglia *et al.* 2016	Prospective	21 months	504 (100)	Open 126 (25) Min inv 378 (75)	327/177 (65/35)	504 (100)	504	28 (5.6)	PCT CRP
Pantel *et al.* 2019	Retrospective	54 months	752 (100)	Open 197 (26) Min inv 555 (74)	604/124 (80/17)***	227 (33)	752	17 (2.3)	CRP
iCral Study Group 2020	Prospective	12 months	1546 (100)	Open 255 (17) Min inv 1291 (83)	n.s.	1064 (68.8)	1546	76 (4.9)	CRP PCT
Messias *et al.* 2020	Retrospective	49 months	64 (71)	n.s.	65/25 (72/28)	31 (34.4)	90	11 (12.2)	CRP
Stephensen *et al.* 2020	Prospective	16 months	833 (100)	n.s.	663/170 (80/20)	584 (70.1)	833	41 (4.9)	CRP
Pantoja Pachajoa *et al.* 2021	Retrospective	46 months	101 (82)	Open 65 (56) Min inv 51 (44)	100/16 (86/14)	86 (74)	116	9 (8)	CRP WBC
Jin *et al.* 2021	Retrospective	23 months	196 (100)	Open 0 (0) Min inv 196 (100)	0/196 (0/100)	196 (100)	196	11 (5.6)	CRP
Baeza-Murcia *et al.* 202	Prospective	8 months	95 (100)	Open 40 (42) Min inv 55 (58)	77/18 (81/19)	75 (78.9)	95	14 (14,7)	CRP PCT

*Min inv, minimally invasive surgery; CRP, C-reactive protein; WBC, white blood cells; PCT, 20 procalcitonin; n.s., not stated; * 133 surgeries, 135 anastomosis; ** 123 colorectal surgeries; *** 21 surgeries were not classified in colonic or rectal surgery in 24 patients*

measured PCT daily in the post-operative period, and four studies (Almeida, *et al.* 2012; Garcia-Granero, *et al.* 2013; Pantoja Pachajoa, *et al.* 2021; Scepanovic, *et al.* 2013) had WBC count data available daily after surgery. Only one study (Ortega-Deballon, *et al.* 2010) reported blinding of surgeons to the results of CRP assays. The included studies had different definitions of CAL (**Table 13**) and not all patients had this complication diagnosed by the same reference standard.

3 - Definition of anastomotic leakage

Definition of CAL according to the included studies showed variations that are presented in **Table 13**. **Tables 14 to 15** represent the results of the qualitative analysis performed. Considering the consensus-based recommendation for the definition of CAL established in the study of van Helsdingen et al. (van Helsdingen, *et al.* 2020), the different definitions presented in the selected studies were divided into three categories: clinical, radiological, and surgical findings.

Table 12. Summary of QUADA-2 results.

	Risk of bias				Applicability		
Reference	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Ortega-Deballon *et al.* 2010	-	-	-	+	-	-	-
Almeida *et al.* 2012	+	?	+	+	-	-	+
Lagoutte *et al.* 2012	-	-	+	+	+	-	-
Garcia Granero *et al.* 2013	-	-	+	+	-	-	-
Scepanovic *et al.* 2013	?	?	?	-	-	-	-
Giaccaglia *et al.* 2014	-	-	+	+	-	-	-
Kostić *et al.* 2015	-	?	+	+	-	-	-
Giaccaglia *et al.* 2016	-	-	+	+	-	-	-
Pantel *et al.* 2019	-	-	?	-	-	-	-
iCral Study Group 2020	-	-	?	-	-	-	-
Messias *et al.* 2020	?	?	-	?	-	-	-
Stephensen *et al.* 2020	?	+	?	?	-	-	-
Pantoja Pachajoa *et al.* 2021	-	?	-	-	-	?	-
Jin *et al.* 2021	?	+	-	-	-	-	-
Baeza-Murcia *et al.* 202	-	?	-	-	-	-	-

– LOW RISK ? UNCLEAR RISK + HIGH RISK

Regarding clinical criteria, only one study (Zoran Kostić*† and Slavković* 2015) covers all the defined subcategories, and among these, drainage of faeces or other suspicious contents was considered in thirteen of the fifteen studies. Most studies did not include three of the four consensus clinical subcategories in the definition. In terms of radiological criteria, six studies integrate the subcategories "extravasation of contrast" and "abscess near anastomosis" in the definition. Six studies state that perianastomotic air is a suggestive sign of CAL, and none of them considered the presence of intraperitoneal air as a diagnostic criterion. Finally, operative findings were considered in eleven studies, and each one mentioned up two subcategories: "signs of peritonitis" and "surgical evidence of dehiscence". In selected studies, neither blind loop nor perianastomotic necrosis were considered as diagnostic criteria for CAL. The CAL rate in the included studies ranged from 2 per cent (Pantel, *et al.* 2019) to 15 per cent (Ortega-Deballon, *et al.* 2010).

4 - Diagnostic WBC accuracy for CAL

The results of random-effects meta-analysis including two studies measuring WBC are shown in Figure 3. Subgroups meta-analysis was performed according to POD2 and 4, with low global heterogeneity (I^2 = 0%; p = 0.82). The pooled average WBC level on each POD for patients with and without CAL are shown in Figure 4. A meta-analysis of the predictive value of WBC for CAL was not possible due to the lack of available data in the selected studies.

Figure 3. Forest plot for WBC data showing the results of random-effects meta-analysis on different post-operative days.

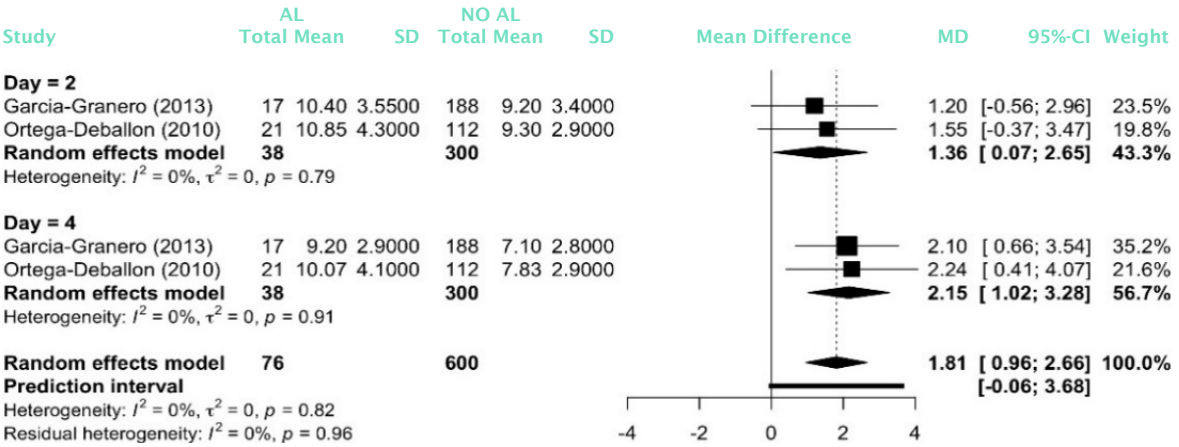


Table 13. Reported definitions of CAL according to each study.

Reference	Definition and diagnosis of anastomotic leak
Ortega-Deballon *et al.* 2010	Presence of one of the following criteria: presence of pus or enteric contents within the drains, presence of abdominal or pelvic collection in the area of the anastomosis on CT scan (performed at the discretion of the attending surgeon), leakage of contrast through the anastomosis during the enema, or evident AL at re-operation for post-operative peritonitis.
Almeida *et al.* 2012	Clinical signs of peritonitis and/or clinical evidence of free faecal fluid within the abdomen or emerging from the drain site. Diagnosis confirmed by abdominal and pelvic CT using intravenous and anorectal contrast.
Lagoutte *et al.* 2012	Presence of one of the following criteria: post-operative peritonitis found at re-operation, purulent or faecaloid wound drainage, presence of air or fluid collection in the anastomotic region on CT.
Garcia-Granero *et al.* 2013	Anastomotic leakages were classified as "major" (need of re-operation or percutaneous radiological drainage, Clavien-Dindo grades III to V) and "minor" (conservative medical treatment, Clavien-Dindo grades I and II). Confirmed either by an X-ray enema with hydrosoluble contrast performed with CT scan, by endoscopy, or intra-operatively.
Scepanovic *et al.* 2013	Clinical presentation of enteric contents within the drains, without imaging performed routinely to search for leakage.
Kostić *et al.* 2015	Presence of purulent or faecal content at the drain site, pelvic abscess, peritonitis, rectovaginal fistula, or the appearance of purulent content from the rectum (per recti). In patients with low colorectal anastomosis, a digital rectal examination was an integral part of the examination to detect a possible anastomotic leak.
Giaccaglia *et al.* 2016	Presence of a faecaloid drain, emission of faecal material from the wound, extravasation of contrast on enema, evidence of post-operative peritonitis at a reintervention and/or the occurrence of fluid, or air in the anastomotic region during a CT scan. Major leakages were considered the ones needing re-operation or percutaneous radiologic drainage (Clavien-Dindo grades III) and minor those in which conservative medical treatment was appropriate (Clavien-Dindo grades I and II).
Pantel *et al.* 2019	Presence of luminal contents through a drain or wound site or abscess cavity, causing inflammation (i.e., fever, leucocytosis, or faecal discharge).

Table 13 (cont). Reported definitions of CAL according to each study.

iCral Study Group 2020	Any deviation from the planned post-operative course related to the anastomosis, presence of pus or enteric fluid in drains or an abdominal/pelvic collection in the area of the anastomosis on CT, contrast leakage through the anastomosis during the administration of an enema, or anastomotic leakage at re-operation for post-operative peritonitis.
Messias *et al.* 2020	Anastomotic leakage was defined using the following clinical and radiologic criteria: 1) presence of air or abscess near the site of anastomosis identified on CT, 2) purulent discharge or enteric secretion through the drain, and 3) clinical signs of peritonitis and/or presence of faecal or purulent discharge during surgical re-approach.
Stephensen *et al.* 2020	A defect in the intestinal wall at the site of the anastomosis requiring operative or radiological intervention.
Pantoja Pachajoa *et al.* 2021	Anastomotic leakage was defined as suture line disruption with intestinal content leakage or abscess formation, associated with fever or abdominal pain, and confirmed by a CT scan or re-operation up to 3 months after colorectal surgery.
Jin *et al.* 2021	Anastomotic leakages were classified as "major" (need of re-operation or percutaneous radiological drainage, Clavien-Dindo grades III to V) and "minor" (conservative medical treatment, Clavien-Dindo grades I and II). All anastomotic leakages were confirmed by fecal fluid drainage, digital rectal examination, signs of peritonitis with high fever, CT scan, endoscopy or operation.
Baeza-Murcia *et al.* 2021	Anastomotic leakage was definite if proven radiologically or clinically and then classified according to the necessary intervention as follows: Grade A, requiring no active intervention (diagnosed radiologically); Grade B, requiring active radiological intervention but manageable without surgical re-intervention; and Grade C, requiring surgical reintervention or showing an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging. The reference test used for AL diagnosing was double- or triple-contrast CT. Patients with poor clinical evolution (fever, prolonged ileus, physical examination suggesting peritoneal irritation, purulent/intestinal output through drain, etc.) underwent the reference test.

CT, computed tomography

Table 14. Qualitative analysis of CAL definitions from the fifteen selected studies: clinical category.

CATEGORY	CLINICAL			
DEFINITIONS	Discharge from the drain	Discharge from the rectum	Rectovaginal fistula	Defect (DRE)
Ortega-Deballon *et al.*	○	●	●	●
Almeida *et al.*	○	●	●	●
Lagoutte *et al.*	○	●	●	●
Scepanovic *et al.*	○	●	●	●
Garcia-Granero *et al.*	●	●	●	●
Giaccaglia *et al.*	○	●	●	●
Kostić *et al.*	○	○	○	○
Giaccaglia *et al.*	○	●	●	●
Pantel *et al.*	○	●	●	●
iCral Study Group	○	●	●	●
Messias *et al.*	○	●	●	●
Stephensen *et al.*	●	●	●	●
Pantoja Pachajoa *et al.*	○	●	●	●
Jin *et al.*	○	◉	●	○
Baeza-Murcia *et al.*	○	◉	●	●

○ Mentioned ● Not mentioned ◉ Mentioned (unclear)

DRE, digital rectal examination

Table 15. Qualitative analysis of CAL definitions from the fifteen selected studies: radiological category.

CATEGORY	RADIOLOGICAL			
DEFINITIONS	Extravasation of contrast	Abcess near anastomosis	Perianastomic air	Free intra-abdominal air
Ortega-Deballon *et al.*	○	○	●	●
Almeida *et al.*	◉	●	●	●
Lagoutte *et al.*	●	○	○	●
Scepanovic *et al.*	●	●	●	●
Garcia-Granero *et al.*	○	●	●	●
Giaccaglia *et al.*	○	○	○	●
Kostić *et al.*	●	●	●	●
Giaccaglia *et al.*	○	○	○	●
Pantel *et al.*	●	●	●	●
iCral Study Group	○	○	●	●
Messias *et al.*	●	○	○	●
Stephensen *et al.*	●	●	●	●
Pantoja Pachajoa *et al.*	○	○	○	●
Jin *et al.*	○	○	○	●
Baeza-Murcia *et al.*	○	◉	◉	●

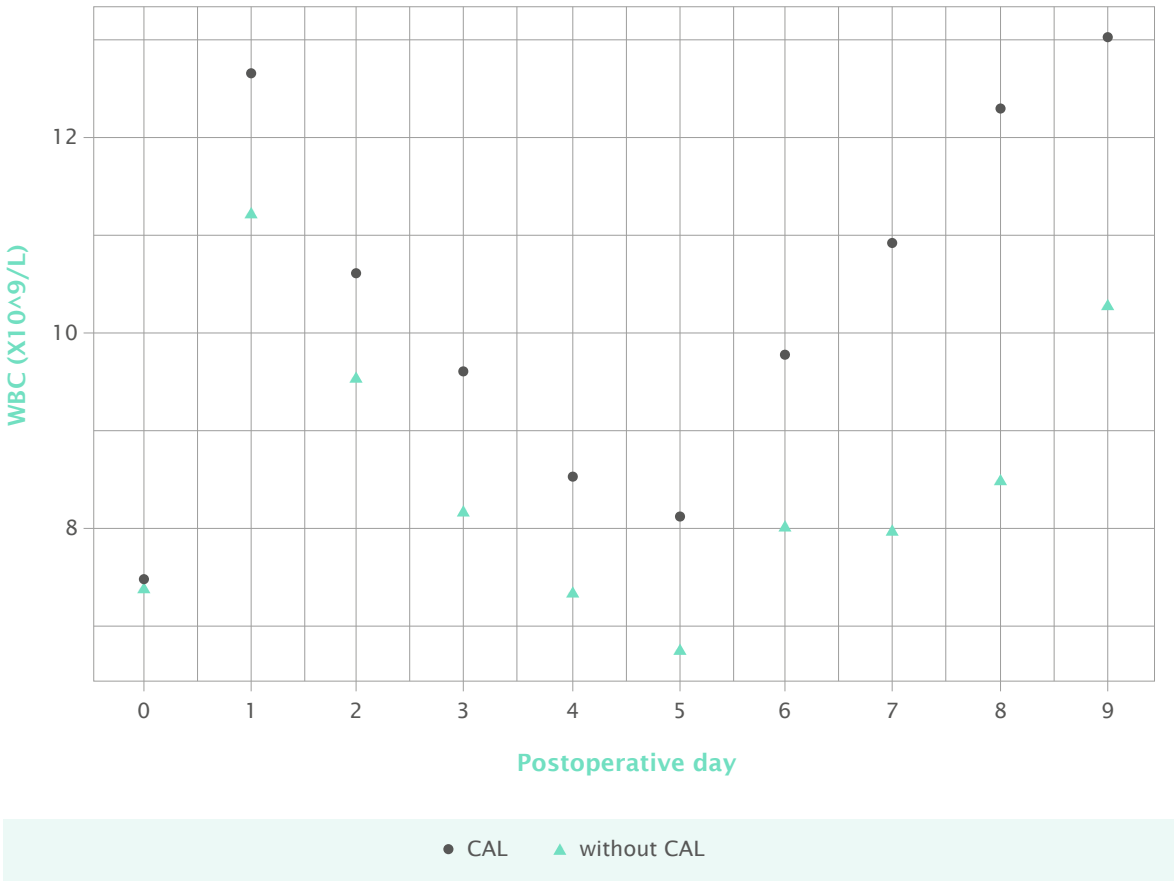
○ Mentioned ● Not mentioned ◉ Mentioned (unclear)

Table 16. Qualitative analysis of CAL definitions from the fifteen selected studies: surgical findings category.

CATEGORY	SURGICAL FINFINGS			
DEFINITIONS	Necrosis of anastomosis	Necrosis of blind loop	Signs of peritonitis	Dehiscence of anastomosis
Ortega-Deballon *et al.*	●	●	●	○
Almeida *et al.*	●	●	●	●
Lagoutte *et al.*	●	●	○	●
Scepanovic *et al.*	●	●	●	●
Garcia-Granero *et al.*	●	●	●	○
Giaccaglia *et al.*	●	●	○	●
Kostić *et al.*	●	●	●	●
Giaccaglia *et al.*	●	●	○	●
Pantel *et al.*	●	●	●	●
iCral Study Group	●	●	●	○
Messias *et al.*	●	●	○	●
Stephensen *et al.*	●	●	●	○
Pantoja Pachajoa *et al.*	●	●	○	○
Jin *et al.*	●	●	○	○
Baeza-Murcia *et al.*	●	●	○	○

○ Mentioned ● Not mentioned ◉ Mentioned (unclear)

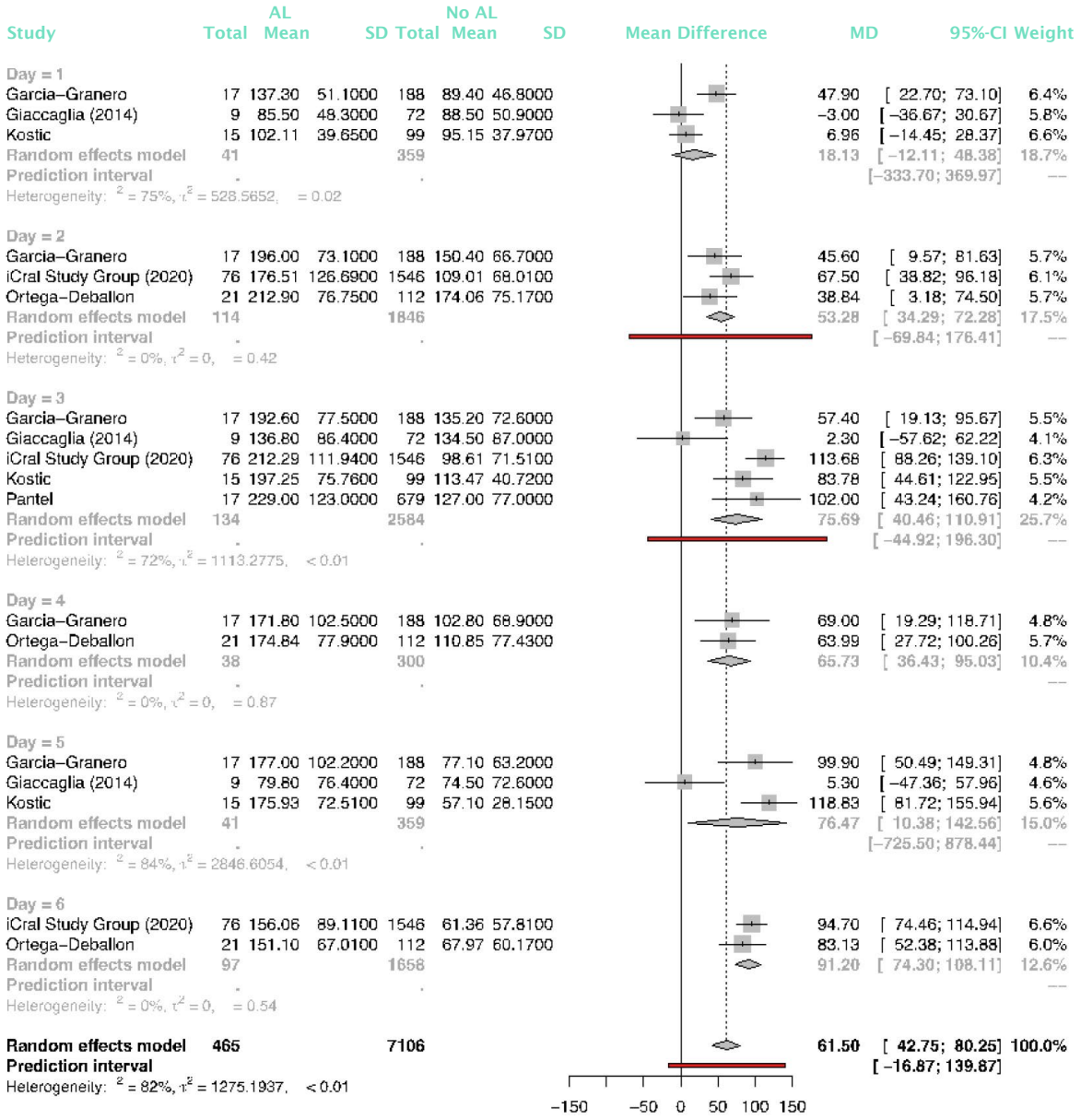
Figure 4. WBC levels in the post-operative period in relation to CAL. Values at each time point represent the pooled median/mean WBC level from the included studies [Ortega-Deballon (2010); Almeida (2012); Garcia-Granero (2013); Scepanovic (2013); Pantoja Pachajoa (2021)], with individual studies weighted by their sample size. CAL, colorectal anastomotic leakage.



4 - Diagnostic CRP accuracy for CAL

The results of random-effects meta-analysis considering the different studies measuring CRP are presented in Figure 5. Subgroups meta-analysis was performed according to POD1 to 7, with a global heterogeneity statistic I^2 values of 82% ($p < 0.01$), which is indicative of high between-study heterogeneity, and a prediction interval that crosses the line of no effect. The comparison of pooled average CRP levels on each POD for patients with and without CAL are presented in Figure 6.

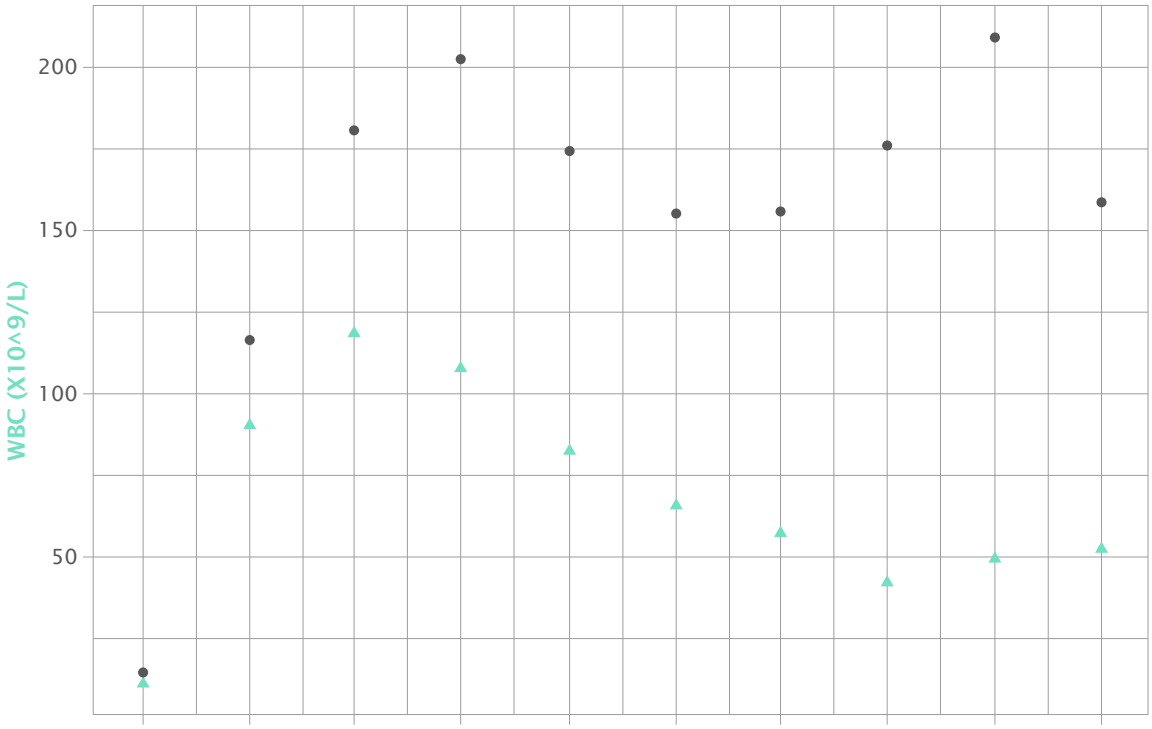
Figure 5. Forest plot for CRP data showing the results of random-effects meta-analysis on different post-operative days.



Ten studies were selected in the subgroups meta-analysis of CRP accuracy for CAL (POD3 to 5), with a pooled prevalence of CAL ranging from 5.9 to 7.7 per cent (Table 16). Pooled AUC values on POD3 and 5 ranged from 77.9 to 87.1% and had similar diagnostic accuracy for CAL (Figure 7). The highest pooled sensitivity and specificity were found on POD5 (79.4 and 80.2% respectively). At these three time-points, pooled PPV and NPV ranged from 21.4 to 30.7%, and from 96.2 to 97.4%, respectively, showing low

and moderate heterogeneity, except for POD3. The positive likelihood ratio (PLR) for CRP varied from 2.7 to 4.1, and the negative LR (NLR) was between 0.30 and 0.36. The derived cut-offs on POD3 and 5 were 150.7 ± 30.5 and 103.5 ± 35.9 mg/L, respectively.

Figure 6. C-reactive protein (CRP) levels in the post-operative period in relation to CAL. Values at each time point represent the pooled median/mean CRP level from the included studies [Ortega-Deballon (2010); Almeida (2012); Lagoutte (2012); Garcia-Granero (2013); Scepanovic (2013); Giaccaglia (2014); Kostic (2015); Giaccaglia (2016); Pantel (2019); iCral Study Group (2020); Messias (2020); Pantoja Pachajoa (2021); Jin (2021); Baeza-Murcia (2021)], with individual studies weighted by their sample size. CAL, colorectal anastomotic leakage.



5 - Diagnostic PCT accuracy for CAL

Random-effects meta-analysis for PCT are shown in **Figure 8** with subgroups meta-analysis for POD1 to 5. Global heterogeneity was moderate (I^2 = 60%; p = 0.13) and the prediction interval crossed the line of no effect. The pooled average PCT level on each POD for patients with and without CAL are shown in **Figure 9**.

Five studies were selected in the subgroups meta-analysis of PCT accuracy for CAL (POD3 and 5), with a pooled prevalence of leakage that ranged from 6.5 to 7.8 per cent (Table 17). Pooled AUC values on POD3 and 5 ranged from 79.3 to 83.1% and had similar diagnostic accuracy for CAL (Figure 10). The highest pooled sensitivity (80.7%) and specificity (84.9%) were found on POD5. At these two time-points, PCT had a low pooled PPV between 26.9 and 36.1 per cent, with moderate and high heterogeneity, and a high pooled NPV of 97.9% on POD 3, presenting low heterogeneity. The PLR for PCT ranged between 3.9 and 5.86, and the NLR ranged from 0.2 to 0.3. Derived cut-offs on POD3 and 5 were 1.8 ± 2.0 and 1.2 ± 1.1 ng/mL, respectively.

Figure 7. Pooled area under the curve for anastomotic leakage at POD 3 (I2 = 0.0%; Q = 4.87; p = 0.899), POD 4 (I2 = 7.7%; Q = 5.42; p = 0.367) and POD 5 (I2 = 55.1%; Q = 15.61; p = 0.029) for CRP. Values are shown with 95 per cent confidence intervals.

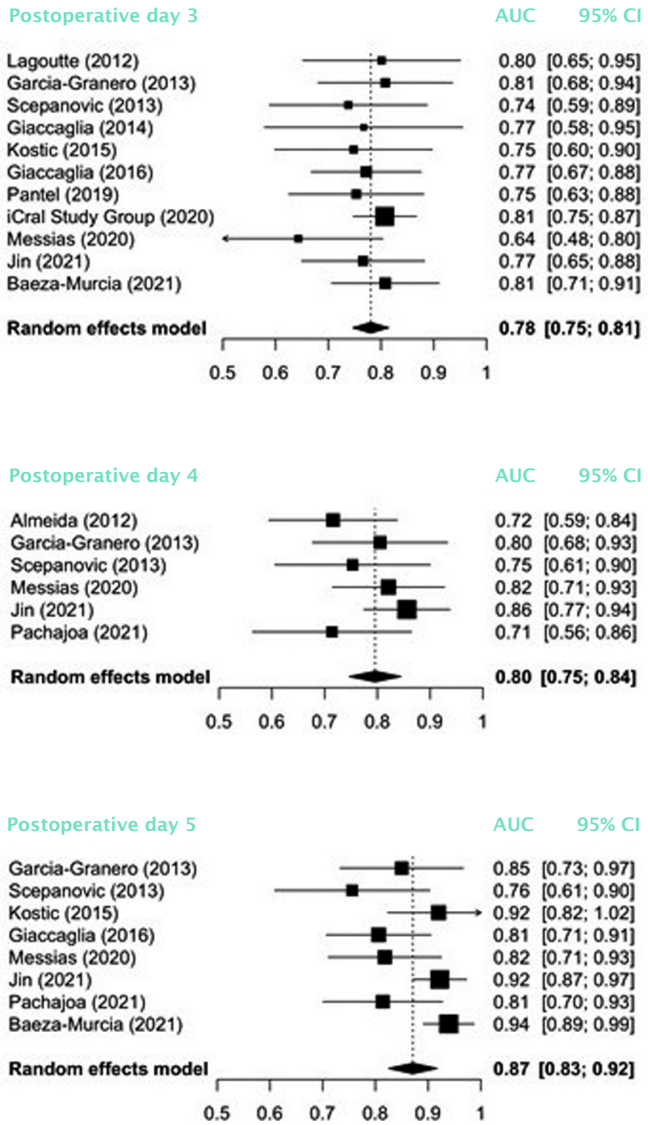


Table 17. Summary estimates for CRP and PCT at different post-operative days. Pooled DOR, sensitivity and specificity, LR+ and LR- were obtained from the summary receiver operating characteristic (bivariate model) for diagnostic test accuracy. Pooled prevalence, area under the curve, positive predictive value and negative predictive value were obtained from standard meta-analysis random forest models. Derived cutoff represents the mean of the cutoff values reported in individual studies.

	CRP (mg/L)			PCT (ng/mL)	
	POD 3#	POD 4$	POD 5‡	POD 3¥	POD 5§
No. Studies (n)	10 (3757)	6 (923)	8 (1380)	5 (2424)	4 (802)
Pooled prevalence of CAL (%)	5.9[a] (4.1; 8.6)	7.7[d] (6.0; 9.9)	7.6[g] (5.7; 10.0)	6.5[j] (3.7; 11.21)	7.8[m] (4.9; 12.2)
Pooled AUC (%)	77.9♔ (74.4; 81.5)	79.6 (74.7; 84.5)	87.1 (82.5; 91.7)	79.3 (74.9; 83.8)	83.1¶ (74.6; 91.5)
Derived Cutoff (Mean±SD)	150.7±30.5	108.2±43.6	103.5±35.9	1.8±2.0	1.2±1.1
Pooled DOR	8.62 (5.76; 12.4)	8.72 (4.05; 16.5)	16.2 (9.1; 26.7)	11.6 (5.3; 22.3)	25.6 (10.6; 52.3)
Pooled sensitivity (%)	73.5 (66.6; 79.4)	77.6 (66.6; 85.7)	79.4 (69.7; 86.6)	73.6 (60.6; 83.4)	80.7 (62.5; 91.3)
Pooled specificity (%)	75.3 (67.5; 81.8)	70.3 (57.8; 80.3)	80.2 (71.7; 86.6)	79.6 (57.8; 91.7)	84.9 (64.8; 94.5)
Pooled PPV (%)	21.4[b] (14.8; 29.8)	22.1[e] (15.3; 30.9)	30.7[h] (23.9; 38.4)	26.9[k] (14.8; 43.8)	36.1[n] (23.5; 50.9)
Pooled NPV (%)	97.0[c] (95.6; 98.0)	96.2[f] (94.1; 97.6)	97.4[i] (96.1; 98.3)	97.9[l] (97.1; 98.5)	5.86 (2.5; 12.5)
Pooled LR-	0.36 (0.28; 0.44)	0.3 (0.2; 0.5)	0.3 (0.2; 0.4)	0.3 (0.2; 0.5)	0.2 (0.1; 0.4)

Values in parentheses represent 95% confidence intervals, unless otherwise stated. AL, anastomotic leakage; AUC, area under the curve; DOR, diagnostic odds ratio; LR+, likelihood ratio positive; LR-, likelihood ratio negative; NPV, negative predictive value; PPV, positive predictive value; SD, standard deviation. # Includes data from Almeida (2012), Garcia-Granero (2013), Scepanovic (2013), Kostic (2015), Giaccaglia (2016), Pantel (2019), iCral Study Group (2020), Messias (2020), Baeza-Murcia (2021), Jin (2021). $ Includes data from Almeida (2012), Garcia-Granero (2013), Scepanovic (2013), Messias (2020), Jin (2021), Pantoja Pachajoa (2021). ‡ Includes data from Garcia-Granero (2013), Scepanovic (2013), Kostic (2015), Giaccaglia (2016), Messias (2020), Baeza-Murcia (2021), Jin (2021), Pantoja Pachajoa (2021). ¥ Includes data from Garcia-Granero (2013), Giaccaglia (2014), Giaccaglia (2016), iCral Study Group (2020), Baeza-Murcia (2021). § Includes data from Garcia-Granero (2013), Giaccaglia (2014), Giaccaglia (2016), Baeza-Murcia (2021). ♔ Data not available in Almeida (2012). ¶ Data not available in Giaccaglia (2014). Heterogeneity: a: I^2 = 82.9% ([70.0%; 90.3%]); Q = 52.78; p <0.0001; b: I^2 = 83.1% ([70.4%; 90.4%]); Q = 53.40; p <0.0001; c: I^2 = 55.0% ([8.4%; 77.9%]); Q = 20.00; p = 0.0179; d: I^2 = 17.9% ([0.0%; 62.7%]); Q = 6.09; p = 0.2972; e: I^2 = 62.6% ([9.1%; 84.6%]); Q = 13.36; p = 0.0202; f: I^2 = 0.0% [0.0%; 73.8%]; Q = 4.84; p = 0.4361; g: I^2 = 50.0% ([0.0%; 77.6%]); Q = 13.99; p = 0.0514; h: I^2 = 48.9% ([0.0%; 77.2%]); Q = 13.71; p = 0.0566. i: I^2 = 0.0% ([0.0%; 56.1%]); Q = 5.17; p = 0.6395; j: I^2 = 86.8.3% ([71.4%; 93.9%]); Q = 30.24; p <0.0001; k: I^2 = 88.8% ([76.5%; 94.6%]); Q = 35.59; p <0.0001; l: I^2 = 0.0% ([0.0%; 67.3%]); Q = 2.54; p = 0.6373; m: I^2 = 67.1% ([3.9%; 88.7%]); Q = 9.11; p = 0.0279; n: I^2 = 65.8% ([0.0%; 88.4%]); Q = 8.77; p = 0.0325; o: I^2 = 0.0% ([0.0%; 79.1%]); Q = 2.19; p = 0.5330.

Figure 8. Forest plot for PCT data showing the results of random-effects meta-analysis on different post-operative days. PCT, procalcitonin.

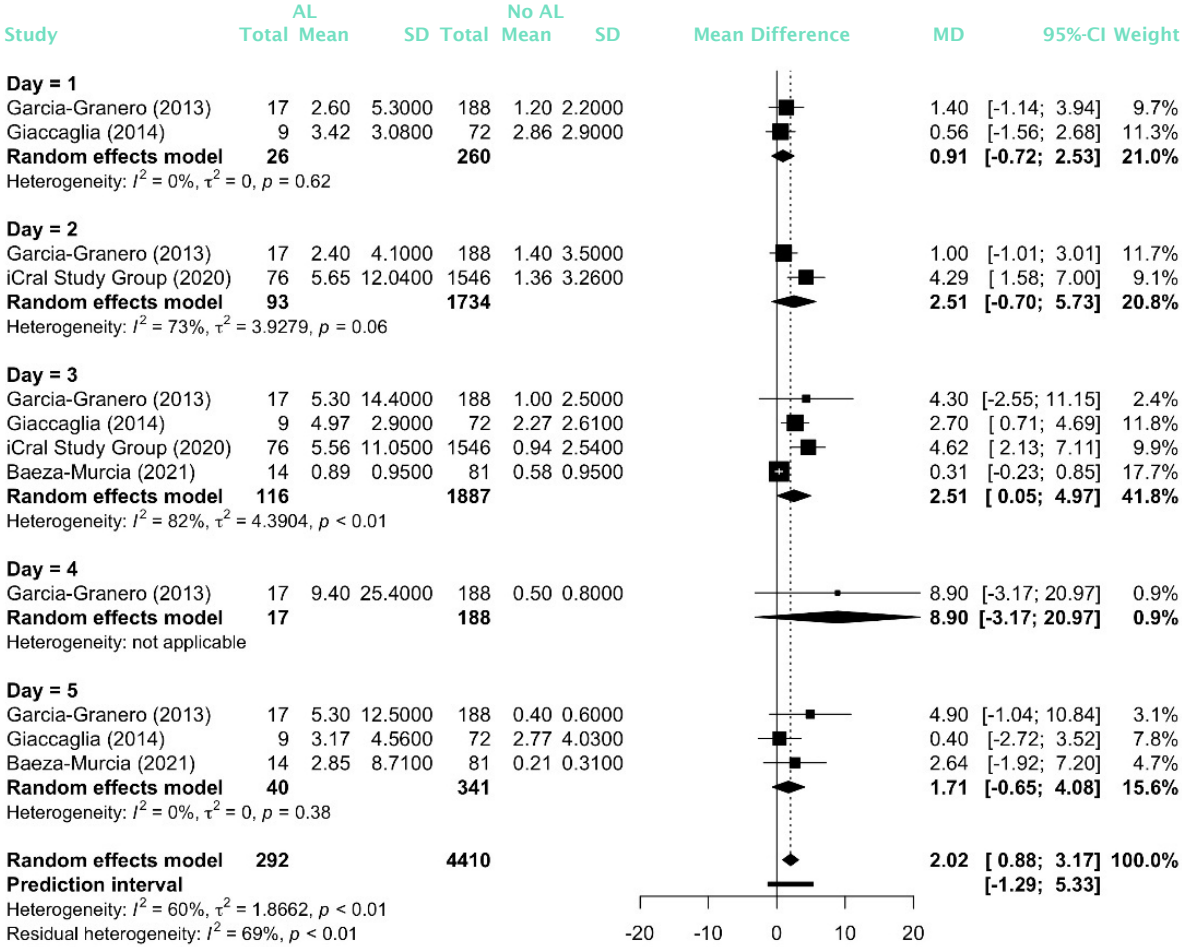


Figure 9. Procalcitonin (PCT) levels in the post-operative period in relation to CAL. Values at each time point represent the pooled median/mean PCT level from the included studies [Lagoutte (2012); Garcia-Granero (2013); Giaccaglia (2014); Giaccaglia (2016); iCral Study Group (2020); Baeza-Murcia (2021)], with individual studies weighted by their sample size.

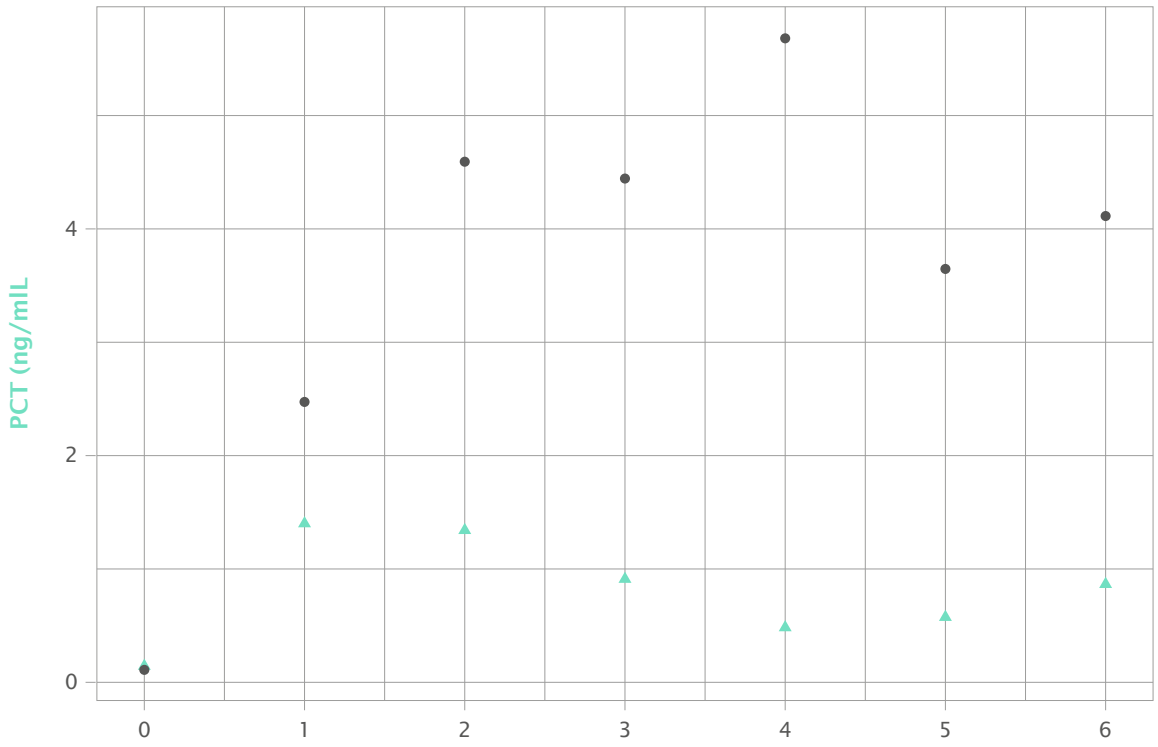
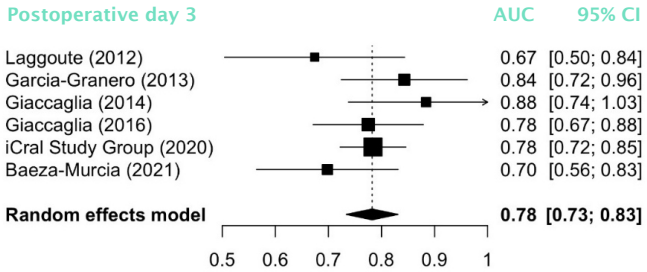


Figure 10. Pooled area under the curve for CAL at POD 3 for PCT (I2 = 16.4%; Q = 5.98; p = 0.308). Values are shown with 95 per cent confidence intervals. PCT, procalcitonin.



D - DISCUSSION

Over the past ten years, few systematic reviews and meta-analyses have evaluated the role of biomarkers in the early diagnosis of anastomotic leakage in colorectal surgery. Su'a *et al*. (Su'a, *et al*. 2017) analyzed both peritoneal drain fluid and systemic biomarkers that are increased in the CAL environment, finding an improvement in predictive accuracy when combining these biomarkers.

This systematic review and meta-analysis demonstrated that the diagnostic accuracy of CRP and PCT was similar on all days and showed higher values on POD5, being superior for CRP with a value of 87.1%. Systemic biomarkers were moderate predictors of CAL when assessed individually. Nevertheless, a combination of biomarkers could increase the predictive accuracy, but data meta-regression was not possible due to the small number of selected studies.

Singh *et al*. (Singh, *et al*. 2014) showed that serum CRP is a useful negative predictive test for detecting anastomotic leakage after colorectal surgery, but not a good positive predictor. In this study, the NPV of serum biomarkers was calculated and proved to be high and useful as a predictive indicator for CAL exclusion. In fact, increased CRP and PCT may result from other clinical conditions, post-operative complications, and systemic inflammatory response. Hence, the clinical usefulness of biomarkers is based on the probability of ruling out an CAL when a patient had a negative test (lower CRP and PCT level) on POD3 and 5. In daily practice, this estimated high NPV is critical for ensuring safe early discharge.

The likelihood ratio is a useful tool for clinical decision-making as these values are test-specific and independent of the prevalence and are more reliable as a single test for an individual patient. Therefore, likelihood ratio provides relevant information applied to a variety of patient characteristics, as it can provide probabilities adjusted to each case, using information obtained from populations, institutions, or surgeon's personal data. The usefulness of likelihood ratio for CAL detection reflects the ability to change a pre-test probability to a new post-test probability, considering the systemic biomarker measured, in relation to the estimated cut-off. In this study, the PLR for PCT showed a good impact on the clinical decision, as a "rule-in" and "rule-out" test for CAL. Moreover, likelihood ratio calculated for CRP presented a moderate impact on the decision-making process, being relevant as a "rule-out" test.

In this random-effects meta-analysis, interstudy heterogeneity varied according to the biomarker measured, being high in the CRP studies. This important limitation can result from the differences in the patient population, study design and risk of bias. Five studies are

retrospective, but only two of the prospective studies did not show investigation bias (blinded surgeons). Furthermore, not all biomarker assays were performed in a standardized manner for the same POD. The qualitative analysis detected inconsistencies in CAL definitions, leading to a relevant verification bias. Both CRP and PCT had a prediction interval that crosses the line of no effect, reflecting the uncertainty expected in the summary effect if a new study is included in the meta-analysis. Only six studies measuring PCT were included, making the prediction interval particularly imprecise. The reduced number of studies assessing WBC and PCT did not support a meta-regression, which would be able to minimize the observed heterogeneity. A further limitation of the studies is that no analytic study was made between colonic and rectal procedures, which might also be responsible for different post-operative inflammatory reactions.

This review distinguishes itself from others that have been published previously. First, we only selected studies including a range of systemic biomarkers, mainly prospective, which can be useful in daily practice. However, rigorous inclusion criteria excluded the only eligible CLP study, and the scarce WBC studies available hampered relevant conclusions. Secondly, we decided not only to conduct a random-effects meta-analysis, but also to present and discuss the predictive interval, assuming its usefulness and potential drawbacks. Finally, a qualitative analysis of CAL definitions in the selected studies was performed, based on the recommendation recently published (van Helsdingen, *et al*. 2020), revealing remarkable conceptual heterogeneity.

The cost-effectiveness of these tests is a critical subject to be considered in further studies. Blood tests included in the post-operative routine are probably cost-effective given the high cost of late treatment of CAL. Furthermore, it is important to assess the combination of biomarkers to raise the accuracy of the test, as well as to define the best time to request them, considering the clinical approach.

Our review and meta-analysis demonstrated that CRP and PCT are moderate predictors of anastomotic leakage in colorectal surgery. It is important for clinicians to be familiar with the role of biomarkers and their benefits. Despite a lack of evidence, it is interesting to note that some biomarkers have been used in clinical practice to predict CAL. In this study, we found higher serum levels of systemic biomarkers in the group of patients presenting CAL. However, these results should be interpreted with caution due to significant heterogeneity among the studies. Many questions remain regarding the usefulness of each biomarker both for early detection of CAL and for assuring safe discharge of patients in this context, making their clinical application challenging.

CHAPTER IV

PROSPECTIVE STUDY

A – INTRODUCTION

Anastomotic leakage is one of the most frequent complications after colorectal surgery, representing a dreaded issue for patients and surgeons. The reported incidence ranges from 0.2% to 27.2%, depending on the study nature, level of anastomosis, or pathology (Boccola, *et al.* 2011; McDermott, *et al.* 2015; Pommergaard *et al.* 2014; Smith, *et al.* 2018; Trencheva, *et al.* 2013). This occurrence is associated with increased morbidity, mortality, re-operation, and healthcare costs (Cousin, *et al.* 2016; Iancu, *et al.* 2008; Matthiessen, *et al.* 2008; Watson, *et al.* 1999). Thus, its clinical relevance should not be underestimated. It also has a negative impact on a patient's quality of life (McDermott, *et al.* 2015; Trencheva, *et al.* 2013).

Early CAL detection is key to decrease related morbidity and mortality; therefore, a prompt and timely diagnosis is crucial (den Dulk, *et al.* 2013; Rojas-Machado, *et al.* 2016; Smith, *et al.* 2018). Initially, it is difficult to distinguish CAL from other post-operative abdominal complications. Surgeons should be aware of subtle clinical signs, and then order additional tests, including serum biomarkers, proper imaging, or even early re-operation. Unfortunately, diagnosis is often delayed, because of a misleading clinical picture, non-systematic assessment, or inconclusive investigations (Doeksen, *et al.* 2007; Marres, *et al.* 2017; Regenbogen, *et al.* 2016; Rojas-Machado, *et al.* 2016; Sutton, *et al.* 2004). Besides clinical parameters, several biomarkers (plasmatic or intraperitoneal), imaging methods (such as abdominal CT scan or WSCE), and scores have been proposed to reduce the time to diagnosis, and to establish an appropriate management pathway (den Dulk, *et al.* 2009; Giaccaglia, *et al.* 2016; Reisinger, *et al.* 2014; Warschkow, *et al.* 2011b).

Plasma C-reactive protein has been proposed as an early predictor of post-operative infectious complications (Facy, *et al.* 2016; Kørner, *et al.* 2009; Silvestre *et al.* 2014; Warschkow, *et al.* 2011b; Welsch, *et al.* 2007). This biomarker is an acute-phase protein, increasing between 6 and 48 hours after surgery, and returning to baseline if inflammation ceases. After this period, a high CRP level is associated with post-operative infectious complications, especially in patients with CAL (Garcia-Granero, *et al.* 2013; Lagoutte, *et al.* 2012; Ortega-Deballon, *et al.* 2010). On the other hand, calprotectin (CLP) is a useful biomarker of inflammation and infection (Cikot, *et al.* 2016; Reisinger, *et al.* 2014). Faecal CLP has been widely used as a marker of gastrointestinal inflammation. However, some authors suggest that high levels of serum CLP could be associated with septic intra-abdominal complication, such as early-stage CAL (Aadland and Fagerhol 2002; Reisinger, *et al.* 2014).

The first part of this research, the results of the prospective study, was published as an original article in the World Journal of Gastroenterology, in a paper entitled *Usefulness of serum C-reactive protein and calprotectin for the early detection of colorectal anastomotic leakage: A prospective observational study*, which is available in the **Appendix 1** (Rama et al. 2022).

B - METHODS

1 - STUDY DESIGN AND POPULATION

A prospective observational, single-center study was conducted, including over 18-year-old adults, undergoing urgent or elective colorectal resection, regardless of the surgical approach (open or laparoscopic), the indication (benign or malignant) or the option for covering stoma. Patients who were, at the time, younger than 18 years old, pregnant, unable to give or provide a written-informed consent were excluded from the study, as well as the ones who did not perform R0 resection with anastomosis or who suffered from inflammatory bowel disease.

The study was conducted in the Colorectal Division of a non-academic hospital, accredited by Joint Comission International®, covering around 500,000 in-habitants. Patients were recruited for 29 months, from 1 March 2017 to 31 August 2019.

This study was carried out in accordance with the Declaration of Helsinki and was approved by the Local Ethical Committees of CHL, after an authorization obtained from the Portuguese Data Protection Authority. This study is registered with the number 9930/2016 (**Appendix 12**). Informed consent was obtained from all individual participants included in the study (**Appendix 13**).

2 - STUDY PROTOCOL AND VARIABLES

Prospective data were collected and recorded in an electronic database according to the study protocol presented in **Appendix 14**. After fulfilling inclusion and exclusion criteria, and obtaining written informed consent, blood samples, demographic, and clinical data [Health-related quality of life score (EQ-5D-5L), nutritional status, comorbidities, Charlson Comorbidity Index (CCI) score, smoke and alcohol habits, allergies, previous abdominal surgery (and number of), use of steroids or immunosuppression in the last six months, pre-operative diagnosis and staging (if malignancy), use of bowel preparation and ASA score] were collected in agreement with pre-operative stage of study protocol. Patients underwent prophylactic antibiotic and bowel preparation, as defined in institutional protocols. Type of anaesthesia, surgical approach and duration, anastomotic technique, blood losses and transfusions required, and operative complications, were recorded.

Follow-up time of the study was 90 days, including data of all post-operative complications, length of hospital stays and readmissions. Early in post-operative period, clinical criteria such as vital signs, abdominal pain and clinical condition were assessed. Clinical condition assessment was performed by senior surgeons and classified in “stable”, “improved” and “deteriorated” accordingly the daily clinical evaluation and progression in the post-operative period, including the subjective appraisal, vital signs, and Glasgow Coma Scale score. Abdominal pain was defined by the presence of pain localised in the abdominal region, identified during daily physical examination performed by senior surgeons, and applying pain visual analogue scale (VAS). Classified in “Absent/low (VAS≤3) / Wound pain (VAS>4) / Localized pain (VAS>4) / Diffuse pain (VAS>4)”.

Five biomarkers were measured in the first five PODs: WBC, ECC, CRP, CLP and PCT. Blood samples were analysed in CHL laboratory, according to the techniques described afterwards. CLP assays were obtained in a deferred manner and the results were not available for the daily decision-making process.

2.1 – White blood cells count / Eosinophils cells count

The determination of WBC and WBC differential, namely the ECC, was carried out in whole blood samples; therefore, a peripheral blood sample was collected by venipuncture into EDTA (Ethylene Diamine Tetra Acetic Acid)-K3 tubes (BD Vacutainer® K3-EDTA). The sample was homogenized by gentle inversion and processed on the *Beckman Coulter® UniCel DxH 800* automated hematology analyzer, in the hematology laboratory department. This analyzer used the complementary data obtained by three methodologies: impedance (to obtain cell volume), radiofrequency conductivity (to analyze internal composition of the cell and nucleus-to-cytoplasm ratio) and light scattering in 5 different angles (to obtain information about cellular granularity). The analyzer was controlled prior to the samples’ processing using controls provided by Beckman Coulter®.

2.2 - C-Reactive Protein

Laboratory analysis of CRP was performed on serum samples, which were obtained by collection of peripheral blood by venipuncture into tubes with separating gel and clot activator (*BD Vacutainer® SST ™ II Advance*) and centrifugation in a refrigerated

centrifuge (3,200 RPM for 10 minutes). An immunoturbidimetric assay was used for quantitative determination of CRP in human serum or plasma (*CRP Latex, Beckman Coulter®*), on an automated clinical chemistry analyzer, *AU5800 from Beckman Coulter®*. During the technique procedure, the patient sample was mixed with a suspension of latex particles coated with goat anti-CRP antibodies; the CRP present in the sample reacted with the reagent's goat anti-CRP antibodies, forming insoluble immune complexes. The turbidity produced by immune complexes, which determines a decrease in the intensity of transmitted light (due to the portion of light that is reflected, absorbed, or scattered), can be measured by the spectrophotometer, and is proportional to the concentration of CRP in the sample. The test is linear within the concentration range of 0.2 – 480 mg/L. Values higher than 5 mg/L were considered pathological. The technique was calibrated (obtaining a 6-point curve) and controlled (using 2 levels of control, normal and pathological) prior to the samples' processing, using specific material supplied by *Beckman Coulter®*.

2.3 – Procalcitonin

Until April 2018, PCT laboratory assay was performed on serum samples, obtained after collection of peripheral blood by venipuncture into tubes with separator gel and clot activator (*BD Vacutainer® SST ™ II Advance*) and centrifugation in a refrigerated centrifuge (3,200 RPM for 10 minutes). The *Elecsys BRAHMS PCT, Roche®* electrochemiluminescence immunoassay for quantitative determination of procalcitonin in human serum or plasma was used on the *Elecsys cobas e411, Roche®* analyser. During the sandwich procedure, the calibrator, control, or user sample was incubated with a biotinylated monoclonal anti-PCT antibody (capture antibody) and a monoclonal anti-PCT antibody labelled with a ruthenium complex (antibody marker), forming a sandwich complex. Streptavidin-coated microparticles are then added, which bind to the complex formed by the biotin-streptavidin interaction. The reaction mixture was then aspirated into a reading cell, where the microparticles magnetically attached to the electrode surface. After a wash to remove unbound elements, an electric current was applied to the electrode, inducing a chemiluminescent reaction that was measured by a photomultiplier. The measured signal was converted to a PCT concentration, in ng/ml, using an analyser-fitted 2-point calibration curve and a lot-dependent reagent-specific curve. This immunoassay has a measuring range of 0.02-100 ng/ml. Since May 2018, the laboratory assay of PCT has been performed

on whole blood samples, obtained after collection by venipuncture into tubes with EDTA K3 anticoagulant (*BD Vacutainer®* K3-EDTA). The assay was performed using Radiometer's Procalcitonin Immunoassay, in the *AQT90 FLex®* equipment, a point-of-care (POC) equipment that uses temporal resolution immunoassay and fluorometry technology. This is an essay that also uses the sandwich technique, already described. During the procedure, the calibrator, control, or user sample was incubated at 37°C in a test well that was coated with a biotinylated monoclonal mouse anti-PCT antibody (capture antibody), immobilized on the streptavidin surface, and a monoclonal mouse anti-PCT antibody labelled with europium (marker antibody), forming a sandwich-like complex. After a wash to remove unbound elements, the time-resolved fluorescence of the europium-labelled sandwich complex was measured, after excitation with a 340nm light. The measured signal was converted to a PCT concentration in ng/ml using the lot-specific analyser-fitted calibration curves of the reagent. The PCT concentration is directly proportional to the measured europium signal. The limit of quantification determined for this assay is 0.12 ng/ml.

PCT values can be interpreted as follows: PCT <0.5 ng/mL represents a low risk of sepsis and/or septic shock; PCT > 2ng/mL represents an increased risk of sepsis and/or septic shock.

2.4 – Calprotectin

Calprotectin is a calcium-binding protein secreted predominantly by neutrophils and monocytes. The heterocomplex consists of the two proteins, S100A8 (calgranulin A) and S100A9 (calgranulin B), also designated as MRP8 and MRP14, respectively. Expression of these proteins in epithelial tissues was first described in context with squamous epithelia and with murine and human wound repair. More recently, an association of CLP expression with adenocarcinomas in humans has emerged. Elevated CLP levels have been found in many sites of inflammation and in the extracellular fluid of patients with many types of inflammatory conditions. The concentration of CLP in blood is increased in patients with rheumatoid arthritis, cystic fibrosis, multiple sclerosis, and HIV infections, while elevated CLP levels have been detected in stool of patients with Crohn's disease and colorectal cancer (Hansson *et al.* 2014; Müller *et al.* 1994; Odink *et al.* 1987; Stríz and Trebichavský 2004; Wilkinson *et al.* 1988). Enhanced expression of CLP is an early event in prostate tumour genesis and may contribute to development and progression or extension of prostate carcinomas

(Hermani *et al.* 2005). Furthermore, they tested the value of CLP as a serum marker for prostate cancer comparing the serum concentrations in cancer patients with healthy controls or patients with benign prostatic hyperplasia. Significantly increased CLP serum levels in prostate cancer were found in prostate cancer patients compared to patients with benign prostate hypertrophy, the latter exhibiting values like those obtained for healthy individuals. Herein, for quantification of serum CLP we used an enzyme linked immunoassay (ELISA) intended for the quantitative determination of CLP in serum and plasma [*Immundiagnostik AG assay IDK® Calprotectin* (MRP8/14)]. The assay utilises the two-site sandwich technique with two selected monoclonal antibodies that bind to human CLP. Standards (0; 3,9; 15,6; 62,5; 250 ng/ml), controls and diluted patient samples were added to wells of microplate coated with a high affine monoclonal anti-human CLP antibody using the Triturus ELISA Instrument, a completely open and fully automated ELISA analyser for testing and processing batches of samples for infectious diseases, autoimmunity, and biological drug monitoring (Griffols, S.A.). During the first incubation step, CLP in the samples is bound by the immobilised antibody. Then a peroxidase labelled conjugate was added to each well to form the following complex: capture antibody- human CLP – peroxidase conjugate. Tetramethylbenzidine (TMB) was used as a substrate for peroxidase. Finally, an acidic stop solution was added to terminate the reaction and to change the solution colour from blue to yellow. The intensity of the yellow colour is directly proportional to the CLP concentration of sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from standard. CLP, present in the patient samples, is determined directly from this curve. The obtained results were multiplied by the dilution factor of 30 to get the actual concentrations. The reference range for CLP in plasma of healthy persons: < 3µg/ml.

Study protocol included systematic additional investigation in any case of clinical deterioration and/or serum biomarkers increase. In such cases, patients underwent further imaging study with abdominopelvic CT scan (and WSCE if colorectal anastomosis). Patients were discharged if they tolerated oral intake, had recover lower gastrointestinal function, had adequate pain control with oral analgesia, absence of signs suggesting sepsis, and fulfilled institutional social criteria.

3 - ENDPOINTS AND DEFINITIONS

In this study, CAL rate was established as primary outcomes of interest, and secondary endpoints were septic post-operative complications, length of hospital stay and ninety-days mortality. Colorectal anastomotic leakage was defined in accordance with the following criteria: 1) Clinical: enteric discharge from abdominal drain or wound, rectovaginal fistula or anastomotic defect found by digital examination; 2) Radiological (CT scan): extravasation of endoluminal administrated contrast, intra-abdominal collection around the anastomosis, presacral abscess near anastomosis or perianastomotic air and free intra-abdominal air; 3) Surgical findings (re-operation): necrosis of anastomosis, or signs of peritonitis and anastomotic defect. Once diagnosed, CAL was classified into two categories: 1) Minor: patients with CAL and Clavien-Dindo grade I or II, requiring no active intervention (radiological or re-operation) [Grade A of the International Study Group of Rectal Cancer (ISREC) definition]; 2) Major: all other patients with CAL (Dindo, *et al*. 2004; Rahbari, *et al*. 2010).

Superficial surgical site infection, or wound infection, was characterized by tissue deposition and multiplication of bacteria, with host reaction, and was diagnosed by the presence of inflammatory signs or purulent discharge from the surgical wound. Post-operative ileus was defined by the combination of at least one of the following signs from the third to the seventh POD, with no improvement: nausea and vomiting; inability to tolerate solid or semi-liquid diet during the preceding 24 hours; no gas or stool for the preceding 24 hours; abdominal distension; radiological evidence of ileus; need for nasogastric tube insertion. Pneumonia was diagnosed by suggestive clinical signs of respiratory infection (e.g., fever, cough, dyspnoea) associated with radiological signs of pulmonary infiltration. Urinary tract infection was defined by positive urine culture associated with urinary symptoms, fever and/or leucocytosis.

4 - STATISTICAL ANALYSIS

Data was explored using standard descriptive statistics and graphical analysis. To compare the equality of biomarkers' means across the three relevant groups of patients (G1 – patients without complications; G2 – patients with complications not related to CAL; G3 – patients with CAL) were adopted (one-way) analysis of variance statistical tests. To assess the association between other categorical variables and the G1 to G3-patients, chi-square tests were conducted.

The evaluation of single biomarker as an appropriate classifier to early detect CAL was performed using Receiver Characteristics Analysis. The Area Under the Curve (AUC) of the ROC graph was the criteria to establish the diagnostic performance of biomarkers studied. To establish the biomarker' threshold value for CAL, Liu's method was used, and its sensitivity (SS) and specificity (SP) was defined (Liu 2012). Negative (NLR) and positive (PLR) likelihood ratios, and negative (NPV) and positive (PPV) predictive values were computed combining the observed incidence of CAL with the estimated SS and SP at the optimal cut-off value.

The added value of combining two different biomarkers, observed on POD3 or POD5, as a classifier to early predict CAL was explored. Regression models (*Probit, Logit and Complementary Log-Log*) were adopted to analyse binary dependent variables, and the observed CAL status (0/1) in a pair-wising of all biomarkers included in the study: WBC, ECC, CRP, PCT and CLP. Several potential classifiers of CAL were built, applying a non-linear combination of two different biomarkers, given by the non-linear regression models estimated. To minimize the risk of overfitting, the "*leave-one-out*" (loo) methodology was adopted (Gareth James 2013). The AUC was the criteria to select the classifier (defined by the model and the combination of two biomarker) with best predictive diagnostic performance. Liu's method was adopted to select the cut-off value for CAL.

The expected reduction in time to CAL diagnosis obtained by using one biomarker or a pairwise combination of biomarkers was estimated. This was the difference between the observed and the expected mean time to CAL diagnosis, if a specific classifier is used. The expected time to CAL diagnosis was computed by using the following expression: $S \times d1 + [(1 - S) \times d2]$, where S is the SS of the classifier, d1 is the POD of the classifier yielding a positive cut-off value for CAL, and d2 is the day of diagnosis if the classifier provides a false-negative result (time to CAL diagnosis estimated in the dataset).

A warning index score for CAL was developed, based on variables of the prospective study dataset. It was named E-CRALL, acronymous for Early ColoRectAL Leakage, and the registration of logo (**Figure 11**) trademark was made. The development of the score-based classifier encompassed five stages:

I - Selection of variables that can help predicting the occurrence of CAL. These variables were all part of the dataset of the prospective study and included demographic data, intra-operative data, as well as biomarker's values and patient's clinical findings

(clinical condition and abdominal pain) data at POD3, 4 and 5 (Table 18).

II - Adoption of methods for selecting, among all variables abovementioned, those that present the most predictive power for CAL. In addition, those methods should also weight each variable. Least squares shrinkage and selection operator (LASSO), applied to binary dependent variables (LASSO Logit and LASSO Probit), was adopted (Gareth James 2013; Hastie *et al.* 2009).

III - Application of LASSO technique in a training sample (random sample of 70% of the dataset) to select the variables with highest predictive power for CAL and to estimate the weight of each variable. Models from POD3 to 5, were estimated, using Logit and Probit alternatives.

IV - Assessment of the performance (AUC) of all 8 models (or score-based classifier), in a testing sample (the remainder 30% of the dataset), was performed.

V - Establishment of discrimination threshold, the probability level for signalling CAL ("red flag" threshold). Three models (score-based classifiers) with the best predictive performance were established, and the value that simultaneously maximizes SS and SP of the classifier, was adopted.

Sensitivity, SP, NPV and PPV were computed as abovementioned to a single biomarker evaluation.

Figure 11. E-CRALL Score logotype, acronymous of Early ColoRectAL Leakage Score

Table 18. Description of all variables included in E-CRALL development.

Variable	Description
Patient's Characteristics	
Gender	= 1 if the patient is male, 0 otherwise
Age	Age of the patient
BMI	Body mass index
CCI	Charlson Comorbidity Index score
ASA	= 1 if ASA is III or IV; 0 otherwise
Intra-operative	
Type of Surgery	= 1 if elective surgery, 0 otherwise
Surgical Approach	= 1 if surgical approach was open; 0 otherwise
Level of anastomosis: ileocolic	= 1 if ileocolic; 0 otherwise.
Level of anastomosis: colocolic	= 1 if colocolic; 0 otherwise.
Level of anastomosis: rectum (médium)	= 1 if ≥ 6cm of anal verge; 0 otherwise.
Level of anastomosis: rectum (low)	= if < 6cm of anal verge; 0 otherwise.
Blood loss	Level of bleeding (in mL)
Intra-operative complications	= 1 if present; = 0 if absent.
Length of procedure	Operative time (in minutes)
Post-operative	
Abdominal pain: absent/low	= 1 if absent/low; 0 otherwise.
Abdominal pain: at wound	= 1 if at wound; 0 otherwise.
Abdominal pain: localized	= 1 if localized; 0 otherwise.
Abdominal pain: diffuse	= 1 if diffuse; 0 otherwise.
Clinical condition	= 1 if deteriorated, 0 otherwise,
ECC	Plasmatic level of ECC (in cell/µL) from POD 1 to 5
CRP	Plasmatic level of CRP (in mg/L) from POD 1 to 5
CLP	Plasmatic level of CLP (in µg/mL) from POD 1 to 5
WBC	Plasmatic level of WBC (in G/L) from POD1 to 5
PCT	Plasmatic level of PCT (in ng/mL) from POD 1 to 5

To support the decision of whether E-CRALL is worth using, and if so, which would be the best timing to (POD3 to 5), a cost-minimisation analysis (CMA) was conducted to compare the standard clinical practice with the adoption of the score. This method of economic evaluation compares the costs of alternative interventions, assuming that the alternatives deliver an equivalent medical effect (Drummond *et al.* 2015). Thus, in a conservative standpoint, was postulated that both approaches provide

the same benefits or effectiveness (identical outcomes). In this setting, the decision problem consists in decide among four mutually exclusive alternatives: Standard clinical practice (no use of E-CRALL) or adoption of E-CRALL on POD 3, 4 or 5. The alternative that minimizes the expected costs was preferred.

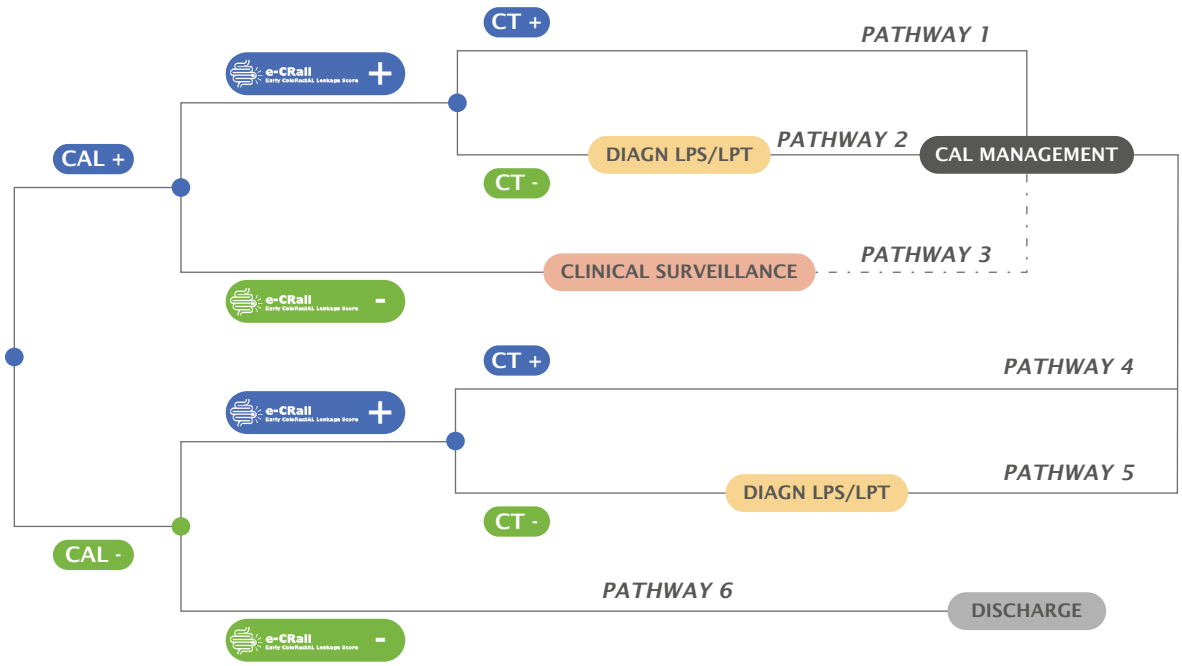
Regarding the standard clinical practice, the estimation of expected costs was: iCAL x Cost_CAL + (1-iCAL) x Cost_NoCAL, where iCAL is the incidence of CAL and Cost_CAL (Cost_NoCAL) is the cost of treatment of a CAL (No CAL) patient. The incidence of CAL is 6.3% (based on prospective study dataset), and costs were defined as public (National Health Service) reimbursement paid to the hospital, which has been used as a surrogate indicator of overall hospital costs. Costs to populate the model were obtained from Ministerial Order nº 254/2018 of 7th, September 2018 (Addendum III). For each patient, the Diagnosis Related Group (DRG) 221 and 223, respective degree of severity and comprehensive cost were identified. To estimate the daily cost, we divided the comprehensive cost by the average LOHS observed.

Concerning the adoption of E-CRALL score, the estimation of expected costs is toughest, involving complex patient pathways and including false-positives and false-negatives generated by the score use. This model helps choosing among different alternative options, from the "no use" of the score to its use on three different PODs (3 to 5). It is worth highlighting that the different pathway options, based on scores with different predictive abilities (different SS and SP) will determine different expected costs. The score that minimizes the expected cost will be chosen.

A Decision Tree model to help estimating the expected costs with E-CRALL score was developed (**Figure 12**), selecting values for branch probabilities and other parameters from the prospective cohort population dataset and respective estimations (e.g., CAL rate, warning score SS and SP). The predictive effect of abdominal and pelvic CT scan was drawn from relevant studies (Gervaz, *et al.* 2013; Kornmann, *et al.* 2013; Marres, *et al.* 2017; Power, *et al.* 2007). The E-CRALL score was assessed from POD3 to POD5, aiming to estimate its ability to save time in CAL detection. After assessing the score, four possible subgroup of patients can be obtained: "True Positive" subgroup, which includes patients with CAL and a "positive" score; "False Negative" subgroup, for CAL patients with a "negative" score; "False Positive" subgroup, consisting of patients without CAL and a positive score, and finally, the "True Negative" subgroup, involving patients without CAL and a negative score (**Figure 12**). In the test setting (E-CRALL adoption), costs of the in-patient hospital

episode were established based on the institutional remuneration tariffs (deduced from Ministerial Order nº 254/2018 of 7th, September 2018 – Addendum III - DRG - 221 and 223) adjusted by LOHS. For pathways 1 to 5 (see also Figure 12), additional readjustments were made, including costs with secondary operative procedures and supplementary CT scans (deduced from Ministerial Order nº 254/2018 of 7th, September 2018 – Addendum IV). Due to scarce evidence of the consequences of false negatives on LOHS, a cut-point with a sensitivity of 100% was selected. This conservative policy was adopted to minimise, not only the impact of false negatives on LOHS, but also the consequences of inappropriate early discharge.

Figure 12. Decision tree model scenario with adoption of E-CRALL score (consider POD 3,4 or 5. independently)



CAL - Colorectal Anastomotic Leakage; **CT** - Computed Tomography;
E-CRALL - Early ColoRectAL Leakage Score; **LPS** - Laparoscopy; **LPT** - Open Surgery (Laparotomy)

The statistical methods of this study were reviewed by Óscar Lourenço from Faculty of Economics, CeBER, University of Coimbra, Portugal. All data management and statistical analysis were conducted using StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC®.

C - RESULTS

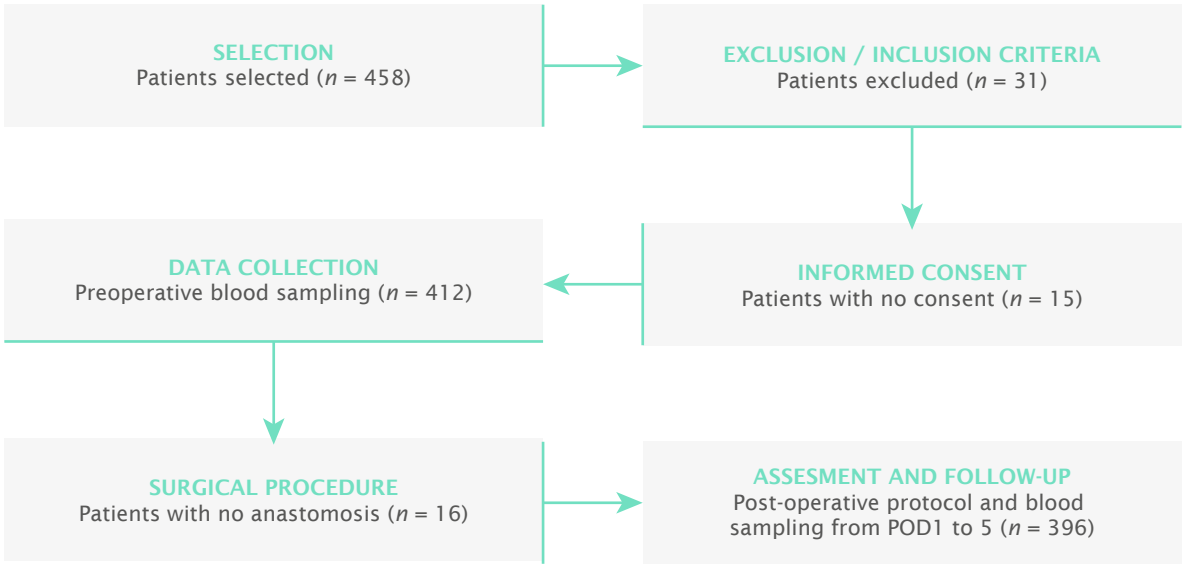
The current section begins by presenting the descriptive statistics and the graphical analysis, thenceforth the predictive value of clinical criteria (abdominal pain and clinical condition) and plasma biomarkers (WBC, ECC, CRP, PCT and CLP) in CAL-patients. After that, we highlighted the combination of biomarkers for early CAL diagnosis and defined the optimized cut-off values of biomarkers for an early discharge of patients. A decision model (warning score) is presented, and a minimization cost analysis performed, for examining the potential benefit of the score application. Finally, these results are employed to explain the hypothesis formulated. Thereby, post-operative monitoring of biomarkers improved the early diagnosis of CAL and reduced the time range to its detection.

1- DESCRIPTIVE STATISTICS

1.1 - PATIENTS AND OUTCOMES

During the study period, were included 458 consecutive patients, who underwent colorectal resection, and 62 (13.5%) were excluded - **Figure 13**.

Figure 13. Flow diagram of patients according to the study protocol.



POD, post-operative day.

Table 19 presents the main patient characteristics, divided into three groups (G1, G2 and G3), as previously defined. Results evidence that age, Charlson Comorbidity Index and ASA score seem to be associated with the occurrence of CAL.

Table 19. Patient demographic and clinical characteristics.

	Group 1 (n=277)	Group 2 (n=94)	Group 3 (n=25)	*P*
Age, mean ± SD	68.8±11.3	72.2±14.5	73.6±13.6	0.02
Gender, n (%)				0.505
Male	161 (58.1)	59 (62.7)	17 (68.0)	
Female	116 (41.9)	35 (37.3)	8 (32.0)	
BMI, mean ± SD	26.8±3.99	26.3±4.05	26.0±3.97	0.33
BMI, n (%)				0.33
17.5 < BMI < 25	95 (35.0)	32 (34.0)	12 (48.0)	
25 ≤ BMI < 30	129 (46.0)	51 (54.0)	9 (36.0)	
BMI ≥ 30	53 (19.0)	11 (12.0)	4 (16.0)	
CCI, mean ± SD	5.12±1.83	5.55±2.38	6.04±2.15	0.03
Prior abdominal surgery, n (%)	77 (27.8)	32 (34.0)	9 (36.0)	0.41
Immunosuppression, n (%)	10 (3.6)	5 (5.3)	0 (0)	0.45
Pre-operative diagnosis malignant, n (%)	272 (98.2)	90 (95.7)	24 (96.0)	0.38
ASA score, n (%)				0.018
I–II	187 (67.5)	47 (50.0)	13 (45.8)	
III–IV	90 (32.5)	47 (50.0)	12 (54.2)	

Group 1, no complications; Group 2, complications not related to CAL; Group 3, CAL; CAL, colorectal anastomotic leakage; SD, standard deviation; BMI, body mass index; CCI, Charlson Classification Index; ASA, American Society of Anesthesiologists.

Main operative characteristics are outlined in Table 20. Eighty-two percent of patients had a laparoscopic approach and the most common procedures performed were right colectomy (n=196; 49.5%) and sigmoid colectomy/rectosigmoid resection (n=74; 18.7%). In our study, surgical approach ($P < 0.001$), volume of blood loss ($P < 0.001$), occurrence of intra-operative complications ($P < 0.001$) and duration of procedure ($P = 0.011$) were significantly related to the development of CAL.

Table 20. Patients operative characteristics.

	Group 1 (n=277)	Group 2 (n=94)	Group 3 (n=25)	*P*
Type of surgery, n (%)				0.071
Elective	238 (86.0)	72 (76.6)	19 (75.0)	
Urgent	39 (14.0)	22 (23.4)	6 (25.0)	
Surgical approach, n (%)				<0.001
Open	25 (9.0)	15 (16.0)	2 (8.0)	
Laparoscopic	238 (86.0)	72 (77.0)	15 (60.0)	
Conversion	14 (5.0)	7 (7.4)	8 (32.0)	
Procedure, n (%)				0.739
Right colectomy[1]	138 (49.8)	47 (50.0)	11 (44.0)	
Left colectomy	17 (6.1)	7 (7.4)	1 (4.0)	
Sigmoid/RS resection	55 (19.8)	15 (15.9)	4 (16.0)	
Low anterior resection	48 (17.3)	16 (17.0)	8 (32.0)	
Other	19 (6.8)	9 (9.6)	1 (4.0)	
Level of anastomosis, n(%)				0.66
Ileocolic	150 (54.1)	50 (53.2)	11 (44.0)	
Colocolic	23 (8.3)	5 (5.3)	1 (4.0)	
≥6 cm from AV	67 (24.2)	25 (26.6)	10 (40.0)	
<6 cm from AV	37 (13.4)	14 (14.9)	3 (12.0)	
Covering stoma, n (%)	23 (8.3)	8 (8.51)	2 (8.0)	0.99
Blood loss, mean±SD, mL	51.6±36.6	58.8±47.7	104.0±191.1	<0.001
Intra-operative complications, n (%)	3 (1.1)	5 (5.3)	4 (16.0)	<0.001
Operative time, mean ± SD, min	141.9 (48.3)	146.2 (50.0)	172.8 (57.2)	0.011

Group 1, no complications; Group 2, complications not related to CAL; Group 3, CAL; CAL, colorectal anastomotic leakage; [1] included ileocecal resection/extended right-sided colectomy; RS, rectosigmoid; AV, anal verge.

Colorectal anastomotic leakage, as previously defined, was diagnosed in 25 out of 396 patients (6.3%) and was more frequent in men rather than women (68% vs. 32%). Twenty-three patients (92.0%) were diagnosed during the first hospital admission. Mean and median time for CAL detection were 9.0±6.8 and 8 days (Interquartile Range=7), respectively. Summary of ninety-days morbidity and mortality were presented in Table 21.

Table 21. Ninety-day post-operative morbidity and mortality.

In	Patients n=(%)		Length of hospital stay mean ± SD, days	
	Group 1 (n=277)	**Group 2** (n=94)	**Group 3** (n=25)	***P***
LOHS, days				<0.001
Mean ± SD	7.4±2.1	14.3±7.4	24.0±14.0	
Median	7	13	21	
90-day morbidity, n (%)				<0.001
Clavien-Dindo I	n.a.	64 (68.1)	0 (0)	
Clavien-Dindo II		14 (14.9)	4 (16.0)	
Clavien-Dindo III		8 (8.5)	16 (64.0)	
Clavien-Dindo IV		8 (8.5)	5 (20.0)	
Readmission, n (%)	15 (5.4)	6 (6.4)	5 (20.0)	0.019
Re-operation, n (%)	4 (1.1)	3 (3.2)	16 (64.0%)	0.005
90-day mortality, n (%)	0 (0)	0 (0)	3 (12.0)	<0.001

Group 1, no complications; Group 2, complications not related to CAL; Group 3, CAL;
CAL, colorectal anastomotic leakage; LOHS, length of hospital stay; n.a., not applicable.

In this study, CAL was significantly associated with a longer hospital stay (median of 21 days in G3-patients vs. 7 and 13 days, in G1 and G2-patients; p< 0.001), readmissions (20% vs. 6.4% and 5.4%) and re-operation rate (12% vs. 3.2% and 1.8%). Based on the Clavien-Dindo classification, complications grades III and IV were significantly higher in G3 patient's cohort (84.0% vs. 17.0%; P < 0.001) – Table 22.

Table 22 outlines intra-operative and post-operative details of CAL patient's group under the classification of CAL (minor vs. major). Seven patients (28.0%) were managed non-operatively and two (8.0%) underwent radiologic drainage of intraabdominal collections. The remaining 16 patients (64.0%) required surgical reintervention. Of the 16 reoperated patients, ten (56%) had an anastomosis takedown with an end stoma, and 6 (44%) received a defunctioning stoma. Ninety-days mortality was 0.8%, representing three patients with CAL.

Table 22. Intra-operative and post-operative details of patients with CAL (minor vs. major).

	Minor CAL (n=7)	Major CAL (n=18)	*P*
Type of anastomosis, n (%)			0.52
Intrabdominal	3 (42.8)	9 (50.0)	
Pelvic	4 (57.2)	9 (50.0)	
Covering stoma, n (%)	1 (14.3)	1 (5.6)	0.47
Abdominal pain			
POD3	1.86	1.94	0.08
POD4	1.57	2.13	**0.04**
POD5	1.86	1.92	**0.03**
Clinical condition			
POD3	1	1.25	0.07
POD4	1.14	1.47	0.13
POD5	1.29	1.58	**0.02**
CRP levels, mg/L			
POD3	178.35	221.02	0.28
POD4	146.30	226.01	0.13
POD5	107.64	251.45	**0.01**
CLP levels, µg/mL			
POD3	2.75	12.99	**<0.001**
POD4	3.34	10.60	**0.01**
POD5	2.52	10.96	**0.004**
CAL diagnosis, median, days	8	5.5	**0.07**
Diagnostic method, n (%)			0.12
Clinical	0 (0)	7 (38.9)	
Abdominopelvic CT	7 (100)	11 (61.1)	
CAL management, n (%)			**<0.001**
Drainage	n.a.	2 (11.1)	
Re-operation		16 (88.9)	
LOHS, mean±SD, days	28.0±17.0	22.4±12.9	0.38

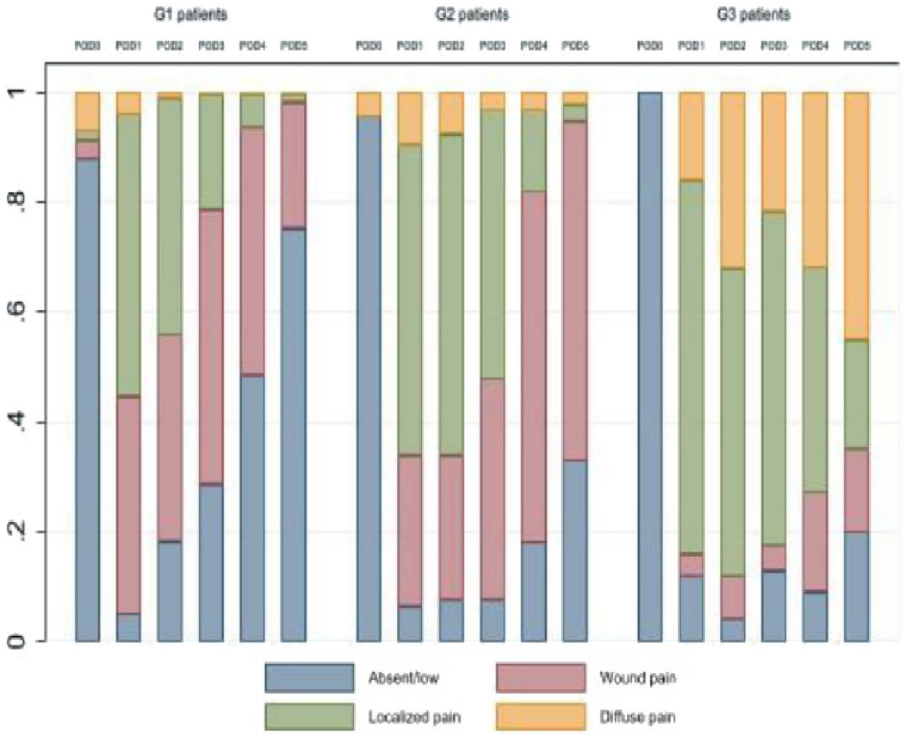
CAL, colorectal anastomotic leakage; POD, post-operative day; CRP, C-reactive protein; CLP, calprotectin; CT, computed tomography; n.a., not applicable; LOHS, length of hospital stay.

1.2 - CLINICAL CRITERIA – POST-OPERATIVE TREND

1.2.1 - Abdominal pain and clinical condition

Abdominal pain was markedly higher, diffuse, and persistent from POD3 onwards in G3-patients, as shown in Figure 14.

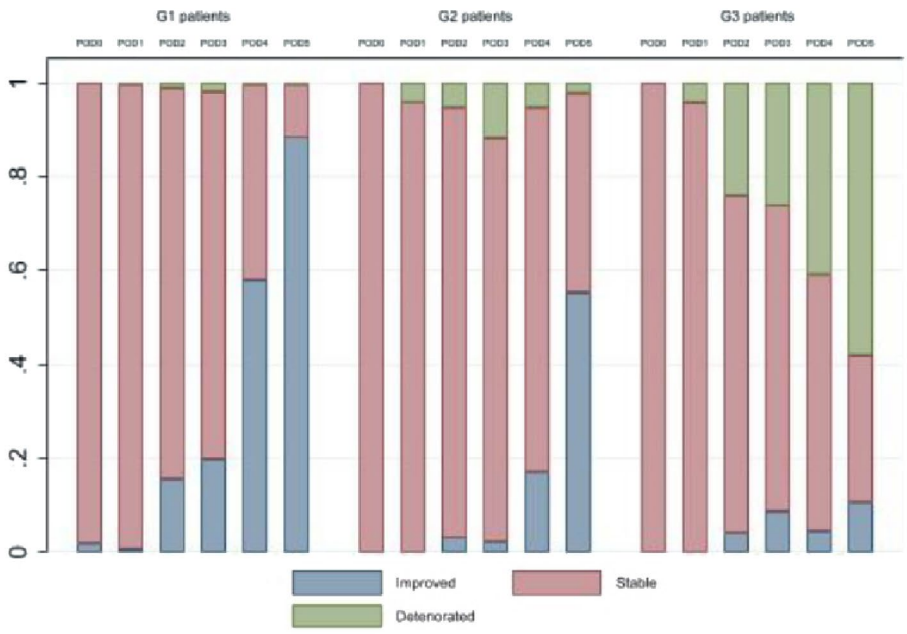
Figure 14. Distribution of rates of abdominal pain, from POD0 to POD5.



G1, no complications; G2, complications not related to CAL; G3, CAL;
CAL, colorectal anastomic leak; POD, post-operative day.

Regarding clinical condition, there was a declining post-operative tendency, significantly worse in G3-patients after POD3 (P = 0.001), comparing to G2-patients - Figure 15.

Figure 15. Distribution of rates of aclinical condition, from POD0 to POD5.



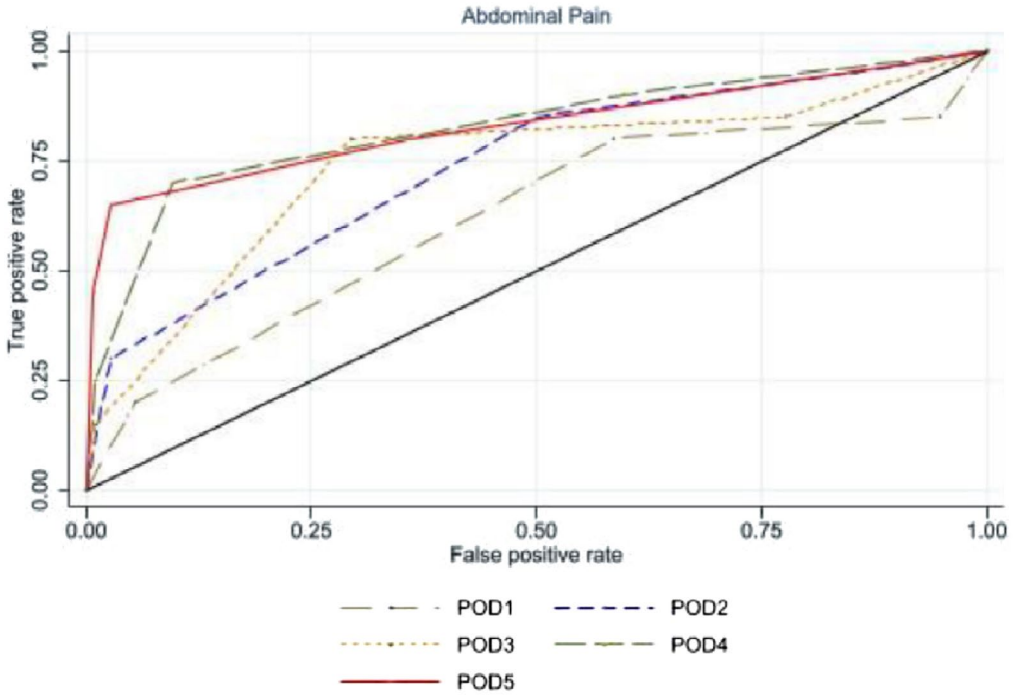
G1, no complications; G2, complications not related to CAL; G3, CAL;
CAL, colorectal anastomic leak; POD, post-operative day.

1.3 - CLINICAL CRITERIA – PREDICTIVE EFFECT

1.3.1 - Abdominal pain and clinical condition

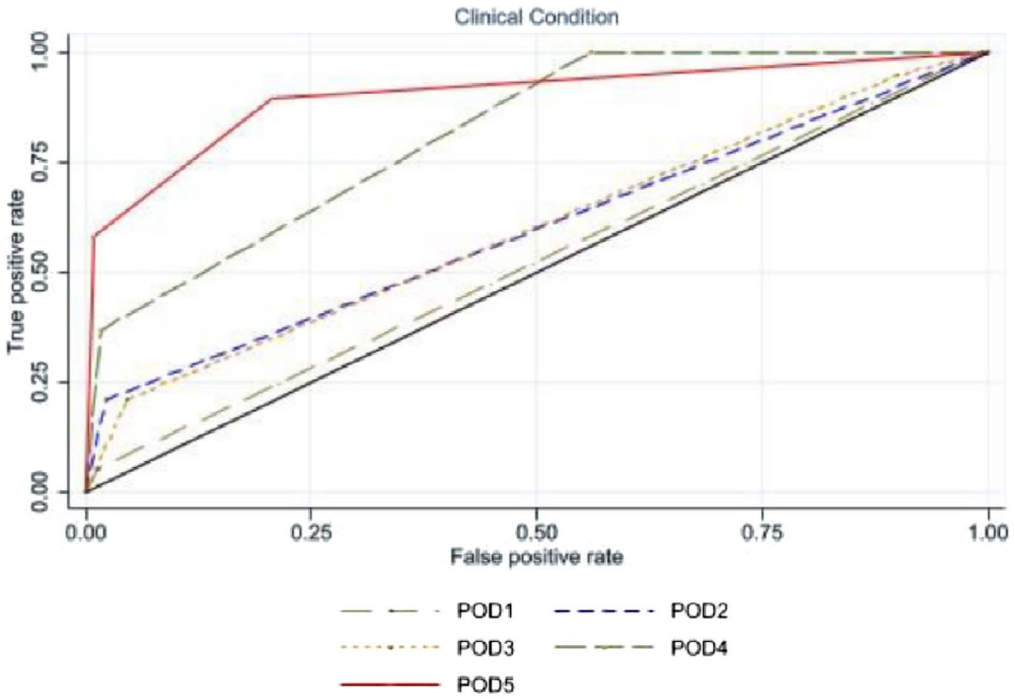
The AUC for abdominal pain from POD3 to 5 was 0.77, 0.84 and 0.83, respectively, as shown in Figure 16 and Table 23. The prediction effect was higher on POD4 with an estimated AUC of 0.84. The AUC for clinical condition from POD3 to 5 was 0.62, 0.81 and 0.90, respectively, as shown in Figure 17 and Table 23. The prediction effect was higher on POD5 with an estimated AUC of 0.90.

Figure 16. Area under the receiver operating characteristic curve (AUC) of CAL for clinical criteria: abdominal pain from POD1 to 5



CAL, colorectal anastomic leak; POD, post-operative day.

Figure 17. Area under the receiver operating characteristic curve (AUC) of CAL for clinical criteria: clinical condition from POD 1 to 5



CAL, colorectal anastomic leak; POD, post-operative day.

Table 23. Summary of predictive performance of the studied clinical criteria.

	AUC	SS	SP	NPV	PPV	PLR	NLR
Abdominal pain							
POD3	0.77	83%	71%	98%	16%	2.84	0.25
POD4	0.84	73%	91%	98%	34%	7.67	0.30
POD5	0.83	65%	97%	98%	60%	23.99	0.36
Clinical condition							
POD3	0.62	26%	95%	95%	29%	6.05	0.77
POD4	0.82	96%	48&	99%	11%	1.83	0.01
POD5	0.90	90%	79&	99%	23%	4.33	0.13

AUROC, area under the receiver operating characteristic curve; SS, sensitivity; SP, specificity; NPV, negative predictive value; PPV, positive predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; POD, post-operative day.

1.4 - BIOMARKERS – POST-OPERATIVE TREND

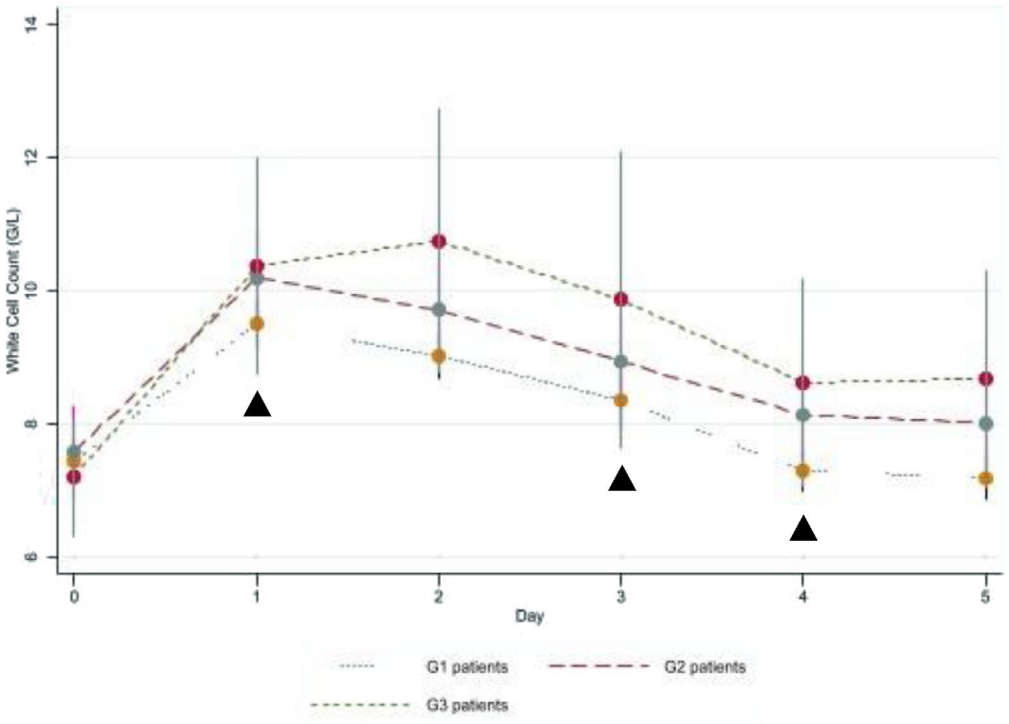
1.4.1 - White blood cell count and eosinophil cell count

During the first five POD, WBC was higher in CAL patients and significant on POD2, 4 and 5 (P = 0.01) instead of ECC that was lower in CAL-patients and significant on POD1 and 5 (P = 0.04 and P = 0.01, respectively), as presented in **Figures 18** and **19**. Overall post-operative course showed a sustained trend in both blood cells count, except for ECC in POD5.

1.4.2 - C-reactive protein

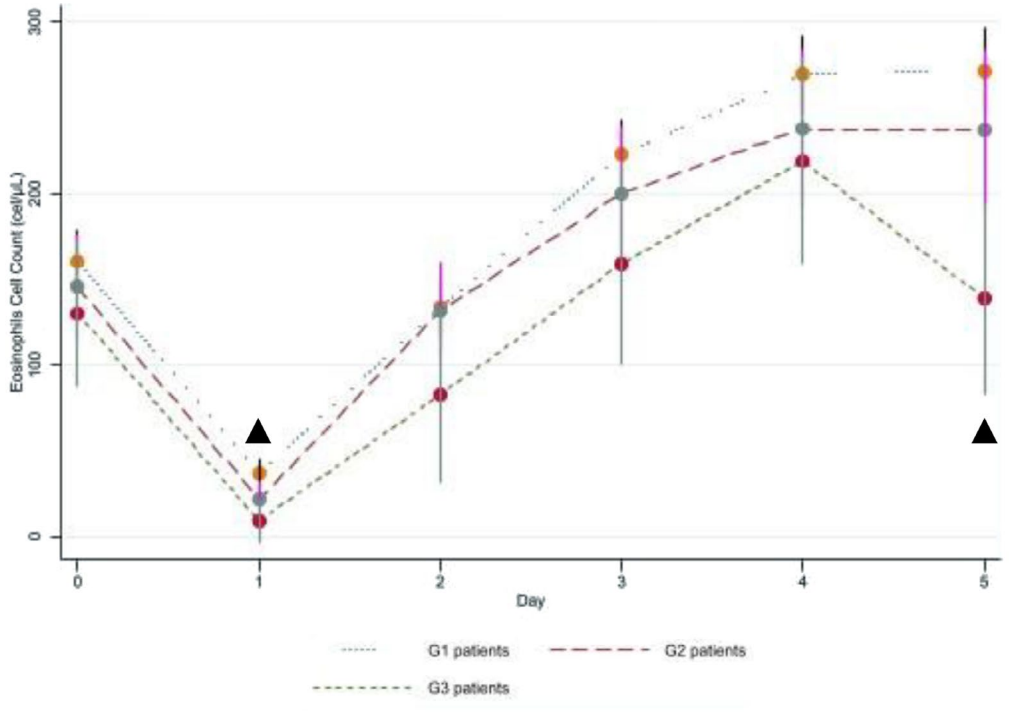
The mean value of CRP increased promptly after surgery, in all groups. C-reactive protein decreased in patients of G1 remaining raised in patients with complicated post-operative course, but significantly higher in G3. On POD5, mean CRP levels in G3 were significantly higher than in G1 (195.5±139.9 mg/L vs. 59.5±43.4 mg/L; P < 0.00001) - **Figure 20**. Patients with major CAL had higher mean CRP levels than those with minor CAL (251.45 mg/dL vs. 107.64 mg/dL; p = 0.01) - **Table 23**.

Figure 18. White blood cell count levels. Values are mean ± standard error



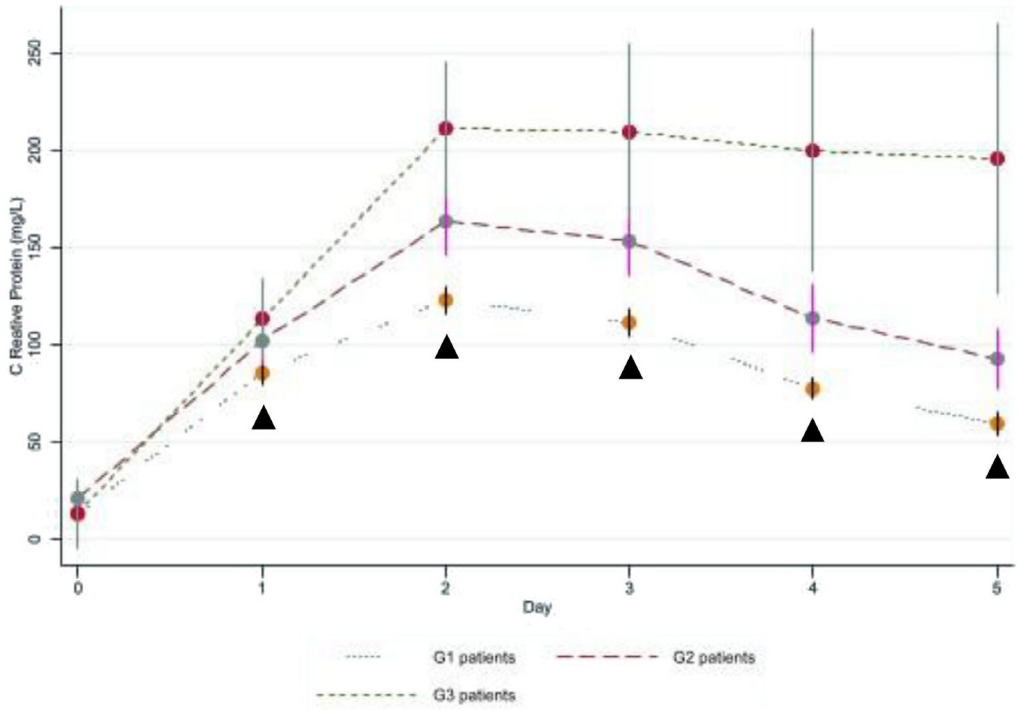
G1, no complications; G2, complications not related to CAL group; G3, CAL group; CAL, colorectal anastomic leak; Δ, *P* statistically significant (*P*<0.05).

Figure 19. Eosinophil cell count levels. Values are mean ± standard error



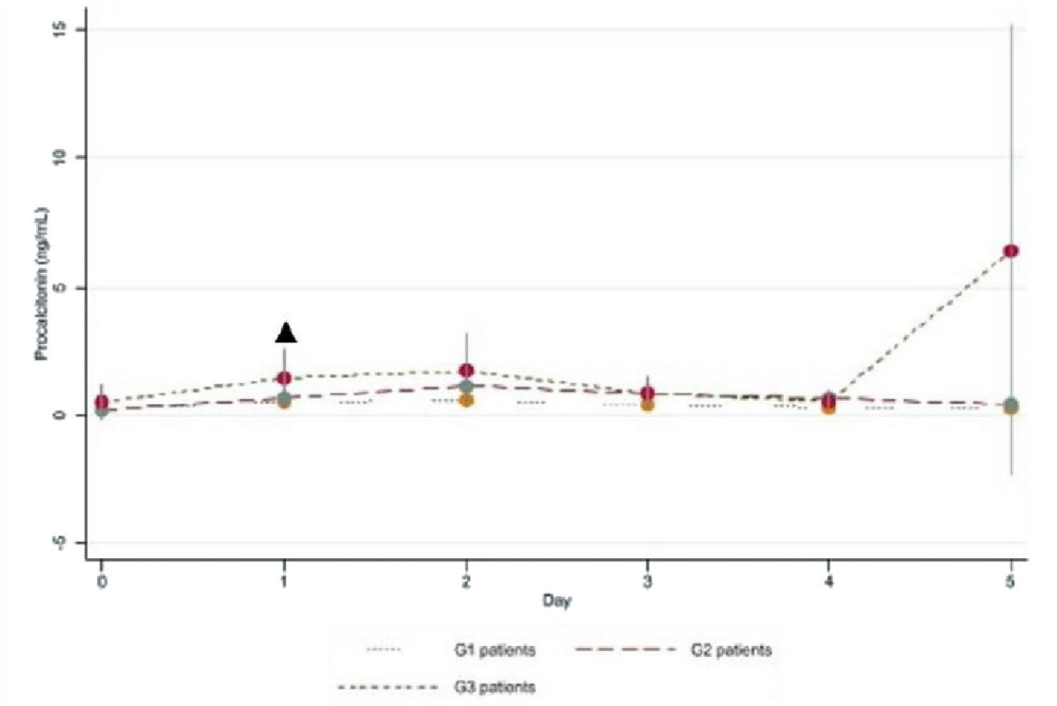
G1, no complications; G2, complications not related to CAL group; G3, CAL group; CAL, colorectal anastomic leak; Δ, *P* statistically significant (*P*<0.05).

Figure 20. C-reactive protein plasma levels. Values are mean ± standard error



G1, no complications; G2, complications not related to CAL; G3, CAL; CAL, colorectal anastomic leak; Δ, *P* statistically significant (*P*<0.05).

Figure 21. Procalcitonin plasma levels. Values are mean ± standard error



G1, no complications; G2, complications not related to CAL group; G3, CAL group; CAL, colorectal anastomic leak; Δ, *P* statistically significant (*P*<0.05).

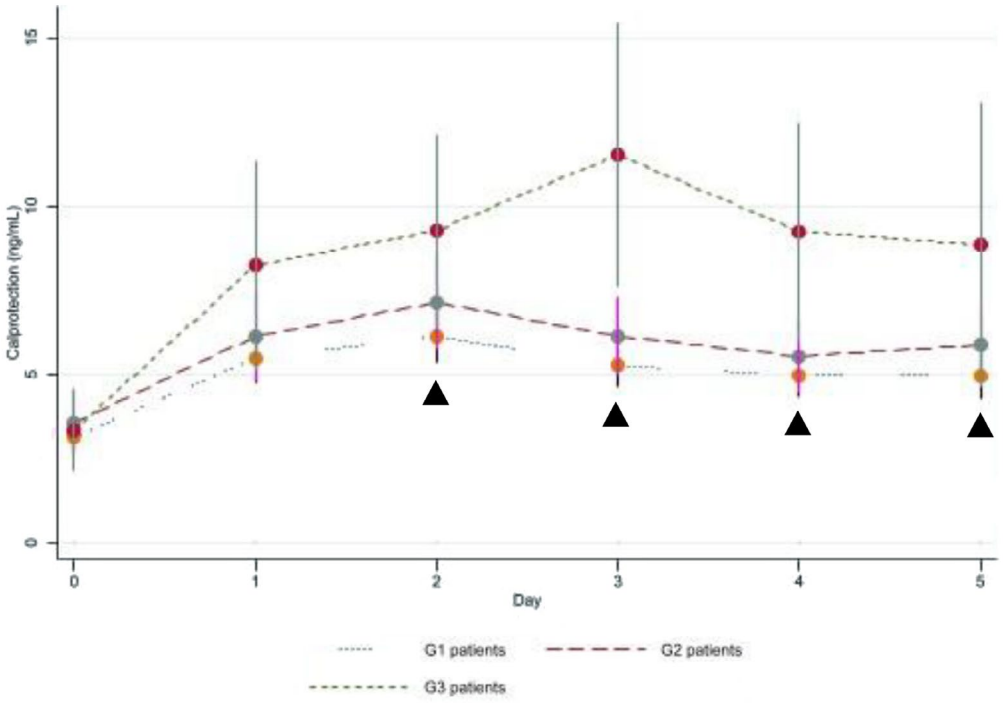
1.4.3 - Procalcitonin

The mean value of PCT increased promptly after surgery, in all groups. Procalcitonin levels tended to be stable from POD3 onwards. Mean values were higher in G3 than in non-CAL patients, but not statistically significant (on POD5, 0.23±0.08 ng/mL vs. 0.22±0.07 ng/mL) - Figure 21.

1.4.4 - Calprotectin

The mean value of CLP increased promptly after surgery, in all groups. In the first five PODs, the mean CLP tendency was following the pattern of CRP, although not so notorious (Figure 22). Mean CLP values were significantly higher in G3 from POD2 onwards. On POD3, mean value on G1 vs. G3 were 5.26±3.58 µg/mL vs. 11.52±6.81 µg/mL (P < 0.00005).

Figure 22. Calprotectin plasma levels. Values are mean ± standard error



G1, no complications; G2, complications not related to CAL group; G3, CAL group; CAL, colorectal anastomic leak; Δ, *P* statistically significant (*P*<0.05).

1.5 - BIOMARKERS – PREDICTIVE EFFECT

1.5.1 - White blood cell count and eosinophil cell count

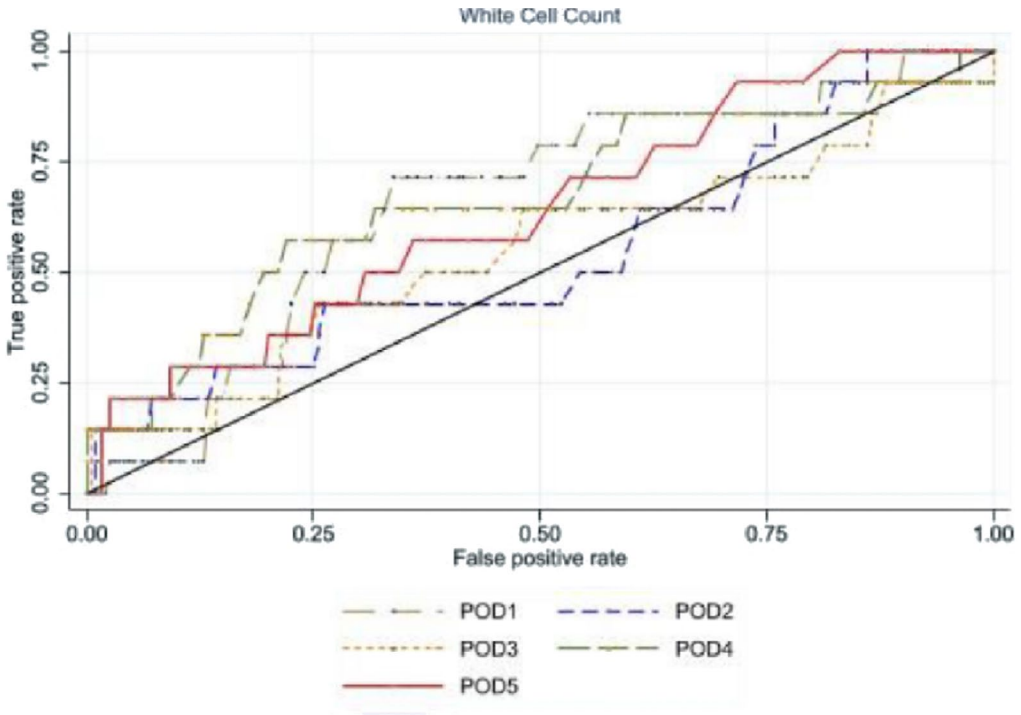
The AUC for WBC and ECC from POD1 to 5 are presented in Figure 23 and 24. The prediction effect of blood cells count was higher on POD5. On POD 5, when the ECC values were greater than 250 cells/µL, the AUC, SS and SP were 0.70, 89.0% and 43.0%, respectively, as shown in Table 24.

Table 24. Summary of the predictive performance of the studied plasma biomarkers.

	AUC	Cut-off value	SS	SP	NPV	PPV	PLR	NLR
WBC (g/L)								
POD3	0.57	9.75	46%	75%	95%	11%	1.84	0.72
POD4	0.60	8.25	52%	68%	96%	10%	1.64	0.70
POD5	0.62	7.55	56%	62%	95%	9%	1.48	0.71
ECC (cells/µL)								
POD3	0.59	150	50%	59%	95%	8%	1.23	0.84
POD4	0.54	150	33%	71%	94%	7%	1.14	0.94
POD5	0.70	250	89%	43%	98%	10%	1.55	0.26
CRP (mg/L)								
POD3	0.76	175.90	64%	83%	97%	20%	3.77	0.44
POD4	0.76	152.40	62%	89%	97%	27%	5.40	0.43
POD5	0.81	96.80	78%	78%	98%	19%	3.48	0.29
PCT (ng/mL)								
POD3	0.57	0.19	68%	47%	96%	8%	1.28	0.68
POD4	0.50	0.31	38%	76%	95%	10%	1.56	0.82
POD5	0.61	0.39	44%	79%	96%	12%	2.10	0.71
CLP (µg/mL)								
POD3	0.78	6.57	71%	72%	97%	15%	2.55	0.40
POD4	0.67	8.34	56%	86%	97%	21%	3.89	0.51
POD5	0.65	6.98	58%	80%	97%	16%	2.84	0.52

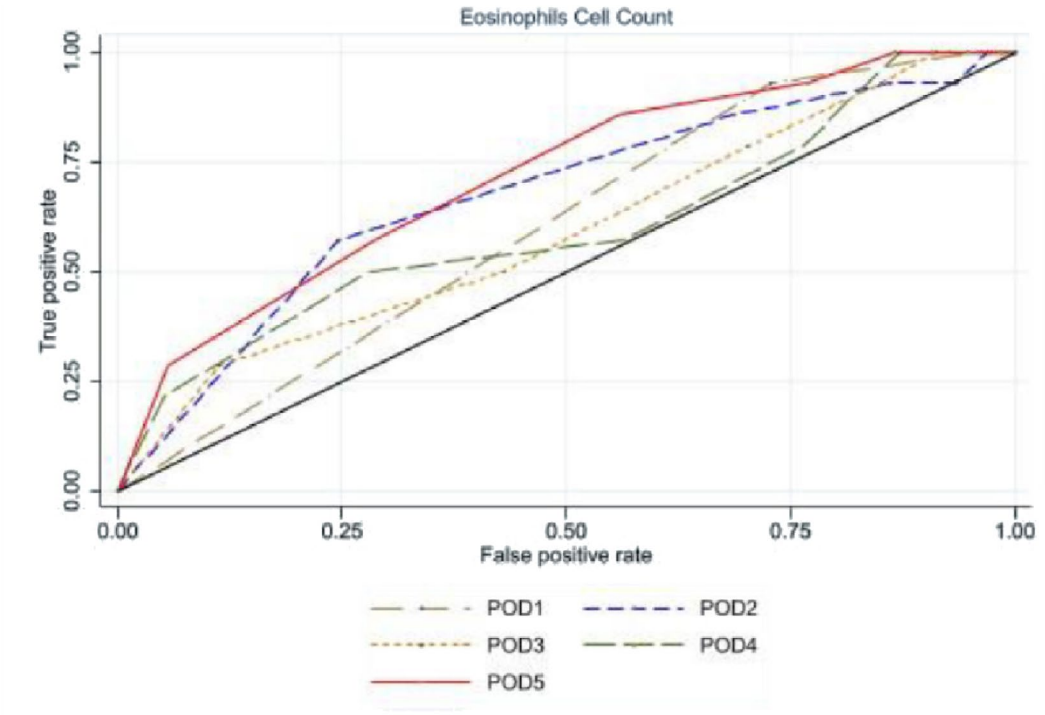
AUROC, area under the receiver operating characteristic curve; SS, sensitivity; SP, specificity; NPV, negative predictive value; PPV, positive predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio, WBC, white blood cell count; POD, post-operative day; ECC, eosinophil cell count; CRP, C-reactive protein; PCT, procalcitonin; CLP, calprotectin.

Figure 23. **Area under the receiver operating characteristic curve of CAL for WBC, from POD 1 to 5.**



CAL, colorectal anastomic leak; POD, post-operative day.

Figure 24. **Area under the receiver operating characteristic curve of CAL for ECC, from POD 1 to 5.**

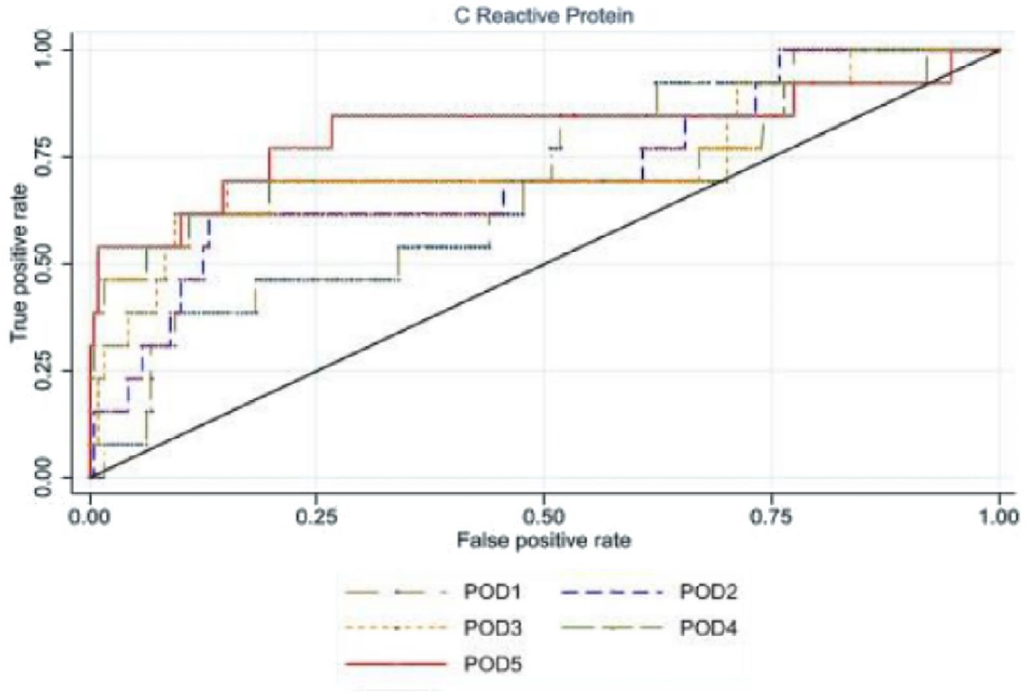


CAL, colorectal anastomic leak; ECC, eosinophil cell count; POD, post-operative day.

1.5.2 - C- Reactive Protein

From POD3 to 5, overall diagnostic accuracy of CRP to detect CAL was expressed by AUC of 0.76, 0.76 and 0.81 (Figure 25). On POD5, optimal cut-off value of 96.8 mg/L was estimated, resulting in a SS and SP of 78%, NPV of 98% and PPV of 19% (Table 25). Concerning patients with major CAL, the AUC of CRP was 0.74 and 0.88, for POD3 and 5, respectively, as shown in Figure 26.

Figure 25. Area under the receiver operating characteristic curve (AUC) of CAL for C-reactive protein from POD1 to 5.



CAL, colorectal anastomic leak; POD, post-operative day.

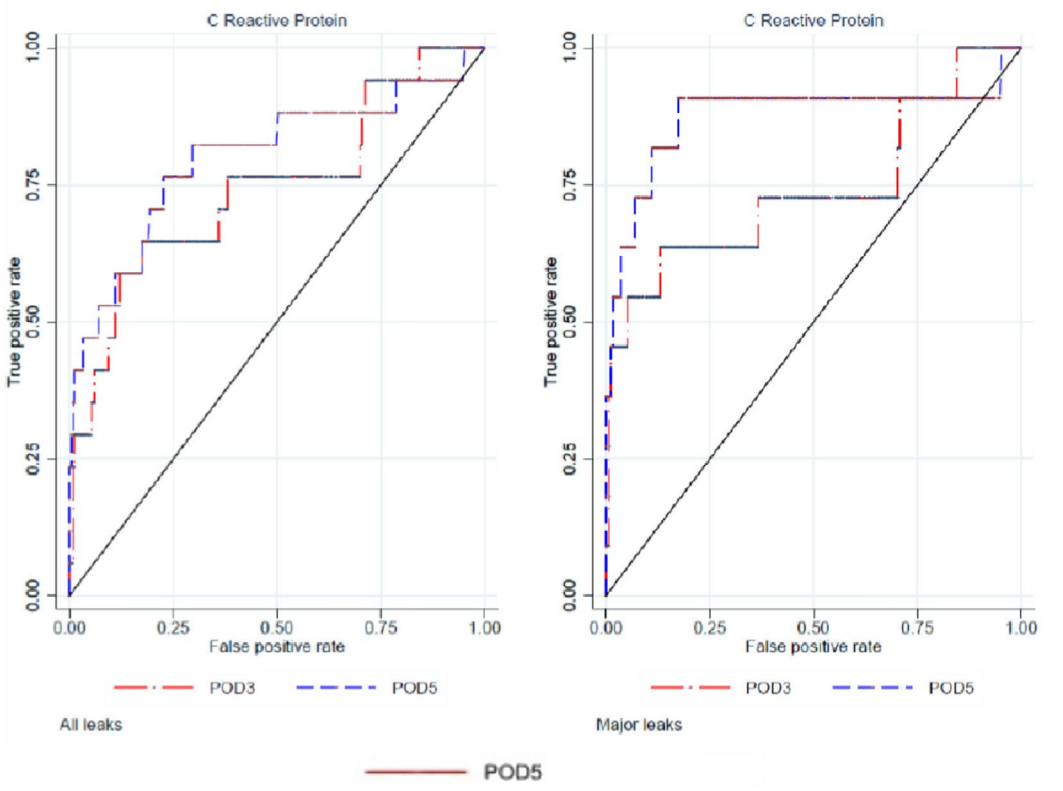
1.5.3 - Procalcitonin

The AUC from POD3 to 5 was 0.57, 0.50 and 0.61, as shown in Figure 27. The best prediction effect was on POD5. When PCT is greater than 0.39 ng/mL, sensitivity and specificity are 44.0% and 79.0%, respectively (Table 24).

1.5.4 - Calprotectin

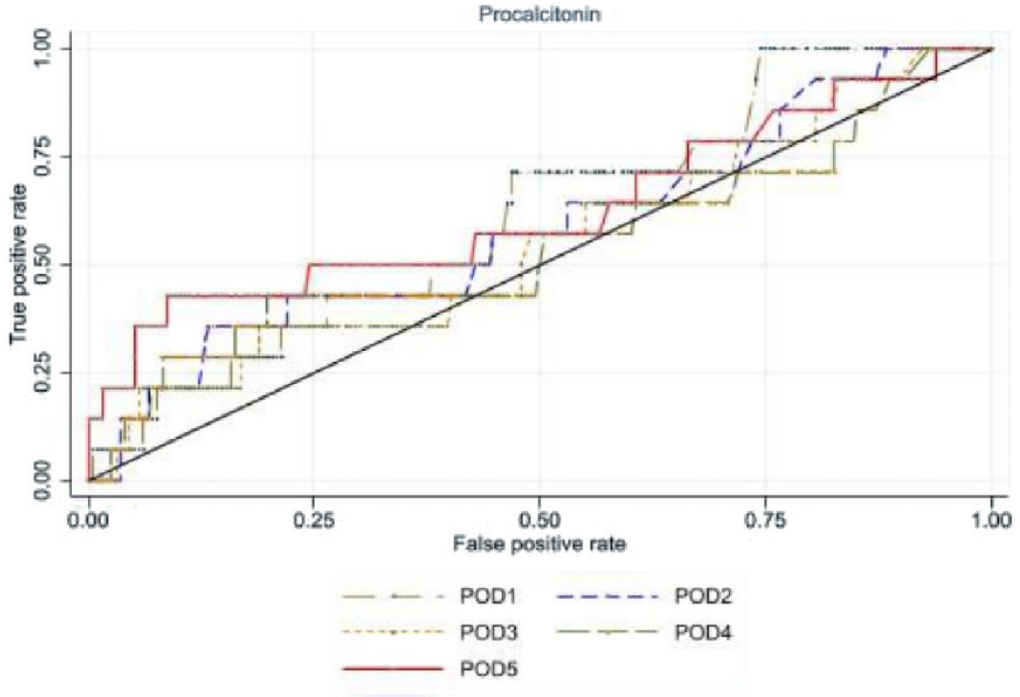
From POD 3 to 5, values of calprotectin AUC were, 0.78, 0.67 and 0.65, respectively as presented in Table 24 and Figure 28. On POD3, a cut-off value of 6.57 µg/mL yielded a sensitivity of 71.0% and a specificity of 72.0% (Table 24). For patients with major CAL, the AUC of CLP was 0.92 and 0.88, for POD3 and 5, respectively - Figure 29.

Figure 26. Area under the receiver operating characteristic curve of CAL for CRP from POD3 to 5 (Left: All leaks; Right: Major leaks)



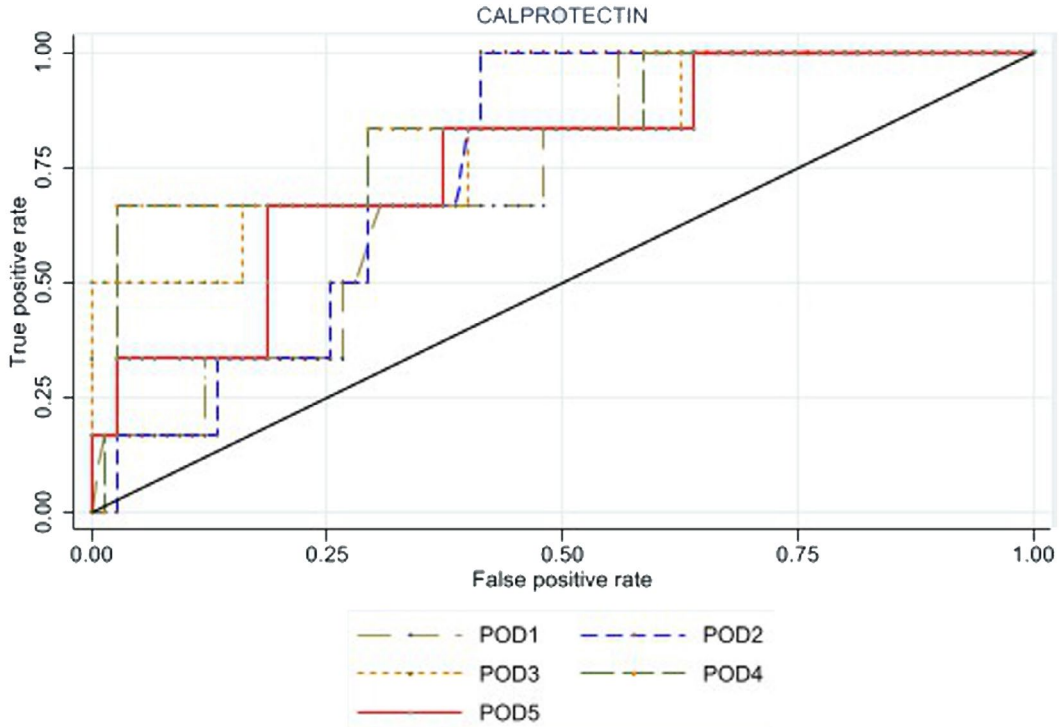
CAL, colorectal anastomic leak; POD, post-operative day.

Figure 27. Area under the receiver operating characteristic curve of CAL for PCT from POD1 to 5.



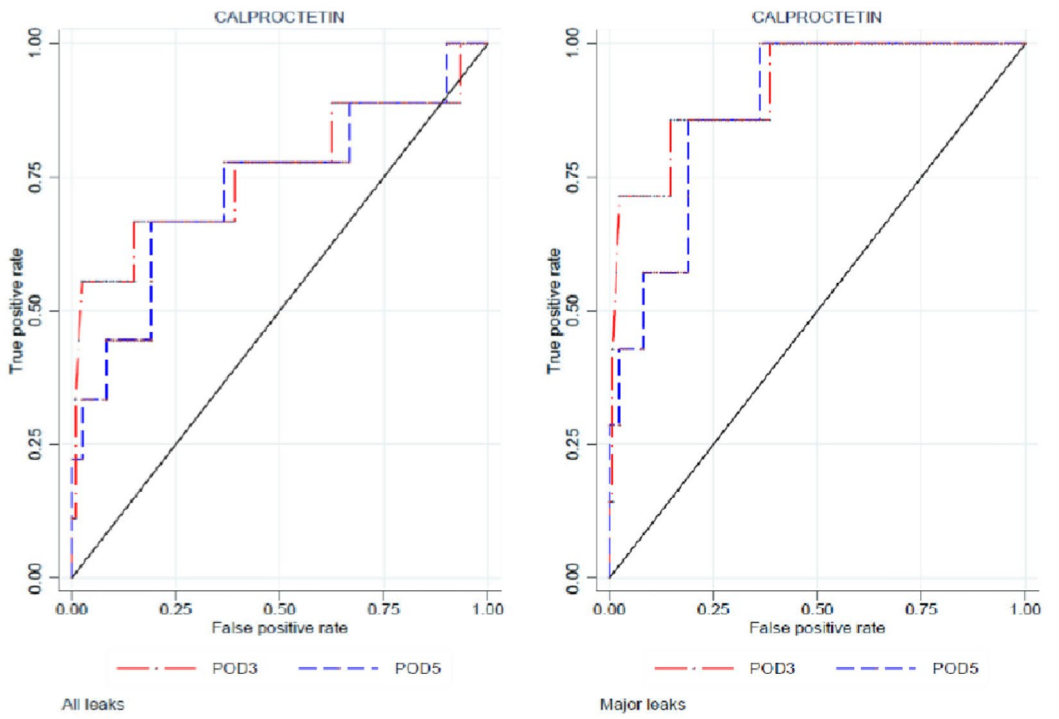
CAL, colorectal anastomic leak; POD, post-operative day.

Figure 28. **Area under the receiver operating characteristic curve (AUC) of CAL for calprotectin from POD1 to 5.**



CAL, colorectal anastomic leak; POD, post-operative day.

Figure 29. **Area under the receiver operating characteristic curve (AUC) of CAL for calprotectin from POD1 to 5 (Left: All leaks; Right: Major leaks).**

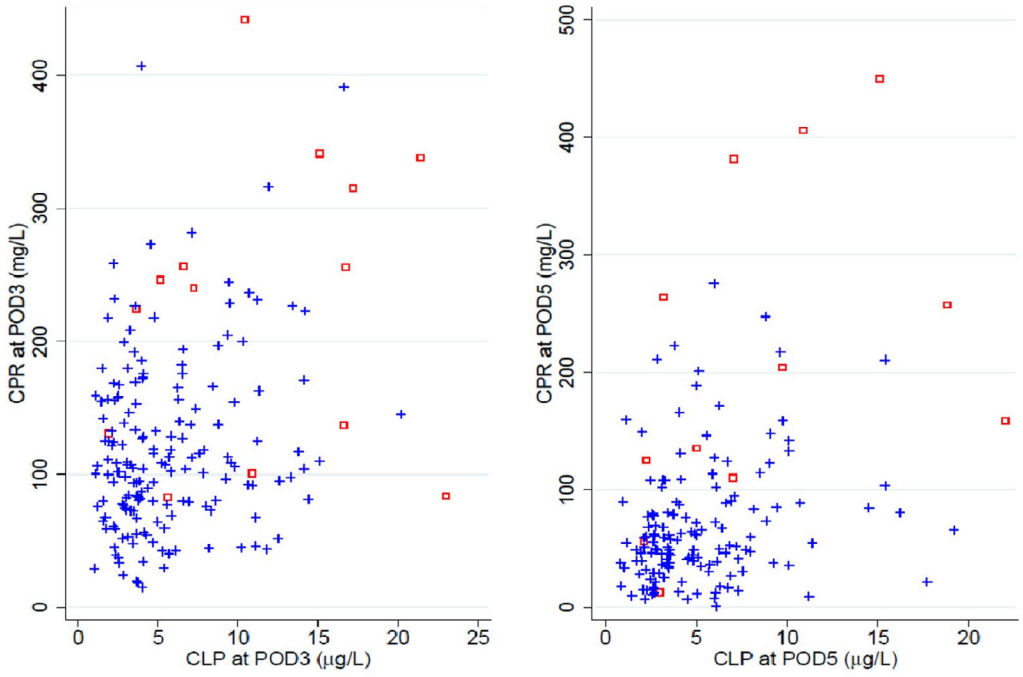


CAL, colorectal anastomic leak; POD, post-operative day.

1.5.5 - Combination of biomarkers

Figure 30 displays two scatter graphs illustrating the correlation of the biomarkers CRP and CLP at POD3 and POD5, and its combined power to predict the occurrence of CAL. Both charts present a clear positive correlation between CRP and CLP, showing also that the combination of these biomarkers can have a high discriminant power between CAL and non-CAL patients. Note the clear tendency for the northwest position of the red squares and the tendency for southwest of the blue crosses.

Figure 30. Combination of CLP and CRP on POD 3 and 5: CAL (red squares) vs no-CAL (blue crosses).



CAL, colorectal anastomic leak; POD, post-operative day.

Tables 25 and 26 present the AUC of several possible classifiers of CAL, built by combining two different biomarkers observed at POD3/POD5. The classifier that showed the best predictive performance was the one that combine CRP and CLP, at POD3, with an AUC of 0.82 (Table 25). This is worth highlighting that on POD5 the combination of CRP and ECC also present a good predictive performance (AUC = 0.81).

Table 25. AUC of pairwise combination of biomarkers on POD3.

Biomarkers combination – POD3				
	CLP	PCT	CRP	ECC
PCT	0.76			
CRP	0.82	0.72		
ECC	0.77	0.52	0.72	
WBC	0.74	0.53	0.72	0.54

POD, post-operative day; CLP, calprotectin; PCT, procalcitonin;
CRP, C-reactive protein; ECC, eosinophil cell count; WBC, white blood cell count.

Table 26. AUC of pairwise combination of biomarkers on POD5.

Biomarkers combination – POD5				
	CLP	PCT	CRP	ECC
PCT	0.60			
CRP	0.78	0.79		
ECC	0.61	0.63	0.81	
WBC	0.57	0.60	0.78	0.67

POD, post-operative day; AUC, area under the curve; CLP, calprotectin; PCT, procalcitonin;
aCRP, C-reactive protein; ECC, eosinophil cell count; WBC, white blood cell count.

The values of CRP and CLP combined through the Probit model at POD3 showed the best predictive ability to predict the occurrence of CAL.

The use of this classifier demands the estimation of the probability of CAL based on the observed values of CRP and CLP, computed as follows:

$$P(CAL) = F(-3.0842 + [0.094 \times CLP\_D3] + [0.0059 \times CRP\_D3])$$

where F is the cumulative standard normal distribution. Applying Liu's method, this classifier had an optimum cut-off point of 0.055, evidencing the existence of CAL above 0.055 on POD3. This classifier has an SS and SP of 86% and 75%, respectively. For a hypothetical patient X on POD3 with CRP and CLP plasma levels of 137.4 mg/L

and 8.75 µg/mL, respectively, the computed probability of CAL is high (score=0.074), therefore, patient X would be classified as CAL

By adopting this classifier, the time to CAL diagnosis is estimated as 3.8 days ([0.86 × 3] + [0.14 × 9.0]), which represents a 5.2-day reduction compared with the baseline results.

1.6 – E-CRALL SCORE

1.6.1 – Score construction

The E-CRALL score is based on the variables displayed in Table 27, which also presents the weights of each variable to the score. Many of the variables were statistically significant and with predictive power to CAL. To compute the score was designed a simple calculator, with a user-friendly interface that requires the selection of POD (from 3 to 5), and the fulfilling of the fields displayed (Link to E-CRALL calculator).

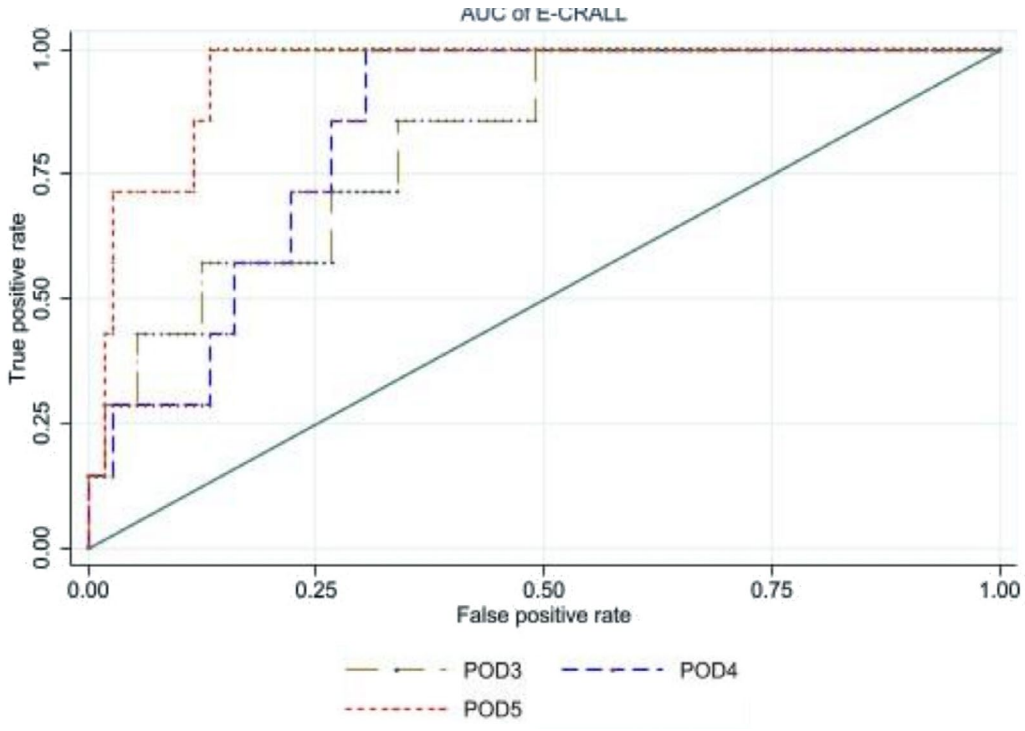
Table 27. Items weighted for the E-CRALL score (from POD3 to 5).

E-CRALL score	POD 3	POD 4	POD 5
Body mass index	-0.05142	-0.02927	Not included
Charlson Comorbidity Index score	0.1403	Not included	Not included
Open surgery	Not included	-0.0196	Not included
ASA score III or IV	0.0764	Not included	Not included
Blood losses (in mL)	0.2418	0.2044	0.1426
Operative time (in minutes)	0.0070	0.0074	0.0041
Anastomosis colocolic	-0.1065	-0.0297	Not included
Intra-operative complications	1.1731	1.378	0.7685
Plasmatic level of CRP (in mg/L)	0.0099	0.0089	0.0066
Plasmatic level of CLP (in µg/mL)	0.1333	0.1809	0.4548
Plasmatic level of ECC (in cell/µL)	Not included	-0.0007	-0.0038
Clinical condition - Improved	Not included	-0.6075	-2.199
Abdominal pain (absent/low)	Not included	-1.1150	-0.2843
Abdominal pain (at wound)	-1.19011	-1.845	-1.5299
Abdominal pain (localized)	Not included	Not included	1.2566

1.6.2 – Predictive ability – E-CRALL score

The predictive ability of E-CRALL warning score was estimated, with an AUC from POD3 to 5 of 0.82, 0.84 and 0.95, respectively, as shown in Figure 31 and Table 28. Clearly, the score applied on POD 5 has the best predictive power [0.95 (CI - 0.90-0.99)].

Figure 31. Area under the receiver operating characteristic curve (AUC) of CAL for E-CRALL score form POD3 to 5.



The cut-off value for applying the E-CRALL score was calculated, defining the threshold for signalling a "CAL patient". Setting the optimal cut-off as the one that maximizes both SS and SP of the classifier, were established the level on POD3 and 5 of 0.0551 and 0.0829, respectively. Considering a discriminant threshold of 5.51 (0.0551 x 100), E-CRALL score on POD3 had a SS, SP, NPV and PPV of 85.7, 66.1, 98.7 and 13.8%, respectively. On POD5, if a threshold of 8.29 (0.0829 x 100) was chosen, 87.4% of anastomotic failures were identified (Table 28).

Table 28. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the E-CRALL score (according to the POD).

E-CRALL score	POD 3	POD 4	POD 5
Threshold	5.51	2.56	8.29
Sensitivity (%)	85.7	100	100
Specificity (%)	66.1	69.6	86.6
PPV	13.8	17.2	32.1
NPV	98.7	100	100
CAL diagnosis (%)	67.2	71.4	87.4
AUC (95%CI)	0.82 (0.67-0.96)	0.84 (0.74-0.94)	0.95 (0.90-0.99)

AUC - Area Under Curve - Receiver Operating Characteristics; CI – Confidence Interval; POD – Post-operative Day;

1.6.3 – Time to CAL diagnosis

The E-CRALL score adoption from POD3 to POD5 allowed to estimate different times to CAL detection, and respective benefits in terms of time saving (Table 29). The E-CRALL score would anticipate CAL diagnosis in 5.2 days, if used on POD 3, and in 2.7 days, if used on POD5.

Time to CAL diagnosis increased over time, being higher on POD5 (6.4 days). Conversely, the earlier was the warning threshold achieved, the higher was the expected time saving. The best time saving was obtained on POD3, with a 5.2-day reduction, compared with the baseline results.

Table 29. Time to CAL diagnosis and time savings, by adopting E-CRALL score, from POD3 to 5.

E-CRALL score	POD 3	POD 4	POD 5
Time to CAL Diagnosis (days)	3.9	5.5	6.4
Expected Time Saving (days)	5.2	3.6	2.7

POD – Post-operative Day

1.7 – COST ANALYSIS

1.7.1 - Prospective monocentric study

In the standard clinical practice, for those patients with CAL (G3 patients), index admission comprehensive costs were markedly greater (286%), in comparison with those without CAL (G1 and G2 patients) - € 9,096 vs. € 3,177, as shown in Table 30.

1.7.2 - E-CRALL score application

In the model setting (Figure 12), after applying the E-CRALL score (on POD5), the adjusted comprehensive costs for each endpoint (pathway 1 to 6) were estimated and summarised in Table 30. In CAL patients, episode comprehensive costs were markedly greater (425%), in comparison with those without CAL (G1 and G2 patients) - € 7,876.36 vs. € 1,852.57.

Table 30. Inpatient episode cost and length of hospital stay (LOHS) - Standard clinical practice (A) vs. E-CRALL score adoption on POD5 (B).

	Non-CAL patients		CAL patients	
	A	B	A	B
Index costs (€)	3,177.00	1,852.57	9,096.00	7,876.36
Index LOHS (days)	9.1	5	24	20

1.7.3 – Cost-minimization analysis

Independent of CAL status, from POD3 to 5, a cost comparison of the two approaches (Standard clinical practice vs. E-CRALL score application) is presented in Table 31. The sooner the E-CRALL was employed, the higher were the patient cost savings. In an overall perspective, the use of E-CRALL warning score was associated with cost savings of € 508,505.44, most of them (93.8%) were at expenses of non-CAL patient's savings - Table 32.

Table 31. Inpatient episode cost analysis, adjust to POD3 to 5 – Standard clinical practice vs. E-CRALL score adoption (B).

	POD 3	POD 4	POD 5
A - Index costs (€)		3,532.14	
B - Index costs (€)	2,169.56	2,192.30	2,215.04

Table 32. Minimization cost analysis – Standard clinical practice vs. E-CRALL score adoption on POD5.

	Non-CAL patients	CAL patients	All patients
E-CRALL score costs [€; (%)]	666,925.2 (76.5)	204,785.36 (23.5)	871,737.56
Standard practice costs [€; (%)]	1143,720 (82.9)	236,496 (17.1)	1380,216
Cost savings [€; (%)]	476,794.8 (93.8)	31,710.64 (6.2)	508,505.44

D - DISCUSSION

This Thesis aims to prove the usefulness of biomarker monitoring in early diagnosis of CAL, helping to reduce the time to its detection. The monocentric prospective study carried out had this precise purpose, considering the relevance of this post-operative complication in daily clinical practice and in research. Despite this, there is still no consistent definition on this topic, mostly due to the heterogeneous clinical presentation. Colorectal anastomotic leakage diagnosis remains a challenge for clinicians, requiring several specific biomarkers measurements, imaging, or re-operation, to the diagnostic process.

Definition and Incidence

This research integrated the consensus-based recommendation for the definition of CAL, published by van Helsdingen *et al.*, improving, in the future, the comparability of study outcomes and quality of hospital care. Clinical and laboratorial parameters, as well as radiological and re-operative findings were included. Consensus was obtained regarding the definition of CAL and the panel recommended the ISREC definition, as general definition, complemented with the Clavien-Dindo classification (van Helsdingen, *et al.* 2020). This aspect is noteworthy and was applied on the qualitative analysis conducted in the selected studies of the meta-analysis (Chapter III), which revealed remarkable conceptual heterogeneity.

In the abovementioned literature review, CAL ranges from 0.2% to 27.2%, with colon and rectum-adjusted rates of 0.2% to 13.2% and 1–27.2%, respectively (Almeida, *et al.* 2012; Golub *et al.* 1997; Matthiessen, *et al.* 2008; Watson, *et al.* 1999). This is the first Portuguese prospective study on this subject and confirmed 6.3% of CAL (n=25). This data is consistent with the international literature.

Pre-operative Risk Factors

Anastomotic failure was more common in men (68%; p=0.505) and in an older population (mean age of 73.6±13.6; p=0.02). Male gender is a classic factor associated with a higher incidence of CAL. In a recent publication, Arron *et al.* presented results from the Dutch Colorectal Audit, including 70,229 colorectal

cancer patients undergoing resection with primary anastomosis between 2011 and 2019. Multivariate analysis established male gender as an independent risk factor for CAL (Arron *et al.* 2021). A systematic review by McDermott *et al.*, confirmed this positive association in both colocolic and colorectal anastomosis (McDermott, *et al.* 2015). From 22 observational studies, involving 105,829 patients, Pommergaard *et al.* identified six with a male gender correlation. Meta-analysis on eleven studies confirmed a pooled OR of 1.48 (95% CI: 1.37-1.60), unchanged after sensitivity analysis after excluding the largest study (Pommergaard, *et al.* 2014). Rationale for this association may stem from male narrow pelvis or hormone-related differences altering gut microcirculation (Ba *et al.* 2004; Jonsson *et al.* 1991; Law *et al.* 2000; Rullier, *et al.* 1998).

In the past, elderly age was considered a contraindication for colorectal resection with primary anastomosis, however the paradigm is changing (Huisman *et al.* 2020; Mamidanna *et al.* 2012). Distinct studies have shown positive association between age over 70 years old and CAL, especially in rectal resection (Fernandes *et al.* 2013; Jung *et al.* 2008), but the most significant systematic review with meta-analysis do not confirm this tendency (McDermott, *et al.* 2015; Pommergaard, *et al.* 2014).

Obesity, defined as BMI equal to or greater than 30 kg/m2 (Lin and Li 2021), is associated with an increase of post-operative complications, including CAL (Biondo, *et al.* 2005; Senagore *et al.* 2003; Volk *et al.* 2011). The present study has shown that obesity is not associated with the development of colorectal anastomotic failure. Likewise, only one out of thirteen observational studies that assessed this risk factor, elected by Pommergaard *et al.*, demonstrated a significant association with CAL. Meta-analysis on three studies estimated a pooled OR of 1.00 (95% CI: 0.93-1.07), supporting that BMI was not associated with CAL, despite the very low quality of the evidence (Pommergaard, *et al.* 2014). However, mainly in minimally invasive procedures, the technical difficulty and risk factor for post-operative complications may be influenced by visceral obesity. Visceral fat area is a better predictive factor for increased post-operative complications, especially CAL, when compared with BMI (Watanabe *et al.* 2014; Yang *et al.* 2015).

In this study, the cumulative burden of comorbidity was assessed by the CCI score, that has shown a significant association with CAL occurrence (mean CCI = 6.04±2.15; p=0.03), in the univariable analysis. Additionally, patients with ASA score > 2 are more frequent amongst those with CAL (p=0.02). Some studies concluded that ASA score >2 is an independent risk factor for anastomotic failure (positive association) (Arron, *et al.* 2021; Bakker, *et al.* 2014; Choi *et al.* 2006; Jestin *et al.* 2008). Pommergaard *et al.* also found a positive association between an ASA score >2 and CAL. Meta-analysis of seven studies confirmed a pooled OR of 1.71 (95% CI: 1.09-2.67), despite the high heterogeneity of the included studies (I2=73%; p = 0.001) (Pommergaard, *et al.* 2014). Nevertheless, its correlation with CAL seems to be superior than other clinical scoring systems, such as CCI (Bakker, *et al.* 2014).

Intra-operative Risk Factors

The increasing use of minimally invasive approaches in colorectal surgery, since the last decade of the 20th century, reflects its clinical advantages and safety (Jayne *et al.* 2010; Kuhry *et al.* 2008; Vennix *et al.* 2014). In this study was found an increased rate of laparoscopic procedures from 73.3% (n=352), in the retrospective cohort, to 82.1% (n=325), in the prospective study. However, the laparoscopic approach did not present association with the CAL occurrence. Currently, minimally invasive surgery has become the gold standard, regardless of the underlying condition. It is worth looking into laparoscopy as a risk factor for CAL. The CLASICC trial assessed the safety and efficacy of laparoscopic vs. open surgery in colorectal cancer management. Regarding the leak rate, there were no significant differences between both approaches for colonic (4% vs. 3%, in open; p>0.05) and rectal (8% vs. 7%, in open; p>0.05) cancer (Jayne, *et al.* 2010). Thenceforth, other authors reached similar conclusions (Anania *et al.* 2021; Athanasiou *et al.* 2016). Limited pelvic access (*"no man´s land"*) and complex anatomy were common arguments for major difficulties during rectal surgery (Ye *et al.* 2021). The laparoscopic approach is associated with a higher hypothetical risk of CAL, especially in obese patients, due to problems on pelvic stapler's negotiation and constrains in anastomosis fashioning (Scheidbach *et al.* 2008; Ye, *et al.* 2021; Zhou *et al.* 2012). Recently, Similis *et al.* compared the different techniques available for proctectomy (open vs. laparoscopic vs. robotic vs. transanal), and no significant differences were found regarding to intra-operative complications, conversion rate, grade III/IV morbidity, re-operation

rate and pathological or oncological outcomes. Moreover, the authors concluded that the approach used does not affect the colorectal leak rate (Simillis *et al.* 2019).

In this monocentric study, colorectal leaks were more frequent in right colectomy (n=11; 44%), corresponding to a leak rate of 5.6%. Colorectal anastomotic leak rate, in this study, compares nearly with three large-scale audits. Data retrieved from the Dutch Surgical Colorectal Audit identified leak rates in the right colectomy (n = 7788) and ileo-caecal resection (n = 240) subgroups of 6.4% and 7.5%, respectively (Bakker, *et al.* 2014). The Spanish ANACO group found an overall leak rate of 8.4% in patients undergoing elective right colectomy for cancer (Frasson, *et al.* 2016). Recently, on ESCP right hemicolectomy audit, anastomotic leak and/or intraperitoneal fluid collection was present in 8.1% of patients (group 2017).

In line with previous literature, this research also found that the volume of blood loss is a risk factor for development of CAL. Furthermore, the need for multiple blood transfusions is a recognized independent risk factor for CAL, probably correlated with its immunosuppressive action, enhancing the rate of post-operative septic complications (Tadros *et al.* 1992; Vasiliu *et al.* 2015). Golub *et al.* conducted a retrospective, multivariate analysis of 764 patients who underwent 813 intestinal anastomoses and found a leak rate of 3.4%. Peri-operative transfusion of more than two units packed red blood cells was recognized as predictive factor for CAL, regardless of the volume losses, hypotensive status or pre-operative hemoglobin level (Golub, *et al.* 1997). A systematic review with meta-analysis carried on by Qu *et al.* included fourteen studies (seven prospective and seven retrospective), in a total of 4,580 patients. From multivariate analysis of operative factors, four studies were significantly associated with increased risk of CAL, including intra-operative transfusions and blood loss greater than 100 mL [OR = 3.79 (2.48 - 5.49), $p < 0.001$)] (Qu *et al.* 2015).

In accordance with the literature, our study identified the occurrence of intra-operative complications, including conversion, and duration of procedure, as risk factors for colorectal leak. Adverse events, such as bleeding, iatrogenic injuries, or the need for conversion, is often associated with an increased leak rate (Frasson, *et al.* 2015;

Kambakamba *et al.* 2014; Yang *et al.* 2009). Kambakamba *et al.* analysed 3,928 patients undergoing elective laparoscopic colorectal resection and identified an overall incidence of intra-operative adverse events of 8.4%. Forty-three percent were surgical complications, mostly iatrogenic solid organ injuries (1.6%) and bleeding (1.6%). The mean conversion rate was 14.9% and patients with intra-operative adverse events had a higher morbidity rate (32.9% vs. 17.2%; $p < 0.001$) (Kambakamba, *et al.* 2014). Similar results were verified by other authors; notwithstanding, conversion was not always associated with an increased risk of CAL (Belizon *et al.* 2006; Casillas *et al.* 2004; Frasson, *et al.* 2015; Yang, *et al.* 2009). Several studies showed that extended surgical procedures were associated with high incidence of colorectal leak rate. This is somewhat linked to higher complexity and intra-operative complication rate, or less technical expertise (2018; García-Granero, *et al.* 2017; Kelly, *et al.* 2014), and with estimated OR ranging from 1.53 to 9.9 (Gervaz *et al.* 2012; Suding *et al.* 2008). Midura *et al.* published a retrospective analysis of 13,684 patients available at the American College of Surgeons NSQIP-2012 database. Colorectal anastomotic leakage was identified in 3.8% of the patients. Surgical procedures longer than 3 hours were correlated with a higher anastomotic leak rate [OR of 1.50, (1.19-1.90), p=0.001] (Midura *et al.* 2015). A similar result was obtained by Nikolian *et al.* from a retrospective cohort of 9,192 patients, with a predicted CAL incidence of 3.0% and 2.5% for pelvic and intra-abdominal anastomoses, respectively. Multivariable analysis demonstrated an association between CAL and extended procedures [OR 1.16, (1.06-1.26), p=0.0009] (Nikolian *et al.* 2017).

Colorectal Anastomotic Leakage and Short-term Outcomes

In the current analysis was recognized that colorectal leak was a major contributor to the short-term morbidity and mortality. Anastomotic leakage occurrence was related to a higher rate of complications, namely septic, extended LOHS, and increased re-operations and readmissions. These findings are in accordance with several studies (Frasson, *et al.* 2015; Frasson, *et al.* 2016; Gessler, *et al.* 2017; Krarup, *et al.* 2015; Sánchez-Guillén *et al.* 2019). Besides, more severe complications according to Clavien-Dindo were seen in CAL patients compared to patients without leakage (Gessler, *et al.* 2017). Minor CAL patients had a higher LOHS, probably resulting from a delay in diagnosis. Subclinical presentation, need for additional investigation and false negative results, or extra morbidity due to extended stays, might explain

this delay. Gessler *et al.* found that one fourth of all CT scans were negative in patients who later developed CAL (SS of 75%). They verified a relevant difference in the meantime to CAL detection. When the CT scan was positive for CAL, it took 8.5 days to the diagnosis, compared to 4.3 days in patients who were diagnosed during a re-operation (Gessler, *et al.* 2017).

Colorectal Anastomotic Leakage Diagnosis

In the present study, ninety percent of patients with CAL were diagnosed during the first hospital admission (n=23). All enrolled patients have been submitted to the study protocol, as previously described. Post-operative follow-up included daily clinical surveillance and plasmatic biomarkers measurement. Whenever a patient was worsening (clinically or analytically) an abdominal and pelvic CT scan was performed, with additional WSCE, if colorectal anastomosis was fashioned. Other authors published similar results, as den Dulk *et al.*, who identified 99% of patients with anastomotic failure during the primary admission applying both the DULK score and the modified version (den Dulk, *et al.* 2013; Giaccaglia, *et al.* 2016; Hyman, *et al.* 2007). Other studies reported a higher rate of delayed CAL detection. Gessler *et al.* debated the question of two different types of leakages (earliest and latest), confirming that 20% of patients had their leakage diagnosed after discharge and at a readmission (Gessler, *et al.* 2017). This prospective study did not confirm this rate of LAL.

Subsequently, will be focused the leading question of this research, detailing the additional objectives proposed, and with the aim to prove the hypothesis of this Thesis: **Does post-operative monitoring of biomarkers improve the early diagnosis of CAL, shortening the time to anastomotic leak detection**.

Usefulness of Clinical Criteria

Clinical criteria demonstrated high diagnostic accuracy (AUC>0.8) on POD4 and 5. Changes in the abdominal pain pattern and worsening of the clinical condition were associated with an increased risk of CAL occurrence. **Both clinical criteria parameters (clinical condition and abdominal pain) seem to be useful early**

markers for this condition, producing the best overall diagnostic accuracy of the parameters analysed. Three large and well-conducted studies on the association between pain and post-operative complications are worth reporting. Boström *et al.* examined a cohort of 3,084 patients and estimated that increased post-operative pain is associated with a high risk of CAL, being an independent marker and suggesting a need for further diagnostic measures (Boström, *et al.* 2021). Two studies reported similar conclusions, even though they were not exclusive for colorectal surgery (Regenbogen, *et al.* 2016; van Boekel, *et al.* 2019). A worse clinical condition and abdominal pain not localized at the wound are two out of four modified DULK score criteria, scoring 1 point each. Using a cut-off value of 1 point, this modified version produced an overall SS and NPV of 97.0% and 99.5%, respectively (den Dulk, *et al.* 2013). We should bring the clinical method to the fore, being aware of the clinical signs of CAL. They are very helpful for the early diagnosis, as "red flags" for further investigation.

John Nicholls highlighted the clinic method today, remembering how the incredible advance of technology has changed all our lives beyond measure (Nicholls 2014). In the same line of thought, there are some things that clinical assessment of the pathology can do better than technology and there are others where technology is not sufficiently accurate to allow an appropriate decision. Digital rectal examination to identify a lower rectal dehiscence may have sufficient accuracy to enable a satisfactory diagnosis. As previously mentioned, digital rectal examination, performed by an experienced surgeon, can provide additional and reliable clinical information compared to WSCE, on the assessment of anastomotic healing (Tang and Seow-Choen 2005). Conversely, CT scan can be insufficiently accurate to CAL detection, due to its high false negative rate (around 30%) (Gervaz, *et al.* 2013; Marres, *et al.* 2017).

Usefulness of Serum Biomarkers

Next, will be discussed the predictive effect of plasma biomarkers (WBC, ECC, CRP, PCT and CLP) for identifying CAL-patients, and the usefulness of cut-off values of CRP, PCT and CLP for early discharge of patients, considering the routine adoption of enhanced recovery programmes.

Serum Biomarkers – Single Use

In the current study, particularly on POD4 and 5, WBC and ECC showed a distinct pattern in patients with and without CAL, with a high NPV (from 94%-98%) but low accuracy, measured by AUC criteria (AUC from 0.54 to 0.70). In the group of patients with CAL, WBC kept often high after the acute inflammatory response, a phenomenon that was notably different from patients without CAL. In most studies, the WBC count is maximum at the time of CAL diagnosis. Garcia-Granero *et al.* estimated a SS, SP, PPV, NPV and AUC, to the cut-off point of 5,910 cells/mm3, of 91.0%, 77%, 19.0%, 99% and 0.82, respectively (Garcia-Granero, *et al.* 2013). The WBC trajectory was analysed in the post-operative period, and regarding the predictive model built, the AUC of 0.76 was established (0.69 - 0.82) (Smith, *et al.* 2018). In a large retrospective study, Warschkow *et al.* found that the WBC level had a fair contribution to the early detection of septic complications, offering a lower diagnostic accuracy if compared to the plasma CRP (Warschkow, *et al.* 2011b). In several other studies, researchers have estimated, from POD5 to 7, an AUC and SS ranging from 0.63 to 0.82 and from 58% to 74%, respectively (Lagoutte, *et al.* 2012; Sutton, *et al.* 2004; van Boekel, *et al.* 2019; Warschkow, *et al.* 2011b; Welsch, *et al.* 2007).

Some researchers have proposed eosinopenia (low plasma ECC) as a useful biomarker in this setting. They concluded that it might help to identify several sepsis-related conditions, distinguished from other causes of SIRS (Abidi, *et al.* 2011; Shaaban, *et al.* 2010; Terradas, *et al.* 2012). It seems to be an interesting biomarker because of its widespread availability and low cost (Garnacho-Montero, *et al.* 2014). Since the flare-up of the coronavirus disease 2019 (COVID-19), eosinopenia, one of the most significant features of COVID-19, has become a hot topic again, due to its probable diagnostic and prognostic value (Li *et al.* 2020; Lin, *et al.* 2021; Xu *et al.* 2020).

Shaaban *et al.* defined an optimum cut-off value of 50 cells/μL, which produced an SS, SP, and NPV of 81%, 65% and 80%, respectively (Shaaban, *et al.* 2010). At hospital admission, ECC <40 cells/μL is an independent prognostic factor for mortality (Abidi, *et al.* 2011; Terradas, *et al.* 2012). A recent meta-analysis included seven studies and a total of 3,842 patients. Eosinopenia, as biomarker, presented a pooled SS and SP, PLR and NLR, and pooled OR of 66%, 68%, 2.09, 0.49 and 4.23, respectively. The AUC was 0.73 (0.68-0.76) and for each subgroup of different eosinopenia cut-off values, the best SS (79%) and SP (83%) was obtained for 40 cells/mm3 and less than 25 cells/mm3, respectively (Lin, *et al.* 2021).

The research reported in this Thesis is original also in assessing the usefulness of ECC for early diagnosis of CAL. The mean ECC level showed a non-significant decline after POD4 in G3 patients, and a fair diagnostic accuracy (AUC from 0.54 to 0.70) when compared with other biomarkers. Nevertheless, ECC, as part as a biomarker toolkit for CAL detection, could still be used as a fast, simple, convenient, and inexpensive biomarker. It should be considered in the decision-making process of future research (Lin, *et al.* 2021).

Serum CRP is the most studied biomarker in the diagnosis of colorectal leak. Its value for early detection has been investigated by several authors over time (den Dulk, *et al.* 2009; Garcia-Granero, *et al.* 2013; Italian ColoRectal Anastomotic Leakage Study 2020; Paliogiannis *et al.* 2021; Terradas, *et al.* 2012). In the current study, the plasma CRP level exhibited a clear tendency to return to normal values from POD3 onwards in patients without CAL (G1 and G2). However, it remained steadily increased in G3 patients, with a markedly high mean value from POD1 to 5. Furthermore, other authors demonstrated a similar trend (Garcia-Granero, *et al.* 2013; Matthiessen, *et al.* 2008; Ortega-Deballon, *et al.* 2010; Smith, *et al.* 2018; Welsch, *et al.* 2007). Smith *et al.* analysed the predictive value of CRP, assessing its trajectory rather than arbitrary values. Regardless of the post-operative day, a single daily rise in CRP of over 50 mg/L had a SS of 91% and a 99.3% NPV, with respect to CAL diagnosis requiring intervention (Smith, *et al.* 2018).

Yeung *et al.* conducted the most comprehensive meta-analysis available in literature, including nearly 7,000 patients pooled from 23 studies. From POD1 to 7, patients with CAL presented a significant higher mean CRP level compared with patients without CAL (P<0.001) (Yeung, *et al.* 2021). **In this study, CRP was the biomarker with higher predictive value for CAL, especially on POD4 and 5 with a maximum AUC of 0.81 (cut-off value of 96.8 mg/L and an NPV of 98%) on POD5**. Similar results have been published by other authors. Ortega-Deballon *et al.* estimated on POD4 an AUC of 0.72 with a cut-off of 125 mg/L, yielding an SS and NPV of 81.8% and 95.8%, respectively (Ortega-Deballon, *et al.* 2010). Garcia-Granero *et al.* reported that the CRP level showed a good predictive ability for major CAL on POD5, with an AUC of 0.85 (cut-off value of 135 mg/L and an NPV of 98%) (Garcia-Granero, *et al.* 2013). In the iCral multicentric prospective observational study, CRP level was a good positive and excellent negative predictor of CAL, with an AUC of 0.81 on POD6 (cut-off value of 81.5 mg/L), and an SS and NPV of 80.9% and 97.7%, respectively (Italian ColoRectal Anastomotic Leakage Study 2020). In the meta-analysis by Yeung

et al. (Yeung, *et al.* 2021), AUC analysis established a threshold CRP level for CAL of 115 mg/L on POD5, with an SS and SP of 100%. All these authors recommended CRP levels to predict CAL, and our group advocate a similar practice and suggest the use of this biomarker to expedite further investigation and treatment (Garcia-Granero, *et al.* 2013; Italian ColoRectal Anastomotic Leakage Study 2020; Ortega-Deballon, *et al.* 2010; Yeung, *et al.* 2021).

Calprotectin, a sign of neutrophil activation, could be an exciting early marker for excessive inflammatory response in major abdominal catastrophes, such as CAL. To the extent of our knowledge, so far, only Reisinger *et al.* have studied the predictive value of CLP in CAL diagnosis (Reisinger, *et al.* 2014). In G3 patients, the mean post-operative CLP level peaked on POD3 and was notably high, persisting thereafter. In our research, on POD3, the AUC (0.78) and SS (71%) were slightly higher than the CRP level, even though they were lower than those obtained in the pioneering study by Reisinger *et al.* (0.92 and 86%, respectively) (Reisinger, *et al.* 2014). One possible explanation could be our comprehensive definition of CAL and the larger sample size. It remains unclear to what extent CLP level is, as an early predictor, better than CRP for detecting CAL. As a neutrophil activation marker, CLP could be increased early after anastomotic failure, compared with CRP, which indicates a delayed systemic inflammatory response. Our study evidenced that CLP is worth evaluating for early diagnosis of CAL.

Some reports about the usefulness of PCT as predictor of CAL was published. Lagoutte *et al.* compared PCT and CRP plasmatic levels after colorectal surgery, from POD1 to POD4. They concluded that serum CRP on POD4 had the best accuracy for CAL diagnosis (AUC 0.869 vs 0.750 for PCT) (Lagoutte, *et al.* 2012). On the other hand, Garcia-Granero *et al.* reported the best accuracy for major CAL diagnosis would be obtained by using plasmatic PCT measurement on POD5, with a AUC, SS, SP, NPV and cut-off value of 0.86, 100%, 72%, 100% and 0.31 ng/mL, respectively (Garcia-Granero, *et al.* 2013).

This study has demonstrated, in the first five POD, that the mean PCT values are marginally higher in G3 patients, but with lower accuracy, SS, and SP than CRP and CLP levels. However, it had a high NPV (>95%), being an adequate and useful marker for an early and safe discharge after colorectal surgery, considering the current ERAS (enhanced recovery after surgery) routine. In contrast to our study, Giaccaglia *et al.* estimated that on POD5, PCT had better accuracy than CRP (0.86 vs. 0.81), as well

as a high NPV (98.3%) (Giaccaglia, *et al.* 2016). A recent meta-analysis published by Su'a *et al.* determined a diagnostic accuracy (AUC) of 0.88 on POD5 and an optimum cut-off value on POD3 and 5 of 0.25 and 680 ng/mL, respectively. The NPV ranged from 95% to 100% (Su'a, *et al.* 2020). In line with these authors, we believe that PCT is a useful predictor for CAL exclusion; as a single test, however, it is worthless for CAL diagnosis.

Serum Biomarkers – Combined Use

Presently, a single biomarker is usually used to CAL detection, as previously outlined. Nevertheless, all these inflammatory indicators can be influenced by several factors or circumstances. If, in fact, these biomarkers are correlated, its combined use for CAL diagnosis can present clear advantages over its single use. Multiple combinations could increase their diagnostic value, in daily clinical practice. In this research, it was verified that, except for plasma CRP on POD5 (AUC>0.80), each individual biomarker was a modest predictor of CAL (Mandrekar 2010). The combination of two or more biomarkers has been considered in previous studies (Giaccaglia, *et al.* 2016; Italian ColoRectal Anastomotic Leakage Study 2020; Reisinger, *et al.* 2014). **In the present study, combination of CRP and CLP values on POD3 increased the diagnostic accuracy, shortening the mean time to CAL diagnosis by 5 days**. This reduction would certainly lead to decreased morbidity and mortality. Reisinger *et al.* confirmed a significant improvement in the diagnostic accuracy (AUC=0.93) with the combination of CRP and CLP plasma levels on POD3, with an SS of 100% and an SP of 89.0%, decreasing the median time to diagnosis by 3 days (Reisinger, *et al.* 2014). Furthermore, Giaccaglia *et al.* found that by adding PCT to CRP on POD5, the diagnostic accuracy improved markedly (AUC=0.90) (Giaccaglia, *et al.* 2016). Similarly, the iCral study demonstrated that the combination of CRP and PCT with a clinical score (DULK score) allowed the exclusion of CAL on POD2 (NPV=99%) (Italian ColoRectal Anastomotic Leakage Study 2020).

Score Approach

An alternative method has been considered to develop a decision system for helping in the early diagnosis of CAL and referred as "Score approach". A "Score approach" can be seen as a user-friendly diagnostic tool, based on observed data, which

generates objective information, in a simple score format, for clinical use in daily practice promoting the timely identification of patients with risk of CAL.

First, a score is a standardized metric that estimates a number (e.g., the probability) that is highly associated with the occurrence of a specific outcome. The proactively adoption of predictive score models may allow early identification of patients who are at highest risk of poor health outcomes, such as CAL, and will benefit them from a proper management. In a CAL detection setting, a few scores have been developed, such as the Dutch leakage (DULK) score (and its modified version) or the Diagnostic Score Leakage (DIACOLE). In addition, a third example is a score developed using artificial intelligence methods (Adams and Papagrigoriadis 2014; den Dulk, *et al.* 2009; den Dulk, *et al.* 2013; Rojas-Machado, *et al.* 2016). **Table 33** summarises the distinctive aspects of the four scores available for CAL diagnosis.

Pioneering by den Dulk *et al.*, their research aimed to develop and test the feasibility and usefulness of a standardised post-operative surveillance protocol in the early diagnosis of CAL, and its impact on mortality. Different post-operative parameters were associated with CAL, but no scoring system had neither been designed nor tested prospectively in a clinical setting. The use of this score showed several benefits, namely the decrease of the delay to CAL detection (median 1.5 day compared to 4 days) and a reduction of CAL mortality, from 39% to 24%, with standardised surveillance (den Dulk, *et al.* 2009). **Concerning the early CAL detection, the E-CRALL, score proposed and tested in our research, demonstrated a substantial reduction on time to CAL detection (from 3.9 to 6.4 days) and expected time savings (from 2.7 to 5.2 days), depending on the day of its application**. Moreover, den Dulk *et al.* proposed a Decision Tree model for CAL diagnosis, indicating which actions should be taken further to each score. One of the most important limitations is methodological. It can be questioned whether all items were weighted properly, the cut-off values were optimal, or why the predictive effect where not estimated, or at least presented. Further improvements of the leakage-score were considered, including a Dutch multicentric prospective study to validate the DULK score and a modified version of DULK score (den Dulk, *et al.* 2009).

More recently a modified DULK score version was developed. The modified version of the DULK aimed to simplify the original version of the score, that was accomplished through the reduction of the number of parameters necessary to compute the score, thus, becoming user-friendly for clinicians in daily clinical practice (den Dulk, *et al.* 2013). Despite the usefulness of the DULK score, the difficulty of its use by clinicians in daily routine has led researchers to propose simplified score systems based on a lower number of parameters/variables. Multiple logistic regression was the adopted methodology, and the variables included in the score calculation were the clinical condition of the patient, abdominal pain location, C-reactive protein level and respiratory rate (den Dulk, *et al.* 2013). At that time, the original DULK score was validated, and its predictive ability estimated. With exception for respiratory rate, the other three parameters were included in the E-CRALL warning score. The predictive ability of both DULK modified version and E-CRALL score was quite similar. However, both score systems were developed based on distinct methodological approaches. Both tools aimed the early recognition of CAL and seem to be useful as a warning sign for further investigation, using for example CT scans with rectal contrast or re-operation.

Concerning the E-CRALL score, it's worth mentioning not only the high AUC after the POD3, evidence of good predictive performance, but also the inclusion of variables from the pre- and intra-operative stages. Two other issues are worth highlighting: first, despite the modified DULK score had been developed and tested, the findings should be confirmed in a different cohort before their full clinical application; second, after external validation, modified DULK score may be useful for standardizing post-operative monitoring, aiding less experienced members in the early CAL-detection (den Dulk, *et al.* 2013). This goal was accomplished by Martin *et al.*, who concluded that the DULK-score is the most reliable instrument for early diagnosis of CAL. They also suggested its integration into risk management health policies, aiming to improve the quality of care according to the FTR concept (Martin, *et al.* 2015).

Artificial intelligence methods, artificial neural networks (ANNs), was used by Adams et., to create a tool capable of accurately identifying patients at risk of developing CAL. Belonging to the field of artificial intelligence, an ANN is a mathematical representation of the human neural architecture, expressing its "learning" and "generalization" capacities (Drew and Monson 2000). They are widely applied in research for modelling non-linear relationships between a set of predictors (input variables) and one or more responses (output variables). In medicine, main applications of

ANNs include image and biochemical analysis, drugs design and diagnostic systems (Adams and Papagrigoriadis 2014; Amato *et al.* 2013; Mochão et al. 2022). Adams *et al.* developed an ANN based score, trained, and validated on a retrospective cohort. Two comparative groups were selected, 20 cases of CAL confirmed at re-operation, and 56 controls, with a post-operative delayed recovery, but without CAL. The score included 19 input variables from the three phases of the surgical process, being, in terms of composition, somehow equivalent to E-CRALL score. Internal validation produced an AUC, SS and SP of 0.89, 85.0 % and 82.1 %, respectively. External validity was estimated in a small prospective consecutive cohort (12 patients), presenting a SP of 83.3 %. These results would suggest a good generalisability and effective prevention of overfitting by the ANN model. The authors concluded that models based on ANNs can assist in early detection of clinical CAL, based on daily clinical data, but not measuring this reduction to CAL detection, as E-CRALL score does (Adams and Papagrigoriadis 2014).

DIACOLE (Diagnostic colorectal leakage) score was built from the results of a systematic review of literature. At the onset, the potential laboratorial and clinical post-operative signs and symptoms of CAL were identified and complemented by a binary meta-analysis of those variables previously identified. Based on meta-analysis data, the weight of each identified factor was estimated. The final diagnostic index was calculated by adding the weight of each risk factor present at the calculation time. Afterwards, formulation of a predictive model based on DIACOLE was performed, and retrospective data were collected from a random case-control sample, for validation purposes. Estimation of predictive model and rating of its predictive capacity were accomplished (Rojas-Machado, *et al.* 2016). DIACOLE diagnostic index showed an AUC of 0.91, comparable with E-CRALL score on POD5 (AUC of 0.95) and considered good warning tools for CAL diagnosis (Rojas-Machado, *et al.* 2016). Finally, the diagnostic threshold of DIACOLE score was established by using the cut-off point that optimises sensitivity and specificity. This estimation process was identical in both scores, even though the E-CRALL tool delivered higher SS and SP (>90%) than DIACOLE (82.9%) (Rojas-Machado, *et al.* 2016). The authors of the Spanish score defined two discriminant thresholds: a lower (level) (> 3.065) advising daily clinical and laboratorial (with complete blood count) re-evaluation, and a higher (> 5.436), recommending imaging (CT scan or WSCE) (Rojas-Machado, *et al.* 2016). On the other hand, E-CRALL score established just one threshold, dependent from the POD, and recommending imaging (CT scan) or early re-operation (in the event of equivocal or negative imaging). Considering both scores calculation(s) seem to be

burdensome, due to assessment concerns, the authors have developed a user-friendly free software to compute the score value (Rojas-Machado, *et al.* 2016).

Table 33. Distinctive aspects of DULK, Adams, DIACOLE and E-CRALL score.

	DULK	Adam *et al.*	DIACOLE	E-CRALL
Pre-operative parameters		X		X
Intra-operative parameters		X		X
Post-operative parameters	X	X	X	X
Method: Points (P) / Threshold (T) / ANN (A)	P	A	T (two)	T (daily)
Predictive ability (AUC)	N.A.	0.89	0.91	0.95(POD5)
Validation: Internal (I) / External (E)	I+E	I+E*	I	I
Early CAL detection	X			X

ANN - Artificial Neural Networks; N.A. – No available;
E* - External validation was obtained from 12 consecutive pilot prospective patients.

Economic Burden of Colorectal Anastomotic Leakage

In terms of economic impact of CAL, this study has clearly demonstrated that the cost of colorectal resection increased markedly after CAL, being significantly greater (286.3%), in comparison with those without CAL (€ 9,096 vs. € 3,177, respectively). This result is in line with the literature reporting's. Ashraf *et al.* found an increase of 154% in mean total in-patient hospital cost, in the 20 patients with anastomotic leakage after anterior resection (£6,233 ± £965 vs. £9605 ± £6908 for non-CAL and CAL patients, respectively) (Ashraf, *et al.* 2013). Similar results were obtained by other authors (Hammond, *et al.* 2014; La Regina, *et al.* 2019; Ribeiro Jr, *et al.* 2019).

One of the aims of this Thesis was also the assessment of the economic value of the use of E-CRALL score. We developed an analytic decision model, a Decision Tree, to estimate the expected costs of E-CRALL score adoption. When comparing expect costs of E-CRALL with the expected costs of standard practice (without E-CRALL), the results clearly pointed to the economic advantage of E-CRALL. As defended elsewhere in this Thesis, we assumed that the health outcomes with E-CRALL and without E-CRALL are similar.

Overall costs were inferior after E-CRALL use, in the model setting, revealing a reduction of 41.7 and 13.4% in in-hospital costs, in non-CAL and CAL patients, respectively, compared with standard clinical practice. **These overall savings were first and foremost explained by the reduction in LOHS, as evidenced by the high proportion of savings that was**

seen in non-CAL group (93.8%). Decision support systems based on inaccurate data are a source of false positive and negative results, with possible adverse impacts on health and financial outcomes. They were incorporated in this analysis, namely potential false positives (i.e., excessive investigations) and negatives (i.e., missed diagnoses). However, in this study, costs related to false positive and negative results had lower impact than the benefits of reduction in Hospital stay. Moreover, reduction on time to CAL diagnosis had a smaller positive economic effect, accounting for 6.2% of cost savings (31,710.64€). So far, a cost minimization analysis was not performed in any other similar scores abovementioned, but these tools may be adopted in daily routine for improving financial outcomes, amongst other benefits.

CHAPTER V

CONCLUSIONS

A - CRITICAL APPRAISAL

This chapter presents the conclusion of the Thesis and discusses some of its strengths, limitations and an overall appraisal of the research conducted.

1 - STRENGTHS OF THE STUDY

The first strength that worth mentioning is the study design: the prospective design and the data collection protocol (data collected and registered by independent collaborators) minimize the observer bias.

Second, using sample size as criteria to assess the research relevance, this is one of the largest monocentric studies published so far. Based on the recent meta-analyses of Yeung *et al.*, only two monocentric prospective studies have enrolled more than 400 patients (Mik *et al.* 2018; Waterland *et al.* 2016; Yeung, *et al.* 2021).

Third, a comprehensive definition of CAL was chosen, similar to the one recently proposed by van Helsdingen *et al.* (van Helsdingen, *et al.* 2020), to include all patients with CAL, minimizing selection bias issues. Minor CAL were not excluded, which might also have affected the predictive effect of the biomarkers.

Furthermore, and for the first time, five plasmatic biomarkers have been analyzed in the same study, including plasma CLP, which was first studied by Reisinger *et al.* (Reisinger, *et al.* 2014).

Fifth, to keep the biomarkers optimum cut-off values in AUC analysis, both standardized and reproducible, Liu's method was adopted. This method defines the optimal cut-off point as the point maximizing the product of SS and SP (Liu 2012). This aspect may explain some differences in biomarkers diagnostic accuracy in the present study.

Sixth, the study protocol was adapted to the daily practice, making its implementation easier in the future. Hence, we included all patients undergoing colorectal resection, even those with a diverting stoma. In addition, clinicians were not blinded to the daily biomarkers results, and might use those data according to the study protocol.

Moreover, we proposed a warning score (E-CRALL) based on the combination of pre, intra and post-operative variables that contributed to predict CAL. The adoption of this decision tool by routine might emphasize the clinic method, not only because it incorporates technology (three biomarkers, as ECC, plasmatic CLP and CRP) but

also information from clinical data and physical examination (pre- and intra-operative aspects, abdominal pain, and clinical condition). Clinicians can be supported by technology but should also include its observation skills, experience, and common sense, in the decision-making process.

Eighth, the E-CRALL score defined a single warning threshold, depending on the POD, and recommending imaging (CT scan) or early re-operation (in case of equivocal or negative imaging), simplifying the approach of CAL diagnosis. Additionally, an early re-operation proposal, as before mentioned, helps reducing the time to CAL detection, and consequently start CAL management promptly. Different authors defend that early re-operation, namely by minimally invasive techniques, for managing complications following colorectal surgery appears to be safe and effective in highly selected patients (Chang, *et al.* 2016; Kirshtein, *et al.* 2008; O'Riordan *et al.* 2013). The key approach for this selection can involve the adoption of E-CRALL score. Besides, a policy of early re-operation in patients with suspected complications enables timely management with expedient resolution, saving time to CAL diagnosis and to discharge (Kirshtein, *et al.* 2008).

On the other hand, it was developed a simple and user-friendly application to compute the score, facilitating this time-consuming task and making implementation easier in daily clinical practice.

Tenth, this is the first Portuguese study which aimed to assess the economic impact of anastomotic leakage in colorectal surgery, based on a cost minimisation study. Moreover, a positive financial effect of the daily clinical score adoption was found. In the literature published, so far, no other CAL predictive score was assessed in terms of its economic value.

2 - LIMITATIONS OF THE STUDY

The prospective study has several limitations. First, the monocentric design may limit external validity of the results.

Second, our sample has some grade of heterogeneity, because the study population included benign and malignant disorders, elective and urgent procedures, and anastomosis within different levels of the colon and rectum.

Third, plasma CLP measurement is expensive, and these kits are not easily accessible

to use in daily clinical practice.

Fourth, it's noteworthy that the E-CRALL score was developed and tested on only one dataset. Therefore, these findings should be considered with caution and need to have an external validation, planned for the multicentric prospective study abovementioned.

Fifth, another limitation is related to the E-CRALL complexity for daily clinical implementation. It includes 13 diverse variables, increasing the workload for health staff: multiple daily evaluation, several blood sampling and laboratory investigation are required, every day.

Furthermore, this study addresses the economic burden of CAL in routine practice, and, in addition, conducted a cost minimization analysis, comparing the expected costs of the adoption of E-CRALL score in daily practice, with the expected cost of standard clinical practice without E-CRALL. To conduct this cost-minimization analysis it was assumed that all alternatives in comparison deliver equivalent health outcomes. This assumption is based on a conservative policy, since health outcomes improves with the early diagnosis (Kirshtein, *et al.* 2008; Spence *et al.* 2021).

Seventh, there was a large divergence in the cost estimation of CAL, depending on the method of its calculation. This prospective study adopted comprehensive costs, as there is the usual practice of public (National Health Service) hospitals reimbursement. These methods may inadvertently underestimate costs, due to under-coding, or in contrast, raise the practice of 'gaming' to get more revenue. Probably, the estimation of personalized cost (tailored approach) by the aggregate of the index costs would be the more appropriate method (Ashraf, *et al.* 2013; Koperna 2003).

Next, it is crucial not only to estimate costs related to a delayed diagnosis, but also the high rate of false positive cases, needless re-operations, or frequent readmissions, amongst other factors. As above mentioned, consequences of false negative cases on LOHS were hard to deal with. A conservative policy was applied, with the adoption of a cut-point with a sensitivity around 100%, to minimize, not only the impact of false negatives on LOHS, but also the consequences of inappropriate early discharge.

Finally, the psychological and physical burden of CAL, as well as the impact on individual and relatives´ quality of life were not considered in this research. This condition limited the type of economic evaluation that was undertaken.

2 – OVERALL APPRAISAL

This Thesis encompasses three core studies, one snapshot study covering the 4-year period before the beginning of the experiment, one systematic review of the literature with meta-analysis, and the prospective study.

First, the retrospective study aimed to assess the incidence and diagnostic criteria of CAL, overall complications, highlighting the significance of FTR philosophy. Clinical criteria were paramount in CAL diagnosis, remaining some questions about optimizing procedures towards its detection. This left the door open for the monocentric prospective study, which evaluated the usefulness of clinical criteria and plasmatic biomarkers in this setting. A sizable sample was included, a more reliable and consensual definition was considered, and a refined statistical analysis was applied. The leakage rate decreased over time, on the retrospective study, and it was even lower on the prospective cohort (7.7% vs. 6.3%, respectively). However, both studies have shown a similar delay on CAL diagnosis (median of 5 and 8 days for major or minor CAL, respectively). In this context, imaging (CT scan) was correlated with a delay in the diagnosis of CAL, mainly due to its low sensitivity, since a negative or unclear result seemed to mislead the attending physician. Based on these findings, in the Decision Tree developed for E-CRALL score application, an early re-operation (whenever possible by a minimally invasive approach) was proposed in patients where imaging was doubtful or negative. In this setting, combinations of plasma CLP and CRP, and E-CRALL score use, were able to reduce the time to CAL diagnosis. To do so, standardized protocol for CAL diagnosis, including a strong warning score, should be recommended. Regarding CAL management, in the retrospective cohort, more patients (81%) required re-operation, while in the prospective study, surgical management was less required.

Results from systematic review and meta-analysis were more in line with the purpose of the prospective study, and suggested biomarkers as moderate predictors of CAL. This systematic review was “pioneer”, due to the qualitative analysis of CAL definition, which deserved further application in the prospective study. In conclusion, both prospective and retrospective studies have shown CAL rates within the estimates found in the meta-analysis. However, in the prospective study, biases identified in the meta-analysis were not clearly verified. Moreover, some questions, as the economic impact of biomarkers usage, were, at least in part, answered.

B - CONCLUDING REMARKS

In this Thesis, was investigated both clinical criteria and systemic biomarkers levels, as early predictors of anastomotic failure, after colorectal surgery. Currently, CAL diagnosis still depend on clinical presentation and imaging studies. Early clinical presentation is often heterogeneous and nonspecific, resulting in postponed CAL detection. Additionally, if appropriate, diagnosis was complemented with CT scan, which demonstrates significant false negative rates and limited sensitivity and accuracy. Oftentimes, CAL does not turn clinically apparent until the first post-operative week, becoming "visible", in some cases, just after discharge.

Supporting this aim, it was tried to answer the main research question proposed, by defining the useful indicators for timely identify a patient with CAL. These data corroborate that **clinical criteria**, namely the progression pattern of abdominal pain and clinical condition, **have added value as a warning sign of CAL**. Plasmatic **levels of CLP and CRP** substantially increase in CAL patients during the first five post-operative days, suggesting **their potential as best early CAL predictors**, while the systemic levels of WBC, ECC and PCT have limited additional value in this regard. For early discharging, optimized cut-off values of CRP, PCT and CLP were defined. With the growing use of enhanced recovery protocols, these data are extremely useful to reinforce discharge criteria after colorectal surgery. Particularly relevant is the direct **combination of CLP and CRP plasma levels, early during the post-operative period (POD3), and its potential to markedly reduce the time to diagnosis of CAL**, and consequently, its expected effect on reduction of morbidity and mortality.

Another purpose of this Thesis was the development of a decision model, with fewer parameters, for prompt detection of CAL. Based on this, **E-CRALL score** was built and showed a **high predictive ability, with SS and NPV of 100% after the POD4 and a significant SP (86.6%) on POD5**. This study internally validates the E-CRALL score for the early diagnosis of CAL, that will be integrated as part of local risk management policy, improving the quality of healthcare. With the assistance of the application to compute the score value, a simple, uniform, and objective assessment to measure the chance of CAL was possible. E-CRALL score should be included in a standard post-operative surveillance programme of CAL, as close as possible to the before-mentioned Decision Tree model. This protocol proposes an early re-operation in case of dubious or negative imaging, to reduce the time to CAL

detection and enabling prompt management. With this intent, the routine adoption of E-CRALL score may help prioritizing, supporting the policy of early re-operation in patients with suspected anastomotic failure.

In terms of economic burden, this study confirms the negative impact of CAL. **Overall costs of colorectal resection increased significantly, almost three times, in patients who developed anastomotic failure**. In this research, overall costs were inferior after E-CRALL use, revealing a noteworthy reduction of in-hospital costs, in patients with or without CAL, as compared with standard clinical practice. Overall savings result mainly from the reduction in LOHS, which is more remarkable in non-CAL group, whereas the reduction on time to CAL diagnosis has a smaller positive economic effect. Potential false positive and negative results were incorporated in the analysis but seem to have lower economic impact than the benefits before-mentioned.

In conclusion, as part of a standard post-operative surveillance protocol, routine monitoring of clinical criteria (abdominal pain and clinical condition), plasma biomarkers, alone or combined (namely CLP and CRP), or through the warning E-CRALL score adoption, allow an early detection of colorectal anastomotic failure, by reducing the time to CAL diagnosis.

CHAPTER VI

FUTURE RESEARCH

Beyond the contribution of this Thesis to the evolution of the knowledge in the field of colorectal anastomotic failure, the research has also revealed some "new avenues" that is worth exploring.

To begin with, and to overcome some of the limitations before identified, a multicentric randomized prospective study should be conducted, and in fact, it is on our research agenda on the short run. The randomized trial (E-CRALL study) will enrol adults over 18 years old who underwent urgent or elective colorectal resection, regardless on the surgical approach (open or laparoscopic), indication (benign or malignant), and option for a protective stoma. The study will be run in, at least, five Portuguese Colorectal Divisions. Patients will be randomized and allocated in two groups: one group will receive the standard post-operative surveillance protocol of CAL, and the other will include the new model, which incorporate the E-CRALL score and the six pathways of the Decision Tree (E-CRALL protocol). The E-CRALL study will establish as primary outcomes the time to CAL diagnosis, LOHS, ninety-days morbidity and mortality, and total costs estimation. Secondary outcomes will include long-term oncological and functional results. A mobile application that is still under construction, will be used in the E-CRALL protocol arm.

After this randomized trial, it will be possible to analyse the external validity of E-CRALL score, clarifying how generalizable the findings of the monocentric prospective study are, namely to other institutions, settings, and time periods. In addition, by confirming a significant reduction on time to CAL detection, short-term outcomes will improve, in particular, post-operative complications rate, LOHS, quality of life, and mortality rate. Consequently, this validated tool may improve risk management in colorectal surgery, and reduce mortality, according to the FTR philosophy. First introduced by Silber et al., this concept reflects the estimated mortality rate in patients who developed a specific post-operative complication, as CAL in this respect (Silber, et al. 1992). This rate differs among distinct institutions and depends on different diagnostic and management strategies. For institutional benchmarking, including the Portuguese Cancer Reference Centers, this new metric can be useful and should be adopted in the near future (Almoudaris, et al. 2011; Johnston et al. 2015).

Currently, it is still unclear whether CAL - after proctectomy - affects long-term functional outcomes. In terms of bowel function, it seems to increase the risk of low anterior resection syndrome, significantly impairing patients' quality of life. Regarding urinary function, data are controversial, whereas sexual activity was compromised.

In case of malignancy, patients developing CAL exhibit poorer long-term oncological results, as increased LR rates and reduced 5-year OS. The negative impact of CAL on survival was previously detailed on Chapter One. Thereby, this new trial may contribute to clarify some of this unsolved questions, gauging the real impact of E-CRALL score adoption in daily practice. Furthermore, the oncological outcomes of the monocentric study conducted, namely 3-year DFS and OS, will be analysed and published during the second semester of 2022.

As previously described, CAL development will often warrant additional surgical procedures, increased LOHS, extra-costs with medicines, imaging or stoma care, hospital re-admission, among others. Besides the potential negative clinical outcomes, there is a substantial economic and healthcare burden to be considered. Full costs analysis will be performed, combining complete in-hospital expenses, as variable (direct) and fixed (overheads) costs. These new trial will include costs of primary and supplementary operative procedures, extra LOHS, critical care length of stay, imaging, pharmacy, and re-admissions. Additionally, costs resulting from supplies, labour, and depreciation of equipment will be recorded and analysed. Then, cost-effectivity and cost-utility studies will be well conducted.

REFERENCES

AADLAND, E. AND M. K. FAGERHOL Faecal calprotectin: a marker of inflammation throughout the intestinal tract. Eur J Gastroenterol Hepatol, Aug 2002, 14(8), 823-825.

ABIDI, K., J. BELAYACHI, Y. DERRAS, M. E. KHAYARI, et al. Eosinopenia, an early marker of increased mortality in critically ill medical patients. Intensive Care Med, Jul 2011, 37(7), 1136-1142.

ADAMS, K. AND S. PAPAGRIGORIADIS Little consensus in either definition or diagnosis of a lower gastro-intestinal anastomotic leak amongst colorectal surgeons. Int J Colorectal Dis, Jul 2013, 28(7), 967-971.

ADAMS, K. AND S. PAPAGRIGORIADIS Creation of an effective colorectal anastomotic leak early detection tool using an artificial neural network. Int J Colorectal Dis, Apr 2014, 29(4), 437-443.

AGILLI, M. AND F. N. AYDIN Methodologic approach to accurate prediction of anastomotic leakage after colorectal surgery using plasma markers for intestinal damage and inflammation. Journal of the American College of Surgeons, May 2015, 220(5), 971.

AHL, R., P. MATTHIESSEN, X. FANG, Y. CAO, et al. β-Blockade in Rectal Cancer Surgery: A Simple Measure of Improving Outcomes. Ann Surg, Jan 2020, 271(1), 140-146.

AKIYOSHI, T., M. UENO, Y. FUKUNAGA, S. NAGAYAMA, et al. Incidence of and risk factors for anastomotic leakage after laparoscopic anterior resection with intracorporeal rectal transection and double-stapling technique anastomosis for rectal cancer. Am J Surg, Sep 2011, 202(3), 259-264.

AKYOL, A. M., J. R. MCGREGOR, D. J. GALLOWAY AND W. D. GEORGE Early post-operative contrast radiology in the assessment of colorectal anastomotic integrity. Int J Colorectal Dis, Sep 1992, 7(3), 141-143.

ALMEIDA, A. B., G. FARIA, H. MOREIRA, J. PINTO-DE-SOUSA, et al. Elevated serum C-reactive protein as a predictive factor for anastomotic leakage in colorectal surgery. Int J Surg, 2012, 10(2), 87-91.

ALMOUDARIS, A. M., E. M. BURNS, R. MAMIDANNA, A. BOTTLE, et al. Value of failure to rescue as a marker of the standard of care following re-operation for complications after colorectal resection. Br J Surg, Dec 2011, 98(12), 1775-1783.

ALVES, A., Y. PANIS, D. TRANCART, J. M. REGIMBEAU, et al. Factors associated with

clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. World J Surg, Apr 2002, 26(4), 499-502.

AMATO, F., A. LÓPEZ, E. M. PEÑA-MÉNDEZ, P. VAŇHARA, et al. Artificial neural networks in medical diagnosis. Journal of Applied Biomedicine, 2013/01/01/ 2013, 11(2), 47-58.

ANANIA, G., A. AREZZO, R. J. DAVIES, F. MARCHETTI, et al. A global systematic review and meta-analysis on laparoscopic vs open right hemicolectomy with complete mesocolic excision. Int J Colorectal Dis, Aug 2021, 36(8), 1609-1620.

ARRON, M. N. N., N. G. GREIJDANUS, R. P. G. TEN BROEK, J. W. T. DEKKER, et al. Trends in risk factors of anastomotic leakage after colorectal cancer surgery (2011–2019): A Dutch population-based study. Colorectal Disease, 2021, 23(12), 3251-3261.

ASHRAF, S. Q., E. M. BURNS, A. JANI, S. ALTMAN, et al. The economic impact of anastomotic leakage after anterior resections in English NHS hospitals: are we adequately remunerating them? Colorectal Disease, 2013, 15(4), e190-e198.

ATHANASIOU, C. D., G. A. MARKIDES, A. KOTB, X. JIA, et al. Open compared with laparoscopic complete mesocolic excision with central lymphadenectomy for colon cancer: a systematic review and meta-analysis. Colorectal Disease, Jul 2016, 18(7), O224-235.

BA, Z. F., Y. YOKOYAMA, B. TOTH, L. W. RUE, 3RD, et al. Gender differences in small intestinal endothelial function: inhibitory role of androgens. Am J Physiol Gastrointest Liver Physiol, Mar 2004, 286(3), G452-457.

BAEZA-MURCIA, M., G. VALERO-NAVARRO, E. PELLICER-FRANCO, V. SORIA-ALEDO, et al. Early diagnosis of anastomotic leakage in colorectal surgery: prospective observational study of the utility of inflammatory markers and determination of pathological levels. Updates Surg, Dec 2021, 73(6), 2103-2111.

BAKKER, I. S., I. GROSSMANN, D. HENNEMAN, K. HAVENGA, et al. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. Br J Surg, Mar 2014, 101(4), 424-432; discussion 432.

BALDUZZI, S., G. RÜCKER AND G. SCHWARZER How to perform a meta-analysis with R: a practical tutorial. Evid Based Ment Health, Nov 2019, 22(4), 153-160.

BASHIR MOHAMED, K., C. H. HANSEN, P.-M. KRARUP, T. FRANSGÅRD, et al. The

impact of anastomotic leakage on recurrence and long-term survival in patients with colonic cancer: A systematic review and meta-analysis. European Journal of Surgical Oncology, 2020/03/01/ 2020, 46(3), 439-447.

BASS, D. A. Behavior of eosinophil leukocytes in acute inflammation. II. Eosinophil dynamics during acute inflammation. J Clin Invest, Oct 1975, 56(4), 870-879.

BASS, D. A., T. A. GONWA, P. SZEJDA, M. S. COUSART, et al. Eosinopenia of acute infection: Production of eosinopenia by chemotactic factors of acute inflammation. J Clin Invest, Jun 1980, 65(6), 1265-1271.

BEARD, J. D., M. L. NICHOLSON, R. D. SAYERS, D. LLOYD, et al. Intra-operative air testing of colorectal anastomoses: a prospective, randomized trial. Br J Surg, Oct 1990, 77(10), 1095-1097.

BECKER, K. L., E. S. NYLÉN, J. C. WHITE, B. MÜLLER, et al. Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab, Apr 2004, 89(4), 1512-1525.

BECKER, K. L., R. SNIDER AND E. S. NYLEN Procalcitonin assay in systemic inflammation, infection, and sepsis: clinical utility and limitations. Crit Care Med, Mar 2008, 36(3), 941-952.

BELIZON, A., C. T. SARDINHA AND M. E. SHER Converted laparoscopic colectomy: what are the consequences? Surg Endosc, 2006/06// 2006, 20(6), 947-951.

BELL, S. W., K. G. WALKER, M. J. RICKARD, G. SINCLAIR, et al. Anastomotic leakage after curative anterior resection results in a higher prevalence of local recurrence. Br J Surg, Oct 2003, 90(10), 1261-1266.

BELLOWS, C. F., L. S. WEBBER, D. ALBO, S. AWAD, et al. Early predictors of anastomotic leaks after colectomy. Tech Coloproctol, Mar 2009, 13(1), 41-47.

BENTES, M., M. D. L. GONSALVES, M. SANTOS AND E. PINA Design and Development of a Utilization Review Program in Portugal. International Journal for Quality in Health Care, 1995, 7(3), 201-212.

BEST, W. R., R. C. MUEHRCKE AND R. M. KARK Studies on adrenocortical eosinopenia: a clinical and statistical evaluation of four-hour eosinophil response test. J Clin Invest, Jul 1952, 31(7), 733-742.

BIONDO, S., D. PARÉS, E. KREISLER, J. M. RAGUÉ, et al. Anastomotic dehiscence after resection and primary anastomosis in left-sided colonic emergencies. Dis Colon Rectum, Dec 2005, 48(12), 2272-2280.

BOCCOLA, M. A., P. G. BUETTNER, W. M. ROZEN, S. K. SIU, et al. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. World J Surg, Jan 2011, 35(1), 186-195.

BOELENS, P. G., F. F. HEESAKKERS, M. D. LUYER, K. W. VAN BARNEVELD, et al. Reduction of post-operative ileus by early enteral nutrition in patients undergoing major rectal surgery: prospective, randomized, controlled trial. Ann Surg, Apr 2014, 259(4), 649-655.

BONE, R. C. The pathogenesis of sepsis. Ann Intern Med, Sep 15 1991, 115(6), 457-469.

BONE, R. C., R. A. BALK, F. B. CERRA, R. P. DELLINGER, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest, Jun 1992, 101(6), 1644-1655.

BOSTRÖM, P., J. SVENSSON, C. BRORSSON AND M. RUTEGÅRD Early post-operative pain as a marker of anastomotic leakage in colorectal cancer surgery. Int J Colorectal Dis, Sep 2021, 36(9), 1955-1963.

BRANAGAN, G. AND D. FINNIS Prognosis after anastomotic leakage in colorectal surgery. Dis Colon Rectum, May 2005, 48(5), 1021-1026.

BRAY, C., L. N. BELL, H. LIANG, R. HAYKAL, et al. Erythrocyte Sedimentation Rate and C-reactive Protein Measurements and Their Relevance in Clinical Medicine. Wmj, Dec 2016, 115(6), 317-321.

BRENNAN, S. S., I. R. PICKFORD, M. EVANS AND A. V. POLLOCK Staples or sutures for colonic anastomoses--a controlled clinical trial. Br J Surg, Dec 1982, 69(12), 722-724.

BROWN, S. R., R. MATHEW, A. KEDING, H. C. MARSHALL, et al. The impact of post-operative complications on long-term quality of life after curative colorectal cancer surgery. Ann Surg, May 2014, 259(5), 916-923.

BRUCE, J., Z. H. KRUKOWSKI, G. AL-KHAIRY, E. M. RUSSELL, et al. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery.

Br J Surg, Sep 2001, 88(9), 1157-1168.

BUCHS, N. C., P. GERVAZ, M. SECIC, P. BUCHER, et al. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis, Mar 2008, 23(3), 265-270.

BURTON, T. P., A. MITTAL AND M. SOOP Nonsteroidal anti-inflammatory drugs and anastomotic dehiscence in bowel surgery: systematic review and meta-analysis of randomized, controlled trials. Dis Colon Rectum, Jan 2013, 56(1), 126-134.

CAILLE, V., P. BOSSI, D. GRIMALDI AND A. VIEILLARD-BARO [Physiopathology of severe sepsis]. Presse Med, Feb 28 2004, 33(4), 256-261; discussion 269.

CALIFF, R. M. Biomarker definitions and their applications. Experimental Biology and Medicine, 2018, 243(3), 213-221.

CAPOLUPO, G. T., T. GALVAIN, V. PARAGÒ, C. TONG, et al. In-hospital economic burden of anastomotic leakage after colorectal anastomosis surgery: a real-world cost analysis in Italy. Expert Review of Pharmacoeconomics & Outcomes Research, 2021, 1-7.

CASILLAS, S., C. P. DELANEY, A. J. SENAGORE, K. BRADY, et al. Does conversion of a laparoscopic colectomy adversely affect patient outcome? Dis Colon Rectum, 2004/10// 2004, 47(10), 1680-1685.

CHANG, K. H., M. G. BOURKE, D. O. KAVANAGH, P. C. NEARY, et al. A systematic review of the role of re-laparoscopy in the management of complications following laparoscopic colorectal surgery. Surgeon, Oct 2016, 14(5), 287-293.

CHOI, H. K., W. L. LAW AND J. W. HO Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. Dis Colon Rectum, Nov 2006, 49(11), 1719-1725.

CHUANG, D., J. S. PADDISON, R. J. BOOTH AND A. G. HILL Differential production of cytokines following colorectal surgery. ANZ J Surg, Sep 2006, 76(9), 821-824.

CIKOT, M., O. KONES, A. GEDIKBASı, A. KOCATAS, et al. The marker C-reactive protein is helpful in monitoring the integrity of anastomosis: plasma calprotectin. Am J Surg, Jul 2016, 212(1), 53-61.

COUSIN, F., P. ORTEGA-DEBALLON, A. BOURREDJEM, A. DOUSSOT, et al. Diagnostic Accuracy of Procalcitonin and C-reactive Protein for the Early Diagnosis of Intra-abdominal

Infection After Elective Colorectal Surgery: A Meta-analysis. Ann Surg, Aug 2016, 264(2), 252-256.

CUCCURULLO, D., F. PIROZZI, A. SCIUTO, U. BRACALE, et al. Relaparoscopy for management of post-operative complications following colorectal surgery: ten years experience in a single center. Surg Endosc, Jul 2015, 29(7), 1795-1803.

DAAMS, F., Z. WU, M. J. LAHAYE, J. JEEKEL, et al. Prediction and diagnosis of colorectal anastomotic leakage: A systematic review of literature. World J Gastrointest Surg, Feb 27 2014, 6(2), 14-26.

DAMEN, N., K. SPILSBURY, M. LEVITT, G. MAKIN, et al. Anastomotic leaks in colorectal surgery. ANZ Journal of Surgery, 2014, 84(10), 763-768.

DAVIDO, B., S. MAKHLOUFI, M. MATT, R. CALIN, et al. Changes in eosinophil count during bacterial infection: revisiting an old marker to assess the efficacy of antimicrobial therapy. Int J Infect Dis, Aug 2017, 61, 62-66.

DEHNI, N., R. D. SCHLEGEL, C. CUNNINGHAM, M. GUIGUET, et al. Influence of a defunctioning stoma on leakage rates after low colorectal anastomosis and colonic J pouch-anal anastomosis. Br J Surg, Aug 1998, 85(8), 1114-1117.

DEN DULK, M., S. L. NOTER, E. R. HENDRIKS, M. A. M. BROUWERS, et al. Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. European Journal of Surgical Oncology (EJSO), 2009/04/01/ 2009, 35(4), 420-426.

DEN DULK, M., M. J. WITVLIET, K. KORTRAM, P. A. NEIJENHUIS, et al. The DULK (Dutch leakage) and modified DULK score compared: actively seek the leak. Colorectal Disease, Sep 2013, 15(9), e528-533.

DI CRISTOFARO, L., C. RUFFOLO, E. PINTO, M. MASSA, et al. Complications after surgery for colorectal cancer affect quality of life and surgeon-patient relationship. Colorectal Disease, Dec 2014, 16(12), O407-419.

DINDO, D., N. DEMARTINES AND P. A. CLAVIEN Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg, Aug 2004, 240(2), 205-213.

DOEKSEN, A., P. J. TANIS, B. C. VROUENRAETS, J. J. LANSCHOT VAN, et al. Factors determining delay in relaparotomy for anastomotic leakage after colorectal resection. World J Gastroenterol, Jul 21 2007, 13(27), 3721-3725.

DOEKSEN, A., P. J. TANIS, A. F. WÜST, B. C. VROUENRAETS, et al. Radiological evaluation of colorectal anastomoses. Int J Colorectal Dis, Sep 2008, 23(9), 863-868.

DREW, P. J. AND J. R. MONSON Artificial neural networks. Surgery, Jan 2000, 127(1), 3-11.

DRUMMOND, M. F., M. J. SCULPHER, K. CLAXTON, G. L. STODDART, et al. Methods for the Economic Evaluation of Health Care Programmes. Edtion ed. Oxford: Oxford: Oxford University Press, 2015. ISBN 9780199665884.

ECKMANN, C., P. KUJATH, T. H. SCHIEDECK, H. SHEKARRIZ, et al. Anastomotic leakage following low anterior resection: results of a standardized diagnostic and therapeutic approach. Int J Colorectal Disease, Mar 2004, 19(2), 128-133.

EDITORA, P. deiscência no Dicionário infopédia de Termos Médicos. In. Dicionário infopédia Porto Editora, 2021, vol. 2021.

ERLINGER, T. P., E. A. PLATZ, N. RIFAI AND K. J. HELZLSOUER C-Reactive Protein and the Risk of Incident Colorectal Cancer. Jama, 2004, 291(5), 585-590.

ESCP Collaborating Group. The impact of stapling technique and surgeon specialism on anastomotic failure after right-sided colorectal resection: an international multicentre, prospective audit. Colorectal Disease, Nov 2018, 20(11), 1028-1040.

FACY, O., B. PAQUETTE, D. ORRY, C. BINQUET, et al. Diagnostic Accuracy of Inflammatory Markers As Early Predictors of Infection After Elective Colorectal Surgery: Results From the IMACORS Study. Ann Surg, May 2016, 263(5), 961-966.

FARAONE, S. V. Interpreting estimates of treatment effects: implications for managed care. P & T : a peer-reviewed journal for formulary management, 2008, 33(12), 700-711.

FERNANDES, R., I. SHAIKH AND S. DOUGHAN Outcomes of elective laparoscopic colorectal operations in octogenarians at a district general hospital in South East England. World J Gastrointest Surg, Jan 27 2013, 5(1), 9-11.

FINGERHUT, A., J. M. HAY, A. ELHADAD, F. LACAINE, et al. Supraperitoneal colorectal anastomosis: hand-sewn versus circular staples--a controlled clinical trial. French Associations for Surgical Research. Surgery, Sep 1995, 118(3), 479-485.

FITZGERALD, G. A. Measure for Measure: Biomarker standards and transparency. Sci Transl Med, Jun 15 2016, 8(343), 343fs310.

FLOODEEN, H., O. HALLBÖÖK, J. RUTEGÅRD, R. SJÖDAHL, et al. Early and late symptomatic anastomotic leakage following low anterior resection of the rectum for cancer: are they different entities? Colorectal Disease, Mar 2013, 15(3), 334-340.

FRANSVEA, P., G. COSTA, L. D'AGOSTINO, G. SGANGA, et al. Redo-laparoscopy in the management of complications after laparoscopic colorectal surgery: a systematic review and meta-analysis of surgical outcomes. Tech Coloproctol, Apr 2021, 25(4), 371-383.

FRASSON, M., B. FLOR-LORENTE, J. L. RODRÍGUEZ, P. GRANERO-CASTRO, et al. Risk Factors for Anastomotic Leak After Colon Resection for Cancer: Multivariate Analysis and Nomogram From a Multicentric, Prospective, National Study With 3193 Patients. Ann Surg, Aug 2015, 262(2), 321-330.

FRASSON, M., P. GRANERO-CASTRO, J. L. RAMOS RODRÍGUEZ, B. FLOR-LORENTE, et al. Risk factors for anastomotic leak and post-operative morbidity and mortality after elective right colectomy for cancer: results from a prospective, multicentric study of 1102 patients. Int J Colorectal Dis, Jan 2016, 31(1), 105-114.

FRYE, J., E. L. BOKEY, P. H. CHAPUIS, G. SINCLAIR, et al. Anastomotic leakage after resection of colorectal cancer generates prodigious use of hospital resources. Colorectal Disease, Nov 2009, 11(9), 917-920.

GANI, F., M. CERULLO, X. ZHANG, J. K. CANNER, et al. Effect of surgeon "experience" with laparoscopy on post-operative outcomes after colorectal surgery. Surgery, Oct 2017, 162(4), 880-890.

GARCIA-GRANERO, A., M. FRASSON, B. FLOR-LORENTE, F. BLANCO, et al. Procalcitonin and C-reactive protein as early predictors of anastomotic leak in colorectal surgery: a prospective observational study. Dis Colon Rectum, Apr 2013, 56(4), 475-483.

GARCÍA-GRANERO, E., F. NAVARRO, C. CERDÁN SANTACRUZ, M. FRASSON, et al. Individual surgeon is an independent risk factor for leak after double-stapled colorectal anastomosis: An institutional analysis of 800 patients. Surgery, Nov 2017, 162(5), 1006-1016.

GARETH JAMES, D. W., TREVOR HASTIE, ROBERT TIBSHIRA *An Introduction to Statistical Learning with Applications in R. Edtion* ed. New York: Springer Science+Business Media, 2013. 426 p. ISBN 978-1-4614-7138-7.

GARNACHO-MONTERO, J., M. J. HUICI-MORENO, A. GUTIERREZ-PIZARRAYA, I. LOPEZ, et

al. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. Crit Care, Jun 05 2014, 18(3), R116.

GE, X., Y. CAO, H. WANG, C. DING, et al. Diagnostic accuracy of the post-operative ratio of C-reactive protein to albumin for complications after colorectal surgery. World J Surg Oncol, Jan 10 2017, 15(1), 15.

GERVAZ, P., C. BANDIERA-CLERC, N. C. BUCHS, M.-C. EISENRING, et al. Scoring system to predict the risk of surgical-site infection after colorectal resection2. Br J Surg, 2012, 99(4), 589-595.

GERVAZ, P., A. PLATON, N. C. BUCHS, T. ROCHER, et al. CT scan-based modelling of anastomotic leak risk after colorectal surgery. Colorectal Disease, 2013, 15(10), 1295-1300.

GESSLER, B., O. ERIKSSON AND E. ANGENETE Diagnosis, treatment, and consequences of anastomotic leakage in colorectal surgery. Int J Colorectal Dis, 2017/04/01 2017, 32(4), 549-556.

GIACCAGLIA, V., P. F. SALVI, M. S. ANTONELLI, G. NIGRI, et al. Procalcitonin Reveals Early Dehiscence in Colorectal Surgery: The PREDICS Study. Ann Surg, May 2016, 263(5), 967-972.

GIACCAGLIA, V., P. F. SALVI, G. V. CUNSOLO, A. SPARAGNA, et al. Procalcitonin, as an early biomarker of colorectal anastomotic leak, facilitates enhanced recovery after surgery. J Crit Care, Aug 2014, 29(4), 528-532.

GIL, H., N. MAGY, F. MAUNY AND J. L. DUPOND [Value of eosinopenia in inflammatory disorders: an “old” marker revisited]. Rev Med Interne, Jul 2003, 24(7), 431-435.

GILBERT, D. N. Use of plasma procalcitonin levels as an adjunct to clinical microbiology. J Clin Microbiol, Jul 2010, 48(7), 2325-2329.

GOLIGHER, J. C., N. G. GRAHAM AND F. T. DE DOMBAL Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg, Feb 1970, 57(2), 109-118.

GOLUB, R., R. W. GOLUB, R. CANTU, JR. AND H. D. STEIN A multivariate analysis of factors contributing to leakage of intestinal anastomoses. Journal of the American College of Surgeons, Apr 1997, 184(4), 364-372.

GRAY, M., J. R. K. MARLAND, A. F. MURRAY, D. J. ARGYLE, et al. Predictive and

Diagnostic Biomarkers of Anastomotic Leakage: A Precision Medicine Approach for Colorectal Cancer Patients. J Pers Med, May 25 2021, 11(6).

GROUP, T. E. S. O. C. C. The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit. Colorectal Disease, Mar 6 2017.

GÜENAGA, K. F., S. A. LUSTOSA, S. S. SAAD, H. SACONATO, et al. Ileostomy or colostomy for temporary decompression of colorectal anastomosis. Cochrane Database Syst Rev, Jan 24 2007, (1), Cd004647.

GUYTON, K. L., N. H. HYMAN AND J. C. ALVERDY Prevention of Perioperative Anastomotic Healing Complications: Anastomotic Stricture and Anastomotic Leak. Adv Surg, Sep 2016, 50(1), 129-141.

HA, G. W., J. H. KIM AND M. R. LEE Oncologic Impact of Anastomotic Leakage Following Colorectal Cancer Surgery: A Systematic Review and Meta-Analysis. Ann Surg Oncol, Oct 2017, 24(11), 3289-3299.

HABIB, K., A. GUPTA, D. WHITE, F. A. MAZARI, et al. Utility of contrast enema to assess anastomotic integrity and the natural history of radiological leaks after low rectal surgery: systematic review and meta-analysis. Int J Colorectal Dis, Aug 2015, 30(8), 1007-1014.

HAMMOND, J., S. LIM, Y. WAN, X. GAO, et al. The burden of gastrointestinal anastomotic leaks: an evaluation of clinical and economic outcomes. J Gastrointest Surg, Jun 2014, 18(6), 1176-1185.

HANSEN, O., W. SCHWENK, H. P. HUCKE AND W. STOCK Colorectal stapled anastomoses. Dis Colon Rectum, 1996/01/01 1996, 39(1), 30-36.

HANSSON, C., C. ERIKSSON AND G. M. ALENIUS S-calprotectin (S100A8/S100A9): a potential marker of inflammation in patients with psoriatic arthritis. J Immunol Res, 2014, 2014, 696415.

HARBARTH, S., K. HOLECKOVA, C. FROIDEVAUX, D. PITTET, et al. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med, Aug 1 2001, 164(3), 396-402.

HASTIE, T., R. TIBSHIRANI AND J. FRIEDMAN. Unsupervised learning. In The elements of statistical learning. Springer, 2009, p. 485-585.

HAYAMI, S., M. UENO, M. KAWAI, T. KURIYAMA, et al. Optimal Timing of Removal of Epidural and Urethral Catheters to Avoid Post-operative Urinary Retention Undergoing Abdominal Surgery. Dig Surg, 2019, 36(3), 261-265.

HAYNES, I. G., M. GOLDMAN, S. H. SILVERMAN, J. ALEXANDER-WILLIAMS, et al. Water-soluble contrast enema after colonic anastomosis. Lancet, Mar 22 1986, 1(8482), 675-676.

HEALD, R. J. AND R. J. LEICESTER The low stapled anastomosis. Dis Colon Rectum, Sep 1981, 24(6), 437-444.

HERMANI, A., J. HESS, B. DE SERVI, S. MEDUNJANIN, et al. Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. Clin Cancer Res, Jul 15 2005, 11(14), 5146-5152.

HIGGINS, J. P., S. G. THOMPSON, J. J. DEEKS AND D. G. ALTMAN Measuring inconsistency in meta-analyses. Bmj, Sep 6 2003, 327(7414), 557-560.

HIRST, N. A., J. P. TIERNAN, P. A. MILLNER AND D. G. JAYNE Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. Colorectal Disease, Feb 2014, 16(2), 95-109.

HO, Y. H. AND M. A. ASHOUR Techniques for colorectal anastomosis. World J Gastroenterol, Apr 7 2010, 16(13), 1610-1621.

HOLL, S., I. FOURNEL, D. ORRY, O. FACY, et al. Should CT scan be performed when CRP is elevated after colorectal surgery? Results from the inflammatory markers after colorectal surgery study. Journal of Visceral Surgery, 2017/02/01/ 2017, 154(1), 5-9.

HOORN, E. J. AND R. ZIETSE Hyponatremia revisited: translating physiology to practice. Nephron Physiol, 2008, 108(3), p46-59.

HUISMAN, M. G., F. GHIGNONE, G. UGOLINI, G. SIDORENKOV, et al. Long-Term Survival and Risk of Institutionalization in Onco-Geriatric Surgical Patients: Long-Term Results of the PREOP Study. J Am Geriatr Soc, Jun 2020, 68(6), 1235-1241.

HUO, Y. R., K. PHAN, D. L. MORRIS AND W. LIAUW Systematic review and a meta-analysis of hospital and surgeon volume/outcome relationships in colorectal cancer surgery. J Gastrointest Oncol, Jun 2017, 8(3), 534-546.

HYMAN, N., T. L. MANCHESTER, T. OSLER, B. BURNS, et al. Anastomotic leaks after intestinal anastomosis: it's later than you think. Ann Surg, Feb 2007, 245(2), 254-258.

HYMAN, N. H. Managing anastomotic leaks from intestinal anastomoses. Surgeon, Feb 2009, 7(1), 31-35.

HYMAN, N. H. Carcinoma of colon. In *Corman's Colon and Rectal Surgery*. Lippincott Williams & Wilkins (LWW), 2012, vol. 1, p. 775.

HYMAN, N. H., T. OSLER, P. CATALDO, E. H. BURNS, et al. Anastomotic leaks after bowel resection: what does peer review teach us about the relationship to post-operative mortality? Journal of the American College of Surgeons, Jan 2009, 208(1), 48-52.

IANCU, C., L. C. MOCAN, D. TODEA-IANCU, T. MOCAN, et al. Host-related predictive factors for anastomotic leakage following large bowel resections for colorectal cancer. J Gastrointestin Liver Dis, Sep 2008, 17(3), 299-303.

INÊS CAMPOS GIL, N. R., DIANA PARENTE, INÊS SALES, PAULO ALVES, PAULO CLARA, AND M. C. SANDRA AMADO, VÍTOR FARIA Intracorporeal versus Extracorporeal Anastomosis in Laparoscopic Right Colectomy: Short-Term Outcomes. Surgery, Gastroenterology and Oncology, 2018, 23(1), 33-39.

ITALIAN COLORECTAL ANASTOMOTIC LEAKAGE STUDY, G. Anastomotic leakage after elective colorectal surgery: a prospective multiCenter observational study on use of the Dutch leakage score, serum procalcitonin and serum C-reactive protein for diagnosis. BJS open, 2020, 4(3), 499-507.

JAYNE, D. G., H. C. THORPE, J. COPELAND, P. QUIRKE, et al. Five-year follow-up of the Medical Research Council CLASICC trial of laparoscopically assisted versus open surgery for colorectal cancer. Br J Surg, Nov 2010, 97(11), 1638-1645.

JESTIN, P., L. PÅHLMAN AND U. GUNNARSSON Risk factors for anastomotic leakage after rectal cancer surgery: a case-control study. Colorectal Disease, Sep 2008, 10(7), 715-721.

JIN, D. AND L. CHEN Early prediction of anastomotic leakage after laparoscopic rectal surgery using creactive protein. Medicine (Baltimore), Jun 4 2021, 100(22), e26196.

JOHNE, B., M. K. FAGERHOL, T. LYBERG, H. PRYDZ, et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. Mol Pathol, Jun 1997, 50(3), 113-123.

JOHNSTON, M. J., S. ARORA, D. KING, G. BOURAS, et al. A systematic review to identify the factors that affect failure to rescue and escalation of care in surgery. Surgery, Apr 2015, 157(4), 752-763.

JONSSON, K., J. A. JENSEN, W. H. GOODSON, 3RD, H. SCHEUENSTUHL, et al. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. Annals of Surgery, 1991, 214(5), 605-613.

JUNG, S. H., C. S. YU, P. W. CHOI, D. D. KIM, et al. Risk factors and oncologic impact of anastomotic leakage after rectal cancer surgery. Dis Colon Rectum, Jun 2008, 51(6), 902-908.

KAMBAKAMBA, P., D. DINDO, A. NOCITO, P. A. CLAVIEN, et al. Intra-operative adverse events during laparoscopic colorectal resection—better laparoscopic treatment but unchanged incidence. Lessons learnt from a Swiss multi-institutional analysis of 3,928 patients. Langenbeck's Archives of Surgery, 2014/03/01 2014, 399(3), 297-305.

KANG, C. Y., W. J. HALABI, O. O. CHAUDHRY, V. NGUYEN, et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer. JAMA Surg, Jan 2013, 148(1), 65-71.

KARAKONSTANTIS, S., N. GRYLLOU, G. PAPAZOGLOU AND C. LYDAKIS Eosinophil count (EC) as a diagnostic and prognostic marker for infection in the internal medicine department setting. Rom J Intern Med, Jun 1 2019, 57(2), 166-174.

KARANJIA, N. D., A. P. CORDER, P. BEARN AND R. J. HEALD Leakage from stapled low anastomosis after total mesorectal excision for carcinoma of the rectum. Br J Surg, Aug 1994, 81(8), 1224-1226.

KARLICZEK, A., D. A. BENARON, P. C. BAAS, C. J. ZEEBREGTS, et al. Intra-operative assessment of microperfusion with visible light spectroscopy for prediction of anastomotic leakage in colorectal anastomoses. Colorectal Disease, Oct 2010, 12(10), 1018-1025.

KÄSER, S. A., R. FURLER, D. C. EVEQUOZ AND C. A. MAURER Hyponatremia is a specific marker of perforation in sigmoid diverticulitis or appendicitis in patients older than 50 years. Gastroenterol Res Pract, 2013, 2013, 462891.

KÄSER, S. A., U. NITSCHE, M. MAAK, C. W. MICHALSKI, et al. Could hyponatremia be a marker of anastomotic leakage after colorectal surgery? A single center analysis of 1,106 patients over 5 years. Langenbecks Arch Surg, Aug 2014, 399(6), 783-788.

KELLY, M., A. BHANGU, P. SINGH, J. E. F. FITZGERALD, et al. Systematic review and meta-analysis of trainee- versus expert surgeon-performed colorectal resection. British Journal of Surgery, 2014, 101(7), 750-759.

KHAN, A. A., J. M. WHEELER, C. CUNNINGHAM, B. GEORGE, et al. The management and outcome of anastomotic leaks in colorectal surgery. Colorectal Disease, Jul 2008, 10(6), 587-592.

KIRSHTEIN, B., A. ROY-SHAPIRA, S. DOMCHIK, S. MIZRAHI, et al. Early relaparoscopy for management of suspected post-operative complications. J Gastrointest Surg, Jul 2008, 12(7), 1257-1262.

KOMEN, N., R. W. DE BRUIN, G. J. KLEINRENSINK, J. JEEKEL, et al. Anastomotic leakage, the search for a reliable biomarker. A review of the literature. Colorectal Disease, Feb 2008, 10(2), 109-115; discussion 115-107.

KONISHI, T., T. WATANABE, J. KISHIMOTO AND H. NAGAWA Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. Journal of the American College of Surgeons, Mar 2006, 202(3), 439-444.

KOPERNA, T. Cost-effectiveness of defunctioning stomas in low anterior resections for rectal cancer: a call for benchmarking. Arch Surg, Dec 2003, 138(12), 1334-1338; discussion 1339.

KØRNER, H., H. J. NIELSEN, J. A. SØREIDE, B. S. NEDREBØ, et al. Diagnostic accuracy of C-reactive protein for intraabdominal infections after colorectal resections. J Gastrointest Surg, Sep 2009, 13(9), 1599-1606.

KORNMANN, V. N., N. TRESKES, L. H. HOONHOUT, T. L. BOLLEN, et al. Systematic review on the value of CT scanning in the diagnosis of anastomotic leakage after colorectal surgery. Int J Colorectal Dis, Apr 2013, 28(4), 437-445.

KRACHT, M., J. M. HAY, P. L. FAGNIEZ AND A. FINGERHUT Ileocolonic anastomosis after right hemicolectomy for carcinoma: stapled or hand-sewn? A prospective, multicenter, randomized trial. Int J Colorectal Dis, Mar 1993, 8(1), 29-33.

KRARUP, P. M., L. N. JORGENSEN, A. H. ANDREASEN AND H. HARLING A nationwide study on anastomotic leakage after colonic cancer surgery. Colorectal Disease, Oct 2012, 14(10), e661-667.

KRARUP, P. M., A. NORDHOLM-CARSTENSEN, L. N. JORGENSEN AND H. HARLING Association of Comorbidity with Anastomotic Leak, 30-day Mortality, and Length of Stay in Elective Surgery for Colonic Cancer: A Nationwide Cohort Study. Dis Colon Rectum, Jul 2015, 58(7), 668-676.

KRISTENSEN, H., A. THYØ, K. JØSSING EMMERTSEN, N. J. SMART, et al. Translation and international validation of the Colostomy Impact score. Colorectal Disease, Jul 2021, 23(7), 1866-1877.

KUHRY, E., W. F. SCHWENK, R. GAUPSET, U. ROMILD, et al. Long-term results of laparoscopic colorectal cancer resection. Cochrane Database Syst Rev, Apr 16 2008, 2008(2), Cd003432.

KVERNENG HULTBERG, D., J. SVENSSON, H. JUTESTEN, J. RUTEGÅRD, et al. The Impact of Anastomotic Leakage on Long-term Function After Anterior Resection for Rectal Cancer. Dis Colon Rectum, 2020, 63(5), 619-628.

LA REGINA, D., M. DI GIUSEPPE, M. LUCCHELLI, A. SAPORITO, et al. Financial Impact of Anastomotic Leakage in Colorectal Surgery. Journal of Gastrointestinal Surgery, 2019/03/01 2019, 23(3), 580-586.

LAGOUTTE, N., O. FACY, A. RAVOIRE, C. CHALUMEAU, et al. C-reactive protein and procalcitonin for the early detection of anastomotic leakage after elective colorectal surgery: pilot study in 100 patients. J Visc Surg, Oct 2012, 149(5), e345-349.

LAW, W. I., K. W. CHU, J. W. HO AND C. W. CHAN Risk factors for anastomotic leakage after low anterior resection with total mesorectal excision. Am J Surg, Feb 2000, 179(2), 92-96.

LEE, H. Procalcitonin as a biomarker of infectious diseases. Korean J Intern Med, May 2013, 28(3), 285-291.

LEE, S. W., D. GREGORY AND C. L. COOL Clinical and economic burden of colorectal and bariatric anastomotic leaks. Surg Endosc, Oct 2020, 34(10), 4374-4381.

LEVY, M. M., M. P. FINK, J. C. MARSHALL, E. ABRAHAM, et al. 2001 SCCM/ESICM/ ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med, Apr 2003, 29(4), 530-538.

LI, Q., X. DING, G. XIA, H. G. CHEN, et al. Eosinopenia and elevated C-reactive protein facilitate triage of COVID-19 patients in fever clinic: A retrospective case-control study. EClinicalMedicine, Jun 2020, 23, 100375.

LIM, S. B., C. S. YU, C. W. KIM, Y. S. YOON, et al. Late anastomotic leakage after low anterior resection in rectal cancer patients: clinical characteristics and predisposing factors. Colorectal Disease, Apr 2016, 18(4), O135-140.

LIMPER, M., M. D. DE KRUIF, A. J. DUITS, D. P. BRANDJES, et al. The diagnostic role of procalcitonin and other biomarkers in discriminating infectious from non-infectious fever. J Infect, Jun 2010, 60(6), 409-416.

LIN, X. AND H. LI Obesity: Epidemiology, Pathophysiology, and Therapeutics. Frontiers in Endocrinology, 2021-September-06 2021, 12(1070).

LIN, Y., J. RONG AND Z. ZHANG Silent existence of eosinopenia in sepsis: a systematic review and meta-analysis. BMC Infect Dis, May 24 2021, 21(1), 471.

LIPSKA, M. A., I. P. BISSETT, B. R. PARRY AND A. E. MERRIE Anastomotic leakage after lower gastrointestinal anastomosis: men are at a higher risk. ANZ J Surg, Jul 2006, 76(7), 579-585.

LIU, X. Classification accuracy and cut point selection. Statistics in Medicine, 2012, 31(23), 2676-2686.

LUO, J., H. WU, Y. JIANG, Y. YANG, et al. The Role of Heart Rate, Body Temperature, and Respiratory Rate in Predicting Anastomotic Leakage following Surgery for Rectal Cancer. Mediators Inflamm, 2021, 2021, 8698923.

MAMIDANNA, R., A. M. ALMOUDARIS AND O. FAIZ Is 30-day mortality an appropriate measure of risk in elderly patients undergoing elective colorectal resection? Colorectal Disease, Oct 2012, 14(10), 1175-1182.

MANDREKAR, J. N. Receiver Operating Characteristic Curve in Diagnostic Test Assessment. Journal of Thoracic Oncology, 2010/09/01/ 2010, 5(9), 1315-1316.

MANILICH, E., J. D. VOGEL, R. P. KIRAN, J. M. CHURCH, et al. Key Factors Associated With Post-operative Complications in Patients Undergoing Colorectal Surgery. Dis Colon Rectum, 2013, 56(1), 64-71.

MARIJNEN, C. A., E. KAPITEIJN, C. J. VAN DE VELDE, H. MARTIJN, et al. Acute side effects and complications after short-term pre-operative radiotherapy combined with total mesorectal excision in primary rectal cancer: report of a multicenter randomized trial. J Clin Oncol, Feb 1 2002, 20(3), 817-825.

MARINATOU, A., G. E. THEODOROPOULOS, S. KARANIKA, T. KARANTANOS, et al. Do anastomotic leaks impair post-operative health-related quality of life after rectal cancer surgery? A case-matched study. Dis Colon Rectum, Feb 2014, 57(2), 158-166.

MARRES, C. C. M., A. W. H. VAN DE VEN, L. G. J. LEIJSSEN, P. C. M. VERBEEK, et al.

Colorectal anastomotic leak: delay in reintervention after false-negative computed tomography scan is a reason for concern. Tech Coloproctol, Sep 2017, 21(9), 709-714.

MARTIN, G., A. DUPRÉ, A. MULLIEZ, F. PRUNEL, et al. Validation of a score for the early diagnosis of anastomotic leakage following elective colorectal surgery. J Visc Surg, Feb 2015, 152(1), 5-10.

MARUSCH, F., A. KOCH, U. SCHMIDT, S. GEIBETALER, et al. Value of a protective stoma in low anterior resections for rectal cancer. Dis Colon Rectum, Sep 2002, 45(9), 1164-1171.

MATTHIESSEN, P., M. HENRIKSSON, O. HALLBÖÖK, E. GRUNDITZ, et al. Increase of serum C-reactive protein is an early indicator of subsequent symptomatic anastomotic leakage after anterior resection. Colorectal Disease, Jan 2008, 10(1), 75-80.

MCDERMOTT, F. D., A. HEENEY, M. E. KELLY, R. J. STEELE, et al. Systematic review of pre-operative, intra-operative and post-operative risk factors for colorectal anastomotic leaks. Br J Surg, Apr 2015, 102(5), 462-479.

MCGINN, F. P., P. C. GARTELL, P. C. CLIFFORD AND F. J. BRUNTON Staples or sutures for low colorectal anastomoses: a prospective randomized trial. Br J Surg, Aug 1985, 72(8), 603-605.

MEALY, K., P. BURKE AND J. HYLAND Anterior resection without a defunctioning colostomy: questions of safety. Br J Surg, Apr 1992, 79(4), 305-307.

MEISNER, M. Update on procalcitonin measurements. Ann Lab Med, Jul 2014, 34(4), 263-273.

MERKEL, S., W. Y. WANG, O. SCHMIDT, O. DWORAK, et al. Locoregional recurrence in patients with anastomotic leakage after anterior resection for rectal carcinoma. Colorectal Disease, May 2001, 3(3), 154-160.

MESSIAS, B. A., R. V. BOTELHO, S. S. SAAD, E. R. MOCCHETTI, et al. Serum C-reactive protein is a useful marker to exclude anastomotic leakage after colorectal surgery. Sci Rep, Feb 3 2020, 10(1), 1687.

MIDURA, E. F., D. HANSEMAN, B. R. DAVIS, S. J. ATKINSON, et al. Risk Factors and Consequences of Anastomotic Leak After Colectomy: A National Analysis. Dis Colon Rectum, 2015, 58(3), 333-338.

MIK, M., L. DZIKI, M. BERUT, R. TRZCINSKI, et al. Neutrophil to Lymphocyte Ratio

and C-Reactive Protein as Two Predictive Tools of Anastomotic Leak in Colorectal Cancer Open Surgery. Dig Surg, 2018, 35(1), 77-84.

MIRNEZAMI, A., R. MIRNEZAMI, K. CHANDRAKUMARAN, K. SASAPU, et al. Increased Local Recurrence and Reduced Survival From Colorectal Cancer Following Anastomotic Leak: Systematic Review and Meta-Analysis. Annals of Surgery, 2011, 253(5), 890-899.

MITRE, E. Eosinophilia: a diagnostic clue for nonbacterial diseases in patients with systemic inflammatory response syndrome. Crit Care Med, Oct 2013, 41(10), 2464-2465.

MODASI, A., D. PACE, M. GODWIN, C. SMITH, et al. NSAID administration post colorectal surgery increases anastomotic leak rate: systematic review/meta-analysis. Surg Endosc, Mar 2019, 33(3), 879-885.

MORKS, A. N., R. J. PLOEG, H. SIJBRAND HOFKER, T. WIGGERS, et al. Late anastomotic leakage in colorectal surgery: a significant problem. Colorectal Disease, 2013, 15(5), e271-e275.

MÜLLER, F., S. S. FRØLAND, P. AUKRUST AND M. K. FAGERHOL Elevated serum calprotectin levels in HIV-infected patients: the calprotectin response during ZDV treatment is associated with clinical events. J Acquir Immune Defic Syndr (1988), Sep 1994, 7(9), 931-939.

NEHRING, S. M., A. GOYAL, P. BANSAL AND B. C. PATEL. C Reactive Protein. In StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2021, StatPearls Publishing LLC., 2021.

NESBAKKEN, A., K. NYGAARD, O. C. LUNDE, J. BLÜCHER, et al. Anastomotic leak following mesorectal excision for rectal cancer: true incidence and diagnostic challenges. Colorectal Dis, Nov 2005, 7(6), 576-581.

NICHOLLS, J. Clinical method today. Colorectal Disease, Feb 2014, 16(2), 81.

NICKSA, G. A., R. V. DRING, K. H. JOHNSON, W. V. SARDELLA, et al. Anastomotic leaks: what is the best diagnostic imaging study? Dis Colon Rectum, Feb 2007, 50(2), 197-203.

NIKOLIAN, V. C., N. S. KAMDAR, S. E. REGENBOGEN, A. M. MORRIS, et al. Anastomotic leak after colorectal resection: A population-based study of risk factors and hospital variation. Surgery, Jun 2017, 161(6), 1619-1627.

NUNO RAMA, M. L., CÂNDIDA G. SILVA, PATRÍCIA MOTTA LIMA, INÊS CAMPOS GIL, MARIA GUARINO, PEDRO OLIVEIRA, MARIA DIXE, ANABELA ROCHA, FERNANDO CASTRO-POÇAS, JOÃO PIMENTEL The Usefulness of Inflammatory Biomarkers to Predict Anastomotic Leakage after Colorectal Surgery: Systematic Review and Meta-Analysis. Surgery, Gastroenterology and Oncology, September,2022 2022, 27, 169-181.

O'RIORDAN, J. M., J. O. LARKIN, B. J. MEHIGAN AND P. H. MCCORMICK Re-laparoscopy in the diagnosis and treatment of post-operative complications following laparoscopic colorectal surgery. Surgeon, Aug 2013, 11(4), 183-186.

ODINK, K., N. CERLETTI, J. BRÜGGEN, R. G. CLERC, et al. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature, Nov 5-11 1987, 330(6143), 80-82.

ORTEGA-DEBALLON, P., F. RADAIS, O. FACY, P. D'ATHIS, et al. C-reactive protein is an early predictor of septic complications after elective colorectal surgery. World J Surg, Apr 2010, 34(4), 808-814.

PALIOGIANNIS, P., S. DEIDDA, S. MASLYANKOV, T. PAYCHEVA, et al. C reactive protein to albumin ratio (CAR) as predictor of anastomotic leakage in colorectal surgery. Surg Oncol, Sep 2021, 38, 101621.

PANTEL, H. J., L. J. JASAK, R. RICCIARDI, P. W. MARCELLO, et al. Should They Stay or Should They Go? The Utility of C-Reactive Protein in Predicting Readmission and Anastomotic Leak After Colorectal Resection. Dis Colon Rectum, Feb 2019, 62(2), 241-247.

PANTOJA PACHAJOA, D. A., M. GIELIS, R. M. PALACIOS HUATUCO, M. N. BENITEZ, et al. Neutrophil-to-lymphocyte ratio vs C-reactive protein as early predictors of anastomotic leakage after colorectal surgery: A retrospective cohort study. Annals of Medicine and Surgery, 2021/04/01/ 2021, 64, 102201.

PASTERNAK, B., P. MATTHIESSEN, K. JANSSON, M. ANDERSSON, et al. Elevated intraperitoneal matrix metalloproteinases-8 and -9 in patients who develop anastomotic leakage after rectal cancer surgery: a pilot study. Colorectal Disease, Jul 2010, 12(7 Online), e93-98.

PEDERSEN, T., O. ROIKJÆR AND P. JESS Increased levels of C-reactive protein and leukocyte count are poor predictors of anastomotic leakage following laparoscopic colorectal resection. Dan Med J, Dec 2012, 59(12), A4552.

PETERS, E. G., M. DEKKERS, F. W. VAN LEEUWEN-HILBERS, F. DAAMS, et al. Relation between post-operative ileus and anastomotic leakage after colorectal resection: a post hoc analysis of a prospective randomized controlled trial. Colorectal Disease, Jul 2017, 19(7), 667-674.

PETERSEN, S., M. FREITAG, G. HELLMICH AND K. LUDWIG Anastomotic leakage: impact on local recurrence and survival in surgery of colorectal cancer. Int J Colorectal Dis, 1998, 13(4), 160-163.

PFÄFFLIN, A. AND E. SCHLEICHER Inflammation markers in point-of-care testing (POCT). Anal Bioanal Chem, Mar 2009, 393(5), 1473-1480.

PHAN, K., L. OH, G. CTERCTEKO, N. PATHMA-NATHAN, et al. Does a stoma reduce the risk of anastomotic leak and need for re-operation following low anterior resection for rectal cancer: systematic review and meta-analysis of randomized controlled trials. J Gastrointest Oncol, Apr 2019, 10(2), 179-187.

PHITAYAKORN, R., C. P. DELANEY, H. L. REYNOLDS, B. J. CHAMPAGNE, et al. Standardized algorithms for management of anastomotic leaks and related abdominal and pelvic abscesses after colorectal surgery. World J Surg, Jun 2008, 32(6), 1147-1156.

PLATELL, C., N. BARWOOD, G. DORFMANN AND G. MAKIN The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Disease, Jan 2007, 9(1), 71-79.

POMMERGAARD, H. C., B. GESSLER, J. BURCHARTH, E. ANGENETE, et al. Pre-operative risk factors for anastomotic leakage after resection for colorectal cancer: a systematic review and meta-analysis. Colorectal Dis, Sep 2014, 16(9), 662-671.

PORTO EDITORA. anastomose no Dicionário infopédia de Termos Médicos. In. Dicionário infopédia: Porto Editora, 2021, vol. 2021.

POWER, N., M. ATRI, S. RYAN, R. HADDAD, et al. CT assessment of anastomotic bowel leak. Clin Radiol, Jan 2007, 62(1), 37-42.

QU, H., Y. LIU AND D. S. BI Clinical risk factors for anastomotic leakage after laparoscopic anterior resection for rectal cancer: a systematic review and meta-analysis. Surg Endosc, Dec 2015, 29(12), 3608-3617.

RAHBARI, N. N., J. WEITZ, W. HOHENBERGER, R. J. HEALD, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the

International Study Group of Rectal Cancer. Surgery, Mar 2010, 147(3), 339-351.

RAMA, N., D. PARENTE, S. C. G., M. NEVES, et al. Anastomotic Leak in Colorectal Cancer Surgery: From Diagnosis to Management or Failure - A Retrospective Cohort Study. Surgery, Gastroenterology and Oncology, 2021, 26(3), 183-190.

RAMA, N. J., P. L. FERREIRA, J. PIMENTEL AND T. JUUL Validation of Portuguese version of the low anterior resection syndrome score. Journal of Coloproctology, 2019/01/01/ 2019, 39(1), 1-8.

RAMA, N. J., M. C. LAGES, M. P. S. GUARINO, Ó. LOURENÇO, et al. Usefulness of serum C-reactive protein and calprotectin for the early detection of colorectal anastomotic leakage: A prospective observational study. World J Gastroenterol, 2022, 28(24), 2758- 2774.

REGENBOGEN, S. E., A. J. MULLARD, N. PETERS, S. BROOKS, et al. Hospital Analgesia Practices and Patient-reported Pain After Colorectal Resection. Ann Surg, Dec 2016, 264(6), 1044-1050.

REISINGER, K. W., M. POEZE, K. W. HULSEWÉ, B. A. VAN ACKER, et al. Accurate prediction of anastomotic leakage after colorectal surgery using plasma markers for intestinal damage and inflammation. Journal of the American College of Surgeons, Oct 2014, 219(4), 744-751.

RIBEIRO JR, U., D. O. TAYAR, R. A. RIBEIRO, P. ANDRADE, et al. The Clinical and Economic Burden of Colorectal Anastomotic Leaks: Middle-Income Country Perspective. Gastroenterology Research and Practice, 2019/04/01 2019, 2019, 2879049.

ROJAS-MACHADO, S. A., M. ROMERO, A. ARROYO, A. ROJAS-MACHADO, et al. Anastomic leak in colorectal cancer surgery. Development of a diagnostic index (DIACOLE). Int J Surg, Mar 2016, 27, 92-98.

ROTHOLTZ, N. A., C. A. ANGERAMO, M. LAPORTE, M. MATZNER PERFUMO, et al. “Early” Re-operation to Treat Complications Following Laparoscopic Colorectal Surgery: The Sooner the Better. Surg Laparosc Endosc Percutan Tech, Aug 18 2021, 31(6), 756-759.

RULLIER, E., C. LAURENT, J. L. GARRELON, P. MICHEL, et al. Risk factors for anastomotic leakage after resection of rectal cancer. Br J Surg, Mar 1998, 85(3), 355-358.

SAMMOUR, T., A. KAHOKEHR, K. ZARGAR-SHOSHTARI AND A. G. HILL A prospective case-control study of the local and systemic cytokine response after laparoscopic

versus open colonic surgery. J Surg Res, Apr 2012, 173(2), 278-285.

SÁNCHEZ-GUILLÉN, L., M. FRASSON, Á. GARCÍA-GRANERO, G. PELLINO, et al. Risk factors for leak, complications and mortality after ileocolic anastomosis: comparison of two anastomotic techniques. Ann R Coll Surg Engl, Nov 2019, 101(8), 571-578.

SANDER, J., M. K. FAGERHOL, J. S. BAKKEN AND I. DALE Plasma levels of the leucocyte L1 protein in febrile conditions: relation to aetiology, number of leucocytes in blood, blood sedimentation reaction and C-reactive protein. Scand J Clin Lab Invest, Jun 1984, 44(4), 357-362.

SANTOS, J. C., JR., J. BATISTA, M. T. SIRIMARCO, A. S. GUIMARÃES, et al. Prospective randomized trial of mechanical bowel preparation in patients undergoing elective colorectal surgery. Br J Surg, Nov 1994, 81(11), 1673-1676.

SCEPANOVIC, M. S., B. KOVACEVIC, V. CIJAN, A. ANTIC, et al. C-reactive protein as an early predictor for anastomotic leakage in elective abdominal surgery. Tech Coloproctol, Oct 2013, 17(5), 541-547.

SCHEIDBACH, H., F. BENEDIX, O. HÜGEL, D. KOSE, et al. Laparoscopic approach to colorectal procedures in the obese patient: risk factor or benefit? Obes Surg, Jan 2008, 18(1), 66-70.

SCHROCK, T. R., C. W. DEVENEY AND J. E. DUNPHY Factor contributing to leakage of colonic anastomoses. Ann Surg, May 1973, 177(5), 513-518.

SCHUETZ, P., A. BEISHUIZEN, M. BROYLES, R. FERRER, et al. Procalcitonin (PCT)-guided antibiotic stewardship: an international experts consensus on optimized clinical use. Clin Chem Lab Med, Aug 27 2019, 57(9), 1308-1318.

SENAGORE, A. J., C. P. DELANEY, K. MADBOULAY, K. M. BRADY, et al. Laparoscopic colectomy in obese and nonobese patients. J Gastrointest Surg, May-Jun 2003, 7(4), 558-561.

SHAABAN, H., S. DANIEL, R. SISON, J. SLIM, et al. Eosinopenia: Is it a good marker of sepsis in comparison to procalcitonin and C-reactive protein levels for patients admitted to a critical care unit in an urban hospital? J Crit Care, Dec 2010, 25(4), 570-575.

SHIMURA, T., Y. TOIYAMA, J. HIRO, H. IMAOKA, et al. Monitoring perioperative serum albumin can identify anastomotic leakage in colorectal cancer patients with curative

intent. Asian J Surg, Jan 2018, 41(1), 30-38.

SHIN, U. S., C. W. KIM, C. S. YU AND J. C. KIM Delayed anastomotic leakage following sphincter-preserving surgery for rectal cancer. Int J Colorectal Dis, Jul 2010, 25(7), 843-849.

SILBER, J. H., S. V. WILLIAMS, H. KRAKAUER AND J. S. SCHWARTZ Hospital and patient characteristics associated with death after surgery. A study of adverse occurrence and failure to rescue. Med Care, Jul 1992, 30(7), 615-629.

SILVESTRE, J., J. REBANDA, C. LOURENÇO AND P. PÓVOA Diagnostic accuracy of C-reactive protein and procalcitonin in the early detection of infection after elective colorectal surgery - a pilot study. BMC Infect Dis, Aug 16 2014, 14, 444.

SIMILLIS, C., N. LAL, S. N. THOUKIDIDOU, C. KONTOVOUNISIOS, et al. Open Versus Laparoscopic Versus Robotic Versus Transanal Mesorectal Excision for Rectal Cancer: A Systematic Review and Network Meta-analysis. Ann Surg, Jul 2019, 270(1), 59-68.

SINGER, M., C. S. DEUTSCHMAN, C. W. SEYMOUR, M. SHANKAR-HARI, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama, Feb 23 2016, 315(8), 801-810.

SINGH, P. P., I. S. ZENG, S. SRINIVASA, D. P. LEMANU, et al. Systematic review and meta-analysis of use of serum C-reactive protein levels to predict anastomotic leak after colorectal surgery. Br J Surg, Mar 2014, 101(4), 339-346.

SMITH, S. A., D. J. ROBERTS, M. E. LIPSON, W. D. BUIE, et al. Post-operative Nonsteroidal Anti-inflammatory Drug Use and Intestinal Anastomotic Dehiscence: A Systematic Review and Meta-Analysis. Dis Colon Rectum, Nov 2016, 59(11), 1087-1097.

SMITH, S. R., P. POCKNEY, R. HOLMES, F. DOIG, et al. Biomarkers and anastomotic leakage in colorectal surgery: C-reactive protein trajectory is the gold standard. ANZ J Surg, May 2018, 88(5), 440-444.

SPENCE, R. T., D. H. HIRPARA, S. DOSHI, F. A. QUERESHY, et al. Anastomotic leak after colorectal surgery: does timing affect failure to rescue? Surg Endosc, Jan 27 2021.

SPINELLI, A., G. ANANIA, A. AREZZO, S. BERTI, et al. Italian multi-society modified Delphi consensus on the definition and management of anastomotic leakage in colorectal surgery. Updates in Surgery, 2020/09/01 2020, 72(3), 781-792.

SPROSTON, N. R. AND J. J. ASHWORTH Role of C-Reactive Protein at Sites of Inflammation

and Infection. Frontiers in Immunology, 2018-April-13 2018, 9(754).

STEARNS, A. T., F. LICCARDO, K.-N. TAN, E. SIVRIKOZ, et al. Physiological changes after colorectal surgery suggest that anastomotic leakage is an early event: a retrospective cohort study. Colorectal Disease, 2019, 21(3), 297-306.

STEPHENSEN, B. D., F. REID, S. SHAIKH, R. CARROLL, et al. C-reactive protein trajectory to predict colorectal anastomotic leak: PREDICT Study. Br J Surg, Dec 2020, 107(13), 1832-1837.

STRAATMAN, J., A. M. HARMSEN, M. A. CUESTA, J. BERKHOF, et al. Predictive Value of C-Reactive Protein for Major Complications after Major Abdominal Surgery: A Systematic Review and Pooled-Analysis. PLoS One, 2015, 10(7), e0132995.

STRÍZ, I. AND I. TREBICHAVSKÝ Calprotectin - a pleiotropic molecule in acute and chronic inflammation. Physiol Res, 2004, 53(3), 245-253.

SU'A, B., S. TUTONE, W. MACFATER, A. BARAZANCHI, et al. Diagnostic accuracy of procalcitonin for the early diagnosis of anastomotic leakage after colorectal surgery: a meta-analysis. ANZ Journal of Surgery, 2020, 90(5), 675-680.

SU'A, B. U., H. L. MIKAERE, J. L. RAHIRI, I. B. BISSETT, et al. Systematic review of the role of biomarkers in diagnosing anastomotic leakage following colorectal surgery. Br J Surg, Apr 2017, 104(5), 503-512.

SUDING, P., E. JENSEN, M. A. ABRAMSON, K. ITANI, et al. Definitive Risk Factors for Anastomotic Leaks in Elective Open Colorectal Resection. Archives of Surgery, 2008, 143(9), 907-912.

SUTTON, C. D., L. J. MARSHALL, N. WILLIAMS, D. P. BERRY, et al. Colo-rectal anastomotic leakage often masquerades as a cardiac complication. Colorectal Disease, Jan 2004, 6(1), 21-22.

SWART, R. M., E. J. HOORN, M. G. BETJES AND R. ZIETSE Hyponatremia and inflammation: the emerging role of interleukin-6 in osmoregulation. Nephron Physiol, 2011, 118(2), 45-51.

TADROS, T., T. WOBBES AND T. HENDRIKS Blood transfusion impairs the healing of experimental intestinal anastomoses. Ann Surg, Mar 1992, 215(3), 276-281.

TAMINI, N., D. BERNASCONI, L. RIPAMONTI, G. LO BIANCO, et al. Clinical Validation of the Comprehensive Complication Index in Colon Cancer Surgery. Cancers (Basel),

Apr 6 2021, 13(7).

TANG, C. L. AND F. SEOW-CHOEN Digital rectal examination compares favourably with conventional water-soluble contrast enema in the assessment of anastomotic healing after low rectal excision: a cohort study. Int J Colorectal Dis, May 2005, 20(3), 262-266.

TANG, C. L., K. Y. YEONG, D. C. NYAM, K. W. EU, et al. Post-operative intra-abdominal free gas after open colorectal resection. Dis Colon Rectum, Aug 2000, 43(8), 1116-1120.

TANG, R., H. H. CHEN, Y. L. WANG, C. R. CHANGCHIEN, et al. Risk factors for surgical site infection after elective resection of the colon and rectum: a single-center prospective study of 2,809 consecutive patients. Ann Surg, Aug 2001, 234(2), 181-189.

TENNANT, F. The physiologic effects of pain on the endocrine system. Pain Ther, Dec 2013, 2(2), 75-86.

TERRADAS, R., S. GRAU, J. BLANCH, M. RIU, et al. Eosinophil count and neutrophil-lymphocyte count ratio as prognostic markers in patients with bacteremia: a retrospective cohort study. PLoS One, 2012, 7(8), e42860.

TRENCHEVA, K., K. P. MORRISSEY, M. WELLS, C. A. MANCUSO, et al. Identifying important predictors for anastomotic leak after colon and rectal resection: prospective study on 616 patients. Ann Surg, Jan 2013, 257(1), 108-113.

VALLANCE, A., S. WEXNER, M. BERHO, R. CAHILL, et al. A collaborative review of the current concepts and challenges of anastomotic leaks in colorectal surgery. Colorectal Disease, Jan 2017, 19(1), O1-o12.

VAN BOEKEL, R. L. M., M. C. WARLÉ, R. G. C. NIELEN, K. C. P. VISSERS, et al. Relationship Between Post-operative Pain and Overall 30-Day Complications in a Broad Surgical Population: An Observational Study. Ann Surg, May 2019, 269(5), 856-865.

VAN HELSDINGEN, C. P., A. C. JONGEN, W. J. DE JONGE, N. D. BOUVY, et al. Consensus on the definition of colorectal anastomotic leakage: A modified Delphi study. World J Gastroenterol, Jun 21 2020, 26(23), 3293-3303.

VAN ROOIJEN, S. J., A. C. JONGEN, Z. Q. WU, J. F. JI, et al. Definition of colorectal anastomotic leakage: A consensus survey among Dutch and Chinese colorectal surgeons. World J Gastroenterol, Sep 7 2017, 23(33), 6172-6180.

VASILIU, E. C., N. O. ZARNESCU, R. COSTEA AND S. NEAGU Review of Risk Factors for Anastomotic Leakage in Colorectal Surgery. Chirurgia (Bucur), Jul-Aug 2015, 110(4), 319-326.

VENNIX, S., R. ABEGG, O. J. BAKKER, P. B. VAN DEN BOEZEM, et al. Surgical re-interventions following colorectal surgery: open versus laparoscopic management of anastomotic leakage. J Laparoendosc Adv Surg Tech A, Sep 2013, 23(9), 739-744.

VENNIX, S., L. PELZERS, N. BOUVY, G. L. BEETS, et al. Laparoscopic versus open total mesorectal excision for rectal cancer. Cochrane Database Syst Rev, Apr 15 2014, (4), Cd005200.

VIGNALI, A., V. W. FAZIO, I. C. LAVERY, J. W. MILSOM, et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. Journal of the American College of Surgeons, Aug 1997, 185(2), 105-113.

VIJAYAN, A. L., VANIMAYA, S. RAVINDRAN, R. SAIKANT, et al. Procalcitonin: a promising diagnostic marker for sepsis and antibiotic therapy. J Intensive Care, 2017, 5, 51.

VOLK, A., S. KERSTING, H. C. HELD AND H. D. SAEGER Risk factors for morbidity and mortality after single-layer continuous suture for ileocolonic anastomosis. Int J Colorectal Disease, Mar 2011, 26(3), 321-327.

WANG, S., J. LIU, S. WANG, H. ZHAO, et al. Adverse Effects of Anastomotic Leakage on Local Recurrence and Survival After Curative Anterior Resection for Rectal Cancer: A Systematic Review and Meta-analysis. World J Surg, Jan 2017, 41(1), 277-284.

WARSCHKOW, R., U. BEUTNER, T. STEFFEN, S. A. MÜLLER, et al. Safe and early discharge after colorectal surgery due to C-reactive protein: a diagnostic meta-analysis of 1832 patients. Ann Surg, Aug 2012, 256(2), 245-250.

WARSCHKOW, R., T. STEFFEN, J. THIERBACH, T. BRUCKNER, et al. Risk factors for anastomotic leakage after rectal cancer resection and reconstruction with colorectostomy. A retrospective study with bootstrap analysis. Ann Surg Oncol, Oct 2011a, 18(10), 2772-2782.

WARSCHKOW, R., I. TARANTINO, M. TORZEWSKI, F. NÄF, et al. Diagnostic accuracy of C-reactive protein and white blood cell counts in the early detection of inflammatory complications after open resection of colorectal cancer: a retrospective study of 1,187 patients. Int J Colorectal Dis, Nov 2011b, 26(11), 1405-1413.

WATANABE, J., K. TATSUMI, M. OTA, Y. SUWA, et al. The impact of visceral obesity on surgical outcomes of laparoscopic surgery for colon cancer. Int J Colorectal Dis, Mar 2014, 29(3), 343-351.

WATERLAND, P., J. NG, A. JONES, G. BROADLEY, et al. Using CRP to predict anastomotic leakage after open and laparoscopic colorectal surgery: is there a difference? Int J Colorectal Dis, Apr 2016, 31(4), 861-868.

WATSON, A. J., Z. H. KRUKOWSKI AND A. MUNRO Salvage of large bowel anastomotic leaks. Br J Surg, Apr 1999, 86(4), 499-500.

WELSCH, T., S. A. MÜLLER, A. ULRICH, A. KISCHLAT, et al. C-reactive protein as early predictor for infectious post-operative complications in rectal surgery. Int J Colorectal Dis, 2007/12/01 2007, 22(12), 1499-1507.

WHITING, P. F., A. W. RUTJES, M. E. WESTWOOD, S. MALLETT, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med, Oct 18 2011, 155(8), 529-536.

WILKINSON, M. M., A. BUSUTTIL, C. HAYWARD, D. J. BROCK, et al. Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues. J Cell Sci, Oct 1988, 91 (Pt 2), 221-230.

WILLIAMS, C. E., C. A. MAKIN, R. G. REEVE AND S. B. ELLENBOGEN Over-utilisation of radiography in the assessment of stapled colonic anastomoses. Eur J Radiol, Jan-Feb 1991, 12(1), 35-37.

WONG, N. Y. AND K. W. EU A defunctioning ileostomy does not prevent clinical anastomotic leak after a low anterior resection: a prospective, comparative study. Dis Colon Rectum, Nov 2005, 48(11), 2076-2079.

XU, G., Y. YANG, Y. DU, F. PENG, et al. Clinical Pathway for Early Diagnosis of COVID-19: Updates from Experience to Evidence-Based Practice. Clin Rev Allergy Immunol, Aug 2020, 59(1), 89-100.

YANG, C., S. D. WEXNER, B. SAFAR, S. JOBANPUTRA, et al. Conversion in laparoscopic surgery: does intra-operative complication influence outcome? Surg Endosc, Nov 2009, 23(11), 2454-2458.

YANG, J., Q. CHEN, L. JINDOU AND Y. CHENG The influence of anastomotic leakage for rectal cancer oncologic outcome: A systematic review and meta-analysis. J Surg

Oncol, Jun 2020, 121(8), 1283-1297.

YANG, T., M. WEI, Y. HE, X. DENG, et al. Impact of visceral obesity on outcomes of laparoscopic colorectal surgery: a meta-analysis. ANZ J Surg, Jul-Aug 2015, 85(7-8), 507-513.

YE, S. P., W. Q. ZHU, Z. X. HUANG, D. N. LIU, et al. Role of minimally invasive techniques in gastrointestinal surgery: Current status and future perspectives. World J Gastrointest Surg, Sep 27 2021, 13(9), 941-952.

YEUNG, D. E., E. PETERKNECHT, S. HAJIBANDEH, S. HAJIBANDEH, et al. C-reactive protein can predict anastomotic leak in colorectal surgery: a systematic review and meta-analysis. Int J Colorectal Dis, Jun 2021, 36(6), 1147-1162.

ZHANG, G., R. LIAN, L. SUN, H. LIU, et al. Redefined hyponatremia as a marker to exclude the diagnosis of anastomotic leakage after colorectal cancer surgery. J Int Med Res, Aug 2020, 48(8), 300060520950565.

ZHOU, Y., L. WU, X. LI, X. WU, et al. Outcome of laparoscopic colorectal surgery in obese and nonobese patients: a meta-analysis. Surg Endosc, Mar 2012, 26(3), 783-789.

ZORAN KOSTIĆ*†, M. P., BOŠKO MILEV*, ZORAN MIJUŠKOVIĆ†‡, DAMJAN AND M. I. SLAVKOVIĆ* Diagnostic value of serial measurement of C-reactive protein in serum and matrix metalloproteinase-9 in drainage fluid in the detection of infectious complications and anastomotic leakage in patients with colorectal resection. VOJNOSANITETSKI PREGLED, 2015, 72(10), 889-898.

ADDENDUM

APPENDIX 1

- **Nuno Rama,** Marlene Lages, Maria Pedro Guarino, Óscar Lourenço, Patrícia Motta Lima, Diana Parente, Cândida G. Silva, Ricardo Castro, Ana Bento, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

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ORIGINAL ARTICLE

Observational Study

Usefulness of serum C-reactive protein and calprotectin for the early detection of colorectal anastomotic leakage: A prospective observational study

Nuno J G Rama, Marlene C C Lages, Maria Pedro S Guarino, Óscar Lourenço, Patrícia C Motta Lima, Diana Parente, Cândida S G Silva, Ricardo Castro, Ana Bento, Anabela Rocha, Fernando Castro-Pocas, João Pimentel

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Nuno J G Rama, Patrícia C Motta Lima, Diana Parente, Colorectal Surgical Division, Leiria Hospital Centre, Leiria 2410-021, Portugal

Nuno J G Rama, Anabela Rocha, Fernando Castro-Pocas, Abel Salazar Biomedical Institute (ICBAS), University of Oporto, Oporto 4099-002, Portugal

Nuno J G Rama, Marlene C C Lages, Maria Pedro S Guarino, Cândida S G Silva, Center for Innovative Care and Health Technology (ciTechCare), Polytechnic of Leiria, Leiria 2410-541, Portugal

Óscar Lourenço, Faculty of Economics, CeBER, University of Coimbra, Coimbra 3000-137, Portugal

Ricardo Castro, Ana Bento, Clinical Pathology Division, Leiria Hospital Centre, Leiria 2410-541, Portugal

Anabela Rocha, Surgical Division, Oporto Hospital Centre, Oporto 4099-001, Portugal

Fernando Castro-Pocas, Department of Gastroenterology, Santo António Hospital, Porto Hospital Center, Porto 4099-001, Portugal

Fernando Castro-Pocas, Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto 4099-001, Portugal

João Pimentel, Faculty of Medicine, University of Coimbra, Coimbra 3004-531, Portugal

João Pimentel, Surgical Division, Montes Claros Hospital, Coimbra 3030-320, Portugal

Corresponding author: Nuno JG Rama, FEBS, MD, MHSc, Associate Professor, Research Assistant Professor, Surgeon, Colorectal Surgical Division, Leiria Hospital Centre, Rua das Olhalvas, Leiria 2410-021, Portugal. ramanuno@gmail.com

Abstract

BACKGROUND

Colorectal anastomotic leakage (CAL) is one of the most dreaded complications

after colorectal surgery, with an incidence that can be as high as 27%. This event is associated with increased morbidity and mortality; therefore, its early diagnosis is crucial to reduce clinical consequences and costs. Some biomarkers have been suggested as laboratory tools for the diagnosis of CAL.

AIM

To assess the usefulness of plasma C-reactive protein (CRP) and calprotectin (CLP) as early predictors of CAL.

METHODS

A prospective monocentric observational study was conducted including patients who underwent colorectal resection with anastomosis, from March 2017 to August 2019. Patients were divided into three groups: G1 – no complications; G2 – complications not related to CAL; and G3 – CAL. Five biomarkers were measured and analyzed in the first 5 postoperative days (PODs), namely white blood cell (WBC) count, eosinophil cell count (ECC), CRP, CLP, and procalcitonin (PCT). Clinical criteria, such as abdominal pain and clinical condition, were also assessed. The correlation between biomarkers and CAL was evaluated. Receiver operating characteristic (ROC) curve analysis was used to compare the accuracy of these biomarkers as predictors of CAL, and the area under the ROC curve (AUROC), specificity, sensitivity, positive predictive value, and negative predictive value (NPV) during this period were estimated.

RESULTS

In total, 25 of 396 patients developed CAL (6.3%), and the mean time for this diagnosis was 9.0 ± 6.8 d. Some operative characteristics, such as surgical approach, blood loss, intraoperative complications, and duration of the procedure, were notably related to the development of CAL. The length of hospital stay was markedly higher in the group that developed CAL compared with the group with complications other than CAL and the group with no complications (median of 21 d *vs* 13 d and 7 d respectively; $P < 0.001$). For abdominal pain, the best predictive performance was on POD4 and POD5, with the largest AUROC of 0.84 on POD4. Worsening of the clinical condition was associated with the diagnosis of CAL, presenting a higher predictive effect on POD5, with an AUROC of 0.9. WBC and ECC showed better predictive effects on POD5 (AUROC = 0.62 and 0.7, respectively). Those markers also presented a high NPV (94%-98%). PCT had the best predictive effect on POD5 (AUROC = 0.61), although it presented low accuracy. However, this biomarker revealed a high NPV on POD3, POD4, and POD5 (96%, 95%, and 96%, respectively). The mean CRP value on POD5 was significantly higher in the group that developed CAL compared with the group without complications (195.5 ± 139.9 mg/L *vs* 59.5 ± 43.4 mg/L; $P < 0.00001$). On POD5, CRP had a NPV of 98%. The mean CLP value on POD3 was significantly higher in G3 compared with G1 (5.26 ± 3.58 μg/mL *vs* 11.52 ± 6.81 μg/mL; $P < 0.00005$). On POD3, the combination of CLP and CRP values showed a high diagnostic accuracy (AUROC = 0.82), providing a 5.2 d reduction in the time to CAL diagnosis.

CONCLUSION

CRP and CLP are moderate predictors of CAL. However, the combination of these biomarkers presents an increased diagnostic accuracy, potentially decreasing the time to CAL diagnosis.

Key Words: Anastomotic leakage; Colorectal; Surgery; Biomarkers; C-reactive protein; Calprotectin

Core Tip: Colorectal anastomotic leakage (CAL) remains a serious postoperative complication. It is associated with high morbidity rates, affecting overall costs and patients' quality of life. Clinical criteria, imaging studies, and biomarkers have been considered to increase diagnostic accuracy. Plasma C-reactive protein, calprotectin, procalcitonin, white blood cell count, and eosinophil cell count have been proposed as predictors of anastomotic leakage. The combination of C-reactive protein and calprotectin after a minimal clinical suspicion of CAL has shown good diagnostic accuracy, allowing clinicians to reduce the time to CAL detection. Regression models can facilitate building a decision model, as the score proposed for the early detection of CAL.

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INTRODUCTION

Colorectal anastomotic leakage (CAL) is one of the most frequent complications after colorectal surgery, representing a dreaded issue for patients and surgeons. The reported incidence ranges from 0.2% to 27.2%, depending on the study nature, level of anastomosis, or pathology[1-5]. This occurrence is associated with increased morbidity, mortality, reoperation, and health care costs[6-9]. Thus, its clinical relevance should not be underestimated. It also has a negative impact on a patient's quality of life[2,4].

Early CAL detection is key to decreasing related morbidity and mortality; therefore, a prompt and timely diagnosis is crucial[5,10,11]. Initially, it is difficult to distinguish CAL from other postoperative abdominal complications. Surgeons should be aware of subtle clinical signs, and then order additional tests including serum biomarkers, proper imaging, or even early reoperation. Unfortunately, diagnosis is often delayed, because of a misleading clinical picture, non-systematic assessment, or inconclusive investigations[11-15]. Besides clinical parameters, several biomarkers (plasma or intraperitoneal), imaging methods such as abdominal computed tomography (CT) scan or water-soluble contrast enema, and scores have been proposed to reduce the time to diagnosis and to establish an appropriate management pathway[16-19].

Plasma C-reactive protein (CRP) has been proposed as an early predictor of postoperative infectious complications[16,20-23]. This biomarker is an acute phase protein, increasing between 6 h and 48 h after surgery, and returning to baseline if inflammation ceases. After this period, a high CRP level is associated with postoperative infectious complications, especially in patients with CAL[24-26]. On the other hand, calprotectin (CLP) is a useful biomarker of inflammation and infection[18,27]. Fecal CLP has been widely used as a marker of gastrointestinal inflammation. However, some authors suggest that high levels of serum CLP could be associated with septic intra-abdominal complications, such as early-stage CAL[18,28].

The aim of this study was to evaluate the utility of plasma CRP and CLP, individually or combined, to shorten the time to CAL diagnosis.

MATERIALS AND METHODS

Study design and population

This was a prospective observational, single-center study that included adults over 18-years-old who underwent urgent or elective colorectal resection, regardless of the surgical approach (open or laparoscopic), indication (benign or malignant), and option for a protective stoma. The study was conducted in the colorectal division of a non-academic hospital accredited by Joint Commission International® and included about 500000 inhabitants. The data were collected between March 1, 2017 and August 31, 2019. The local ethics committee approved the study, and potential participants provided written informed consent before inclusion.

Definitions

CAL was defined in accordance with the following criteria[29]: (1) Clinical: Enteric discharge from abdominal drain or wound, rectovaginal fistula, or anastomotic defect found by digital examination; (2) Radiological (CT): Extravasation of endoluminally administered contrast, intra-abdominal collection around the anastomosis, presacral abscess near the anastomosis or perianastomotic air, and free intra-abdominal air; and (3) Surgical findings (reoperation): Necrosis of the anastomosis or signs of peritonitis and anastomotic defect.

Faced with clinical deterioration and/or serum biomarker increase, patients underwent further imaging with abdominopelvic CT scan (and water-soluble contrast enema if colorectal anastomosis was present). Once diagnosed, anastomotic leakage was classified into two categories: (1) Minor: Patients with CAL and Clavien-Dindo grade I or II, requiring no active intervention (radiological or surgical intervention) (Grade A of the International Study Group of Rectal Cancer definition); and (2) Major: All other patients with CAL[30,31]. Definitions of other postoperative complications, such as pneumonia, urinary tract infection, paralytic ileus, and surgical wound infection, are available in Supplementary material 1A (Definitions).

Exclusion criteria

Patients were excluded from the study if they were younger than 18-year-old, pregnant, unable to give or not providing written informed consent, R0 resection with anastomosis not having been performed, or presence of inflammatory bowel disease.

Study protocol and variables

Prospective data were collected and recorded in an electronic database according to the study protocol (Supplementary material 1B – Study protocol). Five biomarkers were measured in the first 5 postoperative days (POD), including white blood cell (WBC) count, eosinophil cell count (ECC), CRP, CLP, and procalcitonin (PCT). Clinical criteria, such as abdominal pain and clinical condition, were also assessed. Blood samples were analyzed at the Leiria Hospital Centre laboratory, according to the techniques described in Supplementary material 1C (Laboratory). The 90-d follow-up included data of all postoperative complications, the length of hospital stay, and the readmission rate. Discharge criteria are available in the Supplementary material 1B (Study protocol). All patients received prophylactic antibiotic accordingly to hospital infection control committee protocol.

Statistical analysis

Data were analyzed by using standard descriptive statistics and graphical analysis. One-way analysis of variance was performed to compare the differences in mean biomarkers' values across the three relevant groups of patients (G1 – no complications; G2 – complications not related to CAL; and G3 – CAL). Chi-squared tests were conducted to assess the association between other categorical variables and the patients. Receiver operating characteristic (ROC) curve analysis was employed to evaluate each biomarker as an appropriate classifier to detect CAL early. The area under the receiver operating characteristic curve (AUROC) was used to establish the diagnostic performance of the studied biomarker. Liu's method was used to establish the threshold value of each biomarker, and its sensitivity (SS) and specificity (SP) were defined[32]. The negative likelihood ratio (NLR) and positive likelihood ratio (PLR), and the negative predictive value (NPV) and positive predictive value (PPV) were computed by combining the observed incidence of CAL with the estimated SS and SP at the optimum cut-off value.

The added value of combining two different biomarkers, observed on POD3 or POD5, as a classifier to predict early CAL was explored. Regression models (probit, logit, and complementary log-log) were used to analyze binary dependent variables, and the observed CAL status (0/1) in a pairwise manner of all biomarkers included in our study: WBC, ECC, CRP, PCT, and CLP. Several potential classifiers of CAL were built, applying a non-linear combination of two different biomarkers. To minimize overfitting, the "leave-one-out" methodology was adopted[33]. The AUROC graph was used to select the classifier (defined by the model and the combination of two biomarkers) with the best predictive diagnostic performance. Liu's method was adopted to select the cut-off value for CAL.

The expected reduction in time to CAL diagnosis obtained by using one biomarker or a pairwise combination of biomarkers was estimated. This was the difference between the observed and the expected mean time to CAL diagnosis, if a specific classifier is used. The expected time to CAL diagnosis was computed by using the following expression: S × d1 + [(1 – S) × d2], where S is the SS of the classifier, d1 is the POD of the classifier yielding a positive cut-off value for CAL, and d2 is the day of diagnosis if the classifier provides a false-negative result (time to CAL diagnosis estimated in the dataset). The statistical methods of this study were reviewed by Óscar Lourenço from the Faculty of Economics, CeBER, University of Coimbra, Portugal. All data management and statistical analyses were conducted with Stata Statistical software (Release 16; StataCorp, College Station, TX, United States).

RESULTS

Patients and outcomes

During the study period, we included 458 consecutive patients who underwent colorectal resection, and 62 (13.5%) were ruled out [exclusion criteria (n = 31), no consent (n = 15), no anastomosis (n = 16)] as shown in Figure 1. Patient characteristics, divided into three groups (G1, G2, and G3, as previously defined), are shown in Table 1. Age, the Charlson Comorbidity Index, and American Society of Anesthesiologists grade seem to affect CAL onset.

Table 2 summarizes the main operative characteristics. Eighty-two percent of patients had a laparoscopic approach, and the most common procedures performed were right colectomy (n = 196; 49.5%) and sigmoid colectomy/rectosigmoid resection (n = 74; 18.7%). The surgical approach (P < 0.001), the volume of blood loss (P < 0.001), the occurrence of intraoperative complications (P < 0.001), and the duration of the procedure (P = 0.011) were significantly related to the development of CAL.

In this study, CAL developed in 25 of 396 patients (6.3%) and was more frequent in men than women (68% *vs* 32%). Twenty-three patients with CAL (92.0%) were diagnosed during the first hospital admission. The mean ± SD and median time for CAL detection were 9.0 ± 6.8 d and 8 d (interquartile range = 7), respectively. Anastomotic leak was significantly associated with a longer hospital stay

Table 1 Patient demographic and clinical characteristics

	Group 1, *n* = 277	Group 2, *n* = 94	Group 3, *n* = 25	*P* value
Age, mean ± SD	68.8 ± 11.3	72.2 ± 14.5	73.6 ± 13.6	0.02
Sex, *n* (%)				0.505
Male	161 (58.1)	59 (62.7)	17 (68.0)	
Female	116 (41.9)	35 (37.3)	8 (32.0)	
BMI, mean ± SD	26.8 ± 3.99	26.3 ± 4.05	26.0 ± 3.97	0.33
BMI, *n* (%)				0.33
17.5 < BMI < 25	95 (35.0)	32 (34.0)	12 (48.0)	
25 ≤ BMI < 30	129 (46.0)	51 (54.0)	9 (36.0)	
BMI ≥ 30	53 (19.0)	11 (12.0)	4 (16.0)	
CCI, mean ± SD	5.12 ± 1.83	5.55 ± 2.38	6.04 ± 2.15	0.03
Prior abdominal surgery, *n* (%)	77 (27.8)	32 (34.0)	9 (36.0)	0.41
Immunosuppression, *n* (%)	10 (3.6)	5 (5.3)	0 (0)	0.45
Preoperative diagnosis malignant, *n* (%)	272 (98.2)	90 (95.7)	24 (96.0)	0.38
ASA score, *n* (%)				0.018
I–II	187 (67.5)	47 (50.0)	13 (45.8)	
III–IV	90 (32.5)	47 (50.0)	12 (54.2)	

Group 1: No complications; Group 2: Complications not related to colorectal anastomotic leakage (CAL); Group 3: CAL. BMI: Body mass index; CCI: Charlson Comorbidity Index; ASA: American Society of Anesthesiologists.

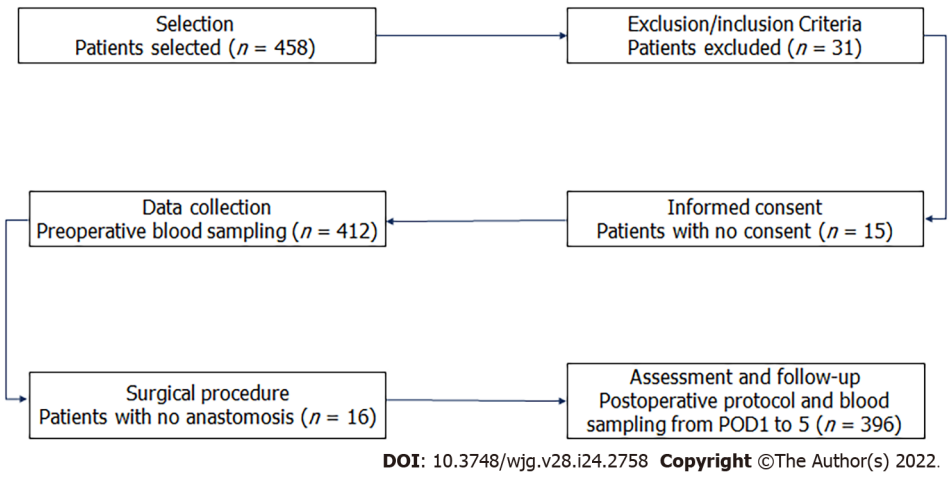

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Figure 1 Flow diagram of patients according to the study protocol. POD1: Postoperative day 1.

(median of 21 d *vs* 7 d and 13 d, in G1 and G2 patients, respectively; $P < 0.001$), the readmission rate (20% *vs* 6.4% and 5.4%), and the reoperation rate (12% *vs* 3.2% and 1.8%). Table 3 provides a summary of 90-d morbidity and mortality rates. Based on the Clavien-Dindo classification, grades III and IV complication were significantly higher in the G3 cohort (84.0% *vs* 17.0%; $P < 0.001$) (Table 4).

Table 5 outlines the intraoperative and postoperative details of patients with CAL (G3) based on the CAL classification (minor *vs* major). Seven patients (28.0%) were managed nonoperatively and two (8.0%) underwent radiologic drainage of intraabdominal collections. The remaining 16 patients (64.0%) required surgical intervention. Of the 16 reoperated patients, 10 (56%) had an anastomosis takedown with an end stoma and 6 (44%) received a defunctioning stoma. The 90-d mortality rate was 0.8%, representing 3 patients with CAL.

Table 2 Patients' operative characteristics

	Group 1, *n* = 277	Group 2, *n* = 94	Group 3, *n* = 25	*P* value
Type of surgery, *n* (%)				0.071
Elective	238 (86.0)	72 (76.6)	19 (75.0)	
Urgent	39 (14.0)	22 (23.4)	6 (25.0)	
Surgical approach, *n* (%)				< 0.001
Open	25 (9.0)	15 (16.0)	2 (8.0)	
Laparoscopic	238 (86.0)	72 (77.0)	15 (60.0)	
Conversion	14 (5.0)	7 (7.4)	8 (32.0)	
Procedure, *n* (%)				0.739
Right colectomy[1]	138 (49.8)	47 (50.0)	11 (44.0)	
Left colectomy	17 (6.1)	7 (7.4)	1 (4.0)	
Sigmoid/RS resection	55 (19.8)	15 (15.9)	4 (16.0)	
Low anterior resection	48 (17.3)	16 (17.0)	8 (32.0)	
Other	19 (6.8)	9 (9.6)	1 (4.0)	
Level of anastomosis, *n* (%)				0.66
Ileocolic	150 (54.1)	50 (53.2)	11 (44.0)	
Colocolic	23 (8.3)	5 (5.3)	1 (4.0)	
≥ 6 cm from AV	67 (24.2)	25 (26.6)	10 (40.0)	
< 6 cm from AV	37 (13.4)	14 (14.9)	3 (12.0)	
Covering stoma, *n* (%)	23 (8.3)	8 (8.51)	2 (8.0)	0.99
Blood loss, mean ± SD, mL	51.6 ± 36.6	58.8 ± 47.7	104.0 ± 191.1	< 0.001
Intraoperative complications, *n* (%)	3 (1.1)	5 (5.3)	4 (16.0)	< 0.001
Operative time in min, mean ± SD	141.9 (48.3)	146.2 (50.0)	172.8 (57.2)	0.011

[1]Included ileocecal resection/extended right-sided colectomy.
Group 1: No complications; Group 2: Complications not related to colorectal anastomotic leakage (CAL); Group 3: CAL. RS: Rectosigmoid; AV: Anal verge.

Clinical criteria – postoperative trend and predictive effect

Abdominal pain: Abdominal pain was markedly higher and persistent from POD3 onwards in G3 patients (Figure 2A). The AUROC for abdominal pain on POD3, POD4, and POD5 was 0.77, 0.84, and 0.83, respectively, as shown in Supplementary Table E (Supplementary material 2A) and Figure 3A. The predictive effect was higher on POD4 with an estimated AUROC of 0.84.

Clinical condition: The clinical condition was worse in G3 compared with G2 patients, and it was significantly different after POD3 (P = 0.001). The overall postoperative trend was a declining clinical condition, as shown in Figure 2B. The AUROC for the clinical condition on POD3, POD4, and POD5 was 0.62, 0.81, and 0.90, respectively, as shown in Supplementary Table E (Supplementary material 2A) and Figure 3B. The prediction effect was higher on POD5 with an estimated AUROC of 0.90.

Biomarkers – postoperative trend and predictive effect

WBC count and ECC: During the first five POD, WBC in G3 patients was higher than that in patients without CAL and was significantly different on POD2, POD4, and POD5 (P = 0.01 for each day). On the other hand, ECC was lower in G3 patients and significantly different on POD1 and POD5 (P = 0.04 and P = 0.01, respectively), as presented in Supplementary Figures 1 and 2 (Supplementary material 2B). Overall, the postoperative course showed a sustained trend for both blood cell counts, except for ECC on POD5. The AUROC for WBC and ECC from POD1 to POD5 is presented in Supplementary Figures 3 and 4, respectively (Supplementary material 2B). The predictive effects of blood cell count were better on POD5. On POD5, when ECC was greater than 250 cells/μL, the AUROC, SS, and SP were 0.70, 89.0%, and 43.0%, respectively, as shown in Table 6.

CRP, PCT, and CLP: The mean values of CRP, PCT, and CLP increased promptly after surgery in all

Table 3 Ninety-day postoperative morbidity and mortality

	Patients, *n* (%)	Length of hospital stay in d, mean ± SD
With complications	119 (30.0)	16.4 ± 9.91
With no complications	277 (70)	7.4 ± 2.10
Noninfectious complications	49 (41.2)	14.2 ± 6.93
Infectious complications		
Surgical wound	36 (30.3)	14.6 ± 8.34
Respiratory tract	10 (8.4)	16.1 ± 7.22
Urinary tract	11 (9.2)	16.2 ± 6.00
Anastomotic leakage classification		
Minor	7 (28)	28.0 ± 17.00
Major	18 (72)	22.4 ± 12.88
Postoperative mortality	3 (0.8)	NA

NA: Not applicable.

Table 4 Short-term outcomes by group

	Group 1, *n* = 277	Group 2, *n* = 94	Group 3, *n* = 25	*P* value
LOHS in d				< 0.001
mean ± SD	7.4 ± 2.1	14.3 ± 7.4	24.0 ± 14.0	
Median	7	13	21	
90-d morbidity, *n* (%)				< 0.001
Clavien-Dindo I	NA	64 (68.1)	0 (0)	
Clavien-Dindo II		14 (14.9)	4 (16.0)	
Clavien-Dindo III		8 (8.5)	16 (64.0)	
Clavien-Dindo IV		8 (8.5)	5 (20.0)	
Readmission, *n* (%)	15 (5.4)	6 (6.4)	5 (20.0)	0.019
Reoperation, *n* (%)	4 (1.1)	3 (3.2)	3 (12.0)	0.005
90-d mortality, *n* (%)	0 (0)	0 (0)	3 (12.0)	< 0.001

Group 1: No complications; Group 2: Complications not related to colorectal anastomotic leakage (CAL); Group 3: CAL. LOHS: Length of hospital stay; NA: Not applicable.

groups. CRP decreased in G1 patients and remained elevated in patients with a complicated postoperative course, but was significantly higher than in G3 patients. On POD5, the mean CRP level in G3 patients was significantly higher than that in G1 patients (195.5 ± 139.9 mg/L *vs* 59.5 ± 43.4 mg/L; $P < 0.00001$) (Figure 4A). Patients with major CAL had a higher mean CRP level than those with minor CAL (251.45 mg/dL *vs* 107.64 mg/dL; $P = 0.01$) (Table 5). On POD3, POD4, and POD5, the overall diagnostic accuracy of CRP to detect CAL was expressed by an AUROC of 0.76, 0.76, and 0.81, respectively (Figure 5A). On POD5, the optimum cut-off value of 96.8 mg/L was estimated, resulting in an SS and SP of 78%, an NPV of 98%, and a PPV of 19% (Table 6).

The PCT level tended to be stable from POD3 onwards. The mean values were higher in G3 patients than in patients without CAL, but without statistical significance [on POD5, 0.23 ± 0.08 ng/mL *vs* 0.22 ± 0.07 ng/mL; Supplementary Figure 5 (Supplementary material 2C)]. The AUROC on POD3, POD4, and POD5 was 0.57, 0.50, and 0.61, respectively, as shown in Supplementary Figure 6 (Supplementary material 2C). The best predictive effect was on POD5. When PCT was greater than 0.39 ng/mL, the SS and SP were 44.0% and 79.0%, respectively (Table 6).

Table 5 Intraoperative and postoperative details of patients with colorectal anastomotic leakage (minor *vs* major)

	Minor CAL, *n* = 7	Major CAL, *n* = 18	*P* value
Type of anastomosis, *n* (%)			0.52
Intrabdominal	3 (42.8)	9 (50.0)	
Pelvic	4 (57.2)	9 (50.0)	
Covering stoma, *n* (%)	1 (14.3)	1 (5.6)	0.47
Abdominal pain			
POD3	1.86	1.94	0.08
POD4	1.57	2.13	0.04
POD5	1.86	1.92	0.03
Clinical condition			
POD3	1	1.25	0.07
POD4	1.14	1.47	0.13
POD5	1.29	1.58	0.02
CRP levels in mg/L			
POD3	178.35	221.02	0.28
POD4	146.30	226.01	0.13
POD5	107.64	251.45	0.01
CLP levels in μg/mL			
POD3	2.75	12.99	< 0.001
POD4	3.34	10.60	0.01
POD5	2.52	10.96	0.004
CAL diagnosis in d, median	8	5.5	0.07
Diagnostic method, *n* (%)			0.12
Clinical	0 (0)	7 (38.9)	
Abdominopelvic CT	7 (100)	11 (61.1)	
CAL management, *n* (%)			< 0.001
Drainage	NA	2 (11.1)	
Reoperation		16 (88.9)	
LOHS in d, mean ± SD	28.0 ± 17.0	22.4 ± 12.9	0.38

CAL: Colorectal anastomotic leakage; POD: Postoperative day; CRP: C-reactive protein; CLP: Calprotectin; CT: Computed tomography; LOHS: Length of hospital stay; NA: Not applicable.

In the first 5 POD, the mean CLP value tended to follow the pattern of CRP, although it was not as pronounced (Figure 4B). The mean CLP value was significantly higher in G3 patients from POD2 onwards. On POD3, the mean values of G1 *vs* G3 patients were 5.26 ± 3.58 μg/mL *vs* 11.52 ± 6.81 μg/mL ($P < 0.00005$). On POD3, POD4, and POD5, the CLP AUROC was 0.78, 0.67, and 0.65, respectively, as presented in Table 6 and Figure 5B. On POD3, a cut-off value of 6.57 μg/mL yielded a sensitivity of 71.0% and a specificity of 72.0% (Table 6).

Finally, when we analyzed the best predictors (CRP and CLP) for major CAL, the AUROC of CRP was 0.74 and 0.88 for POD3 and POD5, respectively. CLP was a better predictor of CAL than CRP at POD3, with an AUROC of 0.92 (Figure 5C and D).

Combination of biomarkers: Tables 7 and 8 present the AUROC of several possible classifiers of CAL, built with the Probit model, on POD3 and POD5, respectively. The combination of CRP and CLP on POD3 showed the best performance, with an AUROC of 0.82 (Table 7). Of note, on POD5, the combination of CRP and ECC also generated good predictive performance (AUROC = 0.81). However, with the aim of early CAL diagnosis, we chose the combination of CRP and CLP on POD3. Thereafter, we determined the probability of CAL, based on the computed equation P (CAL) = F [-3.0842 + (0.094 ×

Table 6 Summary of the predictive performance of the studied plasma biomarkers

	AUROC	Cut-off value	SS	SP	NPV	PPV	PLR	NLR
WBC in g/L								
POD3	0.57	9.75	0.46	0.75	0.95	0.11	1.84	0.72
POD4	0.60	8.25	0.52	0.68	0.96	0.10	1.64	0.70
POD5	0.62	7.55	0.56	0.62	0.95	0.09	1.48	0.71
ECC in cells/μL								
POD3	0.59	150	0.50	0.59	0.95	0.08	1.23	0.84
POD4	0.54	150	0.33	0.71	0.94	0.07	1.14	0.94
POD5	0.70	250	0.89	0.43	0.98	0.10	1.55	0.26
CRP in mg/L								
POD3	0.76	175.90	0.64	0.83	0.97	0.20	3.77	0.44
POD4	0.76	152.40	0.62	0.89	0.97	0.27	5.40	0.43
POD5	0.81	96.80	0.78	0.78	0.98	0.19	3.48	0.29
PCT in ng/mL								
POD3	0.57	0.19	0.68	0.47	0.96	0.08	1.28	0.68
POD4	0.50	0.31	0.38	0.76	0.95	0.10	1.56	0.82
POD5	0.61	0.39	0.44	0.79	0.96	0.12	2.10	0.71
CLP in μg/mL								
POD3	0.78	6.57	0.71	0.72	0.97	0.15	2.55	0.40
POD4	0.67	8.34	0.56	0.86	0.97	0.21	3.89	0.51
POD5	0.65	6.98	0.58	0.80	0.97	0.16	2.84	0.52

AUROC: Area under the receiver operating characteristic curve; SS: Sensitivity; SP: Specificity; NPV: Negative predictive value; PPV: Positive predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; WBC: White blood cell count; POD: Postoperative day; ECC: Eosinophil cell count; CRP: C-reactive protein; PCT: Procalcitonin; CLP: Calprotectin.

Table 7 Area under the receiver operating characteristic curve of pairwise combination of biomarkers on postoperative day 3

	CLP	PCT	CRP	ECC
PCT	0.76			
CRP	0.82	0.72		
ECC	0.77	0.52	0.72	
WBC	0.74	0.53	0.72	0.54

CLP: Calprotectin; PCT: Procalcitonin; CRP: C-reactive protein; ECC: Eosinophil cell count; WBC: White blood cell count.

CLP_D3) + (0.0059 × CRP_D3)], where F is the cumulative standard normal distribution. Applying Liu's method, this classifier had an optimum cut-off point of 0.055, evidencing the existence of CAL above 0.055 on POD3, with an SS and SP of 86% and 75%, respectively. For hypothetical patient X on POD3 with CRP and CLP plasma levels of 137.4 mg/L and 8.75 μg/mL, respectively, the computed probability of CAL is high (score = 0.074). By adopting this classifier, the time to CAL diagnosis is estimated as 3.8 d [(0.86 × 3) + (0.14 × 9.0)], which represents a 5.2-d reduction compared with the baseline results.

Table 8 Area under the receiver operating characteristic curve of pairwise combination of biomarkers on postoperative day 5

	CLP	PCT	CRP	ECC
PCT	0.60			
CRP	0.78	0.79		
ECC	0.61	0.63	0.81	
WBC	0.57	0.60	0.78	0.67

CLP: Calprotectin; PCT: Procalcitonin; CRP: C-reactive protein; ECC: Eosinophil cell count; WBC: White blood cell count.

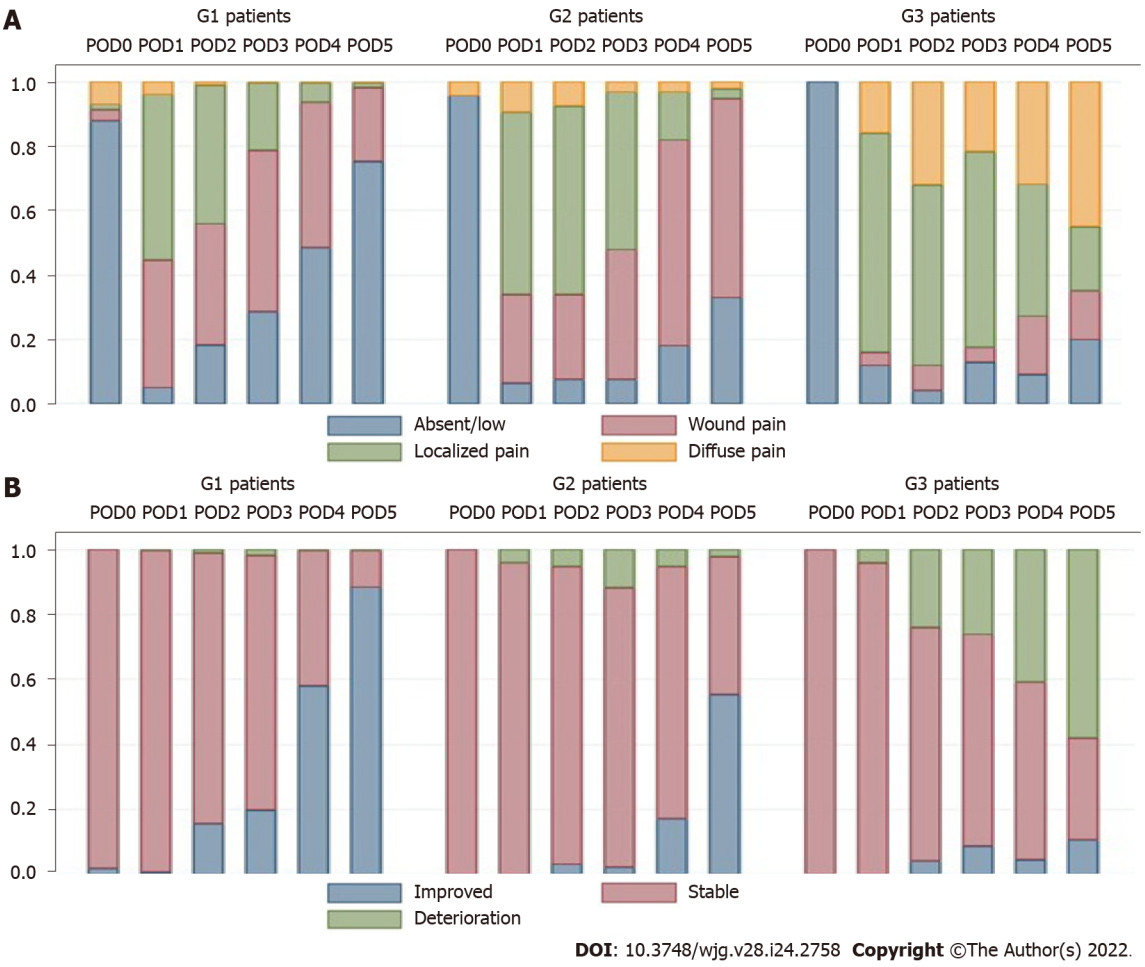

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Figure 2 Distribution of rates of abdominal pain (A) and clinical condition (B). G1: No complications; G2: Complications not related to colorectal anastomotic leakage; G3: CAL. POD: Postoperative day.

DISCUSSION

This study assessed the usefulness of biomarkers for the early detection of CAL. Clinical criteria demonstrated high diagnostic accuracy (AUROC > 0.8) on POD4 and POD5. Changes in the abdominal pain pattern and worsening of the clinical condition were associated with an increased risk of CAL diagnosis. Both clinical criteria seem to be an useful early markers for this condition, producing the best overall diagnostic accuracy of the parameters analyzed. Three large and well-conducted studies on the association between pain and postoperative complications are worth reporting. Boström *et al*[34] examined a cohort of 3084 patients and estimated that increased postoperative pain is associated with a high risk of CAL, being an independent marker and suggesting a need for further diagnostic measures. The other two studies had similar conclusions, although they were not exclusive for colorectal surgery [14,35]. A worse clinical condition and abdominal pain not localized to the wound are two of four modified Dutch leakage (DULK) score criteria, scoring 1 point each. Using a cut-off value of 1 point produced an overall SS and NPV of 97.0% and 99.5%, respectively[10]. We should bring the clinical

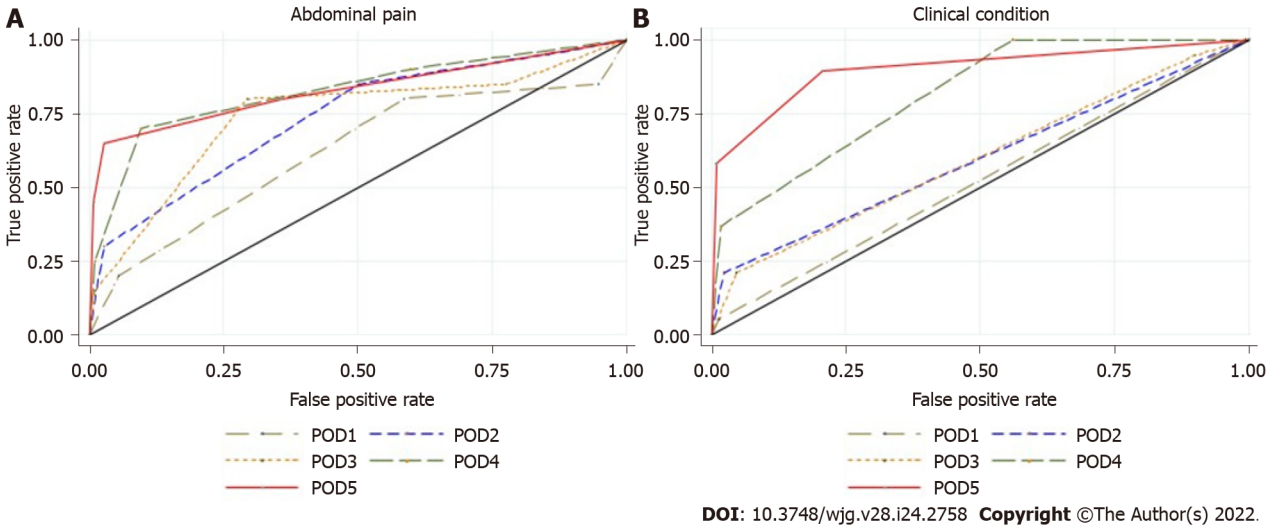

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Figure 3 Area under the receiver operating characteristic curve of colorectal anastomotic leakage for clinical criteria. A: Abdominal pain from postoperative day 1 to postoperative day 5; B: Clinical condition from postoperative day 1 to postoperative day 5. POD: Postoperative day.

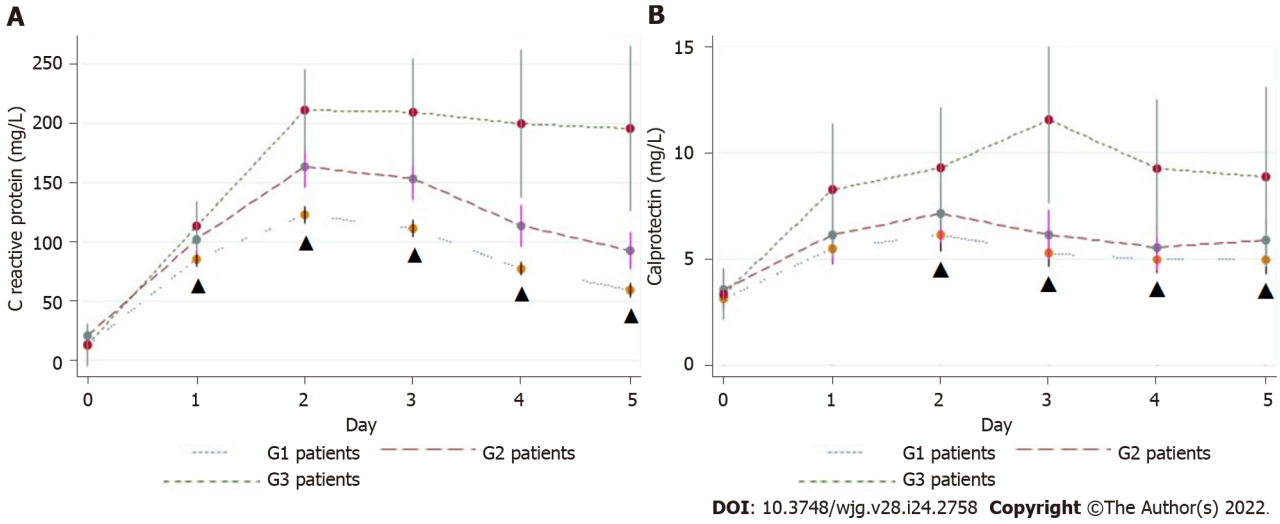

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Figure 4 C-reactive protein (A) and calprotectin (B) levels. Values are the mean ± SE. G1: No complications; G2: Complications not related to colorectal anastomotic leakage; G3: CAL; ▲ : *P* statistically significant ($P < 0.05$).

method to the forefront, being aware of the clinical signs of CAL. They are very helpful for the early diagnosis, as "red flags" for further investigation.

In our study, particularly on POD4 and POD5, WBC and ECC showed a distinct tendency in patients with and without CAL, with a high NPV (from 94%-98%) but low accuracy (AUROC from 0.54 to 0.70). In G3 patients, WBC plateaued after the acute inflammatory response, a phenomenon that was notably different from patients without CAL. In a large retrospective study, Warschkow *et al*[16] found that the WBC level contributed little to the early detection of septic complications, with a lower diagnostic accuracy than plasma CRP. In several other studies, researchers have estimated, from POD5 to POD7, an AUROC and SS ranging from 0.63 to 0.82 and from 58% to 74%, respectively[15,16,20,24,35].

Some researchers have proposed eosinopenia as a biomarker in this scenario. They concluded that it might help to identify several sepsis-related conditions, distinguished from other causes of systemic inflammatory response syndrome. It seems to be an interesting biomarker because of its widespread availability and low cost[36]. Shaaban *et al*[37] defined an optimum cut-off value of 50 cells/μL, which produced an SS, SP, and NPV of 81%, 65% and 80%, respectively. At hospital admission, ECC < 40 cells/μL is an independent prognostic factor for mortality[38,39]. Our study is original in assessing the usefulness of ECC for the early diagnosis of CAL. The mean ECC level showed a non-significant decline after POD4 in G3 patients, and a modest diagnostic accuracy (AUROC from 0.54 to 0.70) when compared with other biomarkers. Nevertheless, ECC could still be used in CAL diagnosis as a fast, simple, convenient, and inexpensive biomarker. It should be considered in the decision-making process

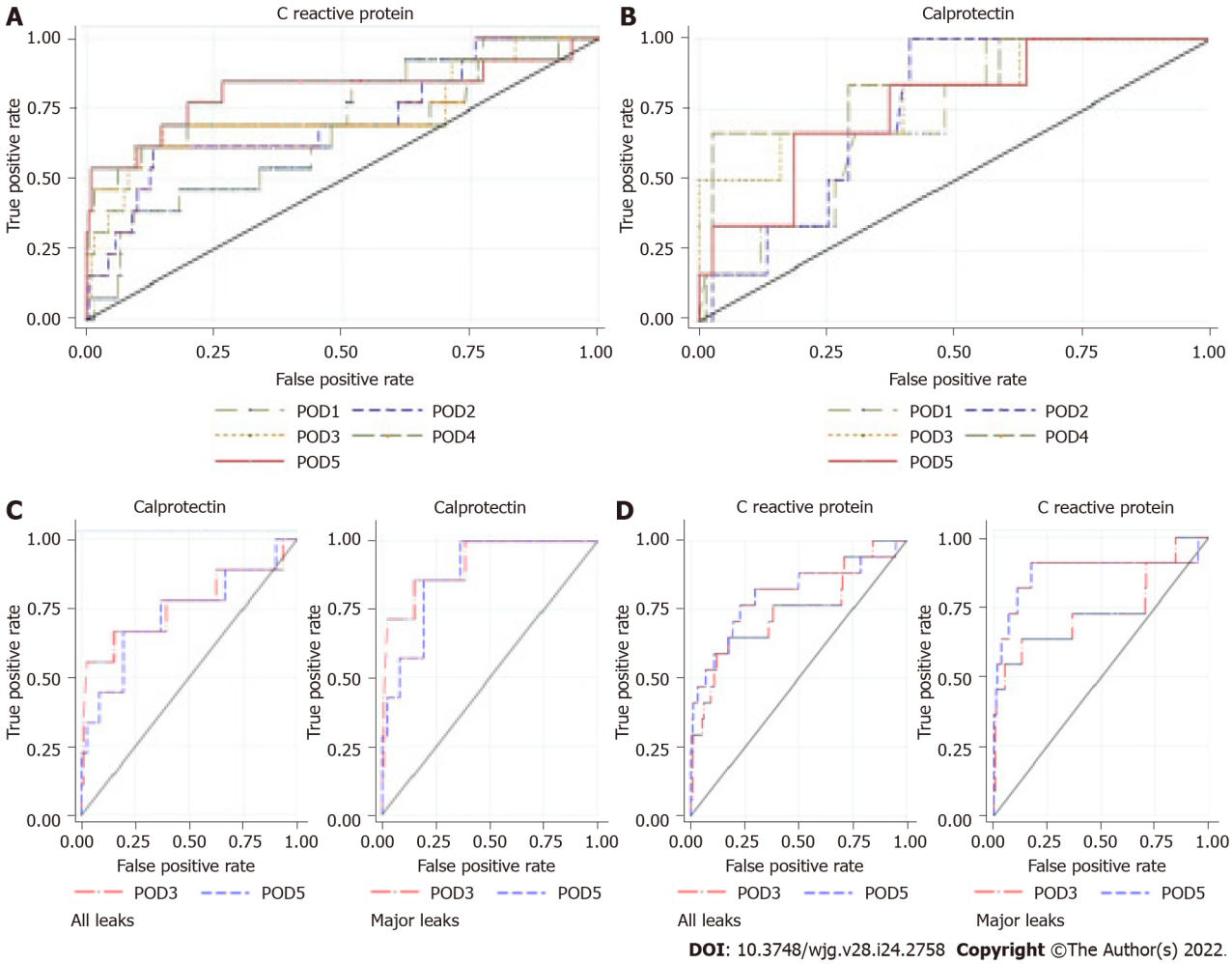

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Figure 5 Area under the receiver operating characteristic curve of colorectal anastomotic leakage. A: For C-reactive protein from postoperative day 1 to postoperative day 5; B: For calprotectin from postoperative day 1 to postoperative day 5; C: For calprotectin from postoperative day 3 to postoperative day 5; D: For C-reactive protein from postoperative day 3 to postoperative day 5. Left: All leaks; Right: Major leaks; POD: Postoperative day.

and future research[40].

The usefulness of CRP as a biomarker for early detection of CAL has been investigated by several groups[19,25,39,41,42]. In this study, the plasma CRP level exhibited a propensity to normalize from POD3 onwards in patients without CAL (G1 and G2). However, it remained steadily increased in G3 patients, with a markedly high mean value from POD1 to POD5. Yeung *et al*[43] performed the most comprehensive meta-analysis available in the literature, including nearly 7000 patients pooled from 23 studies. From POD1 to POD7, patients with CAL had a significantly higher mean CRP level compared with patients without CAL ($P < 0.001$)[43]. In this study, CRP was the best predictor for CAL on POD4 and POD5, with a maximum AUROC of 0.81 (cut-off value of 96.8 mg/L and an NPV of 98%) on POD5. Similar results have been published by other authors. Ortega-Deballon *et al*[26] estimated on POD4 an AUROC of 0.72 with a cut-off of 125 mg/L, yielding an SS and NPV of 81.8% and 95.8%, respectively. Garcia-Granero *et al*[25] reported that CRP level showed a good predictive ability for major CAL on POD5, with an AUROC of 0.85 (cut-off value of 135 mg/L and an NPV of 98%). In the Italian ColoRectal Anastomotic Leakage (iCral) multicentric prospective observational study, the CRP level was a good positive and excellent negative predictor of CAL, with an AUROC of 0.81 on POD6 (cut-off value of 81.5 mg/L), and an SS and NPV of 80.9% and 97.7%, respectively[41]. In the meta-analysis by Yeung *et al*[43], AUROC analysis established a threshold CRP level for CAL of 115 mg/L on POD5, with an SS and SP of 100%. All of these authors recommended CRP levels to predict CAL, and our group advocates a similar practice and suggests the use of this biomarker to expedite further investigation and treatment [25,26,41,43].

CLP, a sign of neutrophil activation, could be a promising early marker for excessive inflammatory response in major abdominal catastrophes, such as CAL. To date, only Reisinger *et al*[18] have studied the predictive value of CLP in CAL diagnosis. In G3 patients, the mean postoperative CLP level peaked on POD3 and was notably higher, persisting thereafter. On POD3, the AUROC (0.78) and SS (71%) were slightly higher than the CRP level, although they were lower than those obtained in the pioneering study by Reisinger *et al*[18] (0.92 and 86%, respectively). One possible explanation could be our compre-

hensive definition of CAL and the larger sample size. It remains unclear to what extent CLP level is an early predictor that is better than CRP for detecting CAL. As a neutrophil activation marker, CLP could be increased early after anastomotic failure, compared with CRP, which indicates a delayed systemic inflammatory response. Our study shows that CLP is worth evaluating for early diagnosis of CAL.

We demonstrated in the first 5 POD, the mean PCT values were marginally higher in G3 patients but with lower accuracy, SS, and SP than CRP and CLP levels. However, it had a high NPV (> 95%), making it an adequate and useful marker for early and safe discharge after colorectal surgery, considering the current enhanced recovery after surgery routine. In contrast to our study, Giaccaglia *et al*[17] estimated that on POD5, PCT had better accuracy than CRP (0.86 *vs* 0.81), as well as a high NPV (98.3%). A recent meta-analysis published by Su'a *et al*[44] determined a diagnostic accuracy of 0.88 on POD5 and an optimum cut-off value on POD3 and POD5 of 0.25 and 680 ng/mL, respectively. The NPV ranged from 95% to 100%. In agreement with these authors, we believe that PCT is a useful negative predictor for CAL; as a single test, however, it is worthless for CAL diagnosis.

We verified that, with the exception of plasma CRP on POD5 (AUROC > 0.80), each biomarker individually was a modest predictor of CAL[45]. The combination of two or more biomarkers has been considered in previous studies[17,18,41]. In this study, the combination of CRP and CLP values on POD3 increased diagnostic accuracy, shortening the mean CAL diagnosis by 5 d. This reduction would likely lead to reduced morbidity and mortality. Reisinger *et al*[18] confirmed a significant improvement in diagnostic accuracy (AUROC = 0.93) with the combination of CRP and CLP plasma levels on POD3, an SS of 100%, and an SP of 89.0%, decreasing the median time to diagnosis by 3 d. Furthermore, Giaccaglia *et al*[17] found that by adding PCT to CRP on POD5, the diagnostic accuracy markedly improved (AUROC = 0.90). Similarly, the iCral study demonstrated that the combination of CRP and PCT with a clinical score (DULK score) allowed the exclusion of CAL on POD2 (NPV = 99%)[41]. We believe that a user-friendly diagnostic tool, combining CLP and CRP levels by this mathematic model, would help the surgeon to diagnose CAL early. Consequently, this biomarkers' combination may be included in a standard postoperative surveillance program, as a warning tool for CAL. In the case of a "positive test", this protocol recommends abdominal and pelvic CT scan or early reoperation in case of imaging-dubious or -negative, to reduce the time to CAL detection and enable prompt management.

Strengths and limitations of the study

One strength of this study was its prospective design and independent data collection model, which minimized observer bias. Second, it was one of the largest monocentric sample size published to date. Based on the recent meta-analyses of Waterland *et al*[46] van Helsdingen *et al*[47], only two monocentric prospective studies have enrolled more than 400 patients. Furthermore, we analyzed five biomarkers, including plasma CLP, which was first studied by Reisinger *et al*[18]. Third, we chose a comprehensive definition of CAL, recently defined by van Helsdingen *et al*[29] to include all patients with CAL, minimizing selection bias. We did not exclude minor CAL from the cohort, which also affected the predictive effect of the analyzed biomarkers. In addition, to keep the biomarkers optimum cut-off values in AUROC analysis both standardized and reproducible, we adopted Liu's method. This method defines the optimum cut-off point as the point maximizing the product of SS and SP[48]. These reasons may explain some differences in biomarkers' diagnostic accuracy in this study. Fourth, we tried to adapt the study protocol to daily practice, making its enforcement easier in the future. Hence, we included all patients undergoing colorectal resection, even those with a diverting ostomy. In addition, clinicians were not blinded to the daily biomarkers' results and might use those data according to the study protocol. Finally, we proposed a predictive tool based on the combination of two biomarkers that improved CAL diagnostic accuracy. Adoption of this tool in daily practice might shorten the time to CAL diagnosis and management. Moreover, the data from this study provide information for the development of more complex mathematical predictive models, including machine learning methods.

This study had several limitations. First, the monocentric design may limit the external validity of the results. Second, our sample had some grade of heterogeneity, because the study population included benign and malignant disorders, elective and urgent procedures, and anastomosis within different levels of the colon and rectum. Third, we designed a phase I diagnostic study and estimated cut-off values for early CAL detection. However, we should change the direction of interpretation, running from the diagnostic test result toward the CAL diagnosis. To address this issue, we are performing a new multicentric prospective phase II diagnostic study, using the predictive tool and defined biomarkers cut-off values[49]. Fourth, plasma CLP measurement is expensive and these kits are not easily accessible in daily clinical practice. Finally, our study did not address the cost-effectiveness of biomarkers' measurement. It is crucial to estimate the economic burden of CAL, including the cost related to a delayed diagnosis, the high rate of false positives, and unjustified reoperations or frequent readmissions.

CONCLUSION

In conclusion, we found that clinical criteria have added value as a warning sign of CAL. On the other

hand, CRP and CLP levels are the best early predictors of CAL. Particularly relevant is the combination of CLP and CRP early during POD3, and its potential to markedly reduce the time to diagnosis of CAL. By reducing the time to CAL diagnosis, reduced morbidity and mortality are expected. Additional studies are needed to confirm the predictive ability of this model on early CAL detection and its utility in routine clinical care.

ARTICLE HIGHLIGHTS

Research background

Colorectal anastomotic leakage (CAL) is a major complication in abdominal surgery. Prompt diagnosis can reduce morbidity and mortality associated with this condition. Serum biomarkers have been proposed as predictors of CAL.

Research motivation

Biomarkers such as C-reactive protein (CRP) and white blood cell (WBC) count are frequently requested in the postoperative period of colorectal surgery. However, the usefulness of these and other biomarkers remains unclear.

Research objectives

To assess the role of CRP, WBC, eosinophil cell count, calprotectin (CLP), and procalcitonin in the first 5 postoperative days (PODs) after colorectal surgery, and thus, discuss in what order these biomarkers can be employed in clinical practice.

Research methods

From March 2017 to August 2019, we measured and analyzed five serum biomarkers daily in 396 patients who underwent colorectal surgery. The area under the receiver operating characteristic curve, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were used to estimate the best predictive diagnostic performance.

Research results

CRP had an NPV of 98% on POD5. The combination of CLP and CRP measurement presented a high diagnostic accuracy (AUCROC = 0.82) on POD3. We identified a reduction of 5.2 d to the diagnosis of CAL.

Research conclusions

The combination of CRP and CLP demonstrated good diagnostic accuracy. These tests can likely be used to reduce time to CAL detection.

Research perspectives

Further studies should test a warning index score built from selected predictive variables as biomarkers CRP and CLP.

FOOTNOTES

Author contributions: Rama NJM, Guarino MPS, and Lourenço Ó designed the study; Lages MCC, Castro R, Bento A, and Parente D coordinated the data collection process; Lourenço Ó and Silva CSG performed the data analyses; Rama NJM, Motta Lima PC and Guarino MPS prepared the manuscript; Rama NJM, Rocha A, Castro-Poças F, and Pimentel J revised the paper critically; All authors read and approved the final manuscript.

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Institutional review board statement: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Local Ethical Committee of the Colorectal Referral Centre, after authorization obtained from the Portuguese Data Protection Authority. This study is registered with the number 9930/2016 and can be consulted at https://drive.google.com/file/d/1BiLxWlvcrqpX4KQrjW4F2codsOOywVF/view?usp=sharing.

Informed consent statement: Informed consent was obtained from all participants included in the study.

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Data sharing statement: For additional data, Dr. Nuno Rama can be contacted by e-mail at ramanuno@gmail.com.

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Country/Territory of origin: Portugal

ORCID number: Nuno J G Rama 0000-0002-1572-2239; Marlene C C Lages 0000-0002-7389-6368; Maria Pedro S Guarino 0000-0001-6079-1105; Óscar Lourenço 0000-0002-3642-4919; Patrícia C Motta Lima 0000-0002-6427-8955; Diana Parente 0000-0003-0271-371X; Cândida S G Silva 0000-0002-7092-1169; Ricardo Castro 0000-0002-5110-0883; Ana Bento 0000-0002-3489-8774; Anabela Rocha 0000-0001-5000-5369; Fernando Castro-Pocas 0000-0002-2268-9107; João Pimentel 0000-0003-1908-8607.

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REFERENCES

1 **Boccola MA**, Buettner PG, Rozen WM, Siu SK, Stevenson AR, Stitz R, Ho YH. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. *World J Surg* 2011; **35**: 186-195 [PMID: 20972678 DOI: 10.1007/s00268-010-0831-7]
2 **Trencheva K**, Morrissey KP, Wells M, Mancuso CA, Lee SW, Sonoda T, Michelassi F, Charlson ME, Milsom JW. Identifying important predictors for anastomotic leak after colon and rectal resection: prospective study on 616 patients. *Ann Surg* 2013; **257**: 108-113 [PMID: 22968068 DOI: 10.1097/SLA.0b013e318262a6cd]
3 **Pommergaard HC**, Gessler B, Burcharth J, Angenete E, Haglind E, Rosenberg J. Preoperative risk factors for anastomotic leakage after resection for colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* 2014; **16**: 662-671 [PMID: 24655784 DOI: 10.1111/codi.12618]
4 **McDermott FD**, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. *Br J Surg* 2015; **102**: 462-479 [PMID: 25703524 DOI: 10.1002/bjs.9697]
5 **Smith SR**, Pockney P, Holmes R, Doig F, Attia J, Holliday E, Carroll R, Draganic B. Biomarkers and anastomotic leakage in colorectal surgery: C-reactive protein trajectory is the gold standard. *ANZ J Surg* 2018; **88**: 440-444 [PMID: 28304142 DOI: 10.1111/ans.13937]
6 **Iancu C**, Mocan LC, Todea-Iancu D, Mocan T, Acalovschi I, Ionescu D, Zaharie FV, Osian G, Puia CI, Muntean V. Host-related predictive factors for anastomotic leakage following large bowel resections for colorectal cancer. *J Gastrointestin Liver Dis* 2008; **17**: 299-303 [PMID: 18836623]
7 **Cousin F**, Ortega-Deballon P, Bourredjem A, Doussot A, Giaccaglia V, Fournel I. Diagnostic Accuracy of Procalcitonin and C-reactive Protein for the Early Diagnosis of Intra-abdominal Infection After Elective Colorectal Surgery: A Meta-analysis. *Ann Surg* 2016; **264**: 252-256 [PMID: 27049766 DOI: 10.1097/SLA.0000000000001545]
8 **Watson AJ**, Krukowski ZH, Munro A. Salvage of large bowel anastomotic leaks. *Br J Surg* 1999; **86**: 499-500 [PMID: 10215823 DOI: 10.1046/j.1365-2168.1999.01096.x]
9 **Matthiessen P**, Henriksson M, Hallböök O, Grunditz E, Norén B, Arbman G. Increase of serum C-reactive protein is an early indicator of subsequent symptomatic anastomotic leakage after anterior resection. *Colorectal Dis* 2008; **10**: 75-80 [PMID: 17666099 DOI: 10.1111/j.1463-1318.2007.01300.x]
10 **den Dulk M**, Witvliet MJ, Kortram K, Neijenhuis PA, de Hingh IH, Engel AF, van de Velde CJ, de Brauw LM, Putter H, Brouwers MA, Steup WH. The DULK (Dutch leakage) and modified DULK score compared: actively seek the leak. *Colorectal Dis* 2013; **15**: e528-e533 [PMID: 24199233 DOI: 10.1111/codi.12379]
11 **Rojas-Machado SA**, Romero M, Arroyo A, Rojas-Machado A, López J, Calpena R. Anastomic leak in colorectal cancer surgery. Development of a diagnostic index (DIACOLE). *Int J Surg* 2016; **27**: 92-98 [PMID: 26827891 DOI: 10.1016/j.ijsu.2016.01.089]
12 **Doeksen A**, Tanis PJ, Vrouenraets BC, Lanschot van JJ, Tets van WF. Factors determining delay in relaparotomy for anastomotic leakage after colorectal resection. *World J Gastroenterol* 2007; **13**: 3721-3725 [PMID: 17659732 DOI: 10.3748/wjg.v13.i27.3721]
13 **Marres CCM**, van de Ven AWH, Leijssen LGJ, Verbeek PCM, Bemelman WA, Buskens CJ. Colorectal anastomotic leak: delay in reintervention after false-negative computed tomography scan is a reason for concern. *Tech Coloproctol* 2017; **21**: 709-714 [PMID: 28929306 DOI: 10.1007/s10151-017-1689-6]
14 **Regenbogen SE**, Mullard AJ, Peters N, Brooks S, Englesbe MJ, Campbell DA Jr, Hendren S. Hospital Analgesia Practices and Patient-reported Pain After Colorectal Resection. *Ann Surg* 2016; **264**: 1044-1050 [PMID: 26756749 DOI: 10.1097/sla.0000000000001541]
15 **Sutton CD**, Marshall LJ, Williams N, Berry DP, Thomas WM, Kelly MJ. Colo-rectal anastomotic leakage often

masquerades as a cardiac complication. *Colorectal Dis* 2004; **6**: 21-22 [PMID: 14692947 DOI: 10.1111/j.1463-1318.2004.00574.x]

16 **Warschkow R**, Tarantino I, Torzewski M, Näf F, Lange J, Steffen T. Diagnostic accuracy of C-reactive protein and white blood cell counts in the early detection of inflammatory complications after open resection of colorectal cancer: a retrospective study of 1,187 patients. *Int J Colorectal Dis* 2011; **26**: 1405-1413 [PMID: 21701807 DOI: 10.1007/s00384-011-1262-0]

17 **Giaccaglia V**, Salvi PF, Antonelli MS, Nigri G, Pirozzi F, Casagranda B, Giacca M, Corcione F, de Manzini N, Balducci G, Ramacciato G. Procalcitonin Reveals Early Dehiscence in Colorectal Surgery: The PREDICS Study. *Ann Surg* 2016; **263**: 967-972 [PMID: 26528879 DOI: 10.1097/SLA.0000000000001365]

18 **Reisinger KW**, Poeze M, Hulsewé KW, van Acker BA, van Bijnen AA, Hoofwijk AG, Stoot JH, Derikx JP. Accurate prediction of anastomotic leakage after colorectal surgery using plasma markers for intestinal damage and inflammation. *J Am Coll Surg* 2014; **219**: 744-751 [PMID: 25241234 DOI: 10.1016/j.jamcollsurg.2014.06.011]

19 **den Dulk M**, Noter SL, Hendriks ER, Brouwers MA, van der Vlies CH, Oostenbroek RJ, Menon AG, Steup WH, van de Velde CJ. Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. *Eur J Surg Oncol* 2009; **35**: 420-426 [PMID: 18585889 DOI: 10.1016/j.ejso.2008.04.009]

20 **Welsch T**, Müller SA, Ulrich A, Kischlat A, Hinz U, Kienle P, Büchler MW, Schmidt J, Schmied BM. C-reactive protein as early predictor for infectious postoperative complications in rectal surgery. *Int J Colorectal Dis* 2007; **22**: 1499-1507 [PMID: 17639424 DOI: 10.1007/s00384-007-0354-3]

21 **Silvestre J**, Rebanda J, Lourenço C, Póvoa P. Diagnostic accuracy of C-reactive protein and procalcitonin in the early detection of infection after elective colorectal surgery - a pilot study. *BMC Infect Dis* 2014; **14**: 444 [PMID: 25132018 DOI: 10.1186/1471-2334-14-444]

22 **Kørner H**, Nielsen HJ, Søreide JA, Nedrebø BS, Søreide K, Knapp JC. Diagnostic accuracy of C-reactive protein for intraabdominal infections after colorectal resections. *J Gastrointest Surg* 2009; **13**: 1599-1606 [PMID: 19479312 DOI: 10.1007/s11605-009-0928-1]

23 **Facy O**, Paquette B, Orry D, Binquet C, Masson D, Bouvier A, Fournel I, Charles PE, Rat P, Ortega-Deballon P; IMACORS Study. Diagnostic Accuracy of Inflammatory Markers As Early Predictors of Infection After Elective Colorectal Surgery: Results From the IMACORS Study. *Ann Surg* 2016; **263**: 961-966 [PMID: 26135691 DOI: 10.1097/SLA.0000000000001303]

24 **Lagoutte N**, Facy O, Ravoire A, Chalumeau C, Jonval L, Rat P, Ortega-Deballon P. C-reactive protein and procalcitonin for the early detection of anastomotic leakage after elective colorectal surgery: pilot study in 100 patients. *J Visc Surg* 2012; **149**: e345-e349 [PMID: 23102916 DOI: 10.1016/j.jviscsurg.2012.09.003]

25 **Garcia-Granero A**, Frasson M, Flor-Lorente B, Blanco F, Puga R, Carratalá A, Garcia-Granero E. Procalcitonin and C-reactive protein as early predictors of anastomotic leak in colorectal surgery: a prospective observational study. *Dis Colon Rectum* 2013; **56**: 475-483 [PMID: 23478615 DOI: 10.1097/DCR.0b013e31826ce825]

26 **Ortega-Deballon P**, Radais F, Facy O, d'Athis P, Masson D, Charles PE, Cheynel N, Favre JP, Rat P. C-reactive protein is an early predictor of septic complications after elective colorectal surgery. *World J Surg* 2010; **34**: 808-814 [PMID: 20049435 DOI: 10.1007/s00268-009-0367-x]

27 **Cikot M**, Kones O, Gedikbası A, Kocatas A, Karabulut M, Temizgonul KB, Alis H. The marker C-reactive protein is helpful in monitoring the integrity of anastomosis: plasma calprotectin. *Am J Surg* 2016; **212**: 53-61 [PMID: 26606896 DOI: 10.1016/j.amjsurg.2015.06.018]

28 **Aadland E**, Fagerhol MK. Faecal calprotectin: a marker of inflammation throughout the intestinal tract. *Eur J Gastroenterol Hepatol* 2002; **14**: 823-825 [PMID: 12172400 DOI: 10.1097/00042737-200208000-00002]

29 **Rahbari NN**, Weitz J, Hohenberger W, Heald RJ, Moran B, Ulrich A, Holm T, Wong WD, Tiret E, Moriya Y, Laurberg S, den Dulk M, van de Velde C, Büchler MW. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery* 2010; **147**: 339-351 [PMID: 20004450 DOI: 10.1016/j.surg.2009.10.012]

30 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]

31 **Hand DJ**. Statistical Evaluation of Diagnostic Performance: Topics in ROC Analysis by Zou KH, Liu A, Bandos AI, Ohno-Machado L, Rockette HE. *Int Stat Rev* 2013; **81**: 335 [DOI: 10.1111/insr.12020_27]

32 **James G**, Witten D, Hastie T, Tibshira R. An introduction to statistical learning with applications in R. 1st ed. New York: Springer Science+Business Media, 2013

33 **Boström P**, Svensson J, Brorsson C, Rutegård M. Early postoperative pain as a marker of anastomotic leakage in colorectal cancer surgery. *Int J Colorectal Dis* 2021; **36**: 1955-1963 [PMID: 34272996 DOI: 10.1007/s00384-021-03984-w]

34 **van Boekel RLM**, Warlé MC, Nielen RGC, Vissers KCP, van der Sande R, Bronkhorst EM, Lerou JGC, Steegers MAH. Relationship Between Postoperative Pain and Overall 30-Day Complications in a Broad Surgical Population: An Observational Study. *Ann Surg* 2019; **269**: 856-865 [PMID: 29135493 DOI: 10.1097/SLA.0000000000002583]

35 **Garnacho-Montero J**, Huici-Moreno MJ, Gutiérrez-Pizarraya A, López I, Márquez-Vácaro JA, Macher H, Guerrero JM, Puppo-Moreno A. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. *Crit Care* 2014; **18**: R116 [PMID: 24903083 DOI: 10.1186/cc13908]

36 **Shaaban H**, Daniel S, Sison R, Slim J, Perez G. Eosinopenia: Is it a good marker of sepsis in comparison to procalcitonin and C-reactive protein levels for patients admitted to a critical care unit in an urban hospital? *J Crit Care* 2010; **25**: 570-575 [PMID: 20435431 DOI: 10.1016/j.jcrc.2010.03.002]

37 **Abidi K**, Belayachi J, Derras Y, Khayari ME, Dendane T, Madani N, Khoudri I, Zeggwagh AA, Abouqal R. Eosinopenia, an early marker of increased mortality in critically ill medical patients. *Intensive Care Med* 2011; **37**: 1136-1142 [PMID: 21369810 DOI: 10.1007/s00134-011-2170-z]

38 **Terradas R**, Grau S, Blanch J, Riu M, Saballs P, Castells X, Horcajada JP, Knobel H. Eosinophil count and neutrophil-

lymphocyte count ratio as prognostic markers in patients with bacteremia: a retrospective cohort study. *PLoS One* 2012; **7**: e42860 [PMID: 22912753 DOI: 10.1371/journal.pone.0042860]

39 **Lin Y**, Rong J, Zhang Z. Silent existence of eosinopenia in sepsis: a systematic review and meta-analysis. *BMC Infect Dis* 2021; **21**: 471 [PMID: 34030641 DOI: 10.1186/s12879-021-06150-3]

40 **Italian ColoRectal Anastomotic Leakage (iCral) Study Group**. Anastomotic leakage after elective colorectal surgery: a prospective multicentre observational study on use of the Dutch leakage score, serum procalcitonin and serum C-reactive protein for diagnosis. *BJS Open* 2020; **4**: 499-507 [PMID: 32134216 DOI: 10.1002/bjs5.50269]

41 **Paliogiannis P**, Deidda S, Maslyankov S, Paycheva T, Farag A, Mashhour A, Misiakos E, Papakonstantinou D, Mik M, Losinska J, Scognamillo F, Sanna F, Feo CF, Porcu A, Xidas A, Zinellu A, Restivo A, Zorcolo L. C reactive protein to albumin ratio (CAR) as predictor of anastomotic leakage in colorectal surgery. *Surg Oncol* 2021; **38**: 101621 [PMID: 34126521 DOI: 10.1016/j.suronc.2021.101621]

42 **Yeung DE**, Peterknecht E, Hajibandeh S, Torrance AW. C-reactive protein can predict anastomotic leak in colorectal surgery: a systematic review and meta-analysis. *Int J Colorectal Dis* 2021; **36**: 1147-1162 [PMID: 33555423 DOI: 10.1007/s00384-021-03854-5]

43 **Su'a B**, Tutone S, MacFater W, Barazanchi A, Xia W, Zeng I, Hill AG. Diagnostic accuracy of procalcitonin for the early diagnosis of anastomotic leakage after colorectal surgery: a meta-analysis. *ANZ J Surg* 2020; **90**: 675-680 [PMID: 31230412 DOI: 10.1111/ans.15291]

44 **Mandrekar JN**. Receiver operating characteristic curve in diagnostic test assessment. *J Thorac Oncol* 2010; **5**: 1315-1316 [PMID: 20736804 DOI: 10.1097/JTO.0b013e3181ec173d]

45 **Mik M**, Dziki L, Berut M, Trzcinski R, Dziki A. Neutrophil to Lymphocyte Ratio and C-Reactive Protein as Two Predictive Tools of Anastomotic Leak in Colorectal Cancer Open Surgery. *Dig Surg* 2018; **35**: 77-84 [PMID: 28132052 DOI: 10.1159/000456081]

46 **Waterland P**, Ng J, Jones A, Broadley G, Nicol D, Patel H, Pandey S. Using CRP to predict anastomotic leakage after open and laparoscopic colorectal surgery: is there a difference? *Int J Colorectal Dis* 2016; **31**: 861-868 [PMID: 26951183 DOI: 10.1007/s00384-016-2547-0]

47 **van Helsdingen CP**, Jongen AC, de Jonge WJ, Bouvy ND, Derikx JP. Consensus on the definition of colorectal anastomotic leakage: A modified Delphi study. *World J Gastroenterol* 2020; **26**: 3293-3303 [PMID: 32684743 DOI: 10.3748/wjg.v26.i23.3293]

48 **Liu X**. Classification accuracy and cut point selection. *Stat Med* 2012; **31**: 2676-2686 [PMID: 22307964 DOI: 10.1002/sim.4509]

49 **Sackett DL**, Haynes RB. The architecture of diagnostic research. *BMJ* 2002; **324**: 539-541 [PMID: 11872558 DOI: 10.1136/bmj.324.7336.539]

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APPENDIX 2

- **Nuno Rama,** Marlene Lages, Cândida G. Silva, Patrícia Mota Lima, Inês Campos Gil, Óscar Lourenço, Maria Pedro Guarino, Pedro Oliveira, Maria Dixe, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

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The Usefulness of Inflammatory Biomarkers to Predict Anastomotic Leakage after Colorectal Surgery: Systematic Review and Meta-Analysis

Nuno Rama[1-5]*, Marlene Lages[2,3], Cândida G. Silva[2,3], Patrícia Motta Lima[1], Inês Campos Gil[1], Maria Guarino[2,3], Pedro Oliveira[4], Maria Dixe[2,3], Anabela Rocha[4], Fernando Castro-Poças[4] and João Pimentel[5]

[1]Colorectal Disease Division, Leiria Hospital Centre, Leiria, Portugal
[2]School of Health Sciences, Polytechnic of Leiria, Leiria, Portugal
[3]Center for Innovative Care and Health Technology (ciTechCare), Polytechnic of Leiria, Leiria, Portugal
[4]School of Medicine and Biomedical Sciences, University of Oporto, Porto, Portugal
[5]Montes Claros Clinic, Coimbra, Portugal

***Corresponding author:**
Nuno JG Rama, FEBS, MD, MHSc
Associate Professor
Research Assistant Professor, Surgeon
Colorectal Surgical Division
Leiria Hospital Centre, Rua das Olhalvas
Leiria 2410-021, Leiria, Portugal
E-mail: ramanuno@gmail.com
Nuno JG Rama, 0000-0002-1572-2239
Marlene CC Lages, 0000-0002-7389-6368
Cândida SG Silva, 0000-0002-7092-1169
Patrícia C Motta Lima, 0000-0002-6427-8955
Inês Campos Gil, 0000-0001-5008-2129
Maria Pedro S Guarino, 0000-0001-6079-1105
Pedro Oliveira, 0000-0002-2470-0795
Maria dos Anjos Dixe, 0000-0001-9035-8548
Anabela Rocha, 0000-0001-5000-5369
Fernando Castro-Poças, 0000-0002-2268-9107
João Pimentel, 0000-0003-1908-8607

ABSTRACT

Aim: Anastomotic leakage (AL) is a severe postoperative complication in colorectal surgery, but its preclinical diagnosis may improve outcomes and increase anastomotic salvage. This study aimed to assess the added value of serum biomarkers for early detection of colorectal AL.
Method: We performed a comprehensive literature review, and a qualitative and quantitative analysis of papers retrieved from MEDLINE, Embase, PubMed, Web of Science, Scopus and the Cochrane Library. We included all studies published before September 2021 assessing the serum biomarkers white blood cells (WBC), C-reactive protein (CRP), procalcitonin (PCT) and calprotectin (CLP) for the early diagnosis of AL.
Results: Fifteen studies that evaluated three different systemic biomarkers in the context of AL were identified, including 5150 patients. Diagnostic test accuracy was estimated for CRP and PCT. On postoperative day (POD) 5, the highest AUC (87.1%) and specificity (80.2%) values were estimated for CRP. Random-effects meta-analysis and total effect sizes estimation for the biomarkers CRP, PCT and WBC were performed according to POD. The concentration of serum biomarkers is significantly higher in patients presenting AL. Regarding the qualitative analysis, there was significant heterogeneity in the inclusion of different subcategories of the consensus definition of colorectal AL in each paper's definition.
Conclusion: The serum biomarkers CRP and PCT are moderate predictors for AL, showing a high heterogeneity among the studies. Combinations of these biomarkers might improve predictive accuracy, but more studies will be necessary to conduct a quality metaregression.
Key words: anastomotic leakage, colorectal, surgery, biomarkers, C-reactive protein, calprotectin

Abbreviations:
AL: anastomotic leakage
AUC: area under the curve
CLP: calprotectin
CRP: C-reactive protein
LR: likelihood ratio
NPV: negative predictive value
PCT: procalcitonin
POD: post-operative day
PPV: positive predictive value
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUADAS: Quality Assessment of Diagnostic Accuracy Studies
ROC: receiver operating characteristic
SD: standard deviation
SIRS: systemic inflammatory response syndrome
WBC: white blood cells

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INTRODUCTION

Minimal access surgery and standardised recovery protocols have improved

patient recovery after colorectal surgery. Regardless of these developments, anastomotic leakage (AL) remains a major complication after colorectal surgery, with a reported incidence ranging from 2 to 7% when surgery is performed by experienced surgeons (1-3), increasing up to 8 to 14% in low colorectal resections (4-6). Early diagnosis of AL is crucial to limit the clinical consequences of this complication, allowing its prompt treatment (4,5). AL contributes to possible patient morbidities, hospital re-admissions and overall healthcare costs. Furthermore, complications such as AL and reoperations are considered a quality indicator in colorectal surgery (6).

Although some risk factors have been identified and reported, it remains difficult to predict the development of AL in individual patients (7). Intraabdominal sepsis can be similar to physiological systemic inflammatory response syndrome (SIRS) to surgery, especially in the immediate postoperative period (8). This leads to a delay in clinical diagnosis, increasing the risk of patients being discharged before diagnosis and then readmitted with AL (7,8). Late detection of AL may lead to the development of sepsis, multiple organ dysfunction or death. Thus, early diagnosis of AL, at the asymptomatic stage, is of paramount importance.

Several studies have suggested the use of serum biomarkers to ease the early detection of postoperative septic complications. In colorectal surgery, some biomarkers have been identified for detecting various stages of early ischaemia, inflammation and necrosis (9). Eosinopenia has been proposed as a biomarker that might help to identify several sepsis-related conditions, distinguished from other causes of SIRS (10). Serum C-reactive protein (CRP) has been shown to have a strong correlation with postoperative complications, including abdominal surgery (11,12). The usefulness of procalcitonin (PCT) has been highlighted as an earlier, more sensitive and more reliable biomarker of AL, even before symptoms appear. Moreover, PCT and CRP have been demonstrated to have a good negative predictive value for AL (13,14). Calprotectin (CLP) can be a biomarker for amplified inflammation early in major abdominal complications. There are currently few studies that have investigated CLP as a predictor for AL. Reisinger et al. showed that CLP is a better biomarker for detecting AL than CRP (15). However, data regarding the diagnostic accuracy of the combination of clinical and laboratory markers for the diagnosis of AL is still scarce. Further studies are needed to ascertain whether the addition of serum biomarkers can improve the early diagnosis of AL. This systematic review and meta-analysis aimed to assess the added value of the serum biomarkers CRP, PCT, CLP and white blood cells (WBC) for the early detection of AL after colorectal surgery.

METHOD

The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Transparent Reporting of Systematic Reviews and Meta-Analysis guideline (16), with PROSPERO registration number 161692.

Literature search

A comprehensive search was performed in MEDLINE, Embase, PubMed, Web of Science, Scopus and Cochrane databases, including the following controlled terms from MeSH: Eosinophils OR C-reactive protein OR Procalcitonin OR Calprotectin AND Colon OR Rectum OR Surgery OR Morbidity. Research articles published until 31st of August 2021, restricted to humans and written in English were considered and included in this study. Review articles were excluded. Additionally, references from the published literature that met the inclusion criteria were identified by searching relevant papers, systematic reviews, and meta-analyses manually. The results of all searches were combined to eliminate duplicate articles. The abstracts obtained by the search were used by two reviewers (N.R. and I.G.) independently to select suitable articles, after which the full-text versions were retrieved and independently reviewed for inclusion by the two reviewers.

Study selection

Studies were assessed for inclusion independently by two authors, and any disagreements over inclusion and exclusion were resolved by consensus. Studies were included if they met the following Population, Intervention, Comparison, Outcomes and Study (PICOS) criteria: (1) patients over the age of 18 years; (2) intervention included colorectal surgical procedure with resection and anastomosis, with or without a protective stoma, regardless of the pathology that motivated the procedure, as well as the elective or urgent character; (3) the comparison group was patients without AL; (4) outcomes assessed were AL rate, area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV); (5) studies with different designs as presented in *table S1 (Supplementary Material)*.

Table S1 - Design of the included studies

Randomised Controlled Trials
Cluster-Randomised Controlled Trials
Non-Randomised Cluster Controlled Trials
Controlled Before and After Studies
Interrupted Time Series
Before-After Study without a Control Group
Comparative Studies with Historical Controls

Data extraction

Data were extracted by three authors (N.R., M.G., M.L.) and entered predefined tables. The primary outcome of interest was AL, defined as reported in the studies included. The measure of diagnostic accuracy, namely, ROC curve, AUC, sensitivity, specificity, PPV and NPV, were recorded in order to perform a diagnostic meta-analysis. Data reported in the text, graphs or figures of the studies were used to obtain the median or mean biomarker values on each postoperative day (POD) for the following patient groups: those with AL, any infectious complication, and no complications. Corresponding authors were contacted to obtain the necessary data when it was not made available from the article or supplementary material.

Quality assessment

Quality assessment of the studies was performed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) 2 tool (17). The QUADAS 2 tool assessed the risk of bias and concerns about applicability in four key domains: patient selection, index test, reference standard, and flow of patients through the study and timing of tests, classifying them as low risk, unclear risk and high risk. The tool was tailored to suit the content of studies and the purpose of this review and applied independently by three authors (N.R., M.G., M.L.).

Data analysis and synthesis

To summarise and compare studies, where available, mean and standard deviation (SD) values for each biomarker in two groups of patients (AL and without AL) were directly pooled and analysed with standardised mean differences (SMDs), mean differences (MDs) and 95% confidence intervals (CIs) (18). Measures of diagnostic accuracy, including area under ROC, AUC, sensitivity, specificity, PPV and NPV, were recorded to enable a diagnostic meta-analysis to be performed. Study-specific estimates were pooled using random-effect models. Two sets of meta-analyses were performed based on the biomarker, and POD.

The statistical heterogeneity among studies was assessed using the I^2 index (19), thus reporting the percentage of variation in the global estimate that was attributable to heterogeneity (I^2 = 25%: low; I^2 = 50%: moderate; I^2 = 75%: high).

Forest plots were created to illustrate the effects in the meta-analysis of the different studies and the global estimation. R (R Core Team, 2020) and RStudio (RStudio Team, 2020) were used to perform all analyses. The R package meta was used to conduct standard meta-analysis (20), and the R package mada was used for meta-analysis of diagnostic accuracy (21). Statistical significance was defined as a p value <0.05.

Qualitative methods were used to analyse the degree of conceptual agreement of the different AL definitions used in the included studies, based on a recently established consensus definition (22). Different conceptual categories of the consensus were considered, and each individual definition was split and whether each category was mentioned was recorded.

RESULTS

A PRISMA flowchart illustrating the selection of articles included in this systematic review is presented in *fig. 1*. Fifteen studies (12–14,23–34) met the defined inclusion criteria and had adequate data to be included in the meta-analysis.

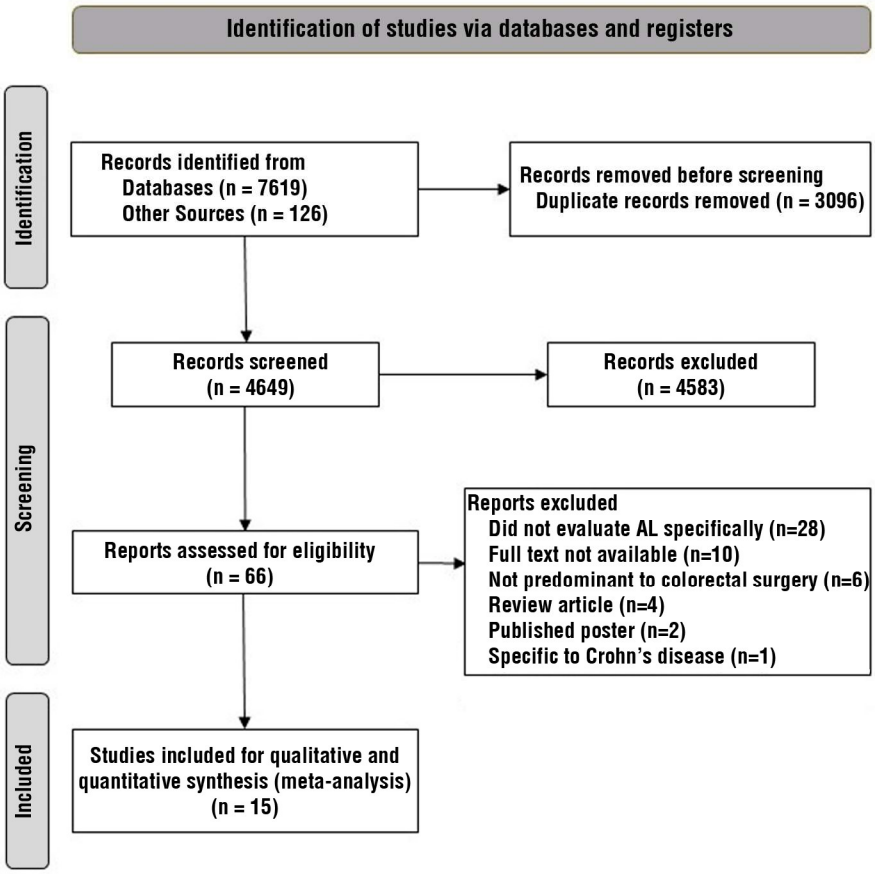
Study characteristics

The characteristics of the fifteen included studies are summarised in *table 1*. All studies included patients undergoing both colonic and rectal surgery. Ten of the fifteen studies were prospective studies.

Risk of bias

The results from the QUADAS-2 assessment are shown in *table 2*. Eight studies (12,23–26,28,30,34) reported measuring CRP routinely during the postoperative period, whereas the other seven (13,14,27, 29,31–33) did not have CRP data available for all patients on each day. Only two studies (28,30) measured PCT daily in the postoperative period, and four studies (12,24,28,34) had WBC count data available daily after surgery. Only one study (29) reported blinding of surgeons to the results of CRP assays. The included studies had different definitions of AL (*table 3*)

Figure 1 - PRISMA flow diagram of the study selection process



and not all patients had this complication diagnosed by the same reference standard.

Definition of anastomotic leakage

Definition of AL according to the included studies showed variations that are presented in *table 3*. *Tables S2* to *S3 (Supplementary Material)* represent the results of the qualitative analysis performed. Considering the consensus-based recommendation for the definition of AL established in the study of van Helsdingen et al. (22), the different definitions presented in the selected studies were divided into three categories: clinical, radiological, and surgical findings. Regarding clinical criteria, only one study (31) covers all of the defined subcategories, and among these, drainage of faeces or other suspicious contents was considered in thirteen of the fifteen studies. Most studies did not include three of the four consensus clinical subcategories in the definition. In terms of radiological criteria, six studies integrate the subcategories "extravasation of contrast" and "abscess near anastomosis" in the definition. Six studies state that perianastomotic air is a suggestive sign of AL, and none of them considered the presence of intraperitoneal air as a diagnostic criterion. Finally, operative findings were considered in eleven studies, and each one mentioned up two subcategories: "signs of peritonitis" and "surgical evidence of dehiscence". In selected studies, neither blind loop nor perianastomotic necrosis were considered as diagnostic criteria for AL. The AL rate in the included studies ranged from 2% (32) to 15% (29).

Diagnostic WBC accuracy for AL

The results of random-effects meta-analysis including two studies measuring WBC are shown in *fig. S1 (Supplementary Material)*. Subgroups meta-analysis was performed according to POD 2 and 4, with low global heterogeneity (I^2 = 0%; p = 0.82). The pooled average WBC level on each POD for patients with and without AL are shown in *fig. S2 (Supplementary Material)*. A meta-analysis of the predictive value of WBC for AL was not possible due to the lack of available data in the selected studies.

Table 1 - Summary of the characteristics of included studies evaluating biomarkers

Reference	Study design	Study interval	Elective, n (%)	Approach, n (%)	Colonic/rectal surgery, n (%)	Operation for cancer, n (%)	n	AL rate, n (%)	Biomarkers assessed
Ortega-Deballon et al. (2010) (29)	Prospective	11 months	133 (100)	Open 117 (88) Min inv 16 (12)	57/78 (42/58)*	82 (61.7)	133	21 (15.5)	CRP WBC
Almeida et al. (2012) (12)	Retrospective	22 months	164 (95)	Open 142 (82) Min inv 31 (18)	138/35 (80/20)	129 (75)	173	24 (13.9)	CRP WBC
Lagoutte et al. (2012) (30)	Prospective	13 months	100 (100)	Open 65 (65) Min inv 35 (35)	68/32 (68/32)	52 (52)	100	13 (13.0)	CRP PCT
Garcia-Granero et al. (2013) (28)	Prospective	17 months	205 (100)	Open 162 (79) Min inv 43 (21)	144/61 (70/30)	150 (73.2)	205	11 (5.4)	PCT CRP WBC
Scepanovic et al. (2013) (34)	Prospective	18 months	156 (100)	Open 156 (100) Min inv 0 (0)	85/38 (69/31)**	151 (96.8)	156	15 (9.6)	CRP WBC
Giaccaglia et al. (2014) (14)	Prospective	12 months	101 (100)	Open 89 (88) Min inv 12 (12)	77/24 (76/24)	93 (92.1)	101	9 (8.9)	PCT PCR WBC
Kostić et al. (2015) (31)	Prospective	20 months	150 (100)	n.s.	85/65 (57/43)	150 (100)	150	15 (10.0)	CRP
Giaccaglia et al. (2016) (13)	Prospective	21 months	504 (100)	Open 126 (25) Min inv 378 (75)	327/177 (65/35)	504 (100)	504	28 (5.6)	PCT CRP
Pantel et al. (2019) (32)	Retrospective	54 months	752 (100)	Open 197 (26) Min inv 555 (74)	604/124 (80/17)***	227 (33)	752	17 (2.3)	CRP
iCral Study Group (2020) (33)	Prospective	12 months	1546 (100)	Open 255 (17) Min inv 1291 (83)	n.s.	1064 (68.8)	1546	76 (4.9)	CRP PCT
Messias et al. (2020) (25)	Retrospective	49 months	64 (71)	n.s.	65/25 (72/28)	31 (34.4)	90	11 (12.2)	CRP
Stephensen et al. (2020) (23)	Prospective	16 months	833 (100)	n.s.	663/170 (80/20)	584 (70.1)	833	41 (4.9)	CRP
Pantoja Pachajoa et al. (2021) (24)	Retrospective	46 months	101 (82)	Open 65 (56) Min inv 51 (44)	100/16 (86/14)	86 (74)	116	9 (8)	CRP WBC
Jin et al. (2021) (26)	Retrospective	23 months	196 (100)	Open 0 (0) Min inv 196 (100)	0/196 (0/100)	196 (100)	196	11 (5.6)	CRP
Baeza-Murcia et al. (2021) (27)	Prospective	8 months	95 (100)	Open 40 (42) Min inv 55 (58)	77/18 (81/19)	75 (78.9)	95	14 (14,7)	CRP PCT

Min inv, minimally invasive surgery; CRP, C-reactive protein; WBC, white blood cells; PCT, 20 procalcitonin; n.s., not stated; * 133 surgeries, 135 anastomosis;
** 123 colorectal surgeries; *** 21 surgeries were not classified in colonic or rectal surgery in 24 patients

Table 2 - Summary of QUADA-2 results

	Risk of bias				Applicability		
Reference	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test standard	Reference
Ortega-Deballon et al. (2010) (29)	-	-	-	+	-	-	-
Almeida et al. (2012) (12)	+	?	+	+	-	-	+
Lagoutte et al. (2012) (30)	-	-	+	+	+	-	-
Garcia-Granero et al. (2013) (28)	-	-	+	+	-	-	-
Scepanovic et al. (2013) (34)	?	?	?	-	-	-	-
Giaccaglia et al. (2014) (14)	-	-	+	+	-	-	-
Kosti□ et al. (2015) (31)	-	?	+	+	-	-	-
Giaccaglia et al. (2016) (13)	-	-	+	+	-	-	-
Pantel et al. (2019) (32)	-	-	?	-	-	-	-
iCral Study Group (2020) (33)	-	-	?	-	-	-	-
Messias et al. (2020) (25)	?	?	-	?	-	-	-
Stephensen et al. (2020) (23)	?	+	?	?	-	-	-
Pantoja Pachajoa et al. (2021) (24)	-	?	-	-	-	?	-
Jin et al. (2021) (26)	?	+	-	-	-	-	-
Baeza-Murcia et al. (2021) (27)	-	?	-	-	-	-	-

–, low risk; ?, unclear risk; +, high risk

Table 3 - Reported definitions of anastomotic leak according to each study

Reference	Definition and diagnosis of anastomotic leak
Ortega-Deballon et al. (2010) (29)	Presence of one of the following criteria: presence of pus or enteric contents within the drains, presence of abdominal or pelvic collection in the area of the anastomosis on CT scan (performed at the discretion of the attending surgeon), leakage of contrast through the anastomosis during the enema, or evident AL at reoperation for postoperative peritonitis.
Almeida et al. (2012) (12)	Clinical signs of peritonitis and/or clinical evidence of free faecal fluid within the abdomen or emerging from the drain site. Diagnosis confirmed by abdominal and pelvic CT using intravenous and anorectal contrast.
Lagoutte et al. (2012) (30)	Presence of one of the following criteria: postoperative peritonitis found at reoperation, purulent or faecaloid wound drainage, presence of air or fluid collection in the anastomotic region on CT.
Garcia-Granero et al. (2013) (28)	Anastomotic leakages were classified as "major" (need of reoperation or percutaneous radiological drainage, Clavien-Dindo grades III to V) and "minor" (conservative medical treatment, Clavien-Dindo grades I and II). Confirmed either by an X-ray enema with hydrosoluble contrast performed with CT scan, by endoscopy, or intraoperatively.
Scepanovic et al. (2013) (34)	Clinical presentation of enteric contents within the drains, without imaging performed routinely to search for leakage.
Giaccaglia et al. (2014) (14)	Presence of one of the following: postoperative peritonitis found at reoperation, faecaloid drain, faecal material from the wound, extravasation of contrast on enema, or the presence of air or fluid in the anastomotic region visualised by CT scan.
Kostić et al. (2015) (31)	Presence of purulent or faecal content at the drain site, pelvic abscess, peritonitis, rectovaginal fistula, or the appearance of purulent content from the rectum (per recti). In patients with low colorectal anastomosis, a digital rectal examination was an integral part of the examination to detect a possible anastomotic leak.
Giaccaglia et al. (2016) (13)	Presence of a faecaloid drain, emission of faecal material from the wound, extravasation of contrast on enema, evidence of post-operative peritonitis at a reintervention and/or the occurrence of fluid, or air in the anastomotic region during a CT scan. Major leakages were considered the ones needing reoperation or percutaneous radiologic drainage (Clavien-Dindo grades III) and minor those in which conservative medical treatment was appropriate (Clavien-Dindo grades I and II).
Pantel et al. (2019) (32)	Presence of luminal contents through a drain or wound site or abscess cavity, causing inflammation (i.e., fever, leucocytosis, or faecal discharge).
iCral Study Group (2020) (33)	Any deviation from the planned postoperative course related to the anastomosis, presence of pus or enteric fluid in drains or an abdominal/pelvic collection in the area of the anastomosis on CT, contrast leakage through the anastomosis during the administration of an enema, or anastomotic leakage at reoperation for postoperative peritonitis.
Messias et al. (2020) (25)	Anastomotic leakage was defined using the following clinical and radiologic criteria: 1) presence of air or abscess near the site of anastomosis identified on CT, 2) purulent discharge or enteric secretion through the drain, and 3) clinical signs of peritonitis and/or presence of faecal or purulent discharge during surgical re-approach.
Stephensen et al. (2020) (23)	A defect in the intestinal wall at the site of the anastomosis requiring operative or radiological intervention.
Pantoja Pachajoa et al. (2021) (24)	Anastomotic leakage was defined as suture line disruption with intestinal content leakage or abscess formation, associated with fever or abdominal pain, and confirmed by a CT scan or re-operation up to 3 months after colorectal surgery.
Jin et al. (2021) (26)	Anastomotic leakages were classified as "major" (need of reoperation or percutaneous radiological drainage, Clavien-Dindo grades III to V) and "minor" (conservative medical treatment, Clavien-Dindo grades I and II). All anastomotic leakages were confirmed by fecal fluid drainage, digital rectal examination, signs of peritonitis with high fever, CT scan, endoscopy or operation.
Baeza-Murcia et al. (2021) (27)	Anastomotic leakage was definite if proven radiologically or clinically and then classified according to the necessary intervention as follows: Grade A, requiring no active intervention (diagnosed radiologically); Grade B, requiring active radiological intervention but manageable without surgical re-intervention; and Grade C, requiring surgical reintervention or showing an intraperitoneal (abdominal or pelvic) fluid collection on postoperative imaging. The reference test used for AL diagnosing was double- or triple-contrast CT. Patients with poor clinical evolution (fever, prolonged ileus, physical examination suggesting peritoneal irritation, purulent/intestinal output through drain, etc.) underwent the reference test.

CT, computed tomography

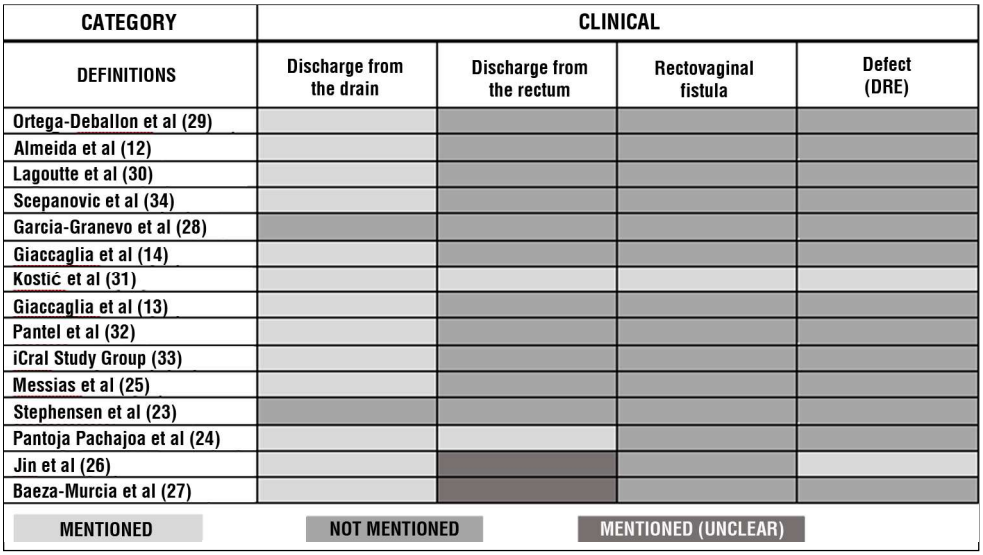


Table S2 - Qualitative analysis of AL definitions from the fifteen selected studies: clinical category. DRE, digital rectal examination

Table S3 - Qualitative analysis of AL definitions from the fifteen selected studies: radiological category.

CATEGORY	RADIOLOGICAL			
DEFINITIONS	Extravasation of contrast	Abscess near anastomosis	Perianastomotic air	Free intra-abdominal air
Ortega-Deballon et al (29)				
Almeida et al (12)				
Lagoutte et al (30)				
Scepanovic et al (34)				
Garcia-Granevo et al (28)				
Giaccaglia et al (14)				
Kostić et al (31)				
Giaccaglia et al (13)				
Pantel et al (32)				
iCral Study Group (33)				
Messias et al (25)				
Stephensen et al (23)				
Pantoja Pachajoa et al (24)				
Jin et al (26)				
Baeza-Murcia et al (27)				

MENTIONED | NOT MENTIONED | MENTIONED (UNCLEAR)

Diagnostic CRP accuracy for AL

The results of random-effects meta-analysis considering the different studies measuring CRP are presented in *fig. 2*. Subgroups meta-analysis was performed according to POD 1 to 7, with a global heterogeneity statistic I^2 values of 85% ($p < 0.01$), which is indicative of high between-study hetero-

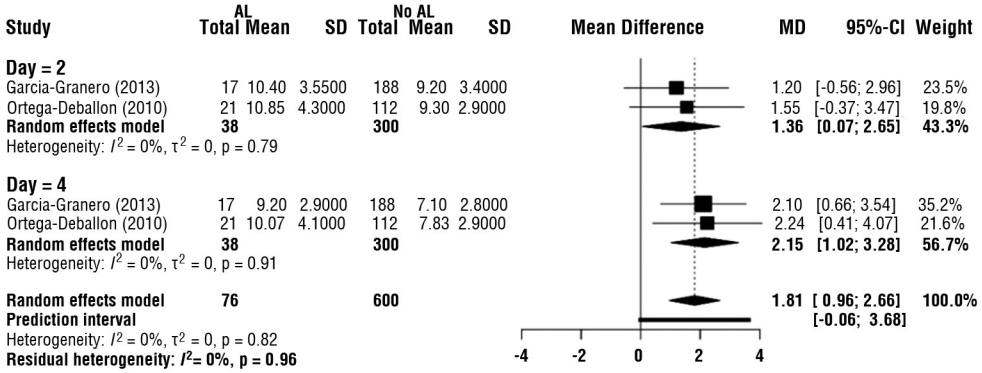


Figure S1 - Forest plot for WBC data showing the results of random-effects meta-analysis on different postoperative days

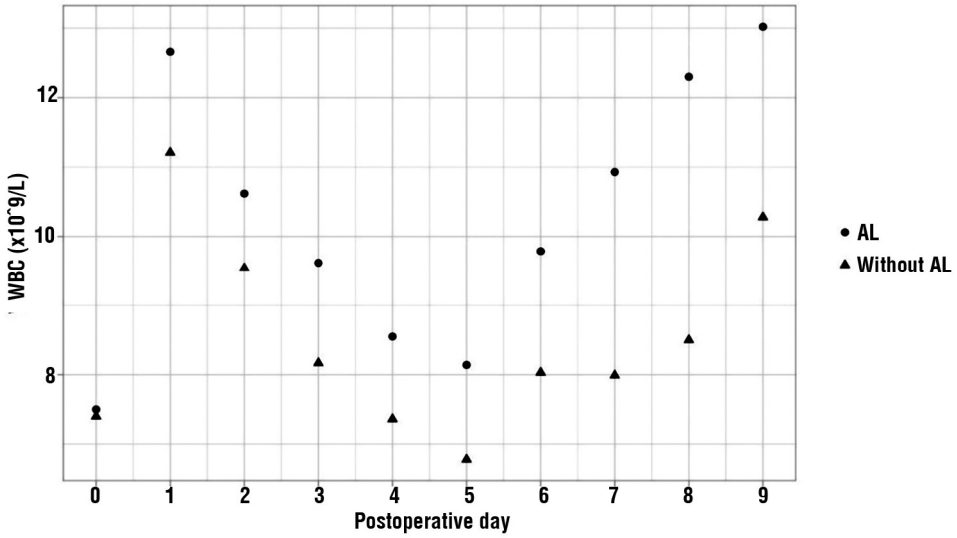


Figure S2 - WBC levels in the postoperative period in relation to AL. Values at each time point represent the pooled median/mean WBC level from the included studies [Ortega-Deballon (2010); Almeida (2012); Garcia-Granero (2013); Scepanovic (2013); Pantoja Pachajoa (2021)], with individual studies weighted by their sample size. AL, anastomotic leakage.

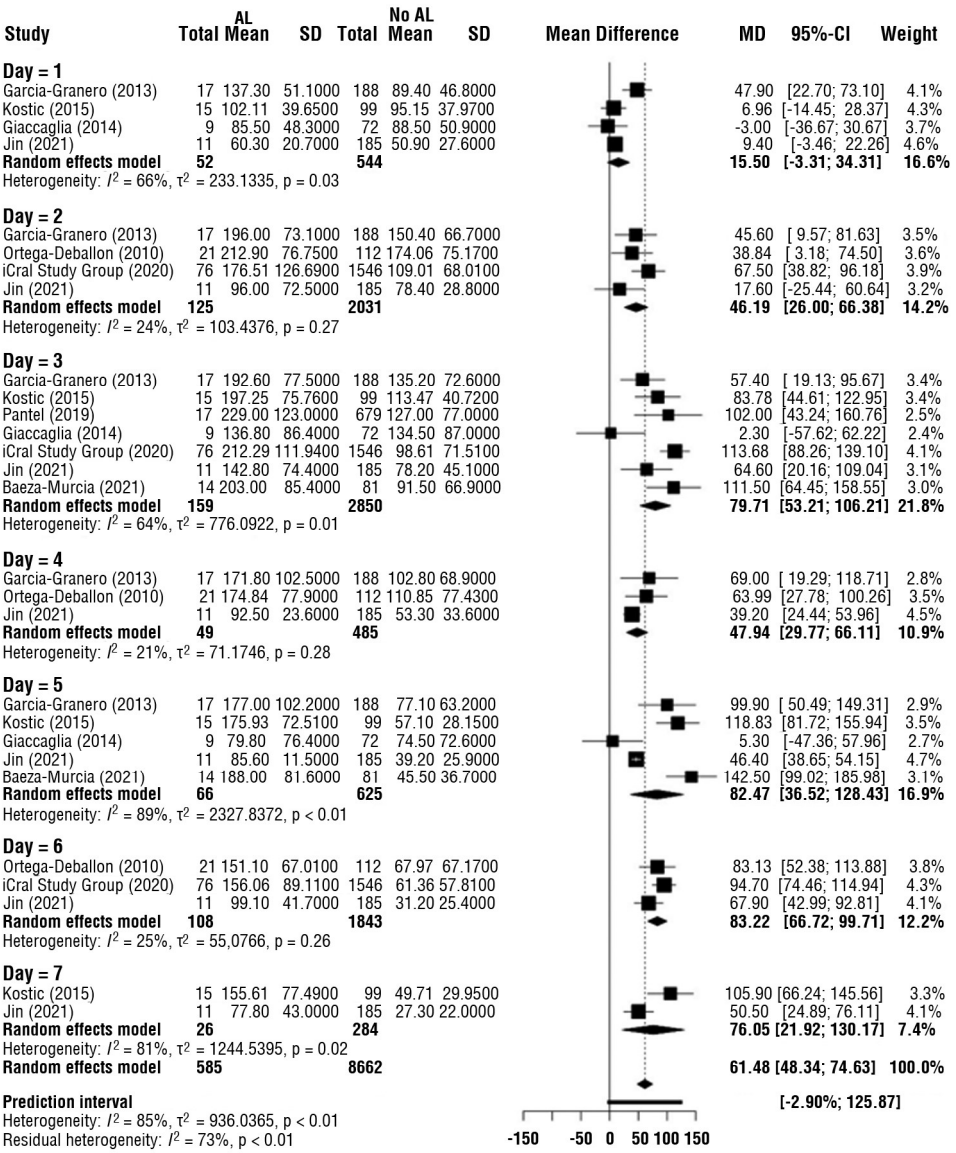


Figure 2 - Forest plot for CRP data showing the results of random-effects meta-analysis on different postoperative days

geneity, and a prediction interval that crosses the line of no effect. The comparison of pooled average CRP levels on each POD for patients with and without AL are presented in *fig. 3*.

Ten studies were selected in the subgroups meta-analysis of CRP accuracy for AL (POD 3 to 5), with a pooled prevalence of AL ranging from 5.9 to 7.7% (*table 4*). Pooled AUC values on POD 3 and 5 ranged from 77.9 to 87.1% and had similar diagnostic accuracy for AL (*fig. S3 - Supplementary Material*). The highest pooled sensitivity and specificity were found on POD 5 (79.4 and 80.2% respectively). At these three time-points, pooled PPV and NPV ranged from 21.4 to 30.7%, and from 96.2 to 97.4%, respectively, showing low and moderate heterogeneity, except for POD 3. The positive likelihood ratio (LR) for CRP varied from 2.7 to 4.1, and the negative LR was between 0.30 and 0.36. The derived cut-offs on POD 3 and 5 were 150.7 ± 30.5 and 103.5 ± 35.9 mg/L, respectively.

Diagnostic PCT accuracy for AL

Random-effects meta-analysis for PCT are shown in *fig. 4* with subgroups meta-analysis for POD 1 to 5. Global heterogeneity was moderate (I^2 = 60%; p = 0.13) and the prediction interval crossed the line of no effect. The pooled average PCT level on each POD for patients with and without AL are shown in *fig. S4* (*Supplementary Material*).

Five studies were selected in the subgroups meta-analysis of PCT accuracy for AL (POD 3 and 5), with a pooled prevalence of leakage that ranged from 6.5 to 7.8% (*table 4*). Pooled AUC values on POD 3 and 5 ranged from 79.3 to 83.1% and had similar diagnostic

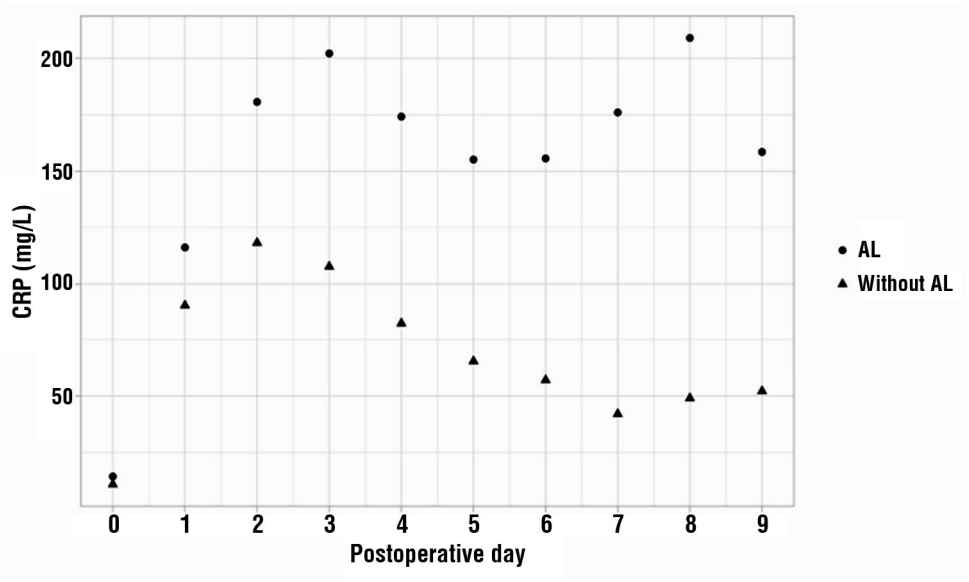


Figure 3 - C-reactive protein (CRP) levels in the postoperative period in relation to AL. Values at each time point represent the pooled median/mean CRP level from the included studies [Ortega-Deballon (2010); Almeida (2012); Lagoutte (2012); Garcia-Granero (2013); Scepanovic (2013); Giaccaglia (2014); Kostic (2015); Giaccaglia (2016); Pantel (2019); iCral Study Group (2020); Messias (2020); Pantoja Pachajoa (2021); Jin (2021); Baeza-Murcia (2021)], with individual studies weighted by their sample size. AL, anastomotic leakage

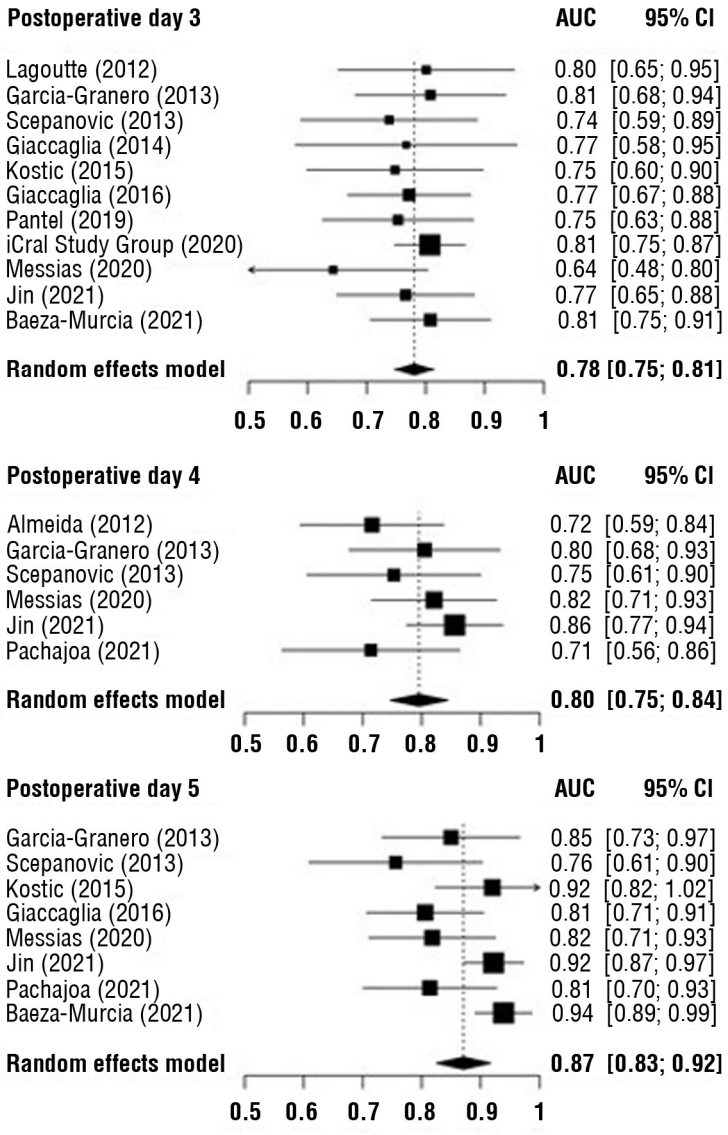


Figure S3 - Pooled area under the curve for anastomotic leakage at POD 3 (I^2 = 0.0%; Q = 4.87; p = 0.899), POD 4 (I^2 = 7.7%; Q = 5.42; p = 0.367) and POD 5 (I^2 = 55.1%; Q = 15.61; p = 0.029) for CRP. Values are shown with 95 per cent confidence intervals.

Table 4 - Summary estimates for CRP and PCT at different postoperative days. Pooled DOR, sensitivity and specificity, LR+ and LR- were obtained from the summary receiver operating characteristic (bivariate model) for diagnostic test accuracy. Pooled prevalence, area under the curve, positive predictive value and negative predictive value were obtained from standard meta-analysis random forest models. Derived cutoff represents the mean of the cutoff values reported in individual studies.

	No. Studies (n)	Pooled prevalence of AL (%)	Pooled AUC (%)	Derived Cutoff (Mean±SD)	Pooled DOR	Pooled sensitivity (%)	Pooled specificity (%)	Pooled PPV (%)	Pooled NPV (%)	Pooled LR+	Pooled LR-
CRP (mg/L)											
POD 3[#]	10 (3757)	5.9[a] (4.1; 8.6)	77.9[♣] (74.4; 81.5)	150.7±30.5	8.62 (5.76; 12.4)	73.5 (66.6; 79.4)	75.3 (67.5; 81.8)	21.4[b] (14.8; 29.8)	97.0[c] (95.6; 98.0)	3.0 (2.31; 3.91)	0.36 (0.28; 0.44)
POD 4[$]	6 (923)	7.7[d] (6.0; 9.9)	79.6 (74.7; 84.5)	108.2±43.6	8.72 (4.05; 16.5)	77.6 (66.6; 85.7)	70.3 (57.8; 80.3)	22.1[e] (15.3; 30.9)	96.2[f] (94.1; 97.6)	2.7 (1.8; 3.9)	0.3 (0.2; 0.5)
POD 5[‡]	8 (1380)	7.6[g] (5.7; 10.0)	87.1 (82.5; 91.7)	103.5±35.9	16.2 (9.1; 26.7)	79.4 (69.7; 86.6)	80.2 (71.7; 86.6)	30.7[h] (23.9; 38.4)	97.4[i] (96.1; 98.3)	4.1 (2.9; 5.7)	0.3 (0.2; 0.4)
PCT (ng/mL)											
POD 3[¥]	5 (2424)	6.5[j] (3.7; 11.21)	79.3 (74.9; 83.8)	1.8±2.0	11.6 (5.3; 22.3)	73.6 (60.6; 83.4)	79.6 (57.8; 91.7)	26.9[k] (14.8; 43.8)	97.9 (97.1; 98.5)	3.9 (1.9; 7.8)	0.3 (0.2; 0.5)
POD 5[§]	4 (802)	7.8[m] (4.9; 12.2)	83.1[¶] (74.6; 91.5)	1.2±1.1	25.6 (10.6; 52.3)	80.7 (62.5; 91.3)	84.9 (64.8; 94.5)	36.1[n] (23.5; 50.9)	97.6[o] (95.9; 98.6)	5.86 (2.5; 12.5)	0.2 (0.1; 0.4)

Values in parentheses represent 95% confidence intervals, unless otherwise stated. AL, anastomotic leakage; AUC, area under the curve; DOR, diagnostic odds ratio; LR+, likelihood ratio positive; LR-, likelihood ratio negative; NPV, negative predictive value; PPV, positive predictive value; SD, standard deviation. # Includes data from Almeida (2012), Garcia-Granero (2013), Scepanovic (2013), Kostic (2015), Giaccaglia (2016), Pantel (2019), iCral Study Group (2020), Messias (2020), Baeza-Murcia (2021), Jin (2021). $ Includes data from Almeida (2012), Garcia-Granero (2013), Scepanovic (2013), Messias (2020), Jin (2021), Pantoja Pachajoa (2021). ‡ Includes data from Garcia-Granero (2013), Scepanovic (2013), Kostic (2015), Giaccaglia (2016), Messias (2020), Baeza-Murcia (2021), Jin (2021), Pantoja Pachajoa (2021), ¥ Includes data from Garcia-Granero (2013), Giaccaglia (2014), Giaccaglia (2016), iCral Study Group (2020), Baeza-Murcia (2021). § Includes data from Garcia-Granero (2013), Giaccaglia (2014), Giaccaglia (2016), Baeza-Murcia (2021). ♣ Data not available in Almeida (2012). ¶ Data not available in Giaccaglia (2014). Heterogeneity: a: I2 = 82.9% ([70.0%; 90.3%]); Q = 52.78; p <0.0001; b: I2 = 83.1% ([70.4%; 90.4%]); Q = 53.40; p <0.0001; c: I² = 55.0% ([8.4%; 77.9%]); Q = 20.00; p = 0.0179; d: I² = 17.9% ([0.0%; 62.7%]); Q = 6.09; p = 0.2972; e: I² = 62.6% ([9.1%; 84.6%]); Q = 13.36; p = 0.0202; f: I² = 0.0% [0.0%; 73.8%]; Q = 4.84; p = 0.4361; g: I² = 50.0% ([0.0%; 77.6%]); Q = 13.99; p = 0.0514; h: I² = 48.9% ([0.0%; 77.2%]); Q = 13.71; p = 0.0566. i: I² = 0.0% ([0.0%; 56.1%]); Q = 5.17; p = 0.6395; j: I² = 86.8.3% ([71.4%; 93.9%]); Q = 30.24; p <0.0001; k: I² = 88.8% ([76.5%; 94.6%]); Q = 35.59; p <0.0001; l: I² = 0.0% ([0.0%; 67.3%]); Q = 2.54; p = 0.6373; m: I² = 67.1% ([3.9%; 88.7%]); Q = 9.11; p = 0.0279; n: I² = 65.8% ([0.0%; 88.4%]); Q = 8.77; p = 0.0325; o: I² = 0.0% ([0.0%; 79.1%]); Q = 2.19; p = 0.5330.

accuracy for AL (*fig. S5 - Supplementary Material*). The highest pooled sensitivity (80.7%) and specificity (84.9%) were found on POD 5. At these two time-points, PCT had a low pooled PPV between 26.9 and 36.1%, with moderate and high heterogeneity, and a high pooled NPV of 97.9% on POD 3, presenting low heterogeneity. The positive LR for PCT ranged between 3.9 and 5.86, and the negative LR ranged from 0.2 to 0.3. Derived cut-offs on POD 3 and 5 were 1.8 ± 2.0 and 1.2 ± 1.1 ng/mL, respectively.

DISCUSSION

Over the past 10 years, few systematic reviews and meta-analyses have evaluated the role of biomarkers in the early diagnosis of AL in colorectal surgery. Su'a et al. (35) analysed both peritoneal drain fluid and systemic biomarkers that are increased in the AL environment, finding an improvement in predictive accuracy when combining these biomarkers.

This systematic review and meta-analysis demonstrated that the diagnostic accuracy of CRP and PCT was similar on all days and showed higher values on POD 5, being superior for CRP with a value of 87.1%. Systemic biomarkers were moderate predictors of AL when assessed individually. Nevertheless, a combination of biomarkers could increase the predictive accuracy, but data meta-regression was not possible due to the small number of selected studies.

Singh et al (7) showed that serum CRP is a useful negative predictive test for detecting AL after colorectal surgery, but not a good positive predictor. In this study, the NPV of serum biomarkers was calculated and proved to be high and useful as a predictive indicator for AL exclusion. In fact, increased CRP and PCT may result from other clinical conditions, postoperative complications, and systemic inflammatory response. Hence, the clinical usefulness of biomarkers is based on the probability of ruling out an AL when a patient had a negative test (lower CRP and PCT level) on POD 3 and 5. In daily practice, this estimated high NPV is critical for

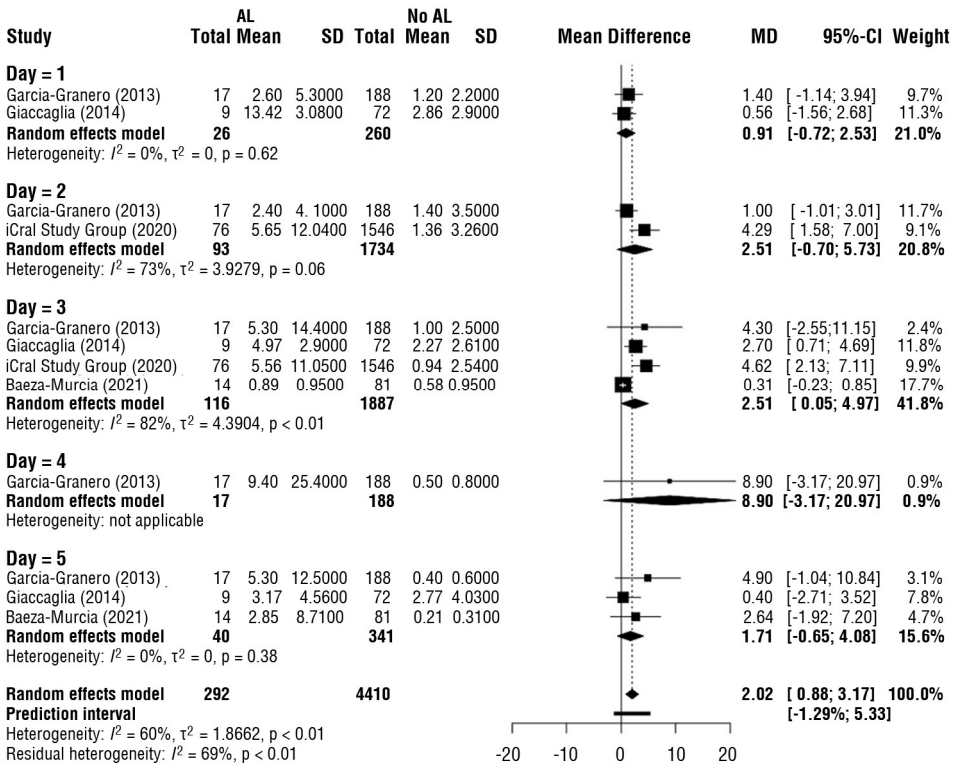


Figure 4 - Forest plot for PCT data showing the results of random-effects meta-analysis on different postoperative days. PCT, procalcitonin.

ensuring safe early discharge.

The LR is a useful tool for clinical decision-making as these values are test-specific and independent of the prevalence and are more reliable as a single test for an individual patient. Therefore, LR provides relevant information applied to a variety of patient characteristics, as it can provide probabilities adjusted to each case, using information obtained from populations, institutions or surgeon's personal data. The usefulness of LR for AL detection reflects the ability to change a pre-test probability to a new post-test probability, considering the systemic biomarker measured, in relation to the estimated cut-off. In this study, the positive LR for PCT showed a good impact on the clinical decision, as a "rule-in" and "rule-out" test for AL. Moreover, LR calculated for CRP presented a moderate impact on the decision-making process, being relevant as a "rule-out" test.

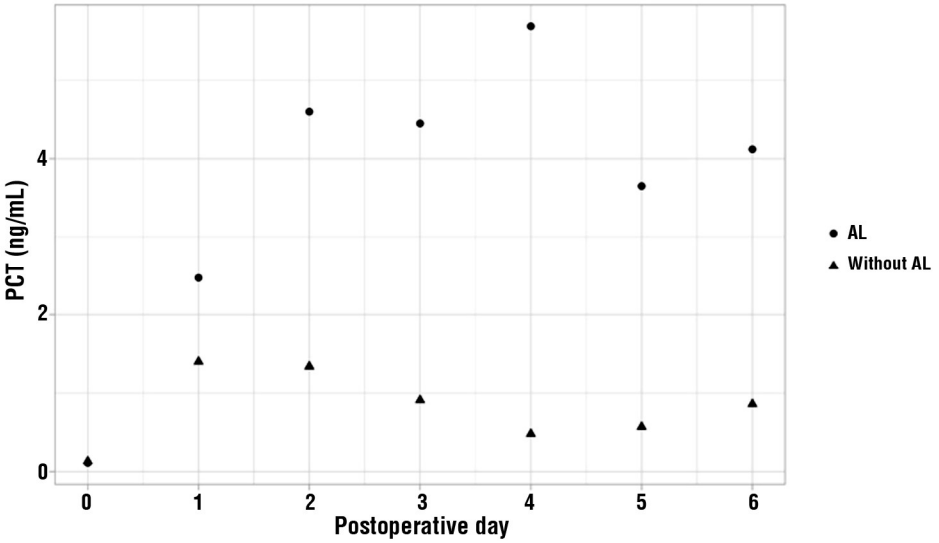


Figure S4 - Procalcitonin (PCT) levels in the postoperative period in relation to AL. Values at each time point represent the pooled median/mean PCT level from the included studies [Lagoutte (2012); Garcia-Granero (2013); Giaccaglia (2014); Giaccaglia (2016); iCral Study Group (2020); Baeza-Murcia (2021)], with individual studies weighted by their sample size. AL, anastomotic leakage.

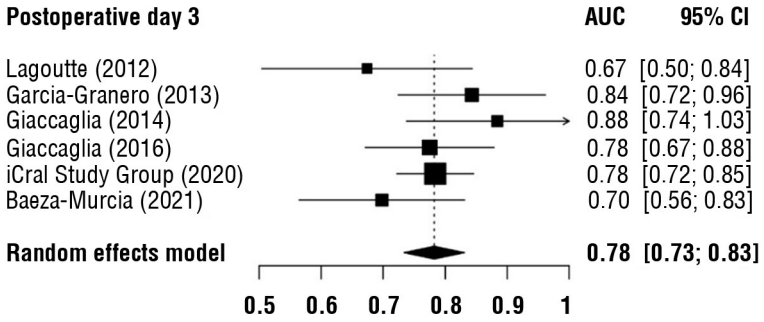


Figure S5 - Pooled area under the curve for anastomotic leakage at POD 3 for PCT (I2 = 16.4%; Q = 5.98; p = 0.308). Values are shown with 95 per cent confidence intervals. PCT, procalcitonin

In this random-effects meta-analysis, interstudy heterogeneity varied according to the biomarker measured, being high in the CRP studies. This important limitation can result from the differences in the patient population, study design and risk of bias. Five studies are retrospective, but only two of the prospective studies did not show investigation bias (blinded surgeons). Furthermore, not all biomarker assays were performed in a standardised manner for the same POD. The qualitative analysis detected inconsistencies in AL definitions, leading to a relevant verification bias. Both CRP and PCT had a prediction interval that crosses the line of no effect, reflecting the uncertainty expected in the summary effect if a new study is included in the meta-analysis. Only six studies measuring PCT were included, making the prediction interval particularly imprecise. The reduced number of studies assessing WBC and PCT did not support a meta-regression, which would be able to minimise the observed heterogeneity. A further limitation of the studies is that no analytic study was made between colonic and rectal procedures, which might also be responsible for different postoperative inflammatory reactions.

This review distinguishes itself from others that have been published previously. First, we only selected studies including a range of systemic biomarkers, mainly prospective, which can be useful in daily practice. However, rigorous inclusion criteria excluded the only eligible CLP study, and the scarce WBC studies available hampered relevant conclusions. Secondly, we decided not only to conduct a random-effects meta-analysis, but also to present and discuss the predictive interval, assuming its usefulness and potential drawbacks. Finally, a qualitative analysis of AL definitions in the selected studies was performed, based on the recommendation recently published (22), revealing remarkable conceptual heterogeneity.

The cost-effectiveness of these tests is a critical subject to be considered in further studies. Blood tests included in the postoperative routine are probably cost-effective given the high cost of late treatment of AL. Furthermore, it is important to assess the combination of biomarkers to raise the accuracy of the test, as well as to define the best time to request them, considering the clinical approach.

Our review and meta-analysis demonstrated that CRP and PCT are moderate predictors of AL in colorectal surgery. It is important for clinicians to be familiar with the role of biomarkers and their benefits. Despite a lack of evidence, it is interesting to note that some biomarkers have been used in clinical practice to predict AL. In this study, we found higher serum levels of systemic biomarkers in the group of patients presenting AL. However, these results should be interpreted with caution due to significant heterogeneity among the studies. Many questions remain regarding the usefulness of each biomarker both for early detection of AL and for assuring safe discharge of patients in this context, making their clinical application challenging.

Statement

Anastomotic leakage (AL) is a life-threatening condition after colorectal surgery. Its early detection is still challenging in clinical practice. This manuscript provides a quantitative analysis for some serum inflammatory biomarkers, suggesting their usefulness for the early detection of AL. Besides, a qualitative analysis of the definition of AL was performed.

Conflicts of interest

We declare no conflicts of interest.

Funding statement

No funding has been received by any author in relation to this article.

Ethical approval

No ethics committee or institutional review board approval.

REFERENCES

1. Matthiessen P, Henriksson M, Hallböök O, Grunditz E, Norén B, Arbman G. Increase of serum C-reactive protein is an early indicator of subsequent symptomatic anastomotic leakage after anterior resection. Color Dis. 2008;10(1):75–80.
2. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. Vol. 102, British Journal of Surgery. John Wiley and Sons Ltd; 2015. p. 462–79.
3. Iancu C, Mocan LC, Todea-Iancu D, Mocan T, Acalovschi I, Ionescu D, et al. Host-related predictive factors for anastomotic leakage following large bowel resections for colorectal cancer. J Gastrointestin Liver Dis. 2008;17(3):299-303.
4. Trencheva K, Morrissey KP, Wells M, Mancuso CA, Lee SW, Sonoda T, et al. Identifying important predictors for anastomotic leak after colon and rectal resection: Prospective study on 616 patients. Ann Surg. 2013;257(1):108–13.
5. Platell C, Barwood N, Dorfmann G, Makin G. The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Dis. 2007;9(1):71-9.
6. Kang CY, Halabi WJ, Chaudhry OO, Nguyen V, Pigazzi A, Carmichael JC, et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer. Arch Surg. 2013;148(1):65-71.
7. Singh PP, Zeng ISL, Srinivasa S, Lemanu DP, Connolly AB, Hill AG. Systematic review and meta-analysis of use of serum C-reactive protein levels to predict anastomotic leak after colorectal surgery. Br J Surg. 2014;101(4):339–46.
8. Sammour T, Kahokehr A, Zargar-Shoshtari K, Hill AG. A prospective case-control study of the local and systemic cytokine response after laparoscopic versus open colonic surgery. J Surg Res. 2012; 173(2):278–85.
9. Chuang D, Paddison JS, Booth RJ, Hill AG. Differential production of cytokines following colorectal surgery. ANZ J Surg. 2006;76(9): 821–4.
10. Garnacho-Montero J, Huici-Moreno MJ, Gutiérrez-Pizarraya A, López I, Márquez-Vácaro J, Macher H, et al. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. Crit Care. 2014;18(3):R116.
11. Straatman J, Harmsen AMK, Cuesta MA, Berkhof J, Jansma EP, van der Peet DL. Predictive value of C-reactive protein for major complications after major abdominal surgery: a systematic review and pooled-analysis. PLoS One. 2015;10(7):e0132995.
12. Almeida AB, Faria G, Moreira H, Pinto-de-Sousa J, Correia-da-Silva P, Maia JC. Elevated serum C-reactive protein as a predictive factor for anastomotic leakage in colorectal surgery. Int J Surg. 2012;10(2):87–91.
13. Giaccaglia V, Salvi PF, Antonelli MS, Nigri G, Pirozzi F, Casagranda B, et al. Procalcitonin reveals early dehiscence in colorectal surgery. the PREDICS study. Ann Surg. 2016;263(5):967-72.
14. Giaccaglia V, Salvi PF, Cunsolo G V., Sparagna A, Antonelli MS, Nigri G, et al. Procalcitonin, as an early biomarker of colorectal anastomotic leak, facilitates enhanced recovery after surgery. J Crit Care. 2014;29(4):528–32.
15. Reisinger KW, Poeze M, Hulsewé KWE, Van Acker BA, Van Bijnen AA, Hoofwijk AGM, et al. Accurate prediction of anastomotic leakage after colorectal surgery using plasma markers for intestinal damage and inflammation. J Am Coll Surg. 2014;219(4):744–51.
16. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535.
17. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011; 155(8): 529-36.
18. Faraone S V. Interpreting estimates of treatment effects: Implications for managed care. P T. 2008;33(12):700.
19. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-60.
20. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. Evid Based Ment Heal. 2019; 22(4):153–60.
21. Doebler P. mada: Meta-Analysis of Diagnostic Accuracy. 2020 [cited 2021 Jul 20]. Available from: https://cran.r-project.org/ package =mada
22. Helsdingen CP van, Jongen ACHM, Jonge WJ de, Bouvy ND, Derikx JPM. Consensus on the definition of colorectal anastomotic leakage: A modified Delphi study. World J Gastroenterol. 2020;26(23): 3293–303.
23. Stephensen BD, Reid F, Shaikh S, Carroll R, Smith SR, Pockney P. C-reactive protein trajectory to predict colorectal anastomotic leak: PREDICT Study. Br J Surg. 2020;107(13):1832-1837.
24. Pantoja Pachajoa DA, Gielis M, Palacios Huatuco RM, Benitez MN, Avila MN, Doniquian AM, et al. Neutrophil-to-lymphocyte ratio vs C-reactive protein as early predictors of anastomotic leakage after colorectal surgery: A retrospective cohort study. Ann Med Surg. 2021;64:102201.
25. Messias BA, Botelho RV, Saad SS, Mocchetti ER, Turke KC, Waisberg J. Serum C-reactive protein is a useful marker to exclude anastomotic leakage after colorectal surgery. Sci Rep. 2020; 10(1):1687.
26. Jin D, Chen L. Early prediction of anastomotic leakage after laparoscopic rectal surgery using creactive protein. Medicine (Baltimore). 2021;100(22):e26196.
27. Baeza-Murcia M, Valero-Navarro G, Pellicer-Franco E, Soria-Aledo V, Mengual-Ballester M, Garcia-Marin JA, et al. Early diagnosis of anastomotic leakage in colorectal surgery: prospective observational study of the utility of inflammatory markers and determination of pathological levels. Updates Surg. 2021;73(6):2103-2111.
28. Garcia-Granero A, Frasson M, Flor-Lorente B, Blanco F, Puga R, Carratalá A, et al. Procalcitonin and C-reactive protein as early predictors of anastomotic leak in colorectal surgery: a prospective observational study. Dis Colon Rectum. 2013;56(4):475-83.
29. Ortega-Deballon P, Radais F, Facy O, D'Athis P, Masson D, Charles PE, et al. C-reactive protein is an early predictor of septic complications after elective colorectal surgery. World J Surg. 2010;34(4): 808–14.
30. Lagoutte N, Facy O, Ravoire A, Chalumeau C, Jonval L, Rat P, et al. C-reactive protein and procalcitonin for the early detection of anastomotic leakage after elective colorectal surgery: pilot study in 100 patients. J Visc Surg. 2012;149(5):e345-9.
31. Kostić Z, Panišić M, Milev B, Mijušković Z, Slavković D, Ignjatović M. Diagnostic value of serial measurement of c-reactive protein in serum and matrix metalloproteinase-9 in drainage fluid in the detection of infectious complications and anastomotic leakage in patients with colorectal resection. Vojnosanit Pregl. 2015;72(10): 889-98.
32. Pantel HJ, Jasak LJ, Ricciardi R, Marcello PW, Roberts PL, Schoetz DJ, et al. Should they stay or should they go? the utility of C-reactive protein in predicting readmission and anastomotic leak after colorectal resection. Dis Colon Rectum. 2019;62(2):241–7.
33. The Italian ColoRectal Anastomotic Leakage (iCral) Study Group. Anastomotic leakage after elective colorectal surgery: a prospective multicentre observational study on use of the Dutch leakage score, serum procalcitonin and serum C-reactive protein for diagnosis. BJS open. 2020;4(3):499–507.
34. Scepanovic MS, Kovacevic B, Cijan V, Antic A, Petrovic Z, Asceric R, et al. C-reactive protein as an early predictor for anastomotic leakage in elective abdominal surgery. Tech Coloproctol. 2013;17(5):541–7.
35. Su'a BU, Mikaere HL, Rahiri JL, Bissett IB, Hill AG. Systematic review of the role of biomarkers in diagnosing anastomotic leakage following colorectal surgery. Br J Surg. 2017;104(5):503–12.

APPENDIX 3

- **Nuno Rama,** Diana Parente, Cândida G. Silva, Miguel Neves, Nuno Figueiredo, Paulo Alves, Paulo Clara, Sandra Amado, Óscar Lourenço, Maria Pedro Guarino, Anabela Rocha, Fernando Castro-Poças, João Pimentel.

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ORIGINAL PAPER

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Anastomotic Leak in Colorectal Cancer Surgery: From Diagnosis to Management or Failure - A Retrospective Cohort Study

Nuno Rama[1,2,3*], Diana Parente[1], Cândida G. Silva[3], Miguel Neves[1], Nuno Figueiredo[4], Paulo Alves[1], Paulo Clara[1], Sandra Amado[1], Óscar Lourenço[5], Maria Pedro Guarino[3], Anabela Rocha[2,6], Fernando Castro-Poças[2,7], João Pimentel[8,9]

[1]Colorectal Cancer Centre, Leiria Hospital Centre (CHL), Leiria, Portugal
[2]Abel Salazar Biomedical Institute (ICBAS), University of Oporto, Oporto, Portugal
[3]Center for Innovative Care and Health Technology (ciTechCare), Polytechnic of Leiria, Portugal
[4]Champalimaud Foundation, Lisbon, Portugal
[5]Faculty of Economics, University of Coimbra, Coimbra, Portugal
[6]Colorectal Centre, Oporto Hospitalar Centre, Oporto, Portugal
[7]Department of Gastroenterology, Oporto Hospitalar Centre, Oporto, Portugal
[8]Colorectal Centre, Coimbra University Hospital Centre, Coimbra, Portugal
[9]Faculty of Medicine, University of Coimbra, Coimbra, Portugal

***Corresponding author:**
Nuno José Gomes Rama, MD
ciTechCare - Campus 5
Rua de Santo André, nº 66-68
2410-541, Leiria, Portugal
Tel: 00351967547698
E-mail: ramanuno@gmail.com

Abbreviations:
AL - Anastomotic Leak.
CR – ColoRectal.
CRC - ColoRectal Cancer.
CT - Computed Tomography.
DULK - Dutch leakage score.
DIACOLE - Diagnostic Leakage score.
FTR - Failure-to-Rescue.
ICU - Intensive Care Unit.
ISGRC - International Study Group of Rectal Cancer.
LOHS - Length of Hospital Stay.

ABSTRACT

Background: Anastomotic leakage (AL) after colorectal resections is a common surgical experience and the most frequent major adverse outcome. Early recognition of AL is critical to reduce mortality. We aim to evaluate the incidence, diagnostic criteria, morbidity, and mortality related with AL.
Methods: This is a cohort, descriptive retrospective, single-centred study of consecutive patients who underwent surgery with a colorectal anastomosis for colorectal cancer, over a 4-year period (2013-2016).
Results: From 2013 to 2016, a total of 480 patients were included. A total of 37 (7.7%) had an anastomotic leakage. AL was diagnosed after 6.8 days in average, most frequently on day 5. 25 out of the 37 patients were diagnosed based on clinical criteria, and the remaining had a CT scan imaging. Clavien-Dindo grade III and IV complications was significantly higher in the AL group (70.2 vs. 7.7%, $p<0.0005$). Mortality was higher in the leakage group (21.6% vs. 4.7%, $p< 0.0005$).
Conclusions: In this study, most patients were diagnosed early based on clinical criteria, and imaging studies were associated with a significant delay in diagnosis. Leakage group had higher morbidity, mortality and rate of reoperations. Early reoperation may have a positive impact in Failure-to-Rescue rate reduction, but additional prospective studies are needed.
Key words: failure-to-rescue, colorectal, surgery, anastomotic leak, mortality

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INTRODUCTION

Colorectal cancer (CRC) remains a public health issue worldwide, ranking third in leading causes of death from cancer in high income countries (1,2).

Surgery is usually required for CRC management, despite significant morbidity and mortality (3,4). Anastomotic leak (AL), a major complication, is not only associated with frequent reoperation, increased length of hospital stay (LOHS) and health-care costs, but also with a higher mortality risk. For AL survivors, an adverse impact on their quality of life is observed (3,5). Incidence of AL may vary from 0.5% to 21% (5-9), depending on the location of the anastomosis, patient co-morbidity profile, pre-operative treatment, and institutional experience (10,11).

Nonspecific signs and symptoms often precede the acute and rapid clinical deterioration of a patient with AL. Once late diagnosis and management increase the likelihood of an undesirable outcome, timely diagnosis is crucial. In daily practice several biomarkers and scores are used for supporting an appropriate clinical decision, that can prevent severe sepsis and death (12-14).

Prevention and treatment of AL have received attention in the last decades. Silber et al. (1992) introduced the Failure-to-Rescue (FTR) concept which reflects the estimated mortality rate in the group of patients who developed a specific postoperative complication (15). FTR differs among distinct institutions and suggests that different therapeutic strategies can influence the patient's survival being useful for institutional benchmarking (16,17). Therefore, as performance indicators for colorectal (CR) surgery, we should not only consider absolute mortality or AL ratios, but also the proportion of patients who died due to a specific complication (15, 18). The main objective of this study is to evaluate the incidence and diagnostic criteria of AL in our cohort, and secondly to assess morbidity, mortality (FTR) and long-term survival impact.

METHODS

Study design and ethics

Retrospective descriptive cohort study, approved by the Local Institutional Ethical Committee, including consecutive patients, who underwent CR resection with anastomosis for CRC from January 2013 to December 2016. All patients were managed in a non-academic Colorectal Referral Centre, which serves an area of 500,000 inhabitants.

The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), the official system of clinical coding in Portugal, was used to classify all patients. The follow-up ended in December 2018 or with death of the patient.

Definitions

Anastomotic leak was confirmed by the presence of one of the following: postoperative peritonitis found at reoperation, faecaloid drainage and presence of air or fluid collection in the anastomotic region on Computed Tomography (CT).

We differentiated two scenarios considering the timing of AL diagnosis: 1) in the same hospital admission; 2) diagnosed after the discharge (deferred AL). Time to AL detection was measured as the number of days between the index operation and diagnosis, according to the criteria. Retrospectively, AL was graded applying the definition and severity grading system developed by the International Study Group of Rectal Cancer (ISGRC) (13).

According to the AL management options, we considered two groups: "Salvage group", composed by patients managed with preservation of bowel continuity with anastomosis repair/refashion and covering stoma; and the "Anastomotic takedown group", when the creation of an end colostomy or ileostomy was necessary.

Surgical approach of the index procedure was divided into three groups: laparoscopy, laparotomy, and conversions (from laparoscopy to open surgery), and LOHS included the second admission, if caused by AL-related complications. Exitus (death) was counted within 30 days of index surgery. Stoma was considered as permanent if it was present at the end of follow-up period.

Exclusion criteria

The following groups of patients were excluded from this study: a) under 18 years old; b) pregnant women; c) mentally disabled; d) under 3 months of follow-up; e) missing data; f) with no anastomosis; g) stoma reversal operation; h) ileo-pouch-anal anastomosis procedures and, i) reoperations.

Included variables

Patient-related demographics, preoperative, intra-operative, and pathologic data were collected from institutional database (SClínico Hospitalar®). Postoperative variables including complications, LOHS, reoperations, intensive care unit (ICU) admissions, death and 30-day readmissions or mortality were also registered.

Statistical analysis

For data analysis, we used descriptive statistics, mean or median, according to the characteristics of the

interest variables. To analyze survival time variables, we used the Kaplan-Meier estimator. Equality of means or proportions between groups were assessed. A t-test was applied to continuous variables. Survival experience was assessed by the Gehan-Breslow-Wilcoxon test (IBM SPSS Statistics version 27.0).

RESULTS

From January 2013 to December 2016, 480 out of 915 patients met the inclusion criteria (*figure 1*), all with CRC and operated in the Colorectal Unit at the Leiria Central Hospital. We excluded procedures for benign disease (n=243; 26.6%), without anastomosis (n=72; 7.9%) and for stoma closure (n=65; 7.1%). Pouch surgery, reintervention or small bowel resection were also not considered.

This cohort (N = 480) is composed mostly by men (n= 287; 59.8%), with colon cancer (n=353; 73,5%) and a mean age of 70.4 ± 12.57 years. Thirty-seven patients developed AL (7.7%) and the rate decreased gradually each year, from 9.1% in 2013 to 5% in 2016 (*figure 2*). Anastomotic leak was more frequent in men (n=26; 70.3%), left colectomy and proctectomy (n=25; 67.5%) and in the laparotomic approach (n=13; 35.1%) or conversion (n=5; 13.5%). Clinical characteristics and different surgical approaches are summarized in *tables 1* and *2*.

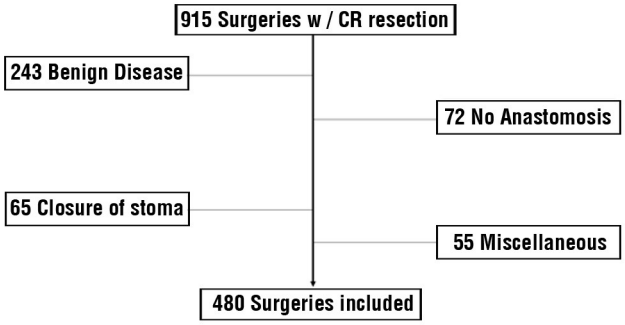

Figure 1 – Flow diagram of patients with inclusion and exclusion criteria

Table 1 – Cohort demographic and clinical characteristics (Leak vs. No leak groups)

	No Anastomotic Leakage (No AL) (N=443; 92.3%)	Anastomotic Leakage (AL) (N=37; 7.7%)	P value (95% CI)
Age (Mean ±SD)	70.25 ± 12.61	72.1 ± 12.05	0.390 (-2.4 to 6.1)
Sex (M/F)	261 (58.9%) /182 (41.1%)	26 (70.3%) / 11 (29.7%)	0.175 (-5.3 to 24.5)
ASA Score			
I – II	270 (60.9%)	24 (64.9%)	0.632 (-12.7 to 18.1)
III – IV	173 (39.1%)	13 (35.1%)	
Stage			
I	148 (33.4%)	9 (24.4%)	0.263 (-7.4 to 20.9)
II	127 (28.7%)	13 (35.1%)	0.411 (-7.6 to 22.9)
III	126 (28.4%)	12 (32.4%)	0.606 (-9.5 to 20.6)
IV	42 (9.5%)	3 (8.1%)	
Comorbidity			
<2	350 (79%)	32 (86.5%)	0,226 (-6.4 to 17.1)
2 or more	93 (21%)	5 (13.5%)	

Table 2 - Cohort demographic and clinical characteristics (Leak vs. No leak groups)

	No Anastomotic Leakage (No AL) (N=443; 92.3%)	Anastomotic Leakage (AL) (N=37; 7.7%)	P value (95% CI)
Timing			
Elective	363 (81.9%)	30 (81.1%)	0.909 (-9.4 to 16.4)
Urgent	80 (18.1%)	7 (18.9%)	
Approach			
Open	97 (21.9%)	13 (35.1%)	0.067 (-0.7 to 16.7)
Laparoscopic	333 (75.2%)	19 (51.4%)	0.002 (8.0 to 39.7)
Conversion	13 (2.9%)	5 (13.5%)	
Procedures			
Right	202 (45.6%)	10 (27.0%)	0.003 (2.0 to 15.9)
Left	128 (28.9%)	13 (35.1%)	0.427 (-7.1 to 22.8)
Rectum	84 (19.0%)	12(32.4%)	0.050 (0.0 to 29.9)
Others	28 (6,5%)	2 (5.5%)	
Covering Stoma			
Yes	53 (11.9%)	10 (27.1%)	0.008 (3.1 to 31.4)
No	390 (88.1%)	27 (72.9%)	

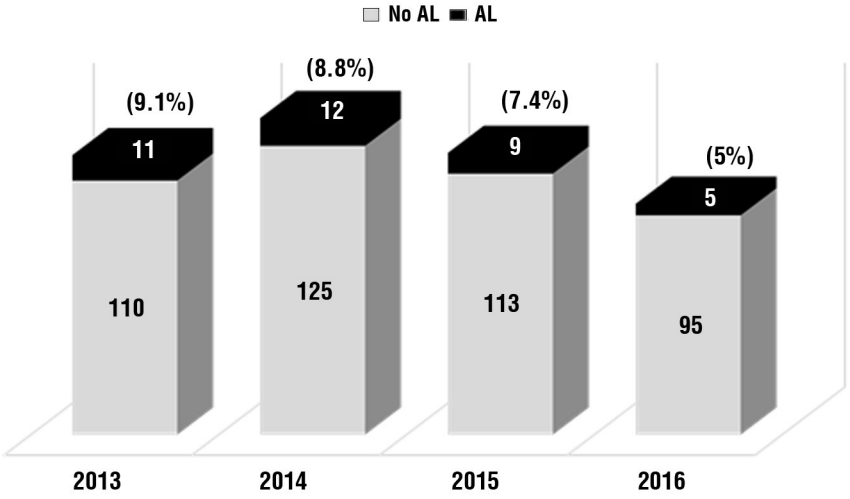

Figure 2 - Anastomotic leakage rate distribution, per year

Thirty-two patients (86.5%) had AL diagnosis at the first hospital admission and five had the diagnosis deferred. Mean time for AL detection was 6.8 days (day 2 to 17) and was most common on day 5. Twenty-five patients were diagnosed based on clinical criteria, including biomarkers (leukocyte and C-Reactive Protein), and in these sub-group, the diagnosis was made earlier (5.6 ± 2.1 days). These patients had a shorter LOHS (26.1 vs. 40.9 days), which is not statistically significant [(p=0.073; 95% CI (-1.0 to 34)]. The remaining twelve required additional exams, such as abdomen-pelvic CT scan and/or lower GI endoscopy. Three out of 12 AL patients scanned did not show unequivocal signs in CT scan. In this subgroup, diagnosis was reached later (8.5 ± 4.2 days), with statistical significance [(p=0.004; 95% CI (0.7 to 4.8)] – *tables 3* and *4*.

Six patients were managed non-operatively and four needed an image-guided drainage of intra-abdominal collections (one by transrectal access). Twenty-four out of 31 patients (64.8%) were submitted to anastomotic takedown and Hartmann's procedure, and six (16.2%) underwent refashion of the anastomosis with covering stoma. Twelve (32.4%) out of the 37 patients required ICU admission and fifteen (40.5%) received parenteral nutrition. Over 34.9 months of follow up, 20 out of 37 patients (54.1%) maintained bowel continuity, including preserved primary or refashioned anastomosis (n=10; 27%) and Hartmann reversal status (n=10; 27%). The main causes for not closing the stoma were patient refusal and morbidity (n=10) and cancer dissemination (n=4). The causes for secondary anastomotic failure were stenosis (n=2) and local recurrence (n=1) - *figure 3*.

Concerning morbidity, the rate of complications was significantly higher in the AL-patient group. Based on

Table 3 - Timing of AL diagnosis

AL DIAGNOSIS	
TIMING (Days):	
• Mean (SD)	6.8 (2.2)
• Median	6
• Mode	5
1ST EPISODE - N (%)	32 (86.5%)
DEFERRED (Readmission) - N (%)	5 / (13.5%)

Table 4 - Methods of AL diagnosis

	Clinic (biomarkers/reoperation) (N=25; 64.9%)	Others (CT scan ± endoscopy) (N=12; 35.1%)	P value (95% CI)
TIMING (days)			
Mean±SD	5.7 ± 2,1	8.5 ± 4.2	0.004
Median	5	8	(0.7 to 4.8)
Max	7	21	
Min	3	4	
LOHS – Days			
Mean±SD	26.1 ± 10.9	40.9 ± 41.5	0.073
Median	21	38	(-1.0 to 34)
Max	97	165	
Min	15	23	

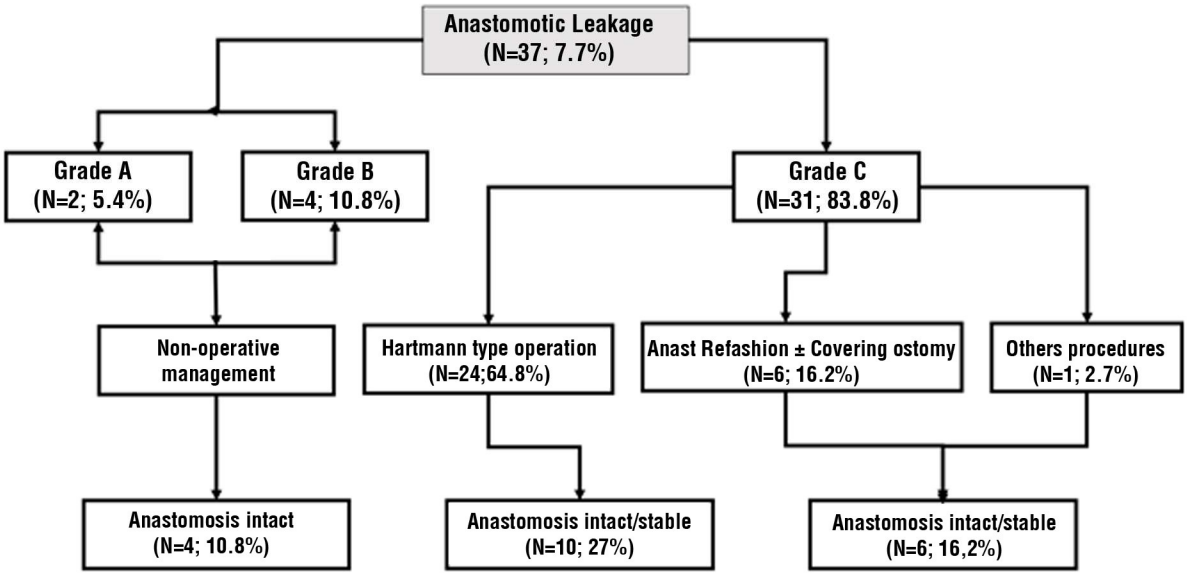

Figure 3 - Management and follow-up of AL patients (according to ISGRC severity grading system)

the Clavien-Dindo classification, 26 out of the 37 patients (70.2%) had grade III and IV complications, vs. 34 patients in the group who had no AL (7.7%) (*table 5*). Mean LOHS was significantly higher in the AL cohort [(10.5 vs. 31.3 days - < 0.0005 (14.9 to 21.9)].

In the first year, need for reoperation and 30-day mortality were more significant in AL-patient group, 83.8% vs. 6.1% (p< 0.0005; 95%CI 6 to 89.4) and 21.6% vs. 4.7% (p< 0.0005; 95%CI 8.1 to 32.9), respectively. Considering the elective cohort, 30-day mortality rate was higher in the AL group (13.5% vs. 1.8%). Furthermore, mortality was lower in the second biennium (2015-16) in both groups (with and without AL), 27.2% vs. 15.5% (p=0.417; 95%CI -17.6 to 34.9) and 6.1% vs. 2.3% (p=0.049; 95%CI – 0.1 to 7.8), respectively.

Concerning the impact of AL on the overall survival (OS), with an average follow-up of 47.4 ± 23.2 months, patients without AL had a 5-year OS (in all stages) of 63.3%, versus 52.9% in the AL-patients group. Comparing Kaplan-Meier's survival curves, the Gehan-Breslow-Wilcoxon test shown statistical significance in OS between the groups (50 ± 6.6 vs. 62.4 ± 1.5 months; p=0.009) – *figure 4*.

Regarding the survival analysis, the 5-year OS was 55.6, 50, 63.6 and 0% for the sub- group with AL complications, versus 76.3, 69.7, 59.7 and 10.5% in the sub-group without AL. Comparing Kaplan-Meier's survival curves, the Gehan-Breslow-Wilcoxon test shown significant differences in survival time between the two groups (p=0.005), at the different stages (*figure 5*).

Table 5 - Postoperative complications according to the Clavien-Dindo classification (Leak vs. no leak group)

	No Anastomotic Leakage (No AL) (N=443; 92.3%)	Anastomotic Leakage (AL) (N=37; 7.7%)	P value (95% CI)
LOHS – days			
Mean (range)	10.5 (3-138)	31.3 (15 -165)	< 0.0005 (14.9 to 21.9)
Median	7	27	
MORBIDITY – n (%)			
Clavien-Dindo I	39 (8.8 %)	2 (5.4%)	0.395 (-5.8 to 9)
Clavien-Dindo II	47 (10.6 %)	1 (2.7%)	0.059 (-0.3 to 11.3)
Clavien-Dindo III	16 (3.6%)	18 (48.6 %)	< 0.0005 (30.2 to 59.5)
Clavien-Dindo IV	18 (4.1 %)	8 (21.6%)	< 0.0005 (8.5 to 34.5)
REOPERATION – n (%)			
(W/in 12 months)	27 (6.1%)	31 (83.8%)	< 0.0005 (6 to 89.4)
30-DAY MORTALITY – n (%)			
Elective	8 (1.8 %)	5 (13.5 %)	< 0.0005 (5.1 to 26.9)
Overall	21 (4.7 %)	8 (21.6 %)	< 0.0005 (8.1 to 32.9)
FOLLOW-UP - months	35.7	34.9	0.818 (-4.7 to 3.9)

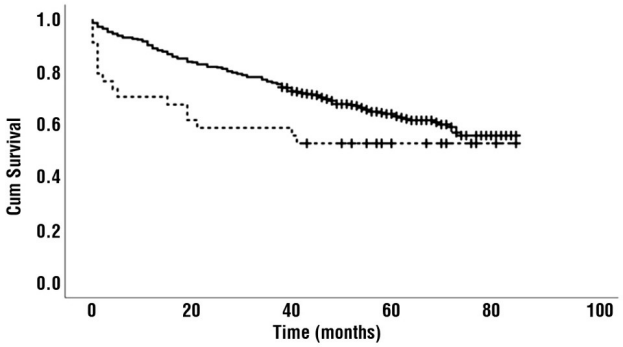

Figure 4 - Kaplan-Meier Overall Survival Curves. Leak group represented as the dashed line and No leak group represented as a straight line. The + symbol represents censored cases

Colon cancer patients who developed AL had a significant lower 5-year OS, 50%, versus 66.3% (p=0.002). This significant difference was not observed in the AL rectal cancer cohort, as the 5-year OS was 55.6% versus 65%, in the no-AL cohort (p>0.05) (*figure 6*).

DISCUSSION

In the literature, AL ranges from 0.5% to 21%, with colon and rectum-adjusted rates of 3–7% and 13–18%, respectively (5, 7-9, 19-22). This is the first retrospective study on this subject in the Portuguese population, and 37 out of 480 patients (7.7%) developed AL. It was higher in left-side anastomosis, in comparison with ileo-colic anastomosis (11.2 vs. 4.7%), decreasing gradually in the second biennium (9.8 to 6.7%). We may correlate this with the increase in surgeon volume, technical and technological progress, among others. Literature highlights this trend, in spite of scarce and controversial evidence (23).

Anastomotic leak may occur in patients without risk factors and non-specific signs often precede rapid and abrupt clinical deterioration. Consequently, early diagnosis is paramount for reducing morbidity and mortality: post-operative clinical assessment is useful but subjective, therefore tools such as the Dutch

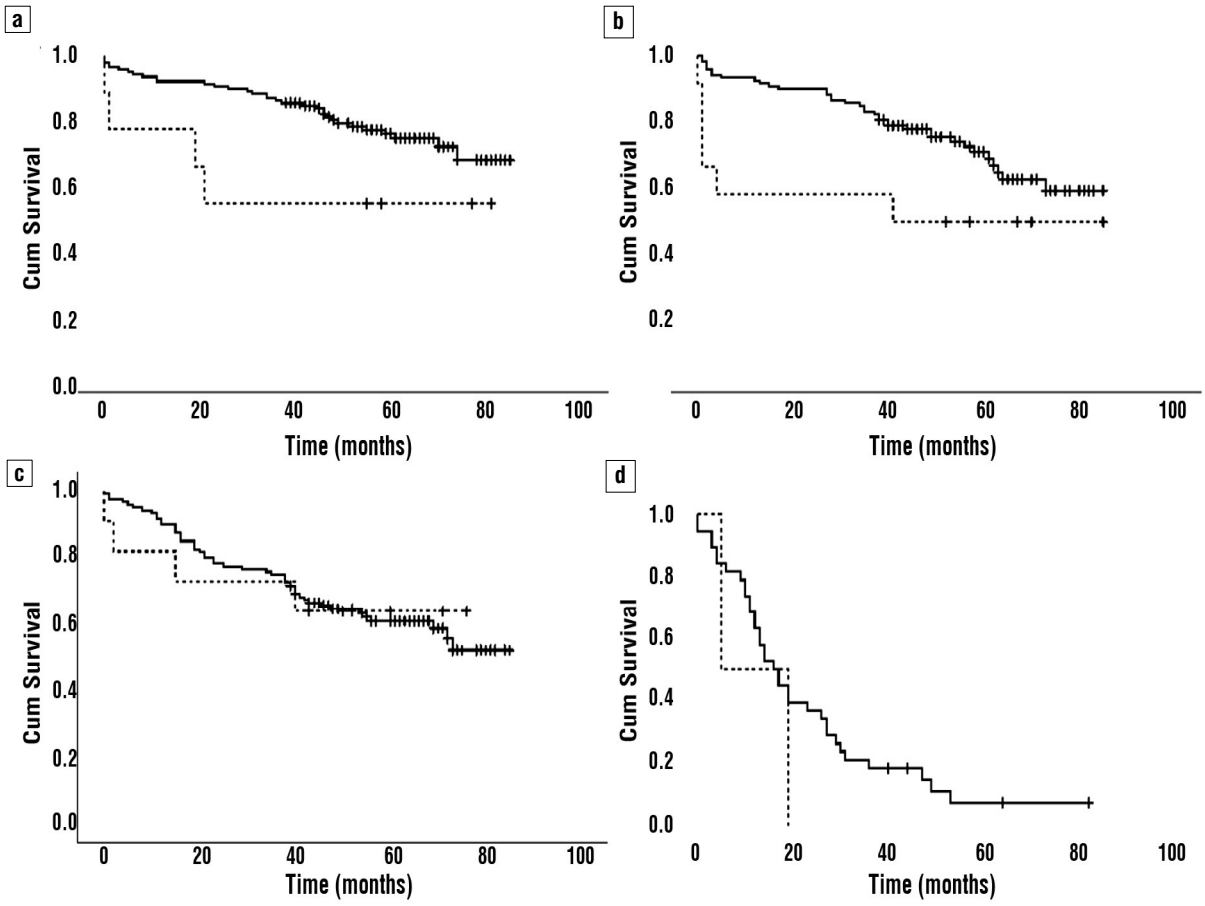

Figure 5- Kaplan-Meier Survival by UICC TNM stage. Panels a. to d. show data for patients in Stages I to IV, respectively. Leak group represented as the dashed line and No leak group represented as a straight line. The + symbol represents censored cases.

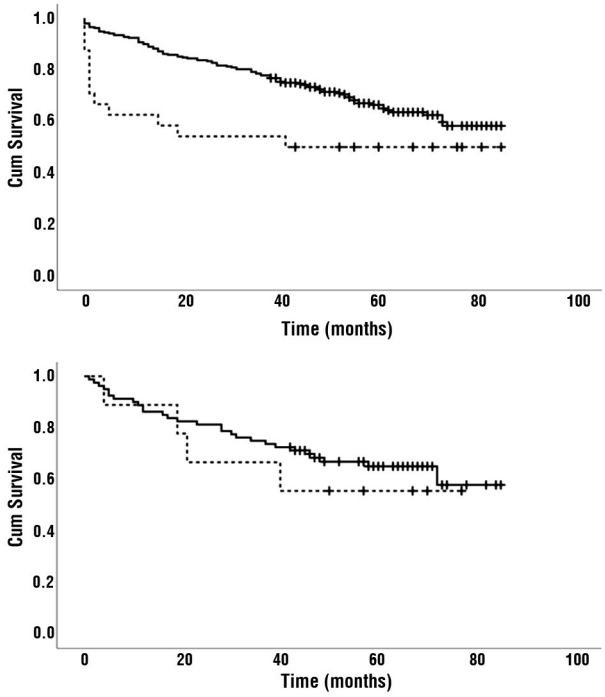

Figure 6 - Kaplan-Meier Survival, by location. Leak group represented as the dashed line and No leak group represented as a straight line. The + symbol represents censored cases

leakage (DULK) or the Diagnostic Leakage (DIACOLE) scores may help selecting patients for additional imaging tests or early reoperation (12,14). In our study, diagnosis was attained mostly at the first hospital admission, more commonly on the fifth postoperative day. Most patients (64.9%) were diagnosed earlier based on clinical criteria. In the remaining patients, diagnosis was complemented with CT scan, with 25% of false negatives but a non-significant delay in diagnosis. In the literature CT scan showed a low sensitivity and accuracy rates, around 60% (24,25). In a recently published study by the iCral group, the original DULK score was shown to be valuable for predicting AL on the second and third days after surgery (22,26,27). Currently we are introducing these predictive tools in daily clinical practice.

High mortality rate was published in large series ranging from 25 to 35%, despite the lower rates presented by Gessler et al. (from 5 to 8.3% at 30 and 90 days, respectively) (22, 25, 28-30). In AL cohort, eight patients (21.6%) died within 30 days, but mortality rate was lower both in elective surgery (13.5%) and in the second biennium (15.5%). This period roughly coincided with the implementation of the CR Unit in the institution. Consequently, FTR should be a useful outcome indicator for assessing the performance of CR surgical teams.

In line with the literature, this study suggests that AL had a negative impact on 5-year OS, excluding the rectal cancer cohort (31-36). However, Mrak et al. and Jörgren et al. did not find such negative correlation in the rectal cancer cohort (37,38), as observed in our series. Heterogeneous samples including different post-operative complications or tumour location may explain these controversial results. In a recent metanalysis with 18 cohort studies and 69,047 patients submitted to colectomy, AL didn't increase local or distant recurrence, but reduced OS (RR 0.85, 95% CI 0.77–0.94)(34).

The limitations of this study depend on its retrospective nature, in particular the quality of records. The size of the sample is another weakness that constrains the statistical strength of the analysis. The strengths are related to the quality of the sample, an unselected and consecutive cohort of patients, from a regional representative CR Unit. Finally, the current study provides information and knowledge that reinforce and improve the informed consent and supports providers in the perioperative decision-making process.

CONCLUSIONS

In this original study in the Portuguese population, two thirds of AL patients were diagnosed earlier based on clinical criteria and AL cohort had higher morbidity and mortality (78.3% and 21.6%, respectively), longer LOHS and rate of reoperations. Both systematic use of scores for AL diagnosis and early re-operation may have a positive impact on FTR rate reduction. This is a useful metric to evaluate different management options, to determine their impact on survival, and to perform institutional benchmarking. Further prospective studies will be useful to obtain added-value evidence in this topic.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

For performing this study ethical approval was obtained.

REFERENCES

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-691. Epub 2016 Jan 27.
2. Marley AR, Nan H. Epidemiology of colorectal cancer. Int J Mol

Epidemiol Genet. 2016;7(3):105-14.
3. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intra-operative and post-operative risk factors for colorectal anastomotic leaks. Br J Surg. 2015;102(5):462-79.
4. Angelucci GP, Sinibaldi G, Orsaria P, Arcudi C, Colizza S. Morbidity and Mortality after Colorectal Surgery for Cancer. Surgical Science. 2013;4(11):520-524.
5. Trencheva K, Morrissey KP, Wells M, Mancuso CA, Lee SW, Sonoda T, et al. Identifying important predictors for anastomotic leak after colon and rectal resection: prospective study on 616 patients. Ann Surg. 2013;257(1):108-13.
6. Veldkamp R, Kuhry E, Hop WCJ, Jeekel J, Kazemier G, Jaap Bonjer H, et al. Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. Lancet Oncol. 2005; 6(7):477-84.
7. Stormark K, Krarup PM, Sjövall A, Søreide K, Kvaløy JT, Nordholm-Carstensen A, et al. Anastomotic leak after surgery for colon cancer and effect on long-term survival. Colorectal Dis. 2020; 22(9):1108-1118.
8. Boccola MA, Buettner PG, Rozen WM, Siu SK, Stevenson ARL, Stitz R, et al. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. World J Surg. 2011;35(1):186-95.
9. Iancu C, Mocan LC, Todea-Iancu D, Mocan T, Acalovschi I, Ionescu D, et al. Host-related predictive factors for anastomotic leakage following large bowel resections for colorectal cancer. J Gastrointestin Liver Dis. 2008;17(3):299-303.
10. Iversen LH, Iversen LH, Harling H, Laurberg S, Wille-Jørgensen P. Influence of caseload and surgical speciality on outcome following surgery for colorectal cancer: a review of evidence. Part 1: short-term outcome. Colorectal Dis. 2007;9(1):28-37.
11. Archampong D, Borowski D, Wille-Jørgensen P, Iversen LH. Workload and surgeon´s specialty for outcome after colorectal cancer surgery. Cochrane Database Syst Rev. 2012;(3): CD005391.
12. den Dulk M, Witvliet MJ, Kortram K, Neijenhuis PA, de Hingh IH, Engel AF, et al. The DULK (Dutch leakage) and modified DULK score compared: actively seek the leak. Colorectal Dis. 2013;15(9): e528-33.
13. Lagoutte N, Facy O, Ravoire A, Chalumeau C, Jonval L, Rat P, et al. C-reactive protein and procalcitonin for the early detection of anastomotic leakage after elective colorectal surgery: pilot study in 100 patients. J Visc Surg. 2012;149(5):e345-9.
14. Rojas-Machado SA, Romero M, Arroyo A, Rojas-Machado A, López J, Calpena R. Anastomic leak in colorectal cancer surgery. Development of a diagnostic index (DIACOLE). Int J Surg. 2016;27: 92-98.
15. Silber JH, Williams SV, Krakauer H, Schwartz JS. Hospital and patient characteristics associated with death after surgery. A study of adverse occurrence and failure to rescue. Med Care. 1992;30(7): 615-29.
16. Almoudaris AM, Burns EM, Mamidanna R, Bottle A, Aylin P, Vincent C, et al. Value of failure to rescue as a marker of the standard of care following reoperation for complications after colorectal resection. Br J Surg. 2011;98(12):1775-83.
17. Johnston MJ, Arora S, King D, Bouras G, Almoudaris AM, Davis R, et al. A systematic review to identify the factors that affect failure to rescue and escalation of care in surgery. Surgery. 2015;157(4): 752-63.
18. Henneman D, van Leersum NJ, Ten Berge M, Snijders HS, Fiocco M, Wiggers T, et al. Failure-to-rescue after colorectal cancer surgery and the association with three structural hospital factors. Ann Surg Oncol. 2013;20(11):3370-6.
19. Cousin F, Ortega-Deballon P, Bourredjem A, Doussot A, Giaccaglia V, Fournel I. Diagnostic Accuracy of Procalcitonin and C-reactive Protein for the Early Diagnosis of Intra-abdominal Infection After Elective Colorectal Surgery: A Meta-analysis. Ann Surg. 2016; 264(2):252-6.
20. Facy O, Paquette B, Orry D, Binquet C, Masson D, Bouvier A, et al. Diagnostic Accuracy of Inflammatory Markers As Early Predictors of Infection After Elective Colorectal Surgery: Results From the IMACORS Study. Ann Surg. 2016;263(5):961-6.
21. Pommergaard HC, Gessler B, Burcharth J, Angenete E, Haglind E, Rosenberg J. Preoperative risk factors for anastomotic leakage after resection for colorectal cancer: a systematic review and meta-analysis. Colorectal Dis. 2014;16(9):662-71.
22. Italian ColoRectal Anastomotic Leakage Study. Anastomotic leakage after elective colorectal surgery: a prospective multicentre observational study on use of the Dutch leakage score, serum procalcitonin and serum C-reactive protein for diagnosis. BJS Open. 2020;4(3): 499-507.
23. Morche J, Mathes T, Pieper D. Relationship between surgeon volume and outcomes: a systematic review of systematic reviews. Syst Rev. 2016;5(1):204.
24. Hirst NA, Tiernan JP, Millner PA, Jayne DG. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. Colorectal Dis. 2014;16(2):95-109.
25. Gessler B, Eriksson O, Angenete E. Diagnosis, treatment, and consequences of anastomotic leakage in colorectal surgery. Int J Colorectal Dis. 2017;32(4):549-556.
26. den Dulk M, Noter SL, Hendriks ER, Brouwers MAM, van der Vlies CH, Oostenbroek RJ, et al. Improved diagnosis and treatment of anastomotic leakage after colorectal surgery. Eur J Surg Oncol. 2009;35(4):420-6. Epub 2008 Jun 27.
27. Martin G, Dupré A, Mulliez A, Prunel F, Slim K, Pezet D. Validation of a score for the early diagnosis of anastomotic leakage following elective colorectal surgery. J Visc Surg. 2015;152(1):5-10.
28. Fraccalvieri D, Biondo S, Saez J, Millan M, Kreisler E, Golda T, et al. Management of colorectal anastomotic leakage: differences between salvage and anastomotic takedown. Am J Surg. 2012;204(5):671-6. Epub 2011 May 19.
29. Alves A, Panis Y, Pocard M, Regimbeau JM, Valleur P. Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg. 1999;189(6):554-9.
30. Eriksen JR, Ovesen H, Gögenur I. Short- and long-term outcomes after colorectal anastomotic leakage is affected by surgical approach at reoperation. Int J Colorectal Dis. 2018;33(8):1097-1105.
31. Khuri SF, Daley J, Henderson W, Hur K, Hossain M, Soybel D, et al. Relation of surgical volume to outcome in eight common operations: results from the VA National Surgical Quality Improvement Program. Ann Surg. 1999;230(3):414-29; discussion 429-32.
32. Jannasch O, Klinge T, Otto R, Chiapponi C, Udelnow A, Lippert H, et al. Risk factors, short and long term outcome of anastomotic leaks in rectal cancer. Oncotarget. 2015;6(34):36884-93.
33. Law WL, Kwok Choi H, Man Lee Y, Wc Ho J. The impact of post-operative complications on long-term outcomes following curative resection for colorectal cancer. Ann Surg Oncol. 2007;14(9): 2559-66.
34. Bashir Mohamed K, Haangard Hansen C, Krarup PM, Fransgţrd T, Tvilling Madsen M, Gögenur I. The impact of anastomotic leakage on recurrence and long-term survival in patients with colonic cancer: A systematic review and meta-analysis. Eur J Surg Oncol. 2020; 46(3): 439-447.
35. Bertelsen CA, Andreasen AH, Jørgensen T, Harling H, Danish Colorectal Cancer Group. Anastomotic leakage after curative anterior resection for rectal cancer: short and long-term outcome. Colorectal Dis. 2010;12(7 Online):e76-81. Epub 2009 Apr 29.
36. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Ann Surg. 2011;253(5):890-9.
37. Jörgren F, Johansson R, Damber L, Lindmark G. Anastomotic leakage after surgery for rectal cancer: a risk factor for local recurrence, distant metastasis and reduced cancer-specific survival? Colorectal Dis. 2011;13(3):272-83.
38. Mrak K, Eberl T, Laske A, Jagoditsch M, Fritz J, Tschmelitsch J. Impact of postoperative complications on long-term survival after resection for rectal cancer. Dis Colon Rectum. 2013;56(1):20-8.

APPENDIX 2

- Inês Gil, **Nuno Rama**, Diana Parente, Inês Sales, Paulo Alves, Paulo Clara, Sandra Amado, Miguel Coelho and Vitor Faria.

"**Intracorporeal versus Extracorporeal Anastomosis in Laparoscopic Right Colectomy: Short-Term Outcomes"**.

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Intracorporeal versus Extracorporeal Anastomosis in Laparoscopic Right Colectomy: Short-Term Outcomes

Inês Campos Gil[1], Nuno Rama[2], Diana Parente[1], Inês Sales[1], Paulo Alves[2], Paulo Clara[2], Sandra Amado[2], Miguel Coelho[3], Vítor Faria[1]

[1]Serviço de Cirurgia 1, Centro Hospitalar de Leiria, Leiria, Portugal
[2]Unidade de Patologia Colorectal,Centro Hospitalar de Leiria, Leiria, Portugal
[3]Serviço de Cirurgia 2, Centro Hospitalar de Leiria, Leiria, Portugal

Corresponding author:
Inês Campos Gil, MD
Serviço de Cirurgia 1 - Hospital de Santo André, Centro Hospitalar de Leiria. Rua das Olhalvas, 2410-197 Leiria, Portugal
E-mail inesmcgil@gmail.com

ABSTRACT

Background: Recently, there has been a growing enthusiasm in developing new techniques of intracorporeal anastomosis following laparoscopic colectomy, which are more challenging than extracorporeal techniques. However, the evidence is still lacking regarding the outcomes' comparison of both procedures.
Methods: We designed a retrospective study comparing intracorporeal and extracorporeal anastomosis following laparoscopic right colectomy. A total of 115 consecutive patients operated for right colon disease were identified, from September 1st 2014 to May 31st 2017. Patient demographics included age, gender, ASA score, past abdominal surgery, anticoagulant and steroid therapy, Diabetes Mellitus and preoperative diagnosis. The analysed outcomes included length of stay, operative time, blood loss, extraction site, postoperative complications (ileus, anastomotic failure and surgical site infection), reoperation rate, readmission rate and 30-day mortality.
Results: The extracorporeal group included 84 and the intracorporeal group 31 patients. The intracorporeal group had less surgical site infections (3,2% versus 27,4%, $p<0,05$). There were no statistically significant differences in operative time, blood loss, ileus, anastomotic failure or mortality.
Conclusion: Our study reveals similar outcomes for both intra- and extracorporeal anastomosis following laparoscopic right colectomy. Therefore, intracorporeal anastomosis seems to be a feasible and safe technique in the hands of experienced laparoscopic colorectal surgeons.
Key words: laparoscopy, colectomy, surgical anastomosis, retrospective study

BACKGROUND

Nowadays, laparoscopic colectomy is considered a safe and effective surgical technique regarding short- and long-term outcomes, as well as specific oncologic outcomes (1). The feasibility and safety of laparoscopic colon resections have been proven by several trials with high level of evidence, such as COST (2), COLOR (3) and CLASICC (4) trials. The advantages of the laparoscopic approach include reduced intraoperative blood loss, reduced intensity of postoperative

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pain, shorter rates of postoperative ileus, decreased overall morbidity and enhanced recovery.

Intra-corporeal (IA) anastomosis techniques are demanding procedures, when compared to extra-corporeal (EA) techniques; requiring advanced training in order to achieve expertise in laparoscopic manual sutures, and a longer learning curve (5). Nevertheless, the growing enthusiasm about minimally invasive approaches has propelled surgeons to develop totally intra-corporeal anastomotic techniques. Its theoretical advantages are the easier handling of structures, lower risk of mesenteric torsion and the ability to choose the incision site for specimen extraction. However, according to some authors, there are some disadvantages, such as a longer operative time, a higher risk of fecal contamination and a more demanding technique (6).

Although the evidence is vast when it comes to compare laparoscopic surgery with EA and hand-assisted or laparotomic surgery, there are only a few comparative trials that state the feasibility and safety of IA techniques (7, 8). A meta-analysis published in 2014 (9), including 484 patients in six case-control trials, compared IA and EA techniques after laparoscopic right colectomy: this showed some encouraging results in the IA group (faster return of bowel movement, shorter length of stay and better cosmetic outcome). However, there were no statistically significant differences in anastomotic failure or early post-operative morbidity. Another meta-analysis (10) recently published which included 12 comparative non-randomised trials concluded that IA technique after laparoscopic right colectomy for colonic cancer showed less overall post-operative morbidity and shorter length of stay; these differences were even more significant in those trials published after 2012, which suggests better results come with a longer learning curve and higher expertise of surgeons. There were no differences in mortality rates, ileus or anastomotic failure.

During our literature research, we did not find any randomised controlled trials comparing EA and IA, and no papers published in Portugal.

Aims

The two aims of this study are: 1) to assess the feasibility and safety of IA technique after laparoscopic right colectomy; 2) to compare operative and post-operative outcomes of IA and EA techniques after laparoscopic right colectomy.

MATERIAL AND METHODS

We designed a retrospective comparative study to assess short-term outcomes of IA and EA techniques after laparoscopic right colectomy. We made a review of 115 consecutive patients who underwent laparoscopic right colectomy between September 1st 2014 and May 31st 2017 at Centro Hospitalar de Leiria. The procedures were performed by four experienced colorectal laparoscopic surgeons of our Colorectal Unit, and the choice of the anastomosis technique (IA or EA) was left at the discretion of each surgeon. Cases were divided into two groups: those with intracorporeal ileo-colic anastomosis (IA) and those with extracorporeal anastomosis (EA).

Demographic data included age, gender, ASA score, previous abdominal surgery, anticoagulant and steroid therapy, history of diabetes mellitus and pre-operative diagnosis.

Intraoperative variables analysed were operative time, blood loss, site and size of incision for specimen extraction.

The variables for early postoperative (30 days) period were length of hospital stay, postoperative complications (ileus, anastomotic failure, intra-abdominal abscess and surgical site infection), reoperation rate, readmission within 30 days and mortality rate within 30 days. In this study, anastomotic failure was defined as fecal or gas leak originated in the anastomosis and either collected inside the abdominal cavity, or exteriorised through the surgical wound or a surgical drain. Cases of fever, abscess, septicemia, peritonitis and/or multiorganic failure in association with imagiologic evidence (CT scan) were also considered as anastomotic failure.

Surgical Technique

The choice of the anastomosis technique (IA or EA) was left at the surgeon's will. Laparoscopic right colectomy was performed employing medial-to-lateral dissection technique, according to the usual standardized procedure of our Colorectal Unit. An extracorporeal anastomosis was constructed after exteriorization and section of the specimen through a median mini-laparotomy or para-umbilical transverse incision, and then creating a stapled side-to-side antiperistaltic anastomosis with a 80mm stapler (functional end-to-end anastomosis). An intracorporeal anastomosis was constructed by firing a 60 mm endostapler in a side-to-side isoperistaltic fashion, followed by manual closure of the enterotomies

using a single layer of mid-term absorbable braided and coated running suture (*fig. 1-4*).

All the patients included in this study received the same antibioprophylaxis and thromboprophylaxis scheme.

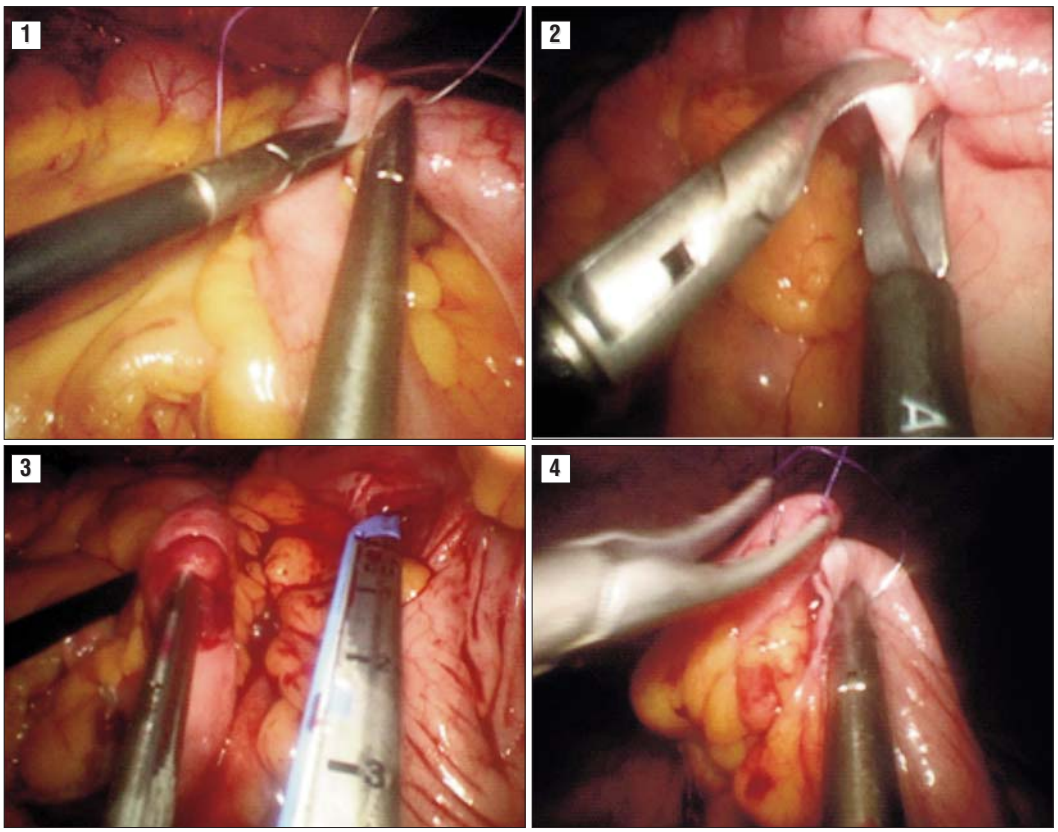
Statistical Analysis

Statistical analysis was performed with the IBM SPSS Statistics v24® software. Demographics and comorbidity data were summarized in *table 1*. The categorical variables are expressed as mean ± standard deviation and continuous variables as n and percentage (%). Statistically significant differences were assessed with t-Student's test for continuous variables and Chi-square or exact Fisher's test for categorical variables. Multivariable analysis was also performed for EA and IA cohorts. Statistical significance was considered for $p < 0,05$, with a confidence interval of 99,5%.

RESULTS

Patient demographics and disease-related characteristics

We reviewed a total of 115 consecutive patients who underwent laparoscopic right colectomy. The EA group included 84 patients and the IA group 31 patients. Mean age was 69 ± 13,2 years for the EA group and 72 ± 12,8 years for the IA group, with similar gender distribution. The majority of patients had a I or II ASA score, with a mean score of 2,38 ± 0,64 for the EA group and 2,52 ± 0,65 for the IA group. In 24,8% of EA group and 16,1% of IA group there was history of previous abdominal surgery. The most frequent pre-operative diagnosis was colonic neoplasm. Mechanic bowel preparation was made in most of the patients of the IA group (90,3%), in contrast with 34,5% of the patients in the EA group; this difference had statistical significance ($p<0,05$, CI 99,5%). In both groups, the



Figures 1 to 4 - Confection of intracorporeal ileocolic anastomosis. After performing the right colectomy with disection in a medial-to-lateral fashion, the specimen is extracted in a organ endobag through a mini-Pfannenstiel incision. Ancorage of bowel in the chosen position for anastomosis with a single reabsorbable suture knot (1). After enterotomy and colotomy incisions (2), introduction of the endostapler for construction of an ileocolic latero-lateral isoperistaltic mechanic anastomosis (3). Closing of the incisions with a manual single layer inverting absorbable suture (4).

Table 1 - Demographic data

Variable	EA (n = 84)	IA (n = 31)	p value
Age (mean ± SD)	69 ± 13,2	72 ± 12,8	NS
Gender (F/M)	38 / 46	14 / 17	NS
ASA score (mean ± SD)	2,38 ± 0,64	2,52 ± 0,65	NS
ASA I or II (n)	52	15	
ASA III (n)	30	15	
ASA IV (n)	2	1	
Previous abdominal surgery	20 (23,8%)	5 (16,1%)	NS
Diabetes Mellitus	18 (21,4%)	7 (22,6%)	NS
Anticoagulation	8 (9,5%)	2 (6,5%)	NS
Steroids	1 (1,2%)	0 (0%)	NS
Preoperative diagnosis			NS
Neoplasm	82 (97,6%)	30 (96,8%)	
Ischaemia	0 (0%)	1 (3,2%)	
Crohn's disease	1 (1,2%)	0 (0%)	
Appendicular plastron	1 (1,2%)	0 (0%)	
Mechanic bowel preparation	29 (34,5%)	28 (90,3%)	p< 0,05
Emergent / Elective surgery	7 / 77 (91,7%)	3 / 28 (90,3%)	NS

majority of patients had elective surgery (91,7% for the EA and 90,3% for the IA group).

Demographics and pre-operative data are summarized in *table 1*.

Operative Outcomes

Intraoperatively we analysed the operative time, estimated blood loss, site and size of surgical incision for specimen extraction; these data are summarized in *table 2*. The mean operative time was similar in both groups (121 ± 27,1 minutes in the IA group versus 125 ± 33,2 minutes in the EA group). The mean estimated blood loss was slightly lower in the IA group (32,9 ± 31,9 mL versus 50,5 ± 58,4mL), although it did not reach statistical significance.

Our study revealed a statistically significant difference in the choice of incision site for specimen extraction, with a clear preference for hypogastric incision in the IA group (90,3%) and mesogastric in the EA group (97,6%) (p<0,05, CI 99,5%). The incision size was similar in both groups.

Short-Term Outcomes and Complications

The analysed variables in the early postoperative period (30 days) were mean length of hospital stay, postoperative complications (ileus, anastomotic failure, intra-abdominal abscess and surgical site infection), and overall mortality. These results are summarized in *table 3*.

The rate of surgical wound infection was significantly lower in the IA group (21,4% vs. 3,2%, p<0,05 and CI 99,5%); moreover, the IA group had a lower rate of abdominal abscess (6% vs. 0%), although with no statistically significant difference for this variable alone.

We also verified a slightly shorter mean length of hospital stay for the IA group (9,3 days ± 5,4 versus 11,3

Table 2 - Intraoperative data. NS – non-significant (p>0,05)

Variable	EA (n = 84)	IA (n = 31)	p value
Mean operative time (minutes)	125 ± 33,2	121 ± 27,1	NS
Mean estimated blood loss (mL)	50,5± 58,4	32,9 ± 31,9	NS
Mean incision size (cm)	5,8	5,2	NS
Incision site Mesogastric	82 (97,6%)	3 (9,7%)	p < 0,05
Hypogastric	2 (2,4%)	28 (90,3%)	p < 0,05

Table 3 - Postoperative outcomes. NS – non-significant (p>0,05)

Variable	EA (n = 84)	IA (n = 31)	p value
Mean length of hospital stay(days)	11,3± 13,3	9,3± 5,4	NS (0.168157)
Postoperative complications			
Ileus (n / %)	17 (20,2%)	5 (16,1%)	NS
Anastomotic failure (n / %)	6 (7,1%)	2 (6,5%)	NS
Intra-abdominal abscess (n / %)	5 (5,9%)	0	NS
Surgical wound infection (n / %)	18 (21,4%)	1 (3,2%)	p< 0,05
Reoperation rate (n / %)	5 (5,9%)	2 (6,5%)	(0,019682)
Readmission within 30 days (n / %)	1 (1,2%)	1 (3,2%)	NS
Mortality (30 days)	2 (2,4%)	1 (3,2%)	NS

± 13,3 days), although not reaching statistical significance. Anastomotic failure rate was similar in both groups (EA 7,1% vs. IA 6,5%).

There were no differences in reoperation rate (6% for EA versus 6,5% for IA group) or readmission within 30 days (1,2% versus 3,2% for EA and IA groups, respectively).

Mortality rate was also similar in both groups.

DISCUSSION

The benefits of laparoscopic colorectal surgery have been proven by multiple randomised trials with high levels of evidence. The growing experience of laparoscopic colorectal surgeons allows a higher range of techniques and choices for the confection of colorectal anastomosis, such as the intracorporeal anastomosis. This type of technique after laparoscopic right colectomy has been used by several groups reporting exciting results; however, these studies are merely observational. It is not proven yet the non-inferiority of intracorporeal versus extracorporeal techniques by large randomised controlled trials.

In the analysis of our 115 patients, there were no demographic differences between the two groups, with a similar mean age and gender distribution. Also, we found no difference in mean ASA score, which means both groups had similar surgical risk. The comorbidities analysis also showed the homogeneity between both groups, with similar rates of previous abdominal surgery, diabetes mellitus and steroid or anticoagulant therapies. The preoperative diagnosis was colonic cancer for most patients, except for 3 cases of benign disease (colonic ischaemia, Crohn's disease and appendicular plastron). There was no difference in preoperative diagnosis for both groups. When it comes to the distribution of emergent/elective procedures, there was no statistically significant difference; in both groups, the majority of patients had elective procedures. Overall, this analysis shows that our cohort was homogeneous, thus diminishing a possible selection bias.

We found no statistically significant differences in intraoperative data, namely mean operative time (125 ± 33,2 minutes for the EA, and 121 ± 27,1 minutes for the IA group) or mean blood loss (50,5 ± 58,4 mL versus 32,9 ± 31,9 mL for EA and IA groups, respectively). There have been multiple studies analysing the differences in operative time between the two techniques, most of them failing to show a significant difference. Chaves et al. (11) reported a shorter operative time for the EA group in an analysis of 25 patients, although not statistically significant. Fabozzi et al. (12) reported a significant decrease in the IA group with 50 patients. Hanna et al. (7) found an improvement in median operative time from 240 minutes in 2005 to 170 minutes in 2014, although failing to reach a statistically significant difference, in a cohort of 71 patients in the IA group. Our study shows similar results for both groups. In the hands of experienced surgeons, mean operative time should be similar for both techniques.

Our study revealed a statistically significant difference in surgical wound infection rate, which was lower in the IA group (21,4% vs. 3,2%, p<0,05 and 99,5% CI), as well as a slightly lower rate of intra-abdominal abscess in the same group (6% vs. 0%), although failing to reach statistical significance. Moreover, when we consider overall local infectious complications (surgical site infection, SSI) we find a statistically significant difference in favour of the IA group (3,2% versus 27,4%, p<0,05 and 99,5% CI).

These findings are supported by several studies reporting significantly lower rates of SSI in the IA group. However, there is no consensus in the literature regarding the reasons to explain these differences, and

we found several possible explanations.

One author (13) explained this difference with the higher tension applied on tissues and traumatic damage of surgical site during specimen extraction in the EA technique. In addition, an IA technique has the advantage of less mobilization of the transverse colon and pancreaticoduodenal block, theoretically resulting in less surgical trauma and therefore less infectious risk.

Theoretically, using a IA technique should lead to an increase in intra-abdominal infections due to the necessity of intraperitoneal opening of contaminated ileon and transverse colon. In an attempt to decrease the risk of fecal contamination when performing the enterotomies for the anastomosis confection, Grams et al. (14) described the use of atraumatic bulldog clamps. Our Unit, on the other hand, used mechanical bowel preparation in order to avoid intraperitoneal fecal spillage after performing the enterotomies. Almost all patients in the IA group received this preparation (90,3%), versus 34,5% in the EA group, with statistically significant difference (p<0,05, 99,5% CI). The remaining patients in the IA group were submitted to emergent procedures, thus not receiving bowel preparation. Both groups received the same antibiotic prophylaxis with a single dose of intravenous cefazolin and metronidazole.

On the other hand, we found a significant difference in the choice of surgical incision site, with a clear preference for a hypogastric incision (mini Pfannenstiel) in the IA group and a mesogastric incision (either mini-laparotomy or transverse para-umbilical incision) in the EA group (p<0,05, 99,5% CI). The mean incision size was similar for both groups (5,8 cm for the EA and 5,2 cm for the IA group). The IA technique allows a larger rate of possibilities for this choice, given the ability to easier manipulation of tissues and therefore the possibility to perform an incision in a lower region of the abdomen. The hypogastric incision offers some advantages previously reported (16), such as better cosmetic results, lower intensity of postoperative pain possibly due to smaller incisions with minimum muscle tear, fewer respiratory complications related to hypoventilation caused by abdominal pain, fewer rates of surgical site infection and fewer rates of incisional herniation (18).

In conclusion, we cannot yet state which factors definitely contribute to a lower rate of surgical site infection in the IA technique, due to the heterogeneity of published studies. In our analysis, the difference found in SSI seems to be related to mechanical bowel preparation (which the majority of IA patients have received), and the choice of extraction site favouring the hypogastric incision.

We found a trend toward a lower anastomotic failure rate in the IA group, although not reaching statistical significance (6,5% versus 7,1%). This finding could be explained with the lower tension and thorough manipulation of bowel and respective mesentery for the confection of the anastomosis, and the lower risk of mesenteric torsion due to the ability of direct visual control. As such, we can consider some advantages of the IA technique regarding a higher flexibility of tissue manipulation and the choice of the incision site, as has been stated by some authors. These advantages could translate into enhanced recovery, decreased complications rate and improved long-term results.

Fabozzi et al (12) reported a significant decrease in the risk of anastomotic leak (p<0,05) in a retrospective study including 50 patients. However, these findings were not replicated by any other retrospective analysis found in the literature. Hannah et al. (7) published a retrospective study, which included 195 patients (86 in the IA and 109 in the EA group), reporting a lower risk of anastomotic failure in the IA group that did not reach statistical significance (AOR 0.29, 95% CI, p<0.05). A meta-analysis (9) of 484 patients (including 272 patients in the IA group and 212 patients in the EA group) did not find any significant difference in anastomotic leak rates (OR 0.98, 95% CI). These findings suggest that a potential benefit or harm of the IA technique regarding anastomotic failure remains unclear.

There were no differences in reoperation rate, with 5 cases (6%) in the EA group and 2 cases (6,5%) in the IA group. Of these, two cases had internal herniation with small bowel obstruction (one case in each group), one had complications of an umbilical herniorraphy (IA group), and four patients in the EA group were reoperated for anastomotic failure with peritonitis.

In our analysis, we considered as length of hospital stay the total time spent in hospital, which included the day the patient was admitted to preoperative preparation and the day of the surgical intervention (D-1 and D0). The mean length of hospital stay was slightly shorter for the IA group (9,3 ± 5,4 days versus 11,3 ± 13,3), although not reaching statistical significance (p>0,05).

In our literature research, we found small trials supporting this difference. Roscio et al. (15) reported a faster return of bowel movement for the patients submitted to IA, with a significant difference, relating this finding to significantly shorter length of hospital stay for the same group. A meta-analysis published in 2017 (10) which included 12 studies published between 2010 and 2015, with a total of 1492 patients, also reveals a difference in the mean length of hospital stay in favor of IA group (MD −0.77 days, 95% CI −1.46 to −0.07); however, due to the cohorts' heterogeneity, this difference did

not reach statistical significance. Nonetheless, a subgroup analysis including only the studies posterior to 2012 stated a statistically significant difference for this particular outcome (0.77 days, 95% CI −1.17 to −0.37), which could be related to a lower rate of postoperative complications in the IA group. Our study showed a difference in postoperative infectious complications, which may have influenced the outcomes in length of hospital stay.

There were two cases of hospital readmission (one in each group), with no statistically significant difference between the two groups.

We found no differences in overall mortality between the two groups, which is consistent with the available literature.

Limitations and Biases

Some limitations of this study must be addressed. The major limitation lies in the study design, which is based on the evaluation of retrospective data, thus lacking randomisation of patients; this could possibly originate a selection bias. Nonetheless, demographic and preoperative data analysis showed no significant differences between the two groups.

Another limitation is related with the small cohort of the study, decreasing the study potency to truly understand its statistical significance.

Also, the follow-up was only of 30 days, focusing on the immediate postoperative outcomes. Our Colorectal Unit intends to design a prospective study addressing long-term outcomes and survival rates, as well as oncologic-specific outcomes.

Nonetheless, we consider these results encouraging, leading us to consider the possibility of stating the non-inferiority of IA technique over the EA technique.

In conclusion, we present our first results of intracorporeal anastomosis after laparoscopic right colectomy, performed by a team of experienced colorectal laparoscopic surgeons. We emphasize the lack of randomised-controlled trials worldwide, and the absence of studies regarding this issue published in Portugal so far.

CONCLUSION

Our study presents similar postoperative results of IA and EA techniques after laparoscopic right colectomy. Possible advantages of the IA technique are the versatility to choose the location of incision site for specimen extraction and lower rates of surgical site infection. Therefore, we can state the safety and feasibility of intracorporeal technique, when performed by experienced colorectal laparoscopic surgeons. We recommend a solid and well-defined learning model in laparoscopy, adapting the IA technique into the practice of surgeons ascending the learning curve.

Disclosure

We have no conflict of interests to declare.

Ethical considerations

Approval for this study was obtained from the Ethical Committee of Centro Hospitalar de Leiria.

REFERENCES

1. Kuhry E, Schwenk WF, Gaupset R, Romild U, Bonjer HJ. Long-term results of laparoscopic colorectal cancer resection. Cochrane Database Syst Rev 2008; (2):CD003432
2. Clinical Outcomes of Surgical Therapy Study Group. A comparison of laparoscopically assisted and open colectomy for colon cancer. Ann Surg 2004, 240(2):205–213
3. Guillou PJ, Quirke P, Thorpe H, Walker J, Jayne DG, Smith AM, et al. Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. Lancet 2005, 365(9472): 1718–1726
4. Colon Cancer Laparoscopic or Open Resection Study Group, Buunen M, Veldkamp R, Hop WC, Kuhry E, Jeekel J, Haglind E, Pahlman L, Cuesta MA, Msika S, Morino M, Lacy A, Bonjer HJ. Survival after laparoscopic surgery versus open surgery for colon cancer: long-term outcome of a randomised clinical trial. Lancet Oncol 2009, 10(1):44–52
5. Jamali FR, Soweid AM, Dimassi H, Bailey C, Leroy J, Mar- escaux J. Evaluating the degree of difficulty of laparo- scopic colorectal surgery. Arch Surg 143(8):762–767
6. Carmichael JC, Stamos MJ. Right hemicolectomy and ileocecectomy: laparoscopic approach. In Bardakcioglu (ed.) Advanced Techniques in Minimally Invasive and Robotic Colorectal Surgery. 1st edition. New York, Springer 2015, pp 37-48.
7. Hanna MH, Hwang GS, Phelan MJ, Bui TL, Carmichael JC, Mills SD, Stamos MJ, Pigazzi A. Laparoscopic right hemicolectomy: short- and long-term outcomes of intracorporeal versus extracorporeal anastomosis. Surg Endosc 2016; 30:3933–3942.
8. Shapiro R, Keler U, Segev L, Sarna S, Hatib K, Hazzan D. Laparoscopic right hemicolectomy with intracorporeal anastomosis: short- and long-term benefits in comparison with extracorporeal anastomosis. Surg Endosc 2016; 30:3823–3829.
9. Carnuccio P, Jimeno J, Pares D. Laparoscopic right colectomy: a systematic review and meta-analysis of observational studies comparing two types of anastomosis. Tech Coloproctol. 2014;18:5–12.
10. Van Oostendorp S, Elfrink A, Borstlap W, Schoonmade L, Sietses C, Meijerink J, Tuynman J. Intracorporeal versus extracorporeal anastomosis in right hemicolectomy: a systematic review and meta-analysis. Surg Endosc 2017; 31(1): 64-77.
11. Chaves JA, Idoate CP, Fons JB, Oliver MB, Rodriguez NP, Delgado AB, Lizoain JLH. A case–control study of extracorporeal versus intracorporeal anastomosis in patients subjected to right laparoscopic hemicolectomy. Cir Esp. 2011;89:24–30.
12. Fabozzi M, Allieta R, Brachet Contul R, Grivon M, Millo P, Lale-Murix E, Nardi Jr. M. Comparison of short- and medium-term results between laparoscopically assisted and totally laparoscopic right hemicolectomy: a case-control study. Surg Endosc. 2010;24: 2085–2091.
13. Chang K, Fakhoury M, Barnajian M, Tarta C, Bergamaschi R. Laparoscopic right colon resection with intracorporeal anastomosis. Surg Endosc. 2013; 27:1730–1736.
14. Grams J, Tong W, Greenstein AJ, Salky B. Comparison of intra-

APPENDIX 3

- Kristensen HØ, Thyø A, Jøssing Emmertsen K, Smart NJ, Pinkney T, Warwick AM, Pang D, Furnée EJB, Verkuijl SJ, **José Rama N**, Domingos H, Maciel J, Solis-Peña A, Espín Basany E, Hidalgo-Pujol M, Biondo S, Sjövall A, Christensen P.

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ORIGINAL ARTICLE

Translation and international validation of the Colostomy Impact score

Helle Ø Kristensen[1] | Anne Thyø[1] | Katrine Jøssing Emmertsen[1,2] | Neil J. Smart[3] | Thomas Pinkney[4] | Andrea M. Warwick[5] | Dong Pang[6] | Edgar J. B. Furnée[7] | Sanne J. Verkuijl[7] | Nuno José Rama[8] | Hugo Domingos[9] | João Maciel[10] | Alejandro Solis-Peña[11] | Eloy Espín Basany[11] | Marta Hidalgo-Pujol[12] | Sebastian Biondo[12] | Annika Sjövall[13,14] | Peter Christensen[1]

[1]Department of Surgery, Danish Cancer Society Centre for Research on Survivorship and Late Adverse Effects After Cancer in the Pelvic Organs, Aarhus University Hospital, Aarhus N, Denmark

[2]Surgical Department, Randers Regional Hospital, Aarhus N, Denmark

[3]Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

[4]Academic Department of Surgery, University of Birmingham, Birmingham, UK

[5]Brisbane Academic Functional Colorectal Unit, QEII Hospital, Brisbane, QLD, Australia

[6]Peking University School of Nursing, Beijing, China

[7]Department of Surgery, Division of Abdominal Surgery, University Medical Center, University of Groningen, Groningen, The Netherlands

[8]Surgery – Colorectal Unit, Centro Hospitalar de Leiria, Leiria, Portugal

[9]Colorectal Surgery Unit, Champalimaud Foundation, Lisboa, Portugal

[10]Colorectal Surgery Unit, Instituto Português de Oncologia- Lisbon, Lisboa, Portugal

[11]Colorectal Surgery Unit, General Surgery Department, Hospital Vall d'Hebron, Universitat Autonoma de Barcelona, Barcelona, Spain

[12]Colorectal Surgery Unit, Bellvitge University Hospital, Barcelona, Spain

[13]Division of Coloproctology, Department of Pelvic Cancer, Karolinska University Hospital, Stockholm, Sweden

[14]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Correspondence
Helle Ø Kristensen, Department of Surgery, Danish Cancer Society Centre for Research on Survivorship and Late Adverse Effects After Cancer in the Pelvic Organs, Aarhus University Hospital, Palle Juul-Jensens Blvd 35, C-119-139, 8200 Aarhus N, Denmark.
Email: hellkren@rm.dk

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Abstract

Aim: Optimal oncological resection in cancers of the lower rectum often requires a permanent colostomy. However, in some patients a colostomy may have a negative impact on health-related quality of life (HRQoL). The Colostomy Impact (CI) score is a simple questionnaire that identifies patients with stoma dysfunction that impairs HRQoL by dividing patients into 'minor' and 'major' CI groups. This aim of this study is to evaluate construct and discriminative validity, sensitivity, specificity and reliability of the CI score internationally, making it applicable for screening and identification of patients with stoma-related impaired HRQoL.

Method: The CI score was translated in agreement with WHO recommendations. Cross-sectional cohorts of rectal cancer survivors with a colostomy in Australia, China, Denmark, the Netherlands, Portugal, Spain and Sweden were asked to complete the CI score, the European Organization for Research and Treatment of Cancer (EORTC) quality of life 30-item core questionnaire, the stoma-specific items of the EORTC quality of life 29-item colorectal-specific questionnaire and five anchor questions assessing the impact of colostomy on HRQoL.

Results: A total of 2470 patients participated (response rate 51%–93%). CI scores were significantly higher in patients reporting reduced HRQoL due to their colostomy than in patients reporting no reduction. Differences in EORTC scale scores between patients with minor and major CI were significant and clinically relevant. Sensitivity was high regarding dissatisfaction with a colostomy. Regarding evaluation of discriminative validity, the CI score relevantly identified groups with differences in HRQoL. The CI score proved reliable, with equal CI scores between test and retest and an intraclass correlation coefficient in the moderate to excellent range.

Conclusion: The CI score is internationally valid and reliable. We encourage its use in clinical practice to identify patients with stoma dysfunction who require further attention.

KEYWORDS

Colostomy, health-related quality of life, rectal cancer, stoma

What does this paper add to the literature?

The Colostomy Impact (CI) score is a patient-reported outcome measure identifying patients with stoma dysfunction that impairs health-related quality of life. This study evaluates construct and discriminative validity, sensitivity, specificity and reliability of the CI score in cross-sectional cohorts of rectal cancer survivors with a colostomy in Australia, China, Denmark, the Netherlands, Portugal, Spain and Sweden.

INTRODUCTION

Surgery is still the cornerstone in curative treatment of cancer of the rectum. In tumours close to the anal verge, formation of a permanent end colostomy is often necessary to obtain an optimal oncological resection. However, a colostomy may have a negative impact on health-related quality of life (HRQoL) in some patients. The boundaries for sphincter-preserving surgery are continuously being pushed, with intersphincteric resections reducing the number of patients requiring an end colostomy. Nevertheless, it is well known that sphincter-preserving surgery carries a risk of low anterior resection syndrome and that the risk of reduced HRQoL from bowel dysfunction increases with decreasing tumour height from the anal verge [1,2]. There is no conclusive evidence that HRQoL in patients with a stoma is inferior to that of patients who have a low anterior resection [3–5]. With the improved 5-year survival rate, there are an increasing number of long-term survivors who have to live with the late effects of their cancer treatment. Increasingly HRQoL has gained acceptance as an important outcome in cancer treatment, and studies have shown that in 19%–23% of patients with a permanent colostomy after rectal cancer surgery the stoma impairs their HRQoL [6,7]. A number of questionnaires have been developed to study stoma-related quality of life [8–10]. However, in view of the need for a short, simple and valid screening tool to identify patients with reduced HRQoL due to stoma dysfunction, the Colostomy Impact (CI) score was developed in 2016 [6]. The unidimensional CI score enables clinicians to identify patients with stoma dysfunction in an efficient, standardized and systematic way in order to initiate targeted measures to improve HRQoL. Once validated, the score will also be valuable for research purposes in standardizing and simplifying the reporting of stoma dysfunction.

The CI score was developed in a Danish population in 2016 and identified patients with reduced HRQoL due to stoma dysfunction with a sensitivity of 85.7% and specificity of 59.5%. The CI score has recently been validated in Danish patients with a colostomy after surgery for benign conditions [11] and is now applicable to a Danish population with a colostomy regardless of the underlying condition. Since cultural and geographical differences may affect the impact of colostomy on patients, the CI score should be meticulously translated and validated thoroughly before translated versions are taken into use.

This validation study reports the translation of the CI score and aims to evaluate construct and discriminative validity as well as sensitivity and specificity of the CI score in Australia, China, Denmark, the Netherlands, Portugal, Spain and Sweden; in addition the test–retest reliability of the CI score was evaluated in Denmark, the Netherlands, Spain and Sweden.

METHOD

Participants

Cross-sectional cohorts of rectal cancer survivors with a permanent colostomy were identified in the participating countries, as shown in Table 1. Inclusion criteria were curative surgery for rectal cancer resulting in a permanent end colostomy and at least 12 months of follow-up. Exclusion criteria were recurrence, inability to complete

TABLE 1 Patient characteristics

	Australia	China	Denmark	The NL	Portugal	Spain	Sweden
n	95	110	1583	117	97	207	258
Population/inclusion method	Hospital database	Hospital's medical record system	National register (DCCG)	Dutch Surgical Colorectal Audit	Hospital database	Hospital database	National register (SCRCR)
Response rate	51%	93%	74%	82%	80%	67%	61%
Mode of administration							
Web-based, *n* (%)	1 (2)	24 (22)	942 (60)	43 (37)	0	0	0
Pen and paper, *n* (%)	53 (94)	83 (78)	641 (40)	73 (63)	29 (30)	46 (23)	258 (100)
Interview, *n* (%)	2 (4)	0	0	0	67 (69)	156 (77)	0
Sex							
Male, *n* (%)	67 (68)	73 (66)	985 (63)	71 (62)	63 (65)	136 (66)	153 (59)
Female, *n* (%)	31 (32)	37 (34)	584 (37)	44 (38)	34 (35)	70 (34)	105 (41)
BMI (kg/m^2), mean (range)	26.9 (16–41)	23.5 (16–32)	27.0 (14–68)	27.1 (16–55)	26.9 (16–35)	26.9 (15–66)	25.9 (15–61)
Age (years), mean (range)	69.7 (35–93)	67.0 (31–93)	74.4 (30–96)	70.7 (37–94)	71.9 (35–97)	76.1 (47–96)	72.7 (36–91)
Follow-up (years), mean (range)	4.5 (1.0–15.0)	4.6 (1.0–39.2)	6.2 (2.1–12.2)	5.7 (1.8–10.7)	5.6 (1.6–16.6)	6.1 (1.0–15.1)	4.1 (1.0–7.9)
Access to stoma nurse							
Yes, *n* (%)	86 (85)	120 (98)	1,180 (77)	101 (89)	77 (87)	160 (78)	190 (77)
No, *n* (%)	10 (10)	3 (2)	47 (3)	7 (6)	4 (4)	32 (16)	24 (10)
Don't know, *n* (%)	5 (5)	0	308 (20)	6 (5)	8 (9)	12 (6)	34 (13)
Stage (IUCC)							
0, *n* (%)	3 (3)	5 (5)	0	0	11 (11)	10 (5)	18 (7)
I, *n* (%)	35 (39)	30 (27)	523 (39)	18 (15)	30 (31)	42 (21)	83 (32)
II, *n* (%)	28 (31)	33 (30)	426 (31)	39 (34)	28 (29)	59 (30)	68 (26)
III, *n* (%)	22 (24)	28 (25)	399 (29)	59 (51)	24 (25)	79 (40)	71 (27)
IV, *n* (%)	2 (2)	14 (13)	5 (1)	0	4 (4)	8 (4)	20 (8)
Procedure							
APE, *n* (%)	70 (76)	105 (97)	1237 (78)	79 (68)	80 (82)	167 (83)	258 (100)
Hartmann's, *n* (%)	6 (7)	2 (2)	346 (22)	21 (18)	9 (9)	14 (7)	0
Pelvic exenteration, *n* (%)	15 (16)	0	0	16 (14)	1 (1)	5 (2)	0
Setting							
Acute, *n* (%)	1 (1)	0	13 (1)	3 (3)	6 (6)	6 (3)	0
Elective, *n* (%)	87 (99)	95 (86)	1570 (99)	113 (97)	90 (93)	195 (97)	258 (100)
ASA score							
1–2, *n* (%)	67 (77)	99 (80)	1356 (86)	108 (93)	57 (59)	131 (66)	179 (69)
≥3, *n* (%)	20 (23)	10 (8)	214 (14)	8 (7)	39 (40)	68 (34)	78 (31)

(Continues)

TABLE 1 (Continued)

	Australia	China	Denmark	The NL	Portugal	Spain	Sweden
Complications							
No complications, *n* (%)	25 (26)	101 (83)	1250 (79)	75 (65)	64 (66)	119 (59)	152 (58)
Clavien–Dindo I–II, *n* (%)	51 (53)	0	29 (2)	21 (18)	16 (17)	50 (25)	71 (27)
Clavien–Dindo III, *n* (%)	9 (9)	6 (5)	57 (4)	18 (15)	14 (14)	25 (12)	33 (13)
Clavien Dindo IV, *n* (%)	2 (2)	0	19 (1)	2 (2)	0	6 (3)	2 (1)
Unknown, *n* (%)	9 (9)	15 (12)	228 (14)	0	3 (3)	1 (1)	2 (1)
Oncological therapy							
Chemotherapy, *n* (%)	7 (8)	1 (1)	225 (35)	1 (1)	5 (5)	10 (5)	12 (5)
Radiotherapy, *n* (%)	1 (1)	1 (1)	39 (6)	30 (26)	7 (8)	14 (7)	119 (46)
Chemo- and radiotherapy, *n* (%)	53 (61)	99 (98)	375 (57)	70 (60)	47 (52)	140 (71)	76 (29)
None, *n* (%)	26 (30)	0	15 (2)	15 (13)	32 (35)	33 (17)	52 (20)

Abbreviations: APE, abdominoperineal excision; ASA, American Society of Anesthesiologists; BMI, body mass index; NL, Netherlands.

questionnaires (dementia, impaired vision, inability to read or write the specific language), age under 18 years and lacking consent. In Denmark, patients were identified from a national registry (Table 1). Eligible patients were sent an electronic invitation and a web-based questionnaire or a paper version of the questionnaire by regular mail with a prepaid envelope, depending on patient preference. Reminders were sent to nonresponders after approximately 3 weeks.

A similar approach was used in Sweden. In the Netherlands, patients treated at the participating hospitals were identified from a national database and approached as described above. Patients in Australia, Spain and Portugal and 91 of the patients from China were identified from local hospital databases or the hospitals' electronic medical record systems and approached in person, by mail or by telephone. In China, convenient inclusion from the hospital's stoma clinic was employed for inclusion of 29 patients.

For test–retest analysis, subgroups of patients in Denmark, Sweden, the Netherlands and Spain were randomly selected and asked to complete the CI score a second time 2–6 weeks after completion of the primary questionnaire, along with a question on changes in stoma function or stoma care since the primary questionnaire. Patients reporting changes in stoma function or returning the second test after more than 6 weeks were excluded.

Questionnaire/booklet

Patients were asked to complete questions regarding sociodemographic factors, lifestyle, stoma care and stoma complications, five anchor questions on the overall impact of colostomy on HRQoL, the CI score, the European Organization for Research and Treatment of Cancer quality of life 30-item core questionnaire (EORTC QLQ-C30) and the stoma-specific items of the EORTC quality of life 29-item colorectal-specific questionnaire (EORTC QLQ-CR29). Disease- and treatment-specific information was collected from hospital charts (Australia, China and the Netherlands) or registers/databases (Denmark Portugal, Spain and Sweden).

The primary anchor question was 'Overall, do you think that the colostomy impairs your quality of life?' The options were 'not at all', 'a little', 'some' and 'a lot'. This question is unvalidated but was part of the basic stoma questionnaire used for the development of the CI score. For the purpose of construct validation, the four categories of this anchor question were merged into two categories: colostomy impact 'none at all/a little' and colostomy impact 'some/a lot'. Four additional anchor questions regarding satisfaction, embarrassment, adaptation and restrictions in daily life were added to explore the construct of the CI score. Each question had four answer options that were dichotomized as described above to calculate the sensitivity and specificity of the CI score of each anchor.

The CI score is a seven-item patient reported outcome measure (PROM) concerning stoma-related problems, symptoms and complications. The CI score was developed using the primary anchor

question to identify factors associated with reduced HRQoL and to obtain a simple unidimensional measure of stoma dysfunction related to reduced HRQoL [6]. Each item has two to five answer options, and a weighted scoring system provides a total sum-score ranging from 0 to 38, with a higher score representing greater stoma dysfunction. A CI score of 0–9 points is categorized as minor CI and a score of 10–38 points is categorized as major CI (Appendix S1 and S2). As missing items hinder calculation of the sum-score, patients with missing items were excluded from further analysis.

The EORTC QLQ-C30 v.3.0 is a 30-item multidimensional generic HRQoL measure for cancer patients. It provides five multi-item functional scales and one multi-item global health status/QoL scale where a higher score represents better functioning, along with three multi-item symptom scales and six single-item measures where a higher score represents worse symptoms. Participants also completed the stoma-specific items of the EORTC QLQ-CR29 – a module for colorectal cancer patients consisting of 29 questions, seven of which concern patients with a stoma. Using linear transformation, scale scores of the EORTC QLQ-C30 were calculated ranging from 0 to 100 according to the scoring manual [12] and the stoma-specific items of the EORTC QLQ CR29 were summarized using linear transformation as a symptom scale ranging from 0 to 100. Missing data were handled according to the scoring manual. The clinical relevance of difference in EORTC QLQ-C30 scale scores between the minor and major CI groups were assessed according to Cocks et al. [13].

Translation and adaptation

The CI score has previously been translated into English [6]. The English version of the CI score, anchor questions and background questions were translated into Chinese, Dutch, Portuguese, Spanish and Swedish following WHO recommendations for translation and cross-cultural adaptation of instruments performed by professional translators. Final versions were reviewed by a colorectal surgeon at each participating centre and adapted where needed.

Validation

This validation study adheres to the Cosmin taxonomy of measurement properties [14]. Since items in the CI score are independent variables of the construct, stoma dysfunction, the conceptual framework is based on a formative model. Content validity was meticulously ensured in the development process, leaving the following measurement properties to be investigated: construct validity, discriminative validity and retest reliability.

Construct validity was assessed by testing hypotheses formulated a priori:

1. Patients reporting inferior HRQoL in the anchor question have a significantly higher CI score than patients reporting better HRQoL.
2. Patients with major CI have significantly lower scores than patients with minor CI on all functional EORTC QLQ-C30 scales and significantly higher scores on all symptom scales. Differences are clinically relevant in global health status, role functioning, social functioning and fatigue.
3. Patients with major CI have significantly higher scores than patients with minor CI on the stoma-specific EORTC QLQ-CR29 scale.
4. The CI score has a sensitivity of 85% and a specificity of 50% for identifying patients with 'some/a lot' CI as measured by the anchor questions.

Discriminative validity was assessed by an a priori formulated hypothesis: any differences in HRQoL between groups as measured by the primary anchor question will be reflected as a significant difference in CI score. Differences in CI score between the following groups were studied: age, sex, body mass index (<25 kg/m^2 or ≥25 kg/m^2), whether costs of stoma care products confer a financial burden on the household, surgical setting (acute or elective), neoadjuvant/adjuvant oncological treatment and postoperative complications (Clavien–Dindo <IIIb or ≥IIIb).

Retest reliability was analysed on an item level and on sum-score. For each item, a change between two adjacent answer categories resulted in a one-point change. A change of more than one category resulted in a two- or three-point change according to the number of categories. The item-level score was calculated as the sum of changed categories. The weighted sum-score was calculated according to the scoring instructions.

Statistical analysis

Analyses of retest reliability and construct validity were performed per country. Discriminative validity was analysed on the cumulated cohort.

The CI scores are presented as medians and interquartile ranges (IQRs) considering skewness of the data. The EORTC data are presented as mean (SD), as is the convention [15]. For significance testing, chi-square tests or Mann–Whitney *U*-tests were performed according to the type of data handled. An a priori power calculation was performed based on detecting a five-point difference in the scales of the EORTC QLQ-C30 with a significance level of 0.05 and power of 80%. Centres including patients corresponding to the power calculation or whose analysis reached significance were included in the analysis of EORTC data. All countries were included in the remaining analysis.

Receiver operating curves (ROCs) were plotted, yielding sensitivity and specificity.

Discriminative validity was assessed using the primary anchor question and the CI score was considered able to detect potential differences if tests of difference in CI score corresponded to the findings using the primary anchor question. Retest reliability was assessed by the Wilcoxon signed-rank test for significance between test and

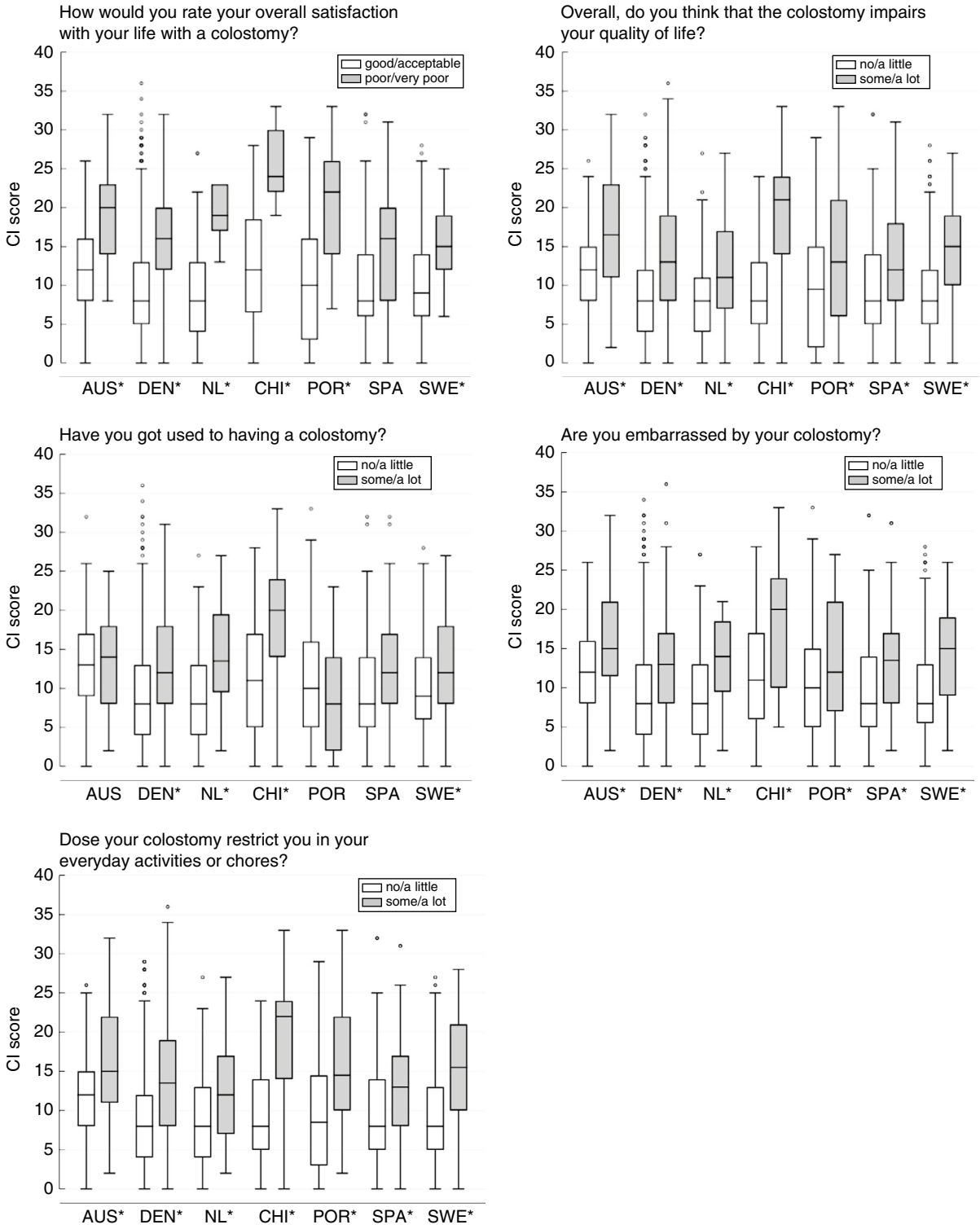

FIGURE 1 Construct validity; Median CI scores and IQR in HRQoL groups determined by anchor questions. Countries where the difference in CI score is significant between anchor groups are marked with (*)

retest, by intraclass correlation coefficients (ICCs) and Bland–Altman plots with limits of agreement (LoA). An ICC less than 0.5 was considered poor reliability, 0.5–0–75 moderate reliability, 0.75–0.9 good reliability and an ICC >0.9 was considered excellent [16].

Data were collected and managed using the REDCap electronic data collection tools hosted at Aarhus University [17,18]. All statistical analyses were performed using Stata 16 (StataCorp LCC). A significance level of 0.05 was chosen.

TABLE 2 Mean scale scores of the EORTC QLQ-C30. The size of the clinical relevance of the differences was assessed according to Cocks et al. [13] and reported as trivial (none), small (light grey ○), medium (grey ●) or large (dark grey ●)

EORTC QLQ-C30	China		Denmark		Spain		Sweden	
	Minor CI, mean (SD)	Major CI, mean (SD)	Minor CI, mean (SD)	Major CI, mean (SD)	Minor CI, mean (SD)	Major CI, mean (SD)	Minor CI, mean (SD)	Major CI, mean (SD)
Global QoL	78.1 (11.6)	62.1 (13.5)	80.2 (19.7)	66.6 (22.3)	72.0 (20.6)	62.0 (22.9)	78.2 (18.1)	63.4 (20.9)
Physical function (PF)	89.1 (6.6)	74.1 (18.0)	87.7 (17.2)	76.1 (22.8)	83.1 (23.2)	70.5 (29.4)	89.0 (15.2)	76.6 (20.8)
Role function (RF)	79.8 (14.3)	66.6 (20.4)	88.2 (20.7)	74.9 (28.4)	86.4 (27.8)	74.6 (33.8)	87.0 (21.8)	74.6 (25.8)
Emotional function (EF)	85.5 (10.9)	76.1 (14.3)	93.6 (12.2)	84.4 (19.5)	86.9 (18.9)	76.7 (24.6)	91.1 (13.6)	78.7 (22.7)
Cognitive function (CF)	94.5 (8.6)	80.0 (12.2)	91.6 (14.5)	83.1 (20.5)	86.0 (19.1)	82.1 (23.4)	92.2 (11.7)	81.4 (23.0)
Social function (SF)	72.8 (15.4)	68.7(19.8)*	91.7 (16.4)	80.9 (24.9)	88.6 (21.1)	77.1 (28.8)	86.8 (21.0)	72.6 (27.3)
Fatigue (FA)	6.2 (8.1)	26.8 (16.4)	16.7 (19.4)	30.8 (25.3)	17.5 (22.7)	29.8 (26.6)	17.3 (18.1)	33.1 (24.8)
Nausea and vomiting (NV)	.77 (5.0)	2.3 (7.7)*	1.8 (8.1)	5.6 (14.1)	2.9 (8.8)	3.8 (31.8)*	1.3 (5.4)	7.6 (16.7)
Pain (PA)	2.7 (7.2)	14.1 (15.9)	5.8 (15.6)	18.6 (25.2)	16.6 (25.5)	25.6 (25.7)	7.5 (17.4)	20.5 (25.1)
Dyspnoea (DY)	.77 (5.0)	12.3 (16.2)	9.8 (20.0)	19.3 (26.5)	12.6 (23.7)	18.6(26.6)*	16.8 (21.4)	30.1 (30.1)
Insomnia (SL)	8.5 (17.9)	23.5 (20.1)	12.6 (21.1)	24.4 (30.2)	22.6 (27.8)	34.0 (31.8)	13.8 (19.9)	26.8 (30.8)
Appetite loss (AP)	1.5 (7.1)	17.4 (18.7)	4.8 (14.4	10.6 (21.7)	8.4 (21.2)	13.6 (23.0)	4.0 (12.5)	10.2 (21.3)
Constipation (CO)	1.5 (7.1)	9.7 (16.3)	3.9 (12.5)	10.6 (21.7)	4.4 (13.8)	12.9 (23.9)	4.9 (14.6)	12.3 (23.4)
Diarrhoea (DI)	6.2 (13.1)	14.3 (22.0)	8.8 (17.0)	19.2 (25.5)	7.6 (16.1)	15.7()23.2	8.1 (16.7)	18.6 (24.9)
Financial difficulties (FI)	10.8 (17.3)	28.7 (21.9)	1.8 (9.3)	6.9 (19.1)	8.2 (21.0)	11.5(25.6)*	4.9 (16.4)	11.8 (25.0)

Abbreviations: CI, Colostomy Impact; EORTC QLQ-C30, European Organization for the Research and Treatment of Cancer (EORTC) quality of life 30-item core questionnaire; QoL, quality of life.

The sizes of the clinical relevance of differences in emotional function (EF) as estimates of clinically relevance were not available. *Difference between minor and major CI groups is not significant.

RESULTS

A total of 2470 patients answered the questionnaire. Patient characteristics by country are presented in Table 1. Response rates were between 51% and 93%.

For the evaluation of construct validity, the CI scores in patients grouped according to the anchor questions are shown in Figure 1. For the primary anchor question and the question regarding restrictions in everyday activities, the differences in CI score were significant in all countries. Differences between groups were significant for all five anchor questions in Denmark, the Netherlands, China and Sweden.

Differences in scale scores and the level of clinical relevance of differences in the EORTC QLQ-C30 between the minor and major CI groups are presented in Table 2 for countries having the number of participants stated in the power calculation. Differences were significant across all scales and measures in all countries except for 'nausea and vomiting', 'insomnia' and 'financial difficulties' in Spain and 'social function' and 'nausea and vomiting' in China. The differences in scale scores for the EORTC QLQ-C30 between the CI groups were all clinically relevant. Figure 2 shows mean scale scores for the EORTC QLQ-C30 by CI group. The stoma-specific scale of the EORTC QLQ-CR29 showed significantly more symptoms in the major than the minor CI group in all countries except for Australia. Differences ranged from 7.4 (Spain) to 14.2 (China).

ROC analyses were performed, yielding sensitivity and specificity of the CI score. Table 3 shows results for the primary anchor question and the question regarding satisfaction with life with a colostomy, the latter showed the best sensitivity in all countries except for Spain. Sensitivity ranged between 42% and 78% for the anchor question concerning adaptation, between 66% and 82% for the anchor question concerning embarrassment and between 60% and 89% for the anchor question concerning restrictions in daily activities (data not shown).

For discriminative validity, the primary anchor question was used to describe differences in HRQoL between subgroups. For groups showing a difference in the rate of patients reporting impaired HRQoL, the CI score correspondingly showed significantly higher CI scores in the groups with inferior HRQoL except for age groups where the difference in CI score did not reach significance (p = 0.0974). Similarly, for subgroups that reported 'some/a lot' impact on HRQoL at equal rates no difference in CI scores were present, with the exception of patients with Clavien–Dindo ≥II complications who had significantly higher CI scores but did not report an impact on HRQoL more often Table 4.

A total of 359 patients answered the retest. Of these, 121 were excluded as they reported change in stoma function since answering the primary questionnaire, leaving 238 eligible patients (75 from Denmark, 75 from Sweden, 45 from Spain and 43 from the Netherlands). No differences were found in item-level score or sum-score between the test and the retest. ICC scores showed moderate reliability in Sweden and the Netherlands for both sum-score (0.663 and 0.701, respectively) and item-level scores (0.640 and 0.749,

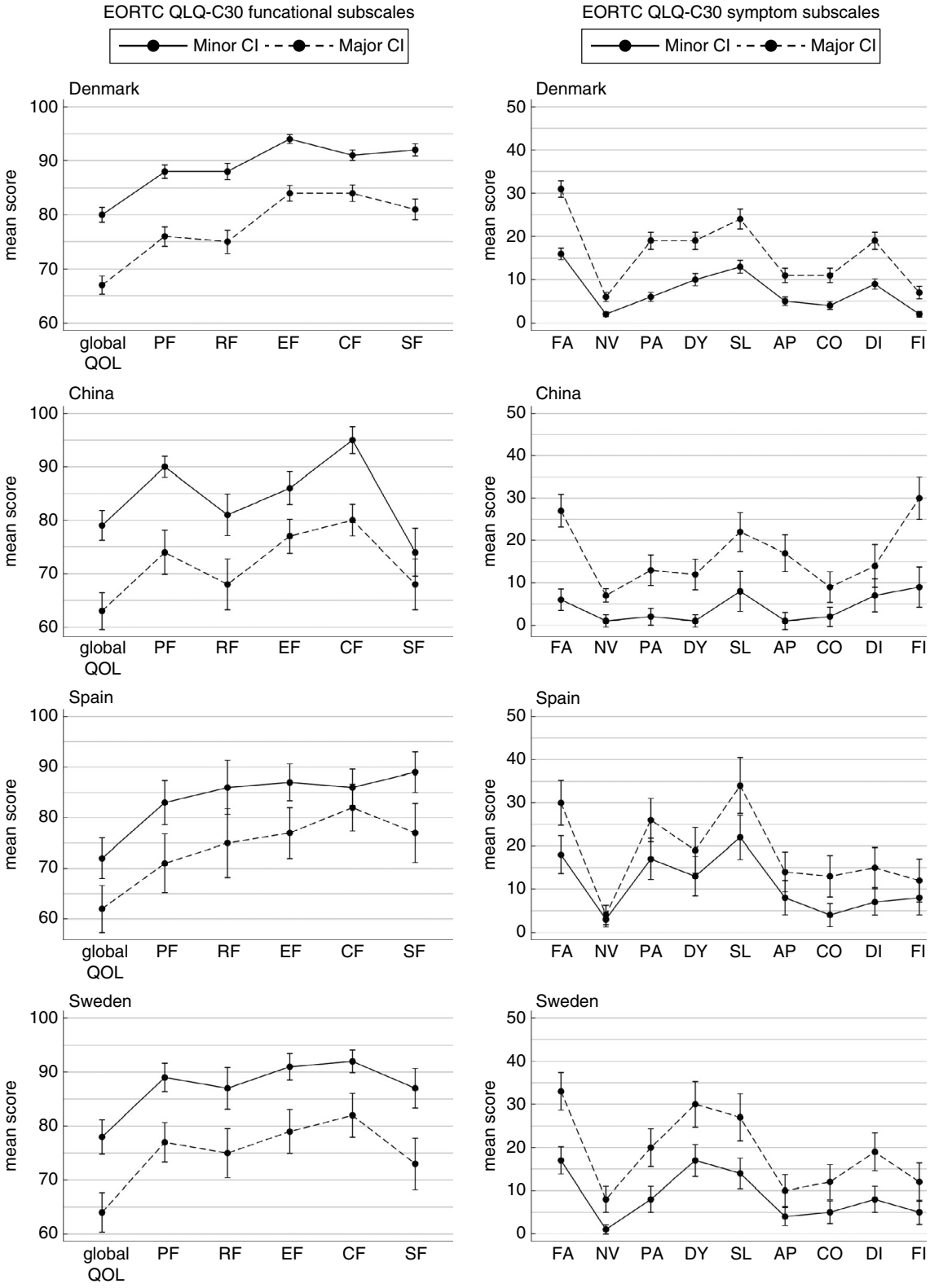

FIGURE 2 EORTC QLQ-C30 scale scores by country (mean +/- SD). Higher functional scores represent better functioning whereas higher symptom scores represent more symptoms. All differences are statistically significant except for NV and SF in China and NV, DY and FI in Spain. All significant differences are clinically relevant

respectively) and for sum-score in Denmark (0.705). Reliability was good on item level in Denmark (0.783) and excellent regarding both sum-score (0.919) and on item level (0.898) in Spain. Bland–Altman plots for assessing test–retest agreement are presented in Figure 3 for the sum-scores. Distributions were acceptable and the 95% LoA representing the individual difference between the test and the

TABLE 3 Sensitivity and specificity of the Colostomy Impact score across countries

	Sensitivity (%)	Specificity (%)	ROC AUC
Overall, do you think that the colostomy impairs your quality of life? Not at all/a little vs. some/a lot			
Australia	79.17	37.1	0.6542
China	85.1	59.0	0.8378
Denmark	68.50	61.50	0.7175
The Netherlands	57.89	60.27	0.6500
Portugal	60.00	50.00	0.6304
Spain	66.67	58.55	0.6600
Sweden	80.8	58.0	0.7403
How would you rate your overall satisfaction with your life with a colostomy? Good/acceptable vs. poor/very poor			
Australia	90.00	35.06	0.7617
China	100.00	43.00	0.9138
Denmark	80.70	55.68	0.7498
The Netherlands	100.00	56.60	0.9009
Portugal	80.00	46.51	0.7860
Spain	64.71	53.76	0.6216
Sweden	84.21	52.89	0.7480

Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic.

retest ranged between −10.27 to 10.48 points (Sweden) and −4.89 to 6.04 points (Spain). LoA on item level was between −3.78 to 3.67 (Sweden) and −1.69 to 2.33 (Spain) (not shown).

DISCUSSION

The CI score is now available in Danish, English, Chinese, Dutch, Portuguese, Spanish and Swedish and this study reports high validity and reliability of the CI score in all included countries.

A thorough assessment of the validity and reliability of a PROM is crucial before a translated version is taken into use because cultural differences may affect the psychometric properties of the instrument. In all countries, the translated versions demonstrated convincing construct validity. CI scores were significantly higher in patients reporting that their colostomy reduced their overall QoL and in patients reporting that colostomy restricted their daily activities. Overall, our hypotheses regarding the anchor questions were satisfied in 30 of 35 tests, demonstrating that the CI score is a valid measure of stoma dysfunction affecting HRQoL. Likewise, differences in EORTC QLQ-C30 scales and measures, including the stoma-specific scale of the EORTC QLQ-CR29, between CI groups were found in 58 out of 64 scales (16 scales per country) and all

	No/a little impact (%)	Some/a lot impact (%)	*P*-value	Median CI (IQR)	*P*-value
Gender					
Male	73.9	26.1		9 (6–15)	
Female	75.4	24.6	0.439	10 (5–15)	0.1298*
Age					
<65 years	70.2	29.8		10 (5–16)	
>65 years	75.4	24.5	0.0196	9 (5–14)	0.0974
BMI					
<25 kg/m^2	77.0	23.0		8 (5–13)	
>25 kg/m^2	73.3	26.7	0.0396	10 (6–15)	0.0000*
Household financial burden					
Unburdened	39.6	60.4		9 (5–14)	
Burdened	23.5	76.5	0.0000	12 (8–19)	0.0000*
Setting					
Acute	65	35		8.5 (3–15)	
Elective	75	25	0.2753	9 (5–14)	0.8942*
Complications					
<Clavien–Dindo III	75	25		9 (5–14)	
≥Clavien–Dindo III	69	31	0.1208	11 (7–17)	0.0002
Oncological treatment					
Any	72.3	26.2		10 (5–15)	
None	76.3	23.7	0.5314	10 (5–15)	0.7903*

Abbreviations: BMI, body mass index; IQR, interquartile range.
Agreement is indicated by an asterisk (*).

TABLE 4 Discriminative validity: the Colostomy Impact (CI) score was hypothesized to provide a significantly different CI score between groups if a difference was present measured by the anchor question and no difference in CI score if the groups reported the same rates of reduced health-related quality of life

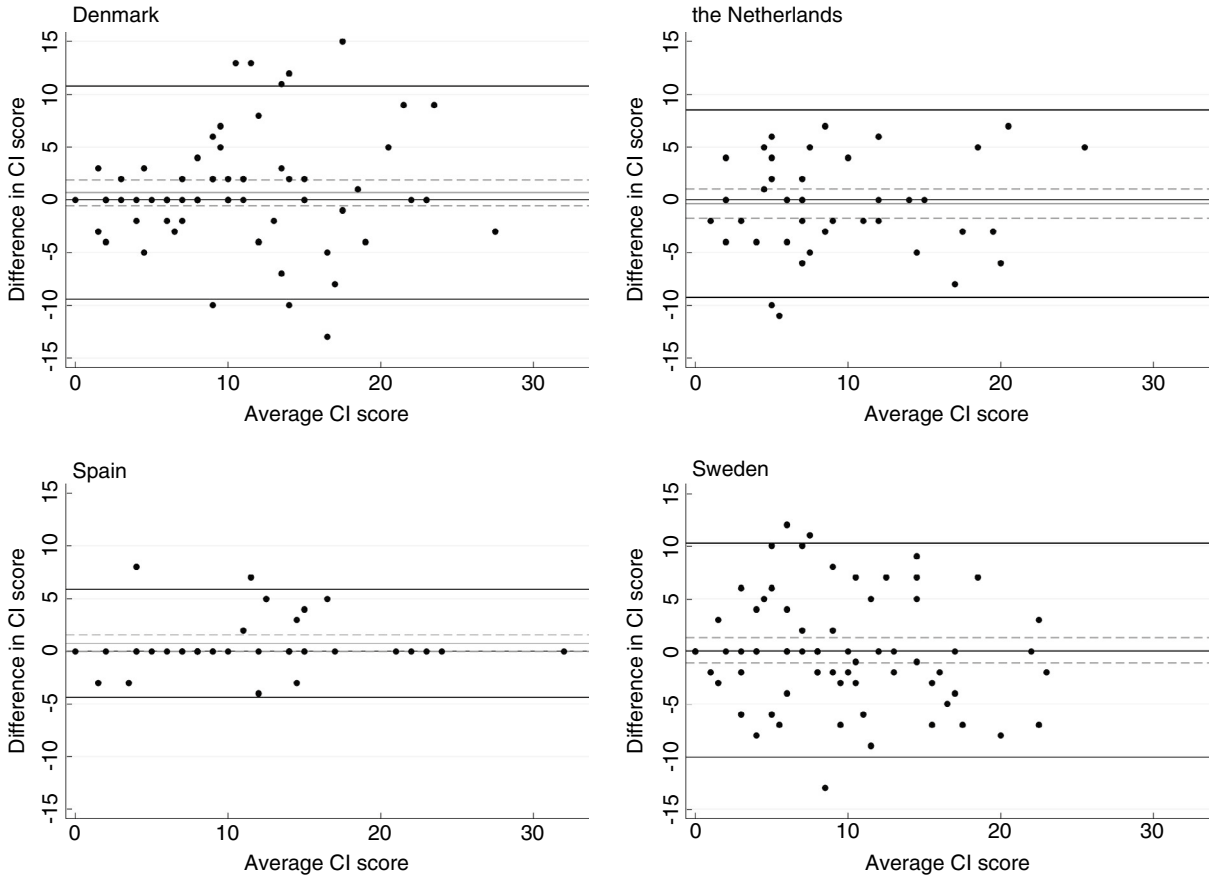

FIGURE 3 Bland Altman plots with difference in sum-score between the test and retest (light grey lines) with 95% CI (dashed light grey lines) and limits of agreement (dark grey lines) for each country

significant differences were clinically relevant, further supporting the construct validity of the CI score.

The EORTC QLQ-C30 is commonly used, and previous reports on colorectal cancer patients with a permanent colostomy correspond well to our results; mean scale scores found in three other cross-sectional studies are similar to the mean scores of the minor and major CI groups found in our study [19–21]. For the three included countries for which reference values of the background population were available, scale scores in the minor CI groups resemble scores of the reference population [22–24], indicating that the CI score categorized patients meaningfully into a minor group resembling the background population and a major group with impaired HRQoL.

The ROC analysis supported the hypothesis regarding sensitivity in one country and specificity in three countries for the primary anchor question. However, when looking at the additional anchor questions, sensitivity of the CI score detected dissatisfaction with life with a stoma with a sensitivity of 80% or more in all countries except Spain. Specificity was lower, which was also the case in the development of the CI score where a high sensitivity was intentionally prioritized. Thus, specificities in the ranges seen here were not surprising and patients with a sum-score in the major CI range (≥10) should have the possibility to decline further evaluation if they are satisfied with their colostomy and QoL. The anchor questions are not validated, but the primary anchor has repeatedly been used in a similar form for the development and validation of PROMs [6,25–27]. The broad phrasing may prompt different interpretations, especially when used in an international setting, which may affect sensitivity assessment. The addition of the other four anchor questions allowed us to further explore the construct measured by the CI score, and the score demonstrated highest sensitivity to dissatisfaction with life with a colostomy and to restrictions in daily activities.

The CI score was able to discriminate between groups that differed with regard to the proportion of patients reporting 'some/a lot' CI in the anchor question as five of our seven hypotheses were supported. Differences in median CI scores were modest (1–3 points) and whether this difference is clinically relevant remains unknown. However, differences in categorization according to the primary anchor question were similarly small.

This study demonstrates that the CI score is a reliable measure over time with no difference in scores between the test and the retest and ICCs in the moderate to excellent range.

We have demonstrated that the CI score is valid and reliable, and we highly encourage its use in the clinical setting where purposeful use of resources is crucial. Follow-up programmes after completed cancer treatment often focus on recurrence. However, concerns about late effects from the cancer treatment should be addressed [28–30]. Systematic use of the CI score will enable clinicians to screen colostomy patients in a quick and reliable way to identify

those suffering from stoma dysfunction so that targeted interventions can be initiated to improve patients' HRQoL.

A strength of this study is the meticulous professional translation of the CI score with adaptation from healthcare professionals ensuring semantically equivalent versions in all languages. Another strength is the use of validated EORTC questionnaires. The cross-sectional design and high number of patients included from each country reduces the risk of selection bias and increases generalizability. The large number of significance tests, however, increases the risk of type I error, which is a limitation to this study and was not taken into account when deciding on the significance level. Furthermore, the cross-sectional design hinders conclusions regarding causality when looking at differences in HRQoL between subgroups and evaluation of the responsiveness and minimal important change in the CI score. Numerous factors may affect HRQoL differently in different cultural settings, which may explain differences in scores between countries seen in this study. Financial concerns, cultural and religious norms and access to healthcare services vary between countries, and the impact of such differences on HRQOL should be further investigated. The CI score has proven to be valid and reliable in seven countries. However, validity cannot necessarily be generalized, and further validation studies are needed if the score is to be translated and used in new countries; future studies evaluating responsiveness and minimal important change of the CI score are encouraged.

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CONFLICT OF INTERESTS

None to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

This is the authors' own original work, and has not been previously published elsewhere.

AUTHOR CONTRIBUTION

Conceptualisation: Peter Christensen, Katrine Jøssing Emmertsen, Anne Thyø, Helle Ø Kristensen. *Methodology*: Peter Christensen, Katrine Jøssing Emmertsen, Anne Thyø, Helle Ø Kristensen, Thomas Pinkney, Neil Smart. *Data curation*: Helle Ø Kristensen, Andrea M. Warwick, Dong Pang, Edgar J. B. Furnée, Sanne J. Verkuijl, Nuno José Rama, Hugo Domingos, João Maciel, Alejando Solis-Peña, Eloy Espín Basany, Marta Hidalgo-Pujol, Sebastian Biondo, Annika Sjövall; statistical analyses, Helle Ø Kristensen. *Writing-original draft preparation*: Helle Ø Kristensen. *Writing–review and editing*: Helle Ø Kristensen, Peter Christensen, Katrine Jøssing Emmertsen, Anne Thyø, Thomas Pinkney, Neil Smart, Andrea M. Warwick, Dong Pang, Edgar J. B. Furnée, Sanne J. Verkuijl, Nuno José Rama, Hugo Domingos, João Maciel, Alejando Solis-Peña, Eloy Espín Basany, Marta Hidalgo-Pujol, Sebastian Biondo, Annika Sjövall. *Supervision*: Peter Christensen, Katrine Jøssing Emmertsen, Anne Thyø. All authors have read and agreed to the published version of the manuscript.

ORCID

Helle Ø Kristensen https://orcid.org/0000-0003-0153-7689
João Maciel https://orcid.org/0000-0003-2496-1642
Sebastian Biondo https://orcid.org/0000-0002-7374-0371

REFERENCES

1. Battersby NJ, Juul T, Christensen P, Janjua AZ, Branagan G, Emmertsen KJ, et al. Predicting the risk of bowel-related quality-of-life impairment after restorative resection for rectal cancer: a Multicenter Cross-Sectional Study. Dis Colon Rectum. 2016;59(4):270–80.
2. Bretagnol F, Rullier E, Laurent C, Zerbib F, Gontier R, Saric J. Comparison of functional results and quality of life between intersphincteric resection and conventional coloanal anastomosis for low rectal cancer. Dis Colon Rectum. 2004;47(6):832–8.
3. Pachler J, Wille-Jørgensen P. Quality of life after rectal resection for cancer, with or without permanent colostomy. Cochrane Database of Syst Rev. 2012;12:CD004323.
4. Kasparek MS, Hassan I, Cima RR, Larson DR, Gullerud RE, Wolff BG. Quality of life after coloanal anastomosis and abdominoperineal resection for distal rectal cancers: sphincter preservation vs quality of life. Colorectal Dis. 2011;13(8):872–7.
5. de Campos-Lobato LF, Alves-Ferreira PC, Lavery IC, Kiran RP. Abdominoperineal resection does not decrease quality of life in patients with low rectal cancer. Clin Sci. 2011;66(6):1035–40.
6. Thyø A, Emmertsen KJ, Pinkney T, Christensen P, Laurberg S. The colostomy impact score: development and validation of a patient reported outcome measure for rectal cancer patients with a permanent colostomy. A population-based study. Colorectal Dis. 2017;19(1):25–33.
7. Kasparek MS, Hassan I, Cima RR, Larson DR, Gullerud RE, Wolff BG. Long-term quality of life and sexual and urinary function after abdominoperineal resection for distal rectal cancer. Dis Colon Rectum. 2012;55(2):147–54.
8. Prieto L, Thorsen H, Juul K. Development and validation of a quality of life questionnaire for patients with colostomy or ileostomy. Health Qual Life Outcomes. 2005;3(c):1–12.
9. Grant M, Ferrell B, Dean G, Uman G, Chu D, Krouse R. Revision and psychometric testing of the City of Hope Quality of Life-Ostomy Questionnaire. Qual Life Res. 2004;13(8):1445–57.
10. Nafees B, Rasmussen M, Lloyd A. The Ostomy-Q: development and psychometric validation of an instrument to evaluate outcomes associated with ostomy appliances. Ostomy Wound Manag. 2017;63(1):12–22.
11. Kristensen HØ, Krogsgaard M, Christensen P, Thomsen T. Validation of the Colostomy Impact score in patients ostomized for a benign condition. Colorectal Dis. 2020;22(12):2270–7.
12. Fayers PM, Aaronson NK, Bjordal K, Groenvold M, Curran D, Bottomley A, et al. EORTC QLQ-C30 Scoring Manual. The EORTC QLQ-C30. Introduction. EORTC QLQ-C30 Scoring Man. 2001;30:1–67.

APPENDIX 4

- **Nuno Rama**, P. Ferreira, T. Jull and J. Pimentel.

"Validation of Portuguese version of the Low Anterior Resection Syndrome Score".

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Original Article

Validation of Portuguese version of the low anterior resection syndrome score

Nuno José Rama[a,b,*], *Pedro Lopes Ferreira*[c,d], *João Pimentel*[e,f], *Therese Juul*[g], *on behalf of the Portuguese PT LARS Collaborative Group*

[a] *Centro Hospitalar de Leiria, Centro Colorretal, Leiria, Portugal*
[b] *Universidade do Porto, ICBAS, Porto, Portugal*
[c] *Universidade de Coimbra, Faculdade de Economia, Coimbra, Portugal*
[d] *Universidade de Coimbra, Centro de Estudos e Investigação em Saúde (CEISUC), Coimbra, Portugal*
[e] *Centro Hospitalar e Universitário de Coimbra, Centro Colorretal, Coimbra, Portugal*
[f] *Universidade de Coimbra, Faculdade de Medicina, Coimbra, Portugal*
[g] *Aarhus University Hospital, Department of Surgery P, Aarhus, Denmark*

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ABSTRACT

Objective: The authors aim to perform a thorough translation with cultural adaptation of the patient reported outcome tool, Low Anterior Resection Syndrome (LARS) Score, to the Portuguese language (LARS-PT) in the Portuguese population with rectal cancer, after proctectomy with anastomosis.

Methods: According to the current international recommendations, we designed this study encompassing three main phases: (i) cultural and linguistic validation to European Portuguese; (ii) feasibility and reliability tests of the version obtained in the previous phase; and (iii) validity tests to produce a final version. The questionnaire was completed by 154 patients from six Portuguese Colorectal Cancer Units, and 58 completed it twice.

Results: Portuguese version of LARS score showed high construct validity. Regarding the test-retest, the global Intraclass Correlation showed very strong test-retest reliability. Looking at all five items, only items 3 and 5 present a moderate correlation. LARS score was able to discriminate symptoms showing worse quality of life, in patients submitted to preoperative radio and chemotherapy.

Conclusions: LARS questionnaire has been properly translated into European Portuguese, demonstrating high construct validity and reliability. This is a precise, reproducible, simple, clear and user-friendly tool for evaluating bowel function in rectal cancer patients after sphincter saving operation.

* *Corresponding author.*
E-mail: ramanuno@gmail.com (N.J. Rama).
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Validação da versão em português do escore da síndrome da ressecção anterior baixa

Palavras-chave:
Neoplasias retais
Disfunção intestinal
Escore da síndrome da ressecção anterior baixa
Qualidade de vida
Validação

RESUMO

Objetivo: Os autores pretendem fazer uma tradução minuciosa e culturalmente adaptada para a língua portuguesa do escore da Síndrome de Ressecção Anterior Baixa (*Low Anterior Resection Syndrome* [LARS]), um instrumento de desfecho relatado pelo paciente, na população portuguesa com câncer retal após proctectomia com anastomose.

Métodos: De acordo com as recomendações internacionais atuais, o estudo foi projetado abrangendo três fases principais: (i) validação cultural e linguística para o português europeu; (ii) testes de viabilidade e confiabilidade da versão obtida na fase anterior; e (iii) testes de validade para produzir a versão final. O questionário foi preenchido por 154 pacientes de seis unidades portuguesas de câncer colorretal e 58 pacientes completaram duas vezes.

Resultados: A versão em português do escore LARS mostrou alta validade de construto. A correlação intra-classe global apresentou confiabilidade muito forte no teste-reteste. Considerando-se todos os cinco itens, apenas os itens 3 e 5 apresentam uma correlação moderada. O escore LARS foi capaz de discriminar sintomas com pior qualidade de vida em pacientes submetidos a radio- e quimioterapia pré-operatória.

Conclusões: O questionário LARS foi traduzido corretamente para o português europeu, demonstrando alta validade de construto e confiabilidade. Trata-se de uma ferramenta precisa, reproduzível, simples, clara e fácil de usar para avaliar a função intestinal em pacientes com câncer retal após operações poupando o esfíncter.

Introduction

Colorectal cancer is the third most frequent diagnosed malignancy followed by prostate in males, breast in females, and by lung cancer in both genders.[1,2] On this matter, one out of three are located in the rectum, one-third on its distal part, and approximately half of patients die from their cancer.[3,4] The incidence and mortality rates vary according to distinct gradients of human development levels, presenting a stabilizing or decreasing trend in highly developed countries, where rates remain amongst the highest in the world.[1]

During the last decades, several improvements in Rectal Cancer (RC) treatment were achieved, but surgery remains the favoured form of treatment. These developments have resulted in markedly increased survival.[5] A tailored treatment was possible since the introduction of routine accurate high-resolution preoperative RC imaging and the standardized proctectomy with Total Mesorectal Excision (TME).[6]

Nowadays, not only oncological outcomes are relevant for colorectal surgeons, but also long-term functional outcomes and Quality of Life (QoL). Knowledge about functional gastrointestinal and genitourinary patient-reported outcomes are crucial in order to select the optimal treatment and to manage functional sequela.[7,8] Despite the rectal reconstruction technique and the use of neoadjuvant therapy, 60% to 90% of patients undergoing proctectomy develop some sort of bowel dysfunction.[9,10]

The syndrome of defecatory dysfunction that occurs after proctectomy, also called "Low Anterior Resection Syndrome (LARS)", is a constellation of symptoms, with variable incidence and degrees, which includes increased bowel frequency, urgency, fragmentation, faecal incontinence, nocturnal defecation, difficulty in discriminating between gas and stools, and incomplete evacuation.[8,11–13]

Several measurement instruments have been used to evaluate bowel dysfunction after anterior resection, but mostly are focused on the incontinence aspect of LARS.[14–18] One of the drawbacks of these tools was the fact that they are based on a linear scale, and the impact on QoL might not be so foreseeable and linear. Additionally these scores only look into one facet of LARS, not considering it as a complex dysfunction.

Recently, Emmertsen and Laurberg developed and validated a symptom-based scoring system, named LARS score that takes into account four aspects of bowel function.[19] This quick, simple and user-friendly self-administered questionnaire objectively measures patient symptoms, and provides information for the LARS management. It consists of five simple questions regarding incontinence for flatus or liquid stool, urgency, clustering and frequency. Scored according to the impact of each of these symptoms in patients' QoL, they are weighted and presented in a summative score ranging from 0 to 42. Patients are ranked into three severity groups: no LARS (0–20 points), minor LARS (21–29 points) and major LARS (30–42 points). Until now, this score has been translated and validated in several languages, worldwide.[11,13,20,21]

The aim of our study was to perform a thorough translation with cultural adaptation of this patient-reported outcome tool (LARS score) to the European Portuguese language (LARS-PT). We assessed its psychometric properties in a Portuguese

sample, in order to build up and validate a suitable tool for daily clinical practice and research in Portugal.

Methods

This study encompassed three main phases: (i) cultural and linguistic validation to European Portuguese; (ii) feasibility and reliability tests of the version obtained in the previous phase; and (iii) validity tests to produce a final version.

After obtaining a written permission from the original author, we followed the forward/backward translation process.[22] The English version of the LARS score was then initially translated into Portuguese by two independent professional translators whose mother tongue was Portuguese. Our group discussed any conceptual discrepancies between the two versions, and we reached a final consensus, the preliminary Portuguese version. A third independent English translator, unfamiliar with the background objectives of the study, then performed a back-translation of this version.

After comparing the original and the backward versions, the investigators revised, checked and agreed upon the Portuguese version. For the face validation process, two clinicians revised this new version and some changes were made accordingly. In addition, a cognitive debriefing sample of ten patients with low literacy level were selected from two participating centres, in order to assess its feasibility, comprehensiveness, length, adequacy, redundancy and text clarity. The final version of LARS-PT was linguistically reviewed to correct possible grammatical errors.

The participants involved were recruited from six Portuguese hospitals, with colorectal cancer units (CRCU), between November 2016 and June 2017. Our study comprised voluntary patients operated for RC, over 18 years old that had undergone either a curative total or Partial Mesorectal Excision (PME), from January 1, 2005 to April 30, 2015. We established a minimum duration of fourteen months after surgery to allow their bowel function to have regained stability. Patients were excluded if they had stoma, disseminated or recurrent disease, any type of bowel dysfunction not related to RC treatment (inflammatory bowel disease, irritable bowel syndrome, amongst others), or mental health problems.

Eligible participants were identified through local medical records of RC patients by the local investigators at each of the participating centres and the patients to be approached were selected randomly from the pool of eligible subjects. The six local clinical researchers collected demographic and clinical information from local databases. Patients received the LARS-PT questionnaire along with an invitation to participate in the study. In addition, we also administered the Portuguese versions of the two quality of life measures EORTC QLQ-C30 and EQ-5D-5L, and a separate "bothersome" question also aiming to assess their QoL ("Overall, how much does your bowel function affect your quality of life?"). The answers from the "bothersome" question were classified according to the inconvenience, where 1 is none and 5–7 is extremely inconvenient.

In most of CRCU, patients who had a T3 tumour with a threatened circumferential margin or T4 tumour (any N) were submitted to neoadjuvant long-course chemoradiotherapy. Moreover, in some CRCU, patients with T3 (any N) cancer or T1 or T2 cancer with node positive underwent short-course radiotherapy (5 × 5 Gy) before surgery. The operative procedure included midline laparotomy or minimally invasive approach, high ligation of the inferior mesenteric vessels, mobilization of the splenic flexure, and colorectal resection with standard TME or PME (depending on the tumour location). All the patients included in the study had negative distal and circumferential margins on histological examination.

In our study, we tested the temporal stability by a randomized subgroup of patients and asked them to fill the LARS-PT questionnaire, between one to two weeks after the completion of the first round. The interviews were face-to-face or by phone, depending upon the local facilities and the resources available. We excluded any retest if the time gap between the completions of both tests was outside the predefined acceptable interval of one to twelve weeks. Furthermore, we did not consider for test-retest analysis, patients who had mentioned a relevant change in bowel function in the revaluation period. Intraclass Correlation Coefficient (ICC) was used and was considered significant if higher than 0.7.[23]

It includes the analysis of the content validity, the construct validity and the criteria validity. The cultural and linguistic adaptation process guarantees the content validity. The construct validity tests whether the theoretical framework of the measurement instrument is confirmed by the Portuguese version. This includes hypotheses regarding known sociodemographic and clinical variables, as well as the correlations with a measurement instrument that measures similar concepts. The criterion validity represents the degree of agreement between the measurement instrument and another reference measure. In this study, we used the previously referred bothersome question.

In this study, all statistical analyses were performed using SPSS v22, considering a significance level of 0.05.

Demographic and clinical variables were analyzed by using descriptive statistics. For comparative analyses, we used nonparametric tests, namely, Mann–Whitney *U* and Kruskal–Wallis *H* tests.

To evaluate the criterion validity, Chi-squared test was used to test the independence between these variables and the LARS classified score.

Results

Both translations of LARS demonstrated minor discrepancies easily solved, and discussion of the back translation corroborated the original meaning of the five questions. Cognitive debriefing involved six males and four females, seven aged 65 or more, and all of them with medium to low education. None of the ten patients revealed difficulties in understanding the items. This guaranteed the content validity of this measure. The final Portuguese version can be found in https://www.escp.eu.com/images/news_and_reports/2018/lars-scoring-tool/Portuguese-Portugal-LARS-Questionnaire.pdf.

From November 2016 to June 2017, 154 patients answered the questionnaire LARS-PT. Demographic and clinical information obtained by the six local clinical researchers is presented in Table 1.

Table 1 – Sociodemographic and clinical sample characteristics.

Variable		n	%
Participants		154	100.0
Gender	Male	89	57.9
	Female	65	42.1
Age (years)	<65 years	60	39.0
	65–74 years	46	29.9
	>75 years	48	31.2
	Mean ± SD	68.1 ± 10.9	
	Min–max	36–89	
Family status	Married	126	82.9
	Single	5	3.3
	Widow	13	8.6
	Divorced/separated	8	5.3
Labour status	Active	38	25.7
	Non-active	110	74.3
Education	Less than basic	14	9.2
	Basic (years 1–9)	100	65.8
	Secondary (years 10–12)	18	11.8
	Higher	20	13.2
Stage, TNM	I	38	28.8%
	II	24	18.2%
	III	70	53.0%
Tumour localization	Upper third	45	31.0%
	Middle third	76	52.4%
	Lower third	24	16.6%
Type of anastomosis	Mechanic	136	94.4%
	Manual	8	5.6%
Neoadjuvant radiotherapy	Yes	71	49.0%
	No	74	51.0%
Length of the postoperative period	<3 years	63	44.1%
	≥3 years	80	55.9%
	Mean ± SD	10.3 ± 3.7	
	Min–max	0.0–10.3	
Type of surgery	TME	97	71.3%
	PME	39	28.7%
LARS score	No LARS	52	34.2%
	Minor LARS	37	24.3%
	Major LARS	63	41.4%
	Mean ± SD	23.9 ± 12.4	
	Min–max	0–42	

From Table 1, is evident that our sample had a slight majority (57.9%) of male patents, only 39.0% of the patients had less than 65 years of age, the majority were married (82.9%), professionally non-active (74.3%), and with less than ten years of education (75.0%).

Their tumour was mainly in Stage III (53.1%) and located in the middle third (52.4%), half underwent neo-adjuvant therapy (51.0%) and the mean length of the postoperative period was about 10 years. The type of mesorectal excision was mainly (71.3%) TME.

LARS scores ranged between 0 and 42 with a mean value of 23.9 ± 12.4, a little bit more than one-third (34.2%) with no LARS, 24.3% with minor LARS and 41.8% with major LARS.

Moreover, Table 2 presents the description of the quality of life indicators of our sample.

From Table 2 we notice that, in general, the patients of this study felt a very good quality of life. This is evident from the EORTC-QLQ-C30 functional scales with mean scores between 83.7 and 86.7, from the quality of life questions with a mean of 73.3, and from both index and VAS scale with mean values, respectively, 0.90 and 74.5. Corroborating with these results, and looking at the intensity of the symptoms, we evidence only a light disturbance from sleep, fatigue, pain, diarrhoea and constipation.

Regarding the test-retest, 58 patients repeated the LARS questionnaire, up to three weeks after the completion of the first questionnaire. Table 3 shows the reliability scores.

The global ICC shows very strong test-retest reliability. Looking at all five items, only items 3 and 5 present a moderate correlation.

Validity

To test the construct validity of LARS we looked at the sociodemographic and clinical variables. The results of the tests are presented in Table 4.

Looking at the results from Table 4, we can notice that the sociodemographic variables (gender, age, family status, and labour status) do not have any influence on the LARS final score. In addition, the length of the postoperative period seems to not have any influence on LARS sores. On the contrary, having neo-adjuvant radiotherapy increases LARS scores.

Still addressing construct validity, we looked at the correlations between LARS scores and the various dimensions of EORTC QLQ-C30 as well as EQ-5D-5L index and the EQ-5D-VAS. The results of the corresponding correlation coefficients are presented in Table 5.

From Table 5, as expected, we can see that the major correlation resides on the dimension 'social function' of the EORTC QLQ-C30's functional scales and, mainly on the symptoms pain, and diarrhoea. Financial impact also showed to have a very significant correlation on LARS scores. On the other hand, quality of life showed a very small correlation and EQ-5D-5L was unable to find any significant correlation with the LARS score.

Finally, the independence test between "bothersome" question and the classified LARS scores revealed a Chi-squared statistics of $X^2 = 16.8$ ($\alpha = 0.002$) showing that LARS classification is coherent with how much bowel function affects quality of life. That is, individuals who reported no bother at all, also had a LARS score less than or equal to 20, meaning no LARS. On the other hand, individuals with major LARS were the ones that mentioned their QoL being largely affected by bowel function.

Discussion

Historically, the most relevant outcomes in RC management were mortality and local recurrence, but currently, the evaluation of functional results and QoL of the patients submitted to LAR is a matter of great importance.

Dysfunctions after proctectomy, mainly in LAR, occur in a great number of patients, and affect not only the bowel

Table 2 – Quality of life scores.

QoL measure	Dimension	Min	Max	Mean	SD
EORTC-QLQ-C30	Physical function	0.0	100.0	83.7	19.6
Functional scales	Role physical	0.0	100.0	85.9	24.9
	Emotional function	25.0	100.0	85.9	16.9
	Cognitive function	16.7	100.0	86.1	17.0
	Social function	0.0	100.0	86.7	22.0
EORTC-QLQ-C30	Fatigue	0.0	88.9	18.0	21.1
Symptom scales	Nausea and vomiting	0.0	50.0	1.2	6.9
	Pain	0.0	100.0	14.9	22.1
	Dyspnoea	0.0	66.7	1.5	8.0
	Sleep disturbance	0.0	100.0	18.5	25.6
	Appetite loss	0.0	66.7	5.0	14.7
	Constipation	0.0	100.0	11.1	20.2
	Diarrhoea	0.0	100.0	12.4	20.9
	Financial impact	0.0	100.0	9.8	19.8
	Quality of life	16.7	100.0	73.3	19.0
EQ-5D-5L	Index	16	1.00	0.90	0.16
	VAS	10	100.0	74.5	0.19

Table 3 – Reliability scores.

Items	ICC	95% CI
Item 1	0.763	0.600–0.860
Item 2	0.863	0.769–0.919
Item 3	0.652	0.413–0.794
Item 4	0.761	0.596–0.859
Item 5	0.669	0.441–0.804
LARS total score	0.864	0.771–0.920

ICC, Intraclass Correlation; CI, Confidence Interval.

function but also the genitourinary function, in high figures, up to 70 or even 90%, when we look to bowel dysfunction. These symptoms often arise immediately after surgery and may decrease over the months, reaching a plateau within the first two years.[24] In fact, up to 80% of patients undergoing a LAR or a very LAR will experience postoperatively a constellation of symptoms collectively referred as LARS.[5,25] Although most of the functional impairments are clinically recovered in the first year after the proctectomy, long-term studies are now reporting the presence of adverse symptoms up to 15 years after resection.[20,26]

LARS score, despite being considered user-friendly, had not been tested in the Portuguese population, yet. Our group followed a rigorous protocol in accordance with current international recommendations, similar to that used in the international validation of the LARS score by Juul et al., to

Table 4 – Sociodemographic and clinical determinants of LARS scores.

Hypothesis	Variable	Value	Mean rank	Statistics	Sig.
H1	Gender	Male	73.7	$U = 2568$	0.354
		Female	80.4		
H2	Age (years)	<65 years	84.3	$H = 3.359$	0.186
		65–74 years	78.1		
		>75 years	68.5		
H3	Family status	Married	76.8	$U = 1599$	0.849
		Non-married	75.0		
H4	Labour status	Active	74.5	$U = 2088$	0.993
		Non-active	74.5		
H5	Neoadjuvant radiotherapy	Yes	81.5	$U = 2022$	0.017
		No	64.8		
H6	Anastomosis	Mechanic	69.8	$U = 184$	0.002
		Manual	117.5		
H7	Length of the postoperative period	≤2 years	69.2	$U = 1406$	0.622
		>2 years	73.8		
H8	Type of surgery	TME	70.3	$U = 1718$	0.405
		PME	64.1		

U, Wilcoxon *W*; *H*, Kruskal–Wallis *H*; Sig, asymptotic Sig (2 tailed).

Table 5 – Criterion validity of LARS.

QoL measure	Dimension	LARS scores	*p*-value
EORTC-QLQ-C30	Physical function	−0.116	0.153
Functional scales	Role physical	−0.125	0.123
	Emotional function	−0.131	0.105
	Cognitive function	−0.122	0.134
	Social function	−0.163	0.044
EORTC-QLQ-C30	Fatigue	0.130	0.110
Symptom scales	Nausea and vomiting	0.062	0.448
	Pain	0.206	0.011
	Dyspnoea	0.015	0.856
	Sleep disturbance	0.086	0.289
	Appetite loss	−0.054	0.507
	Constipation	0.073	0.367
	Diarrhoea	0.353	0.000
	Financial impact	0.189	0.020
	Quality of life	−0.150	0.064
EQ-5D-5L	Index	−0.116	0.153
	VAS	−0.089	0.274

ensure semantic equivalence among different languages and to enable the use in different populations worldwide.[20,21,27] We developed this research in six CRUC with patients coming from five public health system institutions and one private hospital. With this method, we guarantee an adequate, balanced national representativeness, including patients with low educational and income levels. None of them exhibited difficulty to understand the items of the questionnaire during the cultural adaptation, proving the practical feasibility of this tool. Overall, we found a good compliance across all items, which demonstrate the user-friendliness of the LARS score.

In our study, LARS score was easily validated for the Portuguese population of patients with RC, and has shown concluding psychometric properties. Considering the construct validity, we have proved a strong association between the LARS-PT score and the self-reported QoL. Patients with poor QoL, due to impaired bowel function, demonstrated higher numerical values on LARS-PT questionnaire. Moreover, LARS-PT score presented a convergent agreement with overall health and with all EORTC QLQ-C30 functional scales, showing that patients with worse LARS classification have lower QoL reported by EORTC QLQ-C30.

The current study provided some evidence for the good discriminate validity of the measures. That is clearly highly important, since the utility of the LARS-PT score would be hampered without the ability to discriminate between patients with different clinical characteristics, known to diverge in terms of LARS symptoms. In this topic, the Portuguese version of LARS score was able to identify groups with worse intestinal functional outcomes after LAR. Known variables such as gender, age, level of the tumour, preoperative therapy, type of procedure (TME vs. PME), temporary diverting stoma and postoperative period length could impair gastrointestinal function after sphincter saving surgery in RC population.[5,24,28] LARS-PT score showed ability to detect differences between patients submitted or not to neo-adjuvant treatment. In our study, we did not prove that LARS symptoms improve with time. By contrast, there were no statistically significant differences related with gender, age, family status or labour status.

Also criterion validity tested with the bothersome question showed that LARS classification is coherent with how much bowel function affects quality of life ($X^2 = 16.8$; $p = 0.002$).

The evaluation of test-retest reliability of LARS-PT score was done from a sample of 58 patients, with the interval between the two surveys ranging from 10 to 21 days. This interval was deemed appropriate, as it avoids not only the first survey effect but also the changes in bowel function, even though participants who reported a significant change in bowel function between the tests were excluded. After repeating the evaluation, no differences were registered in LARS-PT questions and score. The global ICC estimated (ICC = 0.864) demonstrates a very strong test-retest reliability, and when we look at all five items, only items 3 and 5 present a moderate correlation (ICC of 0.652 and 0.669, respectively).

Limitations of this study were the small sample size and its retrospective observational nature, mainly the fact that the anorectal function was not assessed before surgery. The preoperative use of LARS score and the regular surveillance in the early and late postoperative period may contribute to clarifying some aspects of LARS pathophysiology. Some preoperative factors, like neo-adjuvant therapies, gender, age or tumour location, may affect postoperative function, so it is crucial to guide an appropriate preoperative discussion outlining risk and options. The question is: "Can we predict bowel function before proctectomy?" Recently, Battersby et al. developed the POLARS score, and with this instrument, patients with RC can be preoperatively informed of their likely postoperative bowel function, based on the LARS scores evaluation.[29] Additionally it can be used as an adjunct for clinical assessment prior to the multidisciplinary team discussion, helping to guide treatment decisions.

This study has the advantage of having compared the LARS score with a validated general and symptoms-based QoL instruments such as EQ-5D-5L and EORTC QLQ-C30. As we abovementioned, the majority of instruments used to assess bowel function after LAR, measure only faecal incontinence, omitting other symptoms at least so relevant, and with high correlation with QoL, such as urgency or clustering. These symptoms are most closely correlated with QoL, in a patient-centred perspective. Validation of this tool enables the dissemination of the measurement of bowel function after LAR, employing a quick and comprehensive clinically applicable instrument. Therefore, it will help clinicians to understand the impact of LARS symptoms in QOL, from the patient viewpoint.[10,26,29]

In conclusion, LARS questionnaire has been properly translated into Portuguese, demonstrating high construct validity and reliability. Our LARS version is a precise, reproducible, simple, clear and user-friendly tool for evaluating bowel function in RC patients after sphincter saving operation. Thereby should be systematically applied for both clinical and research settings.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the Portuguese ethical standards: Authorization was obtained from the Portuguese Data Protection Authority (CNPD) and Local Ethical Committee approval. Informed consent was obtained from all patients included in the study.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Portuguese LARS collaborative group

Portuguese LARS Collaborative Group includes:

Writing group: N. Rama, P.L. Ferreira, J. Pimentel and T. Jull.

Local Researchers:

Centro Hospitalar de Leiria: N. Rama, P. Alves, P. Clara, S. Amado, I. Gil, I. Sales

Centro Hospitalar e Universitário de Coimbra: J. Pimentel

Champalimaud Foundation: N. Figueiredo, H. Domingos, P. Vieira

IPO Lisboa: M. Limbert, J. Maciel

Hospital Distrital de Santarém: N. Vilela, L. Ferreira, O. Oliveira

Hospital de Braga: P. Leão, A. Goulart, M. Sousa.

REFERENCES

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66:683–91.
2. Ziv Y, Zbar A, Bar-Shavit Y, Igov I. Low anterior resection syndrome (LARS): cause and effect and reconstructive considerations. Tech Coloproctol. 2013;17:151–62.
3. Valentini V, Beets-Tan R, Borras J, Krivokapic Z, Leer J, Pahlman L, et al. Evidence and research in rectal cancer. Radiother Oncol. 2008;87:449–74.
4. Riihimäki M, Hemminki A, Sundquist J, Hemminki K. Patterns of metastasis in colon and rectal cancer. Sci Rep. 2016.
5. Emmertsen KJ, Chen TY-T, Laurberg S. Functional results after treatment for rectal cancer. J Coloproctol (Rio J). 2014;34: 55–61.
6. Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery – the clue to pelvic recurrence? Br J Surg. 1982;69:613–6.
7. Desnoo L, Faithfull S. A qualitative study of anterior resection syndrome: the experiences of cancer survivors who have undergone resection surgery. Eur J Cancer Care. 2006;15:244–51.
8. Juul T, Battersby NJ, Christensen P, Janjua AZ, Branagan G, Laurberg S, et al. Validation of the English translation of the low anterior resection syndrome score. Colorectal Dis. 2015;17:908–16.
9. Kakodkar R, Gupta S, Nundy S. Low anterior resection with total mesorectal excision for rectal cancer: functional assessment and factors affecting outcome. Colorectal Dis. 2006;8:650–6.
10. Keane C, Wells C, O'Grady G, Bissett IP. Defining low anterior resection syndrome: a systematic review of the literature. Colorectal Dis. 2017;19:713–22.
11. Hou X.-t., Pang D, Lu Q, Yang P, Jin S-l, Zhou Y-j, et al. Validation of the Chinese version of the low anterior resection syndrome score for measuring bowel dysfunction after sphincter-preserving surgery among rectal cancer patients. Eur J Oncol Nurs. 2015;19:495–501.
12. Ribas Y, Aguilar F, Jovell-Fernandez E, Cayetano L, Navarro-Luna A, Munoz-Duyos A. Clinical application of the LARS score: results from a pilot study. Int J Colorectal Dis. 2017;32:409–18.
13. Samalavicius NE, Dulskas A, Lasinskas M, Smailyte G. Validity and reliability of a Lithuanian version of low anterior resection syndrome score. Tech Coloproctol. 2016;20:215–20.
14. de la Portilla F, Calero-Lillo A, Jiménez-Rodríguez RM, Reyes ML, Segovia-González M, Maestre MV, et al. Validation of a new scoring system: rapid assessment faecal incontinence score. World J Gastrointest Surg. 2015;7:203–7.
15. Jorge JM, Wexner SD. Etiology and management of fecal incontinence. Dis Colon Rectum. 1993;36:77–97.
16. Rockwood TH, Church JM, Fleshman JW, Kane RL, Mavrantonis C, Thorson AG, et al. Patient and surgeon ranking of the severity of symptoms associated with fecal incontinence: the fecal incontinence severity index. Dis Colon Rectum. 1999;42:1525–32.
17. Vaizey C, Carapeti E, Cahill J, Kamm M. Prospective comparison of faecal incontinence grading systems. Gut. 1999;44:77–80.
18. Williams NS, Patel J, George BD, Hallan RI, Watkins ES. Development of an electrically stimulated neoanal sphincter. Lancet (London, England). 1991;338:1166–9.
19. Emmertsen KJ, Laurberg S. Low anterior resection syndrome score: development and validation of a symptom-based scoring system for bowel dysfunction after low anterior resection for rectal cancer. Ann Surg. 2012;255:922–8.
20. Juul T, Ahlberg M, Biondo S, Emmertsen KJ, Espin E, Jimenez LM, et al. International validation of the low anterior resection syndrome score. Ann Surg. 2014;259:728–34.
21. Buzatti K. Validação para a Língua Portuguesa do Escore "LARS" de avaliação da Síndrome Pós-ressecção Anterior do Reto [Dissertation]. Brasil: Universidade Federal de Minas Gerais; 2016.
22. Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A, et al. Principles of good practice for the translation and cultural adaptation process for patient-reported outcomes (PRO) measures: report of the ISPOR Task Force for Translation and Cultural Adaptation. Value Health. 2005;8:94–104.
23. Terwee CB, Bot SD, de Boer MR, van der Windt DA, Knol DL, Dekker J, et al. Quality criteria were proposed for measurement properties of health status questionnaires. J Clin Epidemiol. 2007;60:34–42.
24. Bleier JI, Maykel JA. Outcomes following proctectomy. Surg Clin North Am. 2013;93:89–106.
25. Bryant CL, Lunniss PJ, Knowles CH, Thaha MA, Chan CL. Anterior resection syndrome. Lancet Oncol. 2012;13:e403–8.
26. Chen TY, Emmertsen KJ, Laurberg S. Bowel dysfunction after rectal cancer treatment: a study comparing the specialist's versus patient's perspective. BMJ Open. 2014;4:e003374.

APPENDIX 5

- The 2017 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** and al. from Portugal).

"Safety of primary anastomosis following emergency left sided colorectal resection: an international, multi-Center prospective audit".

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Co-author (integrating ESCP collaborative group); Part 1 – Chapter 1.

Original article

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Safety of primary anastomosis following emergency left sided colorectal resection: an international, multi-centre prospective audit

The 2017 European Society of Coloproctology (ESCP) collaborating group

European Society of Coloproctology (ESCP) Cohort Studies Committee, Hospital Universitari i Politècnic la Fe, València, Spain

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Abstract

Introduction Some evidence suggests that primary anastomosis following left sided colorectal resection in the emergency setting may be safe in selected patients, and confer favourable outcomes to permanent enterostomy. The aim of this study was to compare the major postoperative complication rate in patients undergoing end stoma *vs* primary anastomosis following emergency left sided colorectal resection.

Methods A pre-planned analysis of the European Society of Coloproctology 2017 audit. Adult patients (> 16 years) who underwent emergency (unplanned, within 24 h of hospital admission) left sided colonic or rectal resection were included. The primary endpoint was the 30-day major complication rate (Clavien-Dindo grade 3 to 5).

Results From 591 patients, 455 (77%) received an end stoma, 103 a primary anastomosis (17%) and 33 primary anastomosis with defunctioning stoma (6%). In multivariable models, anastomosis was associated with a similar major complication rate to end stoma (adjusted odds ratio for end stoma 1.52, 95%CI 0.83–2.79, $P = 0.173$). Although a defunctioning stoma was not associated with reduced anastomotic leak (12% defunctioned [4/33] *vs* 13% not defunctioned [13/97], adjusted odds ratio 2.19, 95%CI 0.43–11.02, $P = 0.343$), it was associated with less severe complications (75% [3/4] with defunctioning stoma, 86.7% anastomosis only [13/15]), a lower mortality rate (0% [0/4] *vs* 20% [3/15]), and fewer reoperations (50% [2/4] *vs* 73% [11/15]) when a leak did occur.

Conclusions Primary anastomosis in selected patients appears safe after left sided emergency colorectal resection. A defunctioning stoma might mitigate against risk of subsequent complications.

Keywords Surgery, emergency surgery, colon cancer, rectal cancer, gastrointestinal surgery, anastomotic leak, surgical complications, surgical outcomes

What does this paper add to the literature?
Anastomosis after emergency left sided colorectal resection is performed in up to one in five patients. In these highly selected patients, this study suggests that it is safe practice. A defunctioning stoma may mitigate against risk if an anastomotic leak subsequently occurs.

Introduction

In patients undergoing emergency left sided colorectal surgery, resection with end colostomy is a commonly described procedure. Concerns about the safety of any anastomosis in the emergency setting are particularly high in the presence of contamination or an unstable patient [1,2]. Although a stoma avoids the risk of anastomotic leak, it carries with its own morbidity and mortality profile (27–55% and 4–27% respectively) [2]. For patients that undergo end stoma formation, the reversal rate is as low as 44% in published series [3], with a significant impact on long-term quality of life and a risk of stoma-related complications.

Many studies have evaluated primary anastomosis in the emergency setting with generally favourable results. Multiple single-centre, retrospective, observational studies have demonstrated that anastomosis can be safely performed in selected patients within

Correspondence to: Luis Sánchez-Guillén, European Society of Coloproctology (ESCP) Cohort Studies Committee, Hospital Universitari i Politècnic la Fe, Avinguda de Fernando Abril Martorell, 106, 46026 València, Spain.
E-mail: drsanchezguillen@gmail.com

the emergency setting, even in presence of peritonitis [4–6]. However, the number of supporting randomised trials in the literature is low and those that exist are mainly related to peritonitis secondary to perforated diverticulitis, with primary anastomosis often only undertaken by specialised colorectal surgeons [4,7,8].

Decision-making about whether to create a primary anastomosis in selected, stable patients in an emergency setting remains a challenge for the individual surgeon. The decision must take into account patient comorbidities, intraoperative findings, underlying colorectal pathology, clinical status of the patient and expertise of the surgeon [9]. The aim of this multi-centre international study was to examine whether current decision-making in real-world settings supports primary anastomosis as a safe technique in selected patients after emergency left sided colorectal resection.

Methods

Protocol and centres

This prospective, observational, multi-centre study was conducted in line with a pre-specified protocol (http://www.escp.eu.com/research/cohort-studies). All participating centres were responsible for compliance to local approval requirements for ethics approval or indemnity as required. In the UK, the National Research Ethics Service tool recommended that this project was not classified as research, and the protocol was registered as clinical audit in all participating centres. Any unit performing gastrointestinal surgery was eligible to register to enter patients into the study. No minimum case volume, or centre-specific limitations were applied. The study protocol was disseminated to registered members of the European Society of Coloproctology (ESCP), and through national surgical or colorectal societies. This study represents planned analysis of the European Society of Coloproctology 2017 audit database.

Patient eligibility

Adult patients (> 16 years) undergoing left side colectomy or rectal resection, via any operative approach in emergency settings (within 24 h of hospital admission) were extracted from the ESCP 2017 Left Colon, Sigmoid and Rectal Resections Audit database. Any indication for surgery (benign or malignant) were eligible. Patients undergoing planned elective surgery were excluded, as were those undergoing left colorectal resection as part of a more extensive resection (e.g. subtotal colectomy, panproctocolectomy).

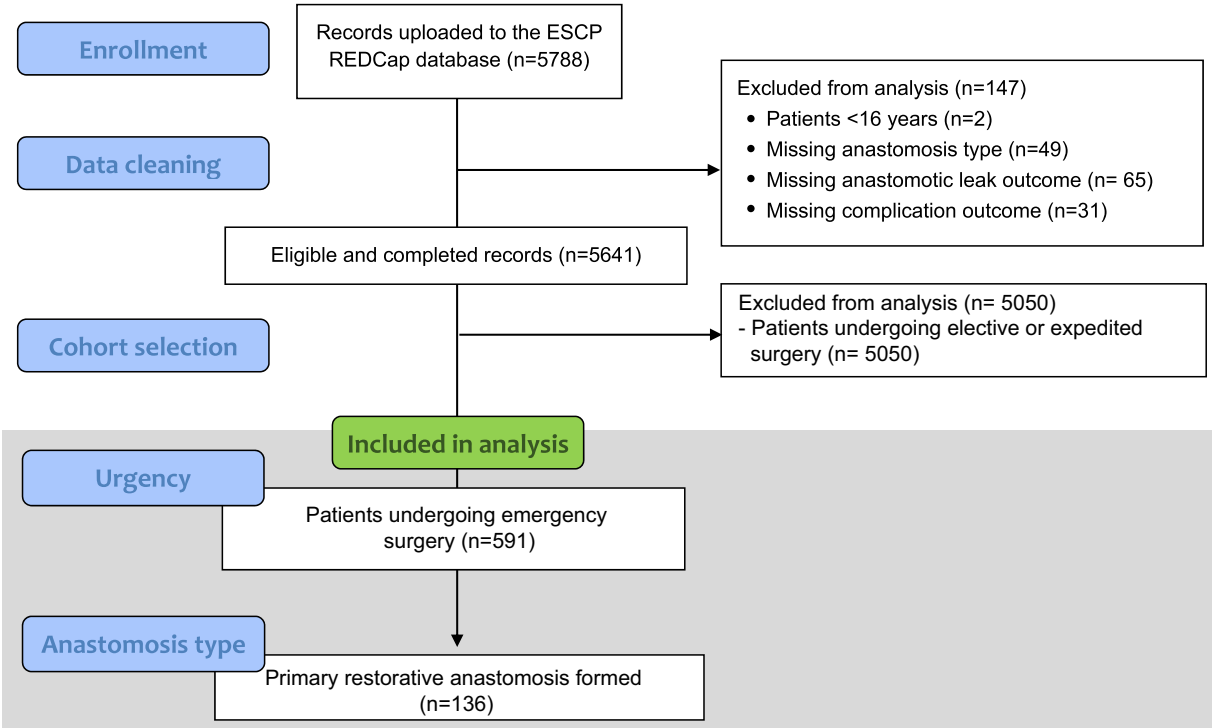

Figure 1 Flowchart for patients included in the analysis of postoperative outcomes of emergency colorectal surgery.

Data capture

Consecutive sampling was performed of eligible patients over an 8-week study period in each included centre. Local investigators commenced data collection on any date between the 1 January 2017 and 15 March 2017, with the last eligible patient being enrolled on 10 May 2017. Small teams of up to five surgeons or surgical trainees worked together to collect prospective data on all eligible patients at each centre. Quality assurance was provided by at least one consultant or attending-level surgeon. Data was recorded contemporaneously and stored on a secure, user-encrypted online platform (REDCap) without using patient identifiable information. Centres were asked to validate that all eligible patients during the study period had been entered, and to attain > 95% completeness of data field entry prior to final submission.

Outcome measure

The primary outcome measure was the 30-day postoperative major complication rate other, defined as Clavien-Dindo classification grade 3–5 (other than anastomotic leak including reoperation, reintervention, unplanned admission to critical care, organ support requirement or death). The secondary outcome measure was anastomotic leak, pre-defined as either (i)

Table 1 Patient and disease characteristics of included patients by anastomotic strategy.

Factor	Levels	Anastomosis, not defunctioned	Anastomosis, defunctioned	End stoma	*P*-value
Total number of patients		103	33	455	
Age group	< 55	26 (25.2)	11 (33.3)	72 (15.8)	0.056
	55–70	35 (34.0)	10 (30.3)	147 (32.3)	
	70–80	25 (24.3)	8 (24.2)	130 (28.6)	
	> 80	17 (16.5)	4 (12.1)	106 (23.3)	
Gender	Female	48 (46.6)	16 (48.5)	221 (48.6)	0.936
	Male	55 (53.4)	17 (51.5)	234 (51.4)	
ASA class	Low risk (ASA 1–2)	55 (53.4)	17 (51.5)	176 (38.7)	0.031
	High risk (ASA 3–5)	47 (45.6)	16 (48.5)	278 (61.1)	
BMI	Normal weight	31 (30.1)	14 (42.4)	135 (29.7)	0.353
	Underweight	1 (1.0)	1 (3.0)	16 (3.5)	
	Overweight	51 (49.5)	14 (42.4)	187 (41.1)	
	Obese	16 (15.5)	4 (12.1)	91 (20.0)	
History of IHD/CVA	No	86 (83.5)	33 (100.0)	363 (79.8)	0.013
	Yes	17 (16.5)	0 (0.0)	92 (20.2)	
History of diabetes mellitus	No	88 (85.4)	29 (87.9)	379 (83.3)	0.922
	Diabetes: any control	15 (14.6)	4 (12.1)	75 (16.5)	
Smoking history	Non-smoker	89 (86.4)	24 (72.7)	343 (75.4)	0.141
	Current	13 (12.6)	9 (27.3)	105 (23.1)	
Indication	Benign	62 (60.2)	26 (78.8)	325 (71.4)	0.042
	Malignant	41 (39.8)	7 (21.2)	130 (28.6)	
Resection type	Colonic only	67 (65.0)	16 (48.5)	271 (59.6)	0.315
	Involved rectum	35 (34.0)	17 (51.5)	183 (40.2)	
Approach	Laparoscopic	22 (21.4)	2 (6.1)	31 (6.8)	< 0.001
	Open	81 (78.6)	30 (90.9)	423 (93.0)	
	Robotic	0 (0.0)	1 (3.0)	0 (0.0)	
Training grade	Consultant	87 (84.5)	29 (87.9)	355 (78.0)	0.165
	Trainee	16 (15.5)	4 (12.1)	100 (22.0)	
Operator type	Colorectal	65 (63.1)	22 (66.7)	239 (52.5)	0.059
	General surgery	38 (36.9)	11 (33.3)	216 (47.5)	
Duration of surgery (minutes)	Mean (SD)	164.3 (73.3)	196.8 (58.2)	153.3 (63.6)	< 0.001

P-value derived from Chi-squared test for categorical variables and Student's t-tests for continuous variables after testing for normality. % shown by column. CVA, cerebrovascular accident; IHD, Ischemic heart disease; IQR, interquartile range; N/A, not applicable; SD, standard deviation. BMI groups are categorised as Underweight (< 18.5), Normal weight (18.5–25), Overweight (25–30), Obese (> 30).

gross anastomotic leakage proven radiologically or clinically, or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging.

Statistical analysis

This report has been prepared in accordance to guidelines set by the STROBE (strengthening the reporting of observational studies in epidemiology) statement for observational studies [10]. Patient, disease and operative characteristics were compared using Student's t-test for normal, continuous data, Mann–Whitney *U* test for non-normal continuous data or Chi-squared test for categorical data. To test the association between the outcome measures and the main explanatory variables of interest (expedited *vs* emergency, end stoma *vs* primary anastomosis), a mixed-effects logistic regression model was fitted. Clinically plausible patient, disease and operation-specific factors were entered into the model for risk-adjustment, treated as fixed effects. These were defined *a priori* within the study protocol, and included irrespective of their significance on univariate analysis. Hospitals were entered into the model as a random-effect, to adjust for hospital-level variation in outcome. Effect estimates are presented as odds ratios (OR) with 95% confidence intervals (95% CI) and two-tailed *P*-values. Model discrimination was quantified using *C*-statistic, or the area under the receiver operating characteristic curve (AUC) of the model. An alpha level of 0.05 was used throughout. Data analysis was undertaken using R Studio V3.1.1 (R Foundation, Boston, MA, USA).

Results

Patients

This study included 591 patients undergoing emergency surgery from 43 countries (Fig. 1). The mean age of patients was 67.4 years (ranging from 18 to 96). 51.8% were male and 57.4% had a high anaesthetic risk class (ASA 3–5). Differences in demographics between patients with anastomosis and end stoma are shown in Table 1. Primary anastomosis was performed in 136 patients (23%) with 33 of these patients receiving a defunctioning stoma. This stoma was a loop ileostomy in 84.8% (28/33), an end/double-barreled ileostomy in 6.1% (2/33) and a loop colostomy in 9.1% (3/33). 30.1% (178/591) of included operations were done for malignancy, with end stoma being most common operative strategy (73.0%, 130/178). Of these, 20.2% were undergoing neoadjuvant therapy prior to their presentation for emergency surgery (short course radiotherapy, 7/36; long course chemoradiotherapy, 18/36; chemotherapy only: 11/36). Primary anastomosis with or without defunctioning stoma was performed less frequently than end stoma in disease affecting the rectum (14.9% and 7.2% *vs* 77.9% respectively). An anastomosis was attempted in 27% (87/326) of patients operated upon by a colorectal surgeon and 18% (49/265) by a general surgeon ($P = 0.059$, Fig. 2).

Major complications (Clavien-Dindo 3 to 5)

Results of analysis for factors associated with the occurrence of major complications are shown in Table 2. An

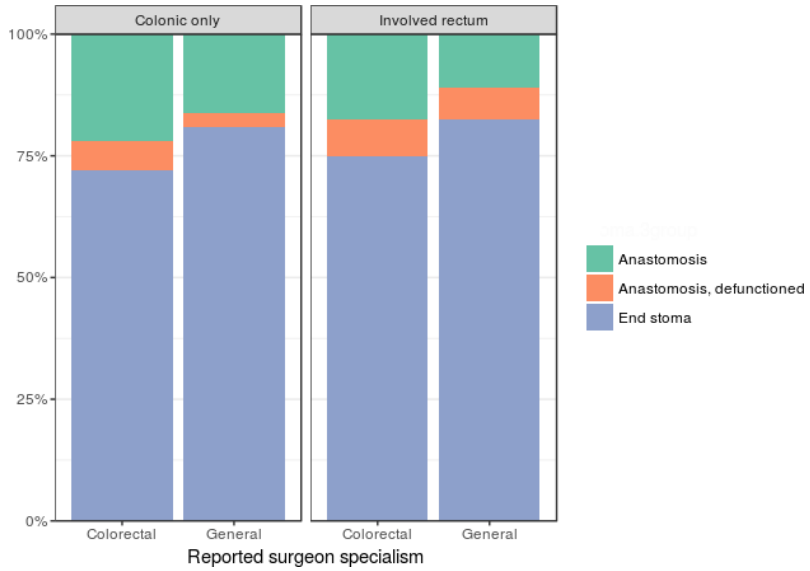

Figure 2 Variation in anastomotic practice between colorectal and general surgeons.

Table 2 Univariable and multilevel models for major postoperative complications.

Factor	Levels	No major complication	Major complication	OR (univariable)	OR (multilevel)
Anastomosis type	Anastomosis, not defunctioned	79 (19.8)	18 (11.6)	– *(Reference)*	– *(Reference)*
	Anastomosis, defunctioned	28 (7.0)	5 (3.2)	0.78 (0.24–2.18, $P = 0.658$)	0.63 (0.20–2.02, $P = 0.442$)
	End stoma	292 (73.2)	132 (85.2)	1.98 (1.17–3.54, $P = 0.015$)	1.52 (0.83–2.79, $P = 0.173$)
Age	< 55	79 (19.8)	26 (16.8)	–	–
	55–70	135 (33.8)	45 (29.0)	1.01 (0.58–1.78, $P = 0.964$)	0.86 (0.46–1.60, $P = 0.635$)
	70–80	104 (26.1)	48 (31.0)	1.40 (0.81–2.48, $P = 0.236$)	1.03 (0.54–1.96, $P = 0.918$)
	> 80	81 (20.3)	36 (23.2)	1.35 (0.75–2.46, $P = 0.320$)	0.91 (0.45–1.82, $P = 0.784$)
Gender	Female	202 (50.6)	65 (41.9)	–	–
	Male	197 (49.4)	90 (58.1)	1.42 (0.98–2.07, $P = 0.067$)	1.66 (1.10–2.51, $P = 0.016$)
ASA class	Low risk (ASA 1–2)	192 (48.1)	40 (25.8)	–	–
	High risk (ASA 3–5)	207 (51.9)	115 (74.2)	2.67 (1.78–4.05, $P < 0.001$)	2.54 (1.59–4.07, $P < 0.001$)
BMI	Normal weight	119 (29.8)	60 (38.7)	–	–
	Underweight	11 (2.8)	7 (4.5)	1.26 (0.44–3.37, $P = 0.647$)	1.37 (0.46–4.07, $P = 0.566$)
	Overweight	194 (48.6)	55 (35.5)	0.56 (0.36–0.86, $P = 0.009$)	0.53 (0.33–0.85, $P = 0.009$)
	Obese	75 (18.8)	33 (21.3)	0.87 (0.52–1.45, $P = 0.603$)	0.76 (0.43–1.33, $P = 0.332$)
History of IHD/CVA	No	330 (82.7)	117 (75.5)	–	–
	Yes	69 (17.3)	38 (24.5)	1.55 (0.99–2.42, $P = 0.054$)	1.12 (0.67–1.87, $P = 0.669$)
History of diabetes mellitus	No	339 (85.0)	126 (81.3)	–	–
	Diabetes: any control	60 (15.0)	29 (18.7)	1.30 (0.79–2.10, $P = 0.292$)	0.99 (0.57–1.72, $P = 0.965$)
Smoking history	Non-smoker	311 (77.9)	120 (77.4)	–	–
	Current	88 (22.1)	35 (22.6)	1.03 (0.65–1.60, $P = 0.894$)	0.94 (0.57–1.55, $P = 0.802$)
Indication	Benign	276 (69.2)	114 (73.5)	–	–
	Malignant	123 (30.8)	41 (26.5)	0.81 (0.53–1.22, $P = 0.312$)	0.85 (0.54–1.34, $P = 0.481$)
Resection type	Colonic only	237 (59.4)	94 (60.6)	–	–
	Involved rectum	162 (40.6)	61 (39.4)	0.95 (0.65–1.38, $P = 0.788$)	1.01 (0.66–1.54, $P = 0.964$)
Approach	Open	354 (88.7)	148 (95.5)	–	–
	Minimally invasive	45 (11.3)	7 (4.5)	0.37 (0.15–0.79, $P = 0.018$)	0.42 (0.17–1.02, $P = 0.055$)
Training grade	Consultant	320 (80.2)	119 (76.8)	–	–
	Trainee	79 (19.8)	36 (23.2)	1.23 (0.78–1.90, $P = 0.373$)	1.01 (0.61–1.65, $P = 0.978$)
Operator type	Colorectal	218 (54.6)	87 (56.1)	–	–
	General surgery	181 (45.4)	68 (43.9)	0.94 (0.65–1.37, $P = 0.751$)	0.97 (0.62–1.51, $P = 0.888$)

Major postoperative complications were pre-defined as Clavien-Dindo grade complications 3 to 5 (re-operation, re-intervention, admission to critical care or death. Odds ratio (OR) presented with 95% confidence intervals. % shown by column. CVA, cerebrovascular accident; IHD, Ischemic heart disease; IQR, interquartile range; N/A, not applicable; SD, standard deviation.

end stoma was significantly associated with increased major postoperative complications upon univariable analysis (OR 1.98 95% CI 1.17–3.54, $P = 0.015$), but this association was not seen following risk adjustment (adjusted odds ratio for end stoma in mixed effects model 1.52, 95%CI 0.83–2.79, $P = 0.173$). In the multilevel model significant predictors for major complications were high ASA risk (grade 3–5) (OR 2.54, 95% CI 1.59–4.07, $P < 0.001$) and male gender (OR 1.66, 95% CI 1.10–2.51, $P = 0.016$). Overweight BMI was associated with a lower major complication rate than a normal BMI (OR 0.53, 95% CI 0.33–0.85, $P = 0.009$), however the location of resection (involving rectum or colonic only) demonstrated no association. The model demonstrated fair discrimination (AUC: 0.71).

Anastomotic leak

Unadjusted outcomes according the anastomotic strategy, stratified by presence of leak, are shown in Table 3. Although a defunctioning stoma was not associated with reduced anastomotic leak (12% defunctioned [4/33] *vs* 13% not defunctioned [13/97],

Table 3 Outcomes of patients undergoing emergency left sided colorectal surgery with or without anastomosis.

Factor	Levels	Anastomosis, defunctioned no leak	Anastomosis, defunctioned with leak	Anastomosis, no leak	Anastomosis, with leak	End stoma	*P*-value
Post-operative complication	No complication	15 (51.7)	0 (0.0)	50 (56.8)	0 (0.0)	159 (34.9)	< 0.001
	Minor complication (Clavien-Dindo 1–2)	12 (41.4)	1 (25.0)	30 (34.1)	2 (13.3)	149 (32.7)	
	Major complication (Clavien-Dindo 3–5)	2 (6.9)	3 (75.0)	8 (9.1)	13 (86.7)	147 (32.3)	
Post-operative mortality	No	28 (96.6)	4 (100.0)	88 (100.0)	12 (80.0)	390 (85.7)	0.001
	Yes	1 (3.4)	0 (0.0)	0 (0.0)	3 (20.0)	65 (14.3)	
Re-operation	No re-operation	28 (96.6)	2 (50.0)	83 (94.3)	4 (26.7)	405 (89.0)	< 0.001
	Re-operation	1 (3.4)	2 (50.0)	5 (5.7)	11 (73.3)	50 (11.0)	
Critical care admission	Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	< 0.001
	None	15 (51.7)	2 (50.0)	63 (71.6)	6 (40.0)	179 (39.3)	
	Planned from theatre	13 (44.8)	2 (50.0)	19 (21.6)	9 (60.0)	216 (47.5)	
	Unplanned from theatre	1 (3.4)	0 (0.0)	5 (5.7)	0 (0.0)	49 (10.8)	
	Unplanned from ward	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	11 (2.4)	
Re-admission	No	28 (96.6)	3 (75.0)	78 (88.6)	14 (93.3)	422 (92.7)	0.746
	Yes	1 (3.4)	1 (25.0)	9 (10.2)	1 (6.7)	28 (6.2)	
	missing	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	5 (1.1)	
Length of stay	Mean (SD)	11 (6.2)	18.5 (9.1)	9 (4.3)	18.7 (6.4)	13.6 (7.8)	< 0.001

P-values derived from Chi-squared test for categorical variables and Student's *T*-test for parametric continuous variables, % shown by column.

adjusted odds ratio 2.19, 95%CI 0.43–11.02, $P = 0.343$), it was associated with fewer major complications (75% [3/4] with defunctioning stoma, 86.7% anastomosis only [13/15]), lower mortality (0% [0/4] *vs* 20% [3/15]), and reoperation 50% [2/4] *vs* 73% [11/15]) when a leak did occur (Fig. 3). The minor complication rate was similar between groups where the anastomosis successfully healed without leak (41.4% defunctioned [12/27] *vs* 34.1% not defunctioned [30/88]) and where an end stoma was formed (32.7% [149/455]). On the univariable analysis (Table 4) previous history of IHD/CVA (OR 5.06, 95% CI 1.50–16.27, $P = 0.007$) was associated with an increased risk of leak, whilst being of middle age was protective (age 55–70 years old; OR 0.10, 95% CI 1.61–100, $P = 0.037$). When a multilevel model was

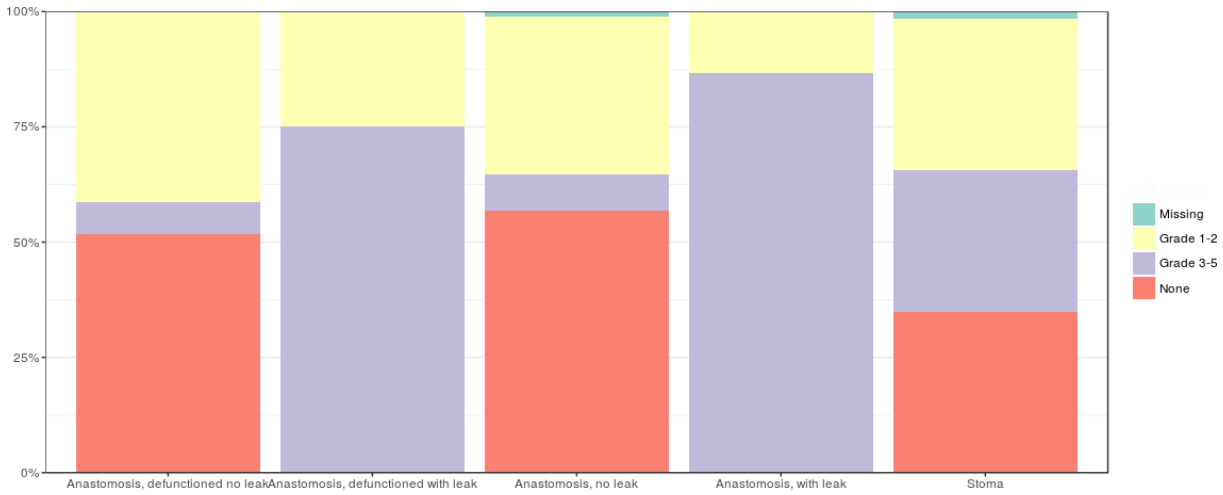

Figure 3 Clavien Dindo complication grade, grouped by anastomotic outcome.

Table 4 Univariable and multilevel models for anastomotic leak amongst patients with anastomosis only.

Factor	Levels	No leak	Leak	OR (univariable)	OR (multilevel)
Defunctioning ileostomy	No	84 (74.3)	13 (76.5)	– *(Reference)*	– *(Reference)*
	Yes	29 (25.7)	4 (23.5)	0.89 (0.24–2.75, $p = 0.851$)	2.19 (0.43–11.02, $P = 0.343$)
Age	< 55	30 (26.5)	7 (41.2)	–	–
	55–70	42 (37.2)	1 (5.9)	0.10 (0.01–0.62, $P = 0.037$)	0.05 (0.00–0.66, $P = 0.023$)
	70–80	28 (24.8)	4 (23.5)	0.61 (0.15–2.25, $P = 0.470$)	0.32 (0.05–1.91, $P = 0.213$)
	> 80	13 (11.5)	5 (29.4)	1.65 (0.42–6.17, $P = 0.458$)	1.03 (0.15–6.96, $P = 0.980$)
Gender	Female	57 (50.4)	6 (35.3)	–	–
	Male	56 (49.6)	11 (64.7)	1.87 (0.66–5.74, $P = 0.249$)	1.50 (0.38–5.87, $P = 0.563$)
ASA class	Low risk (ASA 1–2)	62 (54.9)	7 (41.2)	–	–
	High risk (ASA 3–5)	51 (45.1)	10 (58.8)	1.74 (0.62–5.09, $P = 0.296$)	1.00 (0.20–4.96, $P = 0.996$)
History of IHD/CVA	No	102 (90.3)	11 (64.7)	–	–
	Yes	11 (9.7)	6 (35.3)	5.06 (1.50–16.27, $P = 0.007$)	5.10 (0.75–34.53, $P = 0.095$)
History of diabetes mellitus	No	99 (87.6)	12 (70.6)	–	–
	Diabetes: any control	14 (12.4)	5 (29.4)	2.95 (0.84–9.34, $P = 0.074$)	8.56 (1.16–63.38, $P = 0.035$)
Smoking history	Non-smoker	96 (85.0)	14 (82.4)	–	–
	Current	17 (15.0)	3 (17.6)	1.21 (0.26–4.21, $P = 0.782$)	1.44 (0.25–8.19, $P = 0.678$)
Indication	Benign	75 (66.4)	10 (58.8)	–	–
	Malignant	38 (33.6)	7 (41.2)	1.38 (0.47–3.89, $P = 0.543$)	1.26 (0.29–5.47, $P = 0.753$)
Resection type	Colonic only	64 (56.6)	14 (82.4)	–	–
	Involved rectum	49 (43.4)	3 (17.6)	0.28 (0.06–0.92, $P = 0.055$)	0.18 (0.03–1.00, $P = 0.050$)
Training grade	Consultant	98 (86.7)	12 (70.6)	–	–
	Trainee	15 (13.3)	5 (29.4)	2.72 (0.78–8.55, $P = 0.095$)	1.06 (0.19–5.95, $P = 0.944$)
Operator type	Colorectal	74 (65.5)	9 (52.9)	–	–
	General surgery	39 (34.5)	8 (47.1)	1.69 (0.59–4.75, $P = 0.319$)	2.19 (0.55–8.76, $P = 0.267$)

Overall anastomotic leak was pre-defined as either (i) gross anastomotic leakage proven radiologically or clinically, or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging. Odds ratio (OR) presented with 95% confidence intervals. % shown by column. SD, standard deviation; IQR, Interquartile range; IHD, Ischemic heart disease; CVA, cerebrovascular accident; N/A, not applicable.

fitted (Fig. 4), a history of diabetes also conveyed an increased risk of leak (OR 8.56, 95% CI 1.16–63.38, $P = 0.035$). The model demonstrated good discrimination (AUC: 0.87).

Discussion

This study showed that primary anastomosis was performed in up to one in five patients and appears safe in this highly selected group after emergency left sided colorectal resection (unplanned, within 24 h of hospital admission). A defunctioning stoma was only used in 24% of patients with a primary anastomosis. The exploratory findings of this study, limited by small numbers, suggested that a defunctioning stoma may mitigate against risk if an anastomotic leak occurs. Other patient-related risk characteristics (male gender, high ASA grade) and an open approach were identified as independent risk factors for major postoperative complications. Furthermore, young and elderly age or a history of diabetes were shown as risk factors for anastomotic leak in emergency procedures.

Previously, the simple formation of an end colostomy after resection of the pathology (ubiquitously known as a 'Hartmann's procedure') has been advocated as the gold standard treatment in emergency left colonic resection, to eliminate risk of anastomotic leak [11–13]. In the last 15 years, several studies have questioned this strategy [14,15]. A primary anastomosis is not only feasible, it may even be associated with better postoperative outcomes, both in terms of complications and mortality [16,17]. Given that more than 40% of temporary stomas become permanent, selecting patients correctly for a primary anastomosis is attractive [18–20]. In addition, reversal of Hartmann's can be a technically demanding operation resulting in further morbidity and mortality [21]. These findings support a recent consensus statements and prospective multi-centre randomized trials that suggest primary anastomosis with proximal diversion as an optimal strategy for sigmoid diverticulitis in selected patients with Hinchey 3 or 4 disease [4,22,23]. This current study gives credence to the current situation and confirms that surgeons are making appropriate decisions on a case-by-case level, thereby

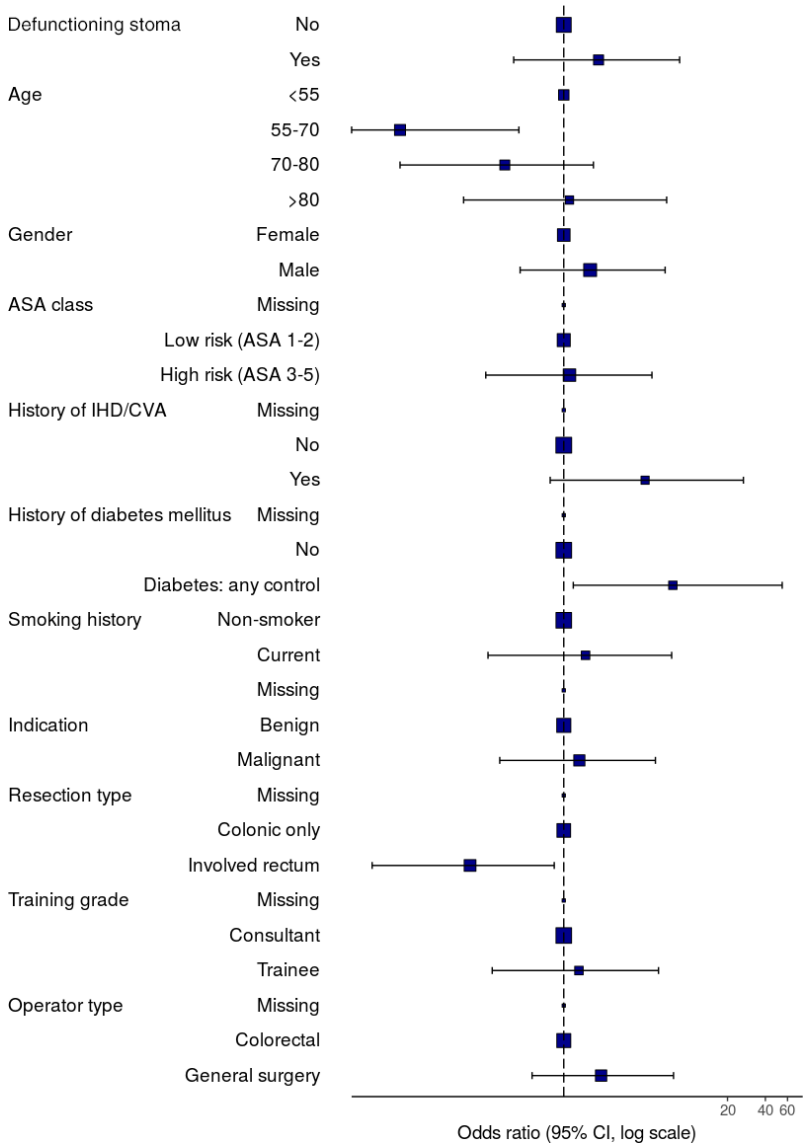

Figure 4 Forest plot demonstrating mixed effects model for factors associated with anastomotic leak in patients undergoing emergency left sided colorectal surgery.

effectively stratifying patients for primary anastomosis or end stoma. It is known that a defunctioning stoma in elective surgery has utility in mitigating the clinical impact of anastomotic leak [24,25]. Loop ileostomies and their closure are not complication-free and several studies have shown that temporary loop ileostomies can become permanent in up to 25% of patients [24–28]. However this study comparably suggests that a defunctioning stoma may mitigate some risk when a leak occurs. This must be interpreted with caution since numbers in this study were low; for example, only four patients with an anastomosis and defunctioning stoma suffered a leak.

There were slightly more primary anastomotic attempts by colorectal *vs* general surgeons in this study. Even though there is no homogenous definition of colorectal surgeon internationally, results of multiple studies confirm the importance of colorectal specialisation in the emergency setting [29,30]. An individual surgeon's personality and their response to perceived operative risk may also influence choice of anastomotic strategy [31]. Further research is needed to determine whether the grade and surgical specialism of the operating (or senior) surgeon, and specialisation and experience of included centres affect both the decision for anastomosis and the subsequent clinical outcome.

There are inherent limitations to the 'snapshot' observational study reported here which we have attempted to overcome in the study design, statistical analysis and interpretation. There is an obvious selection bias in this study, although we planned the analysis around this *a priori*. We aimed to analyse safety of current practice; this study showed that end stoma was more frequently used in older patients, with poor general status, in smokers, and in those with arteriopathy and benign disease. However this paper defines outcomes in the highly selected group of patients undergoing anastomosis, and thus supports surgical decision making in specific cases, rather than in recommending a general change in approach. The low numbers of anastomotic leak and major complication within secondary analyses of the subgroup undergoing anastomosis (< 25% of included patients) makes estimation of effect sizes inaccurate here (reflected by broad confidence intervals). Therefore, this should be seen as exploratory only; the analysis would likely be underpowered to detect a small to moderate effect size. We are also unable to comment on the appropriateness of decision making and have not collected detailed information on parameters that may effect this (for example: contamination (Mannheim Peritonitis Index [32]), previous surgery, intraoperative physiological instability). Most of the literature available on this topic is based on retrospective or single centres data which lacks sufficient detail to allow case-mix adjustment in multivariable models. This study therefore adds to the literature in providing a contemporary perspective using a prospective international observational study design, with a pre-specified protocol and analysis plan. In addition, the variety of centers included (in terms of number of patients, facilities and different technologies available) in this study delivers a realistic picture of the current management of emergency left colorectal resections, reducing selection bias and increasing the external validity of the findings. The different countries and even continents involved ensured the result's validity resolving the demographic differences in diverticulitis and cancer across countries. Finally, the study is limited by short-term follow up to 30 days only; we have not collected data on stoma reversal rates, quality of life or stoma-related complications following surgery. An alternative complication categorisation system such as the Comprehensive Complications Index [33] may also give increased fidelity in comparisons between intermediate term outcomes. Further evaluation of these important parameters following emergency left sided colorectal surgery is warranted.

The data from this study supports current international practice of primary anastomosis following emergency left sided colorectal resection in a highly selected group of patients, demonstrating satisfactory safety and an acceptable morbidity profile. Where an anastomosis is formed, a defunctioning stoma does not appear to reduce the risk of leak, but may mitigate the severity of resultant complications.

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Conflicts of interest

None to declare.

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References

1 Hartmann H. Note sur un procédé nouveau d'extirpation des cancers de la partie du côlon. *Bull Mem Soc Chir Paris* 1923; **49:** 1474–7.
2 Barbieux J, Plumerias F, Hammy A. Current indications for Hartmann procedure. *J Visc Surg* 2016; **153:** 31–8.
3 van de Wall BJ, Draaisma WA, Schouten ES, Broeders IA, Consten EC. Conventional and laparoscopic reversal of the Hartmann procedure: a review of literature. *J Gastrointest Surg* 2010; **14:** 743–52.
4 Bridoux V, Regimbeau JM, Ouaissi M *et al.* Hartmann's procedure or primary anastomosis for generalized peritonitis due to perforated diverticulitis: a prospective multicenter randomized trial (DIVERTI). *J Am Coll Surg* 2017; **225:** 798–805.
5 Salem L, Flum DR. Primary anastomosis or Hartmann's procedure for patients with diverticular peritonitis? A systematic review. *Dis Colon Rectum* 2004; **47:** 1953–64.
6 Trenti L, Biondo S, Golda T *et al.* Generalized peritonitis due to perforated diverticulitis: Hartmann's procedure or primary anastomosis? *Int J Colorectal Dis* 2011; **26:** 377–84.
7 Oberkofler CE, Rickenbacher A, Raptis DA *et al.* A multicenter randomized clinical trial of primary anastomosis or Hartmann's procedure for perforated left colonic diverticulitis with purulent or fecal peritonitis. *Ann Surg* 2012; **256:** 819–26; discussion 26–7.
8 Binda GA, Karas JR, Serventi A *et al.* Primary anastomosis vs nonrestorative resection for perforated diverticulitis with

peritonitis: a prematurely terminated randomized controlled trial. *Colorectal Dis* 2012; **14:** 1403–10.
9 Gibbons G, Tan CJ, Bartolo DC *et al.* Emergency left colonic resections on an acute surgical unit: does subspecialization improve outcomes? *ANZ J Surg* 2015; **85:** 739–43.
10 von Elm E, Altman DG, Egger M *et al.* Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007; **335:** 806–8.
11 Myers E, Hurley M, O'Sullivan GC, Kavanagh D, Wilson I, Winter DC. Laparoscopic peritoneal lavage for generalized peritonitis due to perforated diverticulitis. *Br J Surg* 2008; **95:** 97–101.
12 Sanderson ER. Henri Hartmann and the Hartmann operation. *Arch Surg* 1980; **115:** 792–3.
13 Walklett CL, Yeomans NP. A retrospective case note review of laparoscopic versus open reversal of Hartmann's procedure. *Ann R Coll Surg Engl* 2014; **96:** 539–42.
14 Constantinides VA, Tekkis PP, Athanasiou T *et al.* Primary resection with anastomosis vs. Hartmann's procedure in nonelective surgery for acute colonic diverticulitis: a systematic review. *Dis Colon Rectum* 2006; **49:** 966–81.
15 Zorcolo L, Covotta L, Carlomagno N, Bartolo DC. Safety of primary anastomosis in emergency colo-rectal surgery. *Colorectal Dis* 2003; **5:** 262–9.
16 Gachabayov M, Oberkofler CE, Tuech JJ, Hahnloser D, Bergamaschi R. Resection with primary anastomosis vs nonrestorative resection for perforated diverticulitis with peritonitis: a systematic review and meta-analysis. *Colorectal Dis* 2018; [EPub ahead of print].
17 Schilling MK, Maurer CA, Kollmar O, Buchler MW. Primary vs. secondary anastomosis after sigmoid colon resection for perforated diverticulitis (Hinchey Stage III and IV): a prospective outcome and cost analysis. *Dis Colon Rectum* 2001; **44:** 699–703; discussion 703–5.
18 Fleming FJ, Gillen P. Reversal of Hartmann's procedure following acute diverticulitis: is timing everything? *Int J Colorectal Dis* 2009; **24:** 1219–25.
19 Hallam S, Mothe BS, Tirumulaju R. Hartmann's procedure, reversal and rate of stoma-free survival. *Ann R Coll Surg Engl* 2018; **100:** 301–7.
20 Roig JV, Cantos M, Balciscueta Z *et al.* Hartmann's operation: how often is it reversed and at what cost? A multicentre study. *Colorectal Dis* 2011; **13:** e396–402.
21 Garber A, Hyman N, Osler T. Complications of Hartmann takedown in a decade of preferred primary anastomosis. *Am J Surg* 2014; **207:** 60–4.
22 Feingold D, Steele SR, Lee S *et al.* Practice parameters for the treatment of sigmoid diverticulitis. *Dis Colon Rectum* 2014; **57:** 284–94.
23 Roig JV, Sanchez-Guillen L, Garcia-Armengol JJ. Acute diverticulitis and surgical treatment. *Minerva Chir* 2018; **73:** 163–78.
24 Chow A, Tilney HS, Paraskeva P, Jeyarajah S, Zacharakis E, Purkayastha S. The morbidity surrounding reversal of defunctioning ileostomies: a systematic review of 48 studies including 6,107 cases. *Int J Colorectal Dis* 2009; **24:** 711–23.
25 Garcia-Botello SA, Garcia-Armengol J, Garcia-Granero E *et al.* A prospective audit of the complications of loop ileostomy construction and takedown. *Dig Surg* 2004; **21:** 440–6.
26 Wong NY, Eu KW. A defunctioning ileostomy does not prevent clinical anastomotic leak after a low anterior resection: a prospective, comparative study. *Dis Colon Rectum* 2005; **48:** 2076–9.
27 Kong AP, Kim J, Holt A *et al.* Selective treatment of rectal cancer with single-stage coloanal or ultralow colorectal anastomosis does not adversely affect morbidity and mortality. *Int J Colorectal Dis* 2007; **22:** 897–901.
28 Chun LJ, Haigh PI, Tam MS, Abbas MA. Defunctioning loop ileostomy for pelvic anastomoses: predictors of morbidity and nonclosure. *Dis Colon Rectum* 2012; **55:** 167–74.
29 Biondo S, Kreisler E, Millan M *et al.* Impact of surgical specialization on emergency colorectal surgery outcomes. *Arch Surg* 2010; **145:** 79–86.
30 Patel S, Senagore A. General surgeons vs. colorectal surgeons: who should be doing what to whom? *Semin Colon Rectal Surg* 2013; **24:** 191–4.
31 Moug SJ, Henderson N, Tiernan J *et al.* The colorectal surgeon's personality may influence the rectal anastomotic decision. *Colorectal Dis* 2018; [Epub ahead of print]. https://doi.org/10.1111/codi.14293
32 Wacha H, Linder MM, Feldman U, Wesch G, Gundlach E, Steifensand RA. Mannheim peritonitis index – prediction of risk of death from peritonitis: construction of a statistical and validation of an empirically based index. *Theoretical Surg* 1987; **1:** 169–77.
33 Clavien PA, Vetter D, Staiger RD *et al.* The Comprehensive Complication Index (CCI®): added value and clinical perspectives 3 years "Down the Line". *Ann Surg* 2017; **265:** 1045–50.

Authorship list

Writing group

Luis Sánchez-Guillén (Chair), Dmitri Nepogodiev, Emre Sivrikoz, Tim van Elst, Ana María Minaya-Bravo, Francesco Pata, James Glasbey, Thomas Pinkney, Aneel Bhangu

ESCP cohort studies and audits committee

Alaa El-Hussuna (2017 Audit Lead), Aneel Bhangu, Nicholas Buchs, Christianne Buskens, Sanjay Chaudri, Matteo Frasson, Gaetano Gallo, James Glasbey, Ana María Minaya-Bravo, Dion Morton, Ionut Negoi, Dmitri Nepogodiev, Francesco Pata, Tomas Poskus, Luis Sánchez-Guillén, Baljit Singh, Oded Zmora, Thomas Pinkney (Chair)

APPENDIX 6

- The 2017 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** and al. from Portugal).

"An international multiCenter prospective audit of elective rectal cancer surgery; operative approach versus outcome, including transanal total mesorectal excision (TaTME)".

in Colorectal Disease · September 2018; DOI: 10.1111/codi.14376.

Co-author (integrating ESCP collaborative group); Part 1 – Chapter 1.

Original article

doi:10.1111/codi.14376

An international multicentre prospective audit of elective rectal cancer surgery; operative approach versus outcome, including transanal total mesorectal excision (TaTME)

The 2017 European Society of Coloproctology (ESCP) collaborating group

European Society of Coloproctology (ESCP) Cohort Studies Committee, Department of Surgery, Aalborg University Hospital, Aalborg, Denmark

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Abstract

Introduction Transanal total mesorectal excision (TaTME) has rapidly emerged as a novel approach for rectal cancer surgery. Safety profiles are still emerging and more comparative data is urgently needed. This study aimed to compare indications and short-term outcomes of TaTME, open, laparoscopic, and robotic TME internationally.

Methods A pre-planned analysis of the European Society of Coloproctology (ESCP) 2017 audit was performed. Patients undergoing elective total mesorectal excision (TME) for malignancy between 1 January 2017 and 15 March 2017 by any operative approach were included. The primary outcome measure was anastomotic leak.

Results Of 2579 included patients, 76.2% (1966/2579) underwent TME with restorative anastomosis of which 19.9% (312/1966) had a minimally invasive approach (laparoscopic or robotic) which included a transanal component (TaTME). Overall, 9.0% (175/1951, 15 missing outcome data) of patients suffered an anastomotic leak. On univariate analysis both laparoscopic TaTME (OR 1.61, 1.02–2.48, $P = 0.04$) and robotic TaTME (OR 3.05, 1.10–7.34, $P = 0.02$) were associated with a higher risk of anastomotic leak than non-transanal laparoscopic TME. However this association was lost in the mixed-effects model controlling for patient and disease factors (OR 1.23, 0.77–1.97, $P = 0.39$ and OR 2.11, 0.79–5.62, $P = 0.14$ respectively), whilst low rectal anastomosis (OR 2.72, 1.55–4.77, $P < 0.001$) and male gender (OR 2.29, 1.52–3.44, $P < 0.001$) remained strongly associated. The overall positive circumferential margin resection rate was 4.0%, which varied between operative approaches: laparoscopic 3.2%, transanal 3.8%, open 4.7%, robotic 1%.

Conclusion This contemporaneous international snapshot shows that uptake of the TaTME approach is widespread and is associated with surgically and pathologically acceptable results.

Keywords Rectal cancer, laparoscopic surgery, TME, transanal TME, TaTME, robotic surgery

What does this paper add to the literature?
Approaches to rectal cancer resection vary internationally. One in five patients is undergoing a TaTME approach, with results suggesting equivalent anastomotic leak and positive resection margin rates. Both robotic and TaTME approaches need further evidence to support their impact on major complications. Anastomotic leak rates in low rectal anastomoses remain high, regardless of operative approach.

Introduction

The best technique to achieve safe and effective total mesorectal excision (TME) for rectal cancer continues to pose a significant challenge for surgeons and patients. The ideal technique aims for an intact TME with clear circumferential and distal resection margins [1]. When reconstruction is planned, an anastomotic technique that minimises the risk of leak whilst promoting good function is needed. A significant challenge is posed by cancers in the lowest third of the rectum, particularly in a narrow pelvis. From an abdominal approach, the ability to pass a stapler safely below the tumour is vital to avoid an involved distal resection margin. Similarly, the

Correspondence to: Alaa El-Hussuna, European Society of Coloproctology (ESCP) Cohort Studies Committee, Department of Surgery, Aalborg University Hospital, Hobrovej 18-22, 9100 Aalborg, Denmark.
E-mail: alaanewemail@gmail.com

need for multiple firings of a cross-stapler predisposes to anastomotic leak [2]. Finally, precise placement of circular stapling devices through cross-stapled rectal stumps can be challenging.

Transanal TME (TaTME) has been proposed as a method to improve surgery of mid and low rectal lesions [3,4]. It is typically performed as a hybrid procedure with a minimally invasive (laparoscopic or robotic) abdominal approach, with dissection and ultralow colorectal/coloanal anastomosis through the transanal port to improve visualisation and avoid cross stapling [5] or multiple firings [2,5]. It has the potential to be safer for the distal resection margin by improving access and precision of dissection and stapler placement [2].

TaTME is still evolving (IDEAL Phase 2b) with moderate stability of its components [6,7]. A prolonged learning curve [8] for transanal surgery has been described, with worse outcomes seen in as many as the first fifty cases performed [9]. Consistent with this, early series report anastomotic leak rates as high as 43% [10], with concerning rates of urethral and other solid organ injury. Concerns also exist about circumferential resection margin (CRM) involvement and suboptimal TME specimen grades in its early adoption [9,11]. There is not yet randomised evidence for the benefit of TaTME. A recent large and comprehensive registry study has identified baseline data and showed acceptable leak rates and safety profiles from the included centres [12]. However, it did not have comparative groups to benchmark current practice, and so to supplement this, we planned a study from a wide range of centres to gather comparative data. The primary aim of this study was to describe the safety profile of TaTME compared to other surgical approaches to manage rectal cancer. The secondary aim was to additionally describe the current landscape in terms of uptake of TaTME and the alternate operative approaches for rectal cancer, including open, laparoscopic, and robotic TME.

Method

Protocol and centres

This prospective, observational, multicentre study was conducted in line with a pre-specified protocol (http://www.escp.eu.com/research/cohort-studies). An external pilot of the protocol and data capture system was conducted in five international centres prior to launch, allowing refinement of the study tool and delivery. Any unit performing gastrointestinal surgery was eligible to register to enter patients into the study. No minimum case volume, or centre-specific limitations were applied. The study protocol was disseminated to registered members European Society of Coloproctology (ESCP), and through national surgical or colorectal societies, and represents a pre-planned analysis of the European Society of Coloproctology 2017 audit database.

Study approvals

All participating centres were responsible for compliance to local approval requirements for ethics approval or indemnity as required. In the UK, the National Research Ethics Service tool recommended that this project was not classified as research, and the protocol was registered as clinical audit in all participating centres.

Patient eligibility

Adult patients (> 16 years) undergoing elective (planned) rectal resection with or without a primary anastomosis were extracted from the main audit database. Only operations performed for a malignant pathology within the rectum, up to the rectosigmoid junction were included. For the abdominal component, open, laparoscopic and robotic procedures were all eligible. Transanal and non-transanal approaches were acceptable. Rectal resections performed as part of a more extensive resection (e.g. panproctocolectomy) were excluded.

Data capture

Consecutive sampling was performed of eligible patients over an 8-week study period in each included centres. Local investigators commenced data collection on any date between the 1 January 2017 and 15 March 2017, with the last eligible patient being enrolled on 10 May 2017. This study adopted the UK National Research Collaborative model for data collection and follow-up. Small teams of up to five surgeons or surgical trainees worked together to collect prospective data on all eligible patients at each centre. Quality assurance was provided by at least one consultant or attending-level surgeon. Data was recorded contemporaneously and stored on a secure, user-encrypted online platform (REDCap) without using patient identifiable information. Centres were asked to validate that all eligible patients during the study period had been entered, and to attain > 95% completeness of data field entry prior to final submission.

Outcome measure

The primary outcome measure was overall anastomotic leak, pre-defined as either (i) gross anastomotic leakage proven radiologically or clinically, or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging. The secondary outcome measures were the postoperative major complication rate; defined as Clavien-Dindo classification grade 3–5 (reoperation, reintervention, unplanned admission to critical care, organ support requirement or death), post-operative length of stay (in whole days); with day of surgery as day zero, the intraoperative serious adverse event (SAE) rate, and the circumferential resection margin involvement rate; defined as tumour tissue ≤ 1 mm from the resection margin.

Statistical analysis

This report has been prepared in accordance to guidelines set by the STROBE (strengthening the reporting of observational studies in epidemiology) statement for observational studies [13]. Patient, disease and operative characteristics were compared by type of surgical approach (open, laparoscopic – transanal (TaTME), laparoscopic – not transanal, robotic – transanal (TaTME), robotic – not transanal) and by the presence or absence of the primary outcome measure (anastomotic leak or intraperitoneal collection) using Student's t-test for normal, continuous data, Mann-Whitney U test for non-normal continuous data or Chi-squared test for categorical data. To test the association between overall anastomotic leak and approach (the main explanatory variable) two models were fitted: the first was a mixed-effects logistic regression model using the whole dataset, the second was a propensity score-matched group of patients who did and did not undergo TaTME in a 1:2 ratio. In the mixed-effects model, clinically plausible patient, disease and operation-specific factors were entered into the model for risk-adjustment, treated as fixed effects. These were defined *a priori* within the study protocol, and included irrespective of their significance on univariate analysis. Hospital was entered into the model as a random-effect, to adjust for hospital-level variation in outcome. Propensity score matching was used to estimate the effect of approach (transanal versus not transanal perineal approach) by accounting for confounding co-variables that might predict patient selection. Nearest neighbour matching was used with scores calculated from variables selected a priori for model adjustment (age, gender, anastomotic height, AJCC stage), and outputs were examined using jitter plots and Chi-squared testing to observe any significant differences between groups. A second propensity-score matched multivariable logistic regression model was then fitted to explore the association of operative approach and anastomotic leak. Effect estimates are presented as odds ratios (OR) with 95% confidence intervals (95% CI) and two-tailed *P*-values. An alpha level of 0.05 was used throughout. Model discrimination was tested by calculating a C-statistic (analogous to the area under the Receiver Operating Curve (AUC); 0.5: no discrimination; 0.6, adequate; 0.7, good; 0.8 excellent). Multiple imputation was not required as the data completeness rate was very high for data points used for propensity score matching. Data analysis was undertaken using R Studio V3.1.1 (R Foundation, Boston, MA, USA).

Results

Patient demographics

Figure 1 shows inclusion of patients within this study. A total of 2579 patients were included from 355 centres across 49 countries. The mean age of the cohort was 66 years (18–98 years), of which 27.7% (715/2579) had low, 26.0% (670/2579) had middle and 46.3% (1194/2579) had high rectal anastomoses. 62.7% were men (1617/2579) and 36.5% (942/2579) underwent neoadjuvant therapy, of which 72.1% (679/942) had long course chemoradiotherapy. A majority of tumours were either T2 (21.8%, 563/2579) or T3 (51.8%, 1337/2579), N0 (58.4%, 1505/2579) and M0 (87.7%, 2262/2579). The abdominoperineal resection rate was 15.4% (396/2579, Fig. 2) and resection with end stoma formation was 8.4% (217/2579). Of those that had an anastomosis (76.2%, 1966/2579), 92.1% (1811/1966) had a stapled anastomosis.

Patient, disease and operative characteristics by operative approach

There was variation in the selection of patients for different approaches to rectal cancer surgery (Table 1). Of patients undergoing restorative surgery, 15.9% (312/1966) of patients from 189 centres underwent surgery with a transanal perineal approach and minimally invasive abdominal approach (TaTME), ranging from one to 15 submitted cases per centre. 6.4% (126/1966) of patients from 40 centres had robotic surgery (ranging from one to 18 submitted case per centre). In patients undergoing TaTME, the anastomosis was was stapled in 73.7% (230/312) and hand-sewn in 26.3% (82/312). The proportion of males undergoing transanal and robotic approaches was

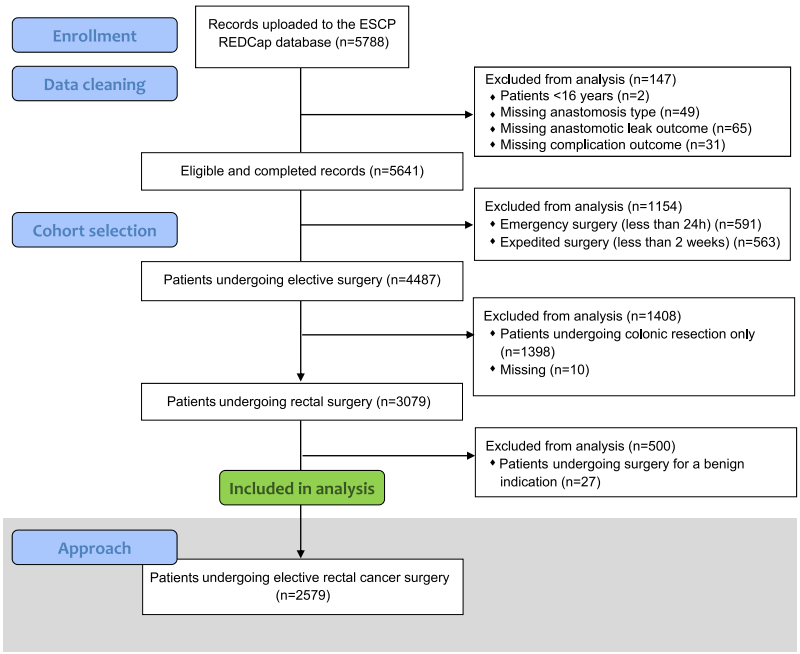

Figure 1 Flowchart for patients included in the analysis of approaches to elective rectal cancer surgery.

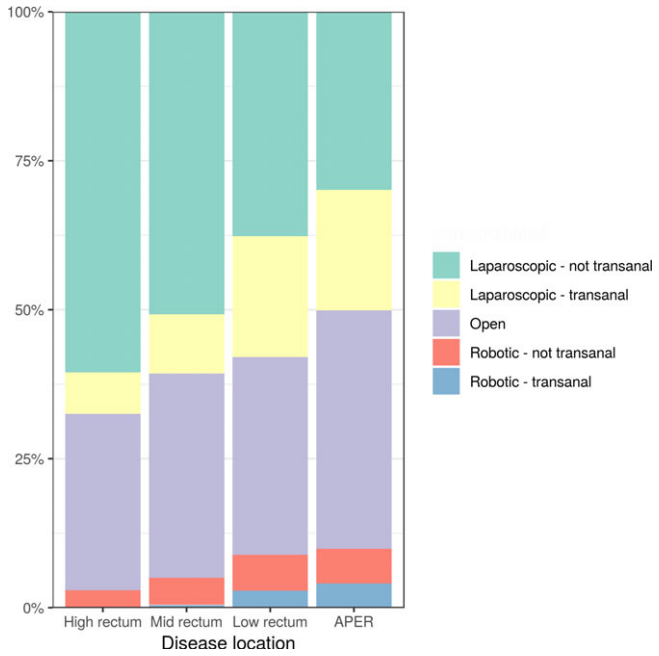

Figure 2 Selection of approach by tumour height in elective rectal cancer surgery.

slightly higher when compared to the other procedures (68.4%, 68.3%, 64.4% *vs* 61.8%, 60.6% respectively; $P = 0.06$). Transanal or robotic approaches were significantly more likely to be selected in low risk ASA 1-2 patients and earlier stage disease.

Anastomotic leak

Within the patients undergoing restorative anastomosis, the overall leak rate was 9.0% (175/1951, with 15 missing outcome data (< 1%)). In the unadjusted data, the

Table 1 Patient, disease and operation characteristics by approach.

Factor	Levels	Laparoscopic not transanal	Laparoscopic transanal	Open	Robotic not transanal	Robotic transanal	*P*-value
Operation type	Primary anastomosis	952 (81.0)	280 (76.3)	608 (70.0)	95 (77.2)	31 (68.9)	< 0.001
	ELAPE	35 (3.0)	25 (6.8)	46 (5.3)	6 (4.9)	1 (2.2)	
	APER	83 (7.1)	51 (13.9)	121 (13.9)	15 (12.2)	13 (28.9)	
	Hartmanns	106 (9.0)	11 (3.0)	93 (10.7)	7 (5.7)	0 (0.0)	
Anastomosis height	High rectum	398 (33.8)	46 (12.5)	195 (22.5)	18 (14.6)	1 (2.2)	< 0.001
	Mid rectum	336 (28.6)	66 (18.0)	227 (26.2)	30 (24.4)	3 (6.7)	
	Low rectum	318 (27.0)	171 (46.6)	280 (32.3)	51 (41.5)	24 (53.3)	
	APER	124 (10.5)	84 (22.9)	166 (19.1)	24 (19.5)	17 (37.8)	
Patient characteristics							
Age	< 55	172 (14.6)	68 (18.5)	135 (15.6)	18 (14.6)	8 (17.8)	0.918
	55–70	521 (44.3)	161 (43.9)	387 (44.6)	57 (46.3)	19 (42.2)	
	70–80	339 (28.8)	103 (28.1)	257 (29.6)	35 (28.5)	13 (28.9)	
	> 80	143 (12.2)	34 (9.3)	89 (10.3)	13 (10.6)	5 (11.1)	
	Missing	1 (0.1)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	
Gender	Female	449 (38.2)	116 (31.6)	342 (39.4)	39 (31.7)	16 (35.6)	0.066
	Male	727 (61.8)	251 (68.4)	526 (60.6)	84 (68.3)	29 (64.4)	
ASA class	Missing	20 (1.7)	2 (0.5)	4 (0.5)	0 (0.0)	0 (0.0)	< 0.001
	Low risk (ASA 1-2)	787 (66.9)	261 (71.1)	516 (59.4)	89 (72.4)	32 (71.1)	
	High risk (ASA 3-5)	369 (31.4)	104 (28.3)	348 (40.1)	34 (27.6)	13 (28.9)	
BMI	Normal weight	338 (28.7)	114 (31.1)	274 (31.6)	49 (39.8)	13 (28.9)	0.681
	Underweight	23 (2.0)	9 (2.5)	21 (2.4)	2 (1.6)	0 (0.0)	
	Overweight	504 (42.9)	150 (40.9)	357 (41.1)	45 (36.6)	19 (42.2)	
	Obese	281 (23.9)	87 (23.7)	201 (23.2)	26 (21.1)	13 (28.9)	
	Missing	30 (2.6)	7 (1.9)	15 (1.7)	1 (0.8)	0 (0.0)	
History of IHD/CVA	Missing	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.041
	No	998 (84.9)	325 (88.6)	704 (81.1)	103 (83.7)	41 (91.1)	
	Yes	176 (15.0)	42 (11.4)	164 (18.9)	20 (16.3)	4 (8.9)	
History of diabetes mellitus	Missing	3 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.35
	No	995 (84.6)	302 (82.3)	736 (84.8)	111 (90.2)	36 (80.0)	
	Diabetes: any control	178 (15.1)	65 (17.7)	132 (15.2)	12 (9.8)	9 (20.0)	
Smoking history	Non-smoker	997 (84.8)	300 (81.7)	723 (83.3)	107 (87.0)	40 (88.9)	0.122
	Current	167 (14.2)	61 (16.6)	143 (16.5)	16 (13.0)	5 (11.1)	
	Missing	12 (1.0)	6 (1.6)	2 (0.2)	0 (0.0)	0 (0.0)	
Disease characteristics							
Neoadjuvant therapy	Missing	9 (0.8)	2 (0.5)	15 (1.7)	0 (0.0)	0 (0.0)	< 0.001
	Chemotherapy only	36 (3.1)	10 (2.7)	38 (4.4)	1 (0.8)	1 (2.2)	
	Long course CRTx	266 (22.6)	142 (38.7)	215 (24.8)	32 (26.0)	24 (53.3)	
	Short course radiotherapy	74 (6.3)	23 (6.3)	62 (7.1)	17 (13.8)	1 (2.2)	
	None	791 (67.3)	190 (51.8)	538 (62.0)	73 (59.3)	19 (42.2)	
MRI T stage	Missing	45 (3.8)	3 (0.8)	20 (2.3)	1 (0.8)	0 (0.0)	< 0.001
	T1	109 (9.3)	32 (8.7)	56 (6.5)	9 (7.3)	2 (4.4)	
	T2	242 (20.6)	68 (18.5)	196 (22.6)	44 (35.8)	13 (28.9)	
	T3	625 (53.1)	213 (58.0)	421 (48.5)	52 (42.3)	26 (57.8)	
	T4	155 (13.2)	51 (13.9)	175 (20.2)	17 (13.8)	4 (8.9)	

Table 1 (Continued).

Factor	Levels	Laparoscopic not transanal	Laparoscopic transanal	Open	Robotic not transanal	Robotic transanal	*P*-value
MRI N stage	Missing	38 (3.2)	3 (0.8)	17 (2.0)	1 (0.8)	0 (0.0)	< 0.001
	N0	699 (59.4)	248 (67.6)	443 (51.0)	79 (64.2)	36 (80.0)	
	N1	339 (28.8)	91 (24.8)	286 (32.9)	39 (31.7)	5 (11.1)	
	N2	100 (8.5)	25 (6.8)	122 (14.1)	4 (3.3)	4 (8.9)	
MRI M stage	Missing	30 (2.6)	3 (0.8)	17 (2.0)	0 (0.0)	0 (0.0)	0.239
	M0	1022 (86.9)	326 (88.8)	759 (87.4)	112 (91.1)	43 (95.6)	
	M1	124 (10.5)	38 (10.4)	92 (10.6)	11 (8.9)	2 (4.4)	
MRI AJCC stage	Missing	46 (3.9)	4 (1.1)	18 (2.1)	0 (0.0)	0 (0.0)	< 0.001
	Stage 1	301 (25.6)	93 (25.3)	186 (21.4)	44 (35.8)	14 (31.1)	
	Stage 2	357 (30.4)	140 (38.1)	236 (27.2)	29 (23.6)	21 (46.7)	
	Stage 3	348 (29.6)	92 (25.1)	336 (38.7)	39 (31.7)	8 (17.8)	
	Stage 4	124 (10.5)	38 (10.4)	92 (10.6)	11 (8.9)	2 (4.4)	
MRI EMVI	Missing	127 (10.8)	42 (11.4)	80 (9.2)	7 (5.7)	4 (8.9)	0.366
	No	954 (81.1)	295 (80.4)	721 (83.1)	111 (90.2)	36 (80.0)	
	Yes	95 (8.1)	30 (8.2)	67 (7.7)	5 (4.1)	5 (11.1)	
MRI CRM	Missing	136 (11.6)	44 (12.0)	88 (10.1)	7 (5.7)	5 (11.1)	0.353
	No	909 (77.3)	289 (78.7)	674 (77.6)	106 (86.2)	36 (80.0)	
	Yes	131 (11.1)	34 (9.3)	106 (12.2)	10 (8.1)	4 (8.9)	
Other operation characteristics							
Anastomotic technique	No anastomosis	224 (19.0)	87 (23.7)	260 (30.0)	28 (22.8)	14 (31.1)	< 0.001
	Handsewn	19 (1.6)	66 (18.0)	54 (6.2)	1 (0.8)	15 (33.3)	
	Stapled	933 (79.3)	214 (58.3)	554 (63.8)	94 (76.4)	16 (35.6)	
Operator type	Missing	1 (0.1)	2 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	< 0.001
	Colorectal	1010 (85.9)	333 (90.7)	704 (81.1)	105 (85.4)	38 (84.4)	
	General surgery	165 (14.0)	32 (8.7)	164 (18.9)	18 (14.6)	7 (15.6)	

P-value derived from χ^2 test for categorical variables. % shown by column.
CRM, Circumferential resection margin (</> 1 mm); CVA, Cerebrovascular accident; EMVI, Extramural vascular invasion; IHD, Ischemic heart disease; IQR, Interquartile range; MRI, Pre-neoadjuvant therapy, and/or baseline Magnetic Resonance Imaging staging; N/A, Not applicable; SD, Standard deviation.

anastomotic leak rate was higher in TaTME (12.9%, 45/311, one missing outcome data (< 1%)) than non-transanal TME (8.9%, 135/1520; Fig. 3). The highest leak rate was seen in robotic surgery, and more major complications were seen in transanal and robotic surgery (Table 2). In the univariate analysis both laparoscopic TaTME (OR 1.61, 1.02–2.48, $P = 0.04$) and robotic TaTME (OR 3.05, 1.10–7.34, $P = 0.02$) were associated with a higher risk of anastomotic leak than non-transanal laparoscopic TME. Once adjusted for confounders (Table 3, Fig. 4), transanal surgery was no longer significantly associated with leak (OR 1.23, 0.77–1.97, $P = 0.39$ and OR 2.11, 0.79–5.62, $P = 0.14$ respectively), whilst low rectal anastomosis (OR 2.72, 1.55-4.77, $P < 0.001$) and male gender (OR 2.29, 1.52–3.44, $P < 0.001$) were strongly associated. The model demonstrated fair discrimination (AUC: 0.70). Propensity score matching gave balanced groups (Table 4). In the propensity matched multivariable model (Table 5), transanal approach was not associated with overall anastomotic leak (OR 1.14, 0.70–1.81, $P = 0.595$). However, male gender (OR 2.88, 1.64–5.38, $P < 0.001$) and low rectal anastomosis (OR 3.92, 1.74–10.52, $P = 0.002$) again remained strong predictors for anastomotic leak.

Circumferential resection margin

In the unadjusted data, restorative surgery had a lower CRM positivity rate (36/1733, with 232 missing outcome data (11.8%)) than non-restorative (58/549) operations (2.3% versus 10.6%). Overall, there was a low CRM positive rates across all approach types to rectal resection with restorative anastomosis (0–4.7%, Table 2). For the low rectum, robotic surgery had a lower positive margin rate than laparoscopic surgery (0/19 with a transanal perineal approach, and 1/27 with a non-transanal approach;

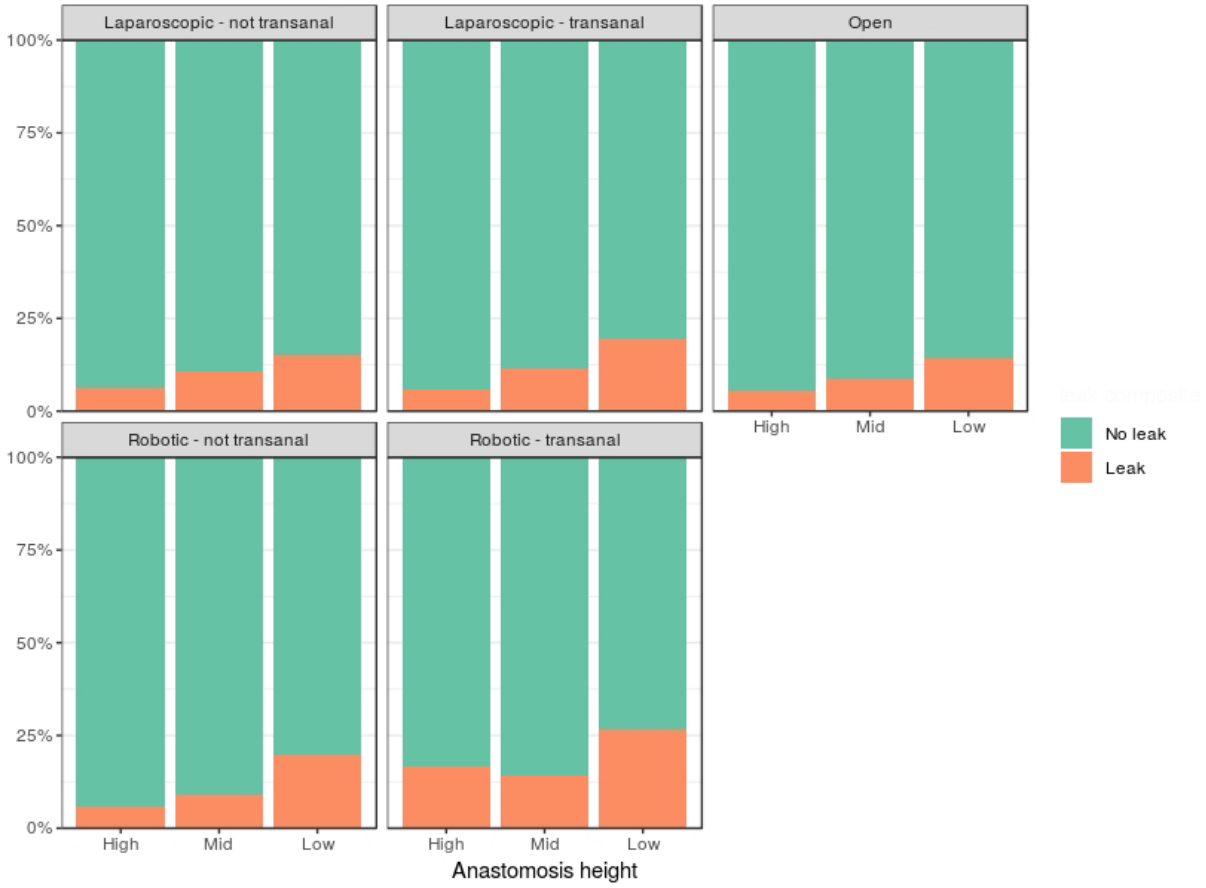

Figure 3 Leak rates by approach and tumour height.

Table 6). However, in a mixed-effects model (Table 7), none of the operative approaches were significantly associated with margin positivity except for non-restorative surgery. The model demonstrated fair discrimination (AUC: 0.72).

Discussion

This study supports the use of a TaTME approach for rectal cancer resection, with comparable postoperative outcomes and pathological safety compared to other approaches. This is in line with recent evidence on TaTME delivery across Europe [12,14,15]. The leak rate was higher than previously reported, at 12.9%, which at univariable level was significantly higher than other techniques. Once adjusted for confounders, this variability was largely a result of anastomosis in the lowest part of the rectum; transanal surgery became non-significant in mixed-effects and propensity-score matched models. By including other techniques within this study, it allows individual surgeons and units to benchmark practice and consider their own selection of patients. TaTME was more commonly used in men, in those undergoing long course chemoradiotherapy and in those with low tumours. This parallels current recommendations for the selection of patients, demonstrating appropriate adoption of this technique within included centres [5,16].

Leak rates after transanal (TaTME) surgery have been reported as 4.7% to 9.1% in recent systematic reviews [5,11] and 6.7% in a subsequent large international registry [17]. We add to this literature by providing an unselected, 'real-world' view of implementation of TaTME internationally in a prospective setting, with risk-adjustment of outcome data with mixed-effects modelling. The higher unadjusted leak rate identified in the present study may reflect learning curve effects from centres being at variable stages of adoption of the technique. It may also reflect the fact that we only included malignant conditions. An important variability between studies still exists in how anastomotic leakage is defined and detected. By comparing leakage to a simultaneous

Table 2 Short-term intraoperative and postoperative outcomes by approach.

Factor	Levels	Laparoscopic not transanal	Laparoscopic transanal	Open	Robotic not transanal	Robotic transanal	*P*-value
Postoperative outcomes							
Anastomotic leak	No leak	873 (74.2)	242 (65.9)	560 (64.5)	87 (70.7)	24 (53.3)	< 0.001
	Leak	79 (6.7)	38 (10.4)	48 (5.5)	8 (6.5)	7 (15.6)	
	No anastomosis	224 (19.0)	87 (23.7)	260 (30.0)	28 (22.8)	14 (31.1)	
Complication grade	Missing	6 (0.5)	1 (0.3)	4 (0.5)	2 (1.6)	2 (4.4)	< 0.001
	Grade 1-2	257 (21.9)	93 (25.3)	241 (27.8)	24 (19.5)	11 (24.4)	
	Grade 3-5	120 (10.2)	58 (15.8)	101 (11.6)	17 (13.8)	8 (17.8)	
	None	793 (67.4)	215 (58.6)	522 (60.1)	80 (65.0)	24 (53.3)	
Pathological margin	CRM involved	38 (3.2)	14 (3.8)	41 (4.7)	1 (0.8)	0 (0.0)	0.134
	CRM not involved	1011 (86.0)	317 (86.4)	750 (86.4)	109 (88.6)	37 (82.2)	
	Missing	127 (10.8)	36 (9.8)	77 (8.9)	13 (10.6)	8 (17.8)	
Length of stay	Mean (SD)	8.4 (5.6)	10 (6.9)	10.7 (5.5)	7.7 (5.8)	9.9 (7.5)	< 0.001
Intraoperative outcomes							
Any intraoperative complication	No	1124 (95.6)	354 (96.5)	834 (96.1)	120 (97.6)	38 (84.4)	0.003
	Yes	52 (4.4)	13 (3.5)	34 (3.9)	3 (2.4)	7 (15.6)	
Vascular injury	No	1161 (98.7)	363 (98.9)	857 (98.7)	121 (98.4)	43 (95.6)	0.455
	Yes	15 (1.3)	4 (1.1)	11 (1.3)	2 (1.6)	2 (4.4)	
Bowel injury	No	1163 (98.9)	365 (99.5)	858 (98.8)	121 (98.4)	42 (93.3)	0.01
	Yes	13 (1.1)	2 (0.5)	10 (1.2)	2 (1.6)	3 (6.7)	
Other organ injury	No	1152 (98.0)	360 (98.1)	854 (98.4)	123 (100.0)	41 (91.1)	0.005
	Yes	24 (2.0)	7 (1.9)	14 (1.6)	0 (0.0)	4 (8.9)	

P-value derived from χ^2 test for categorical variables. % shown by column.
CRM, Circumferential resection margin (</> 1 mm); CVA, Cerebrovascular accident; IHD, Ischemic heart disease; IQR, Interquartile range; N/A, Not applicable; SD, Standard deviation.

cohort of laparoscopic, open and robotic resections from the same centres, we can explore and control for case selection variability by approach and mitigate against concerns of reporting bias. Reassuringly, male gender and low tumour height were strongly predictive factors for leak in our mixed effects models, which is consistent with current knowledge [18–20]. Whilst our data gives evidence for safety in the current dissemination of TaTME, structured training with proctorship from experienced proponents remains essential.

Improved pathological and oncological outcomes are a potential benefit of TaTME. The positive resection margin rate in restorative surgery from this study (4.0%) is consistent with previous reports, including the transanal component [5]. Fleshman *et al.* [21] previously reported a significantly lower difference rate of CRM involvement with TaTME when compared with laparoscopic TME. In contrast, the Bordeaux randomized trial found a significantly greater rate of CRM involvement for laparoscopic TME when compared to TaTME (18.0% *vs* 4.0%, $P = 0.025$) although this did not mean a decrease in local recurrence (long term oncological outcomes) [22]. The low positive CRM rates seen with robotic surgery in the lower rectum within the present study are likely to represent a degree of case selection at a site level; results from randomised trials in TaTME and robotic rectal cancer surgery are awaited.

This study also provides valuable information for other resection techniques. Recent randomised trials have suggested laparoscopic TME may lack oncological safety compared to open surgery in the mid and low rectum (ALaCaRT and ACOSOG) [21,22]. The present study shows pathological equivalence of laparoscopic and open approaches, with a selection variability evident that suggests surgeons are carefully and correctly selecting patients for each approach; this is consistent with COLOR II, COREAN and CLASiCC trials [18,19,23]. There were relatively few robotic cases in this cohort. Where robotics was performed, the positive CRM and conversion rates were lower when compared to laparoscopic techniques. The ROLARR trial with 471 patients did not show differences between laparoscopic and robotic for positive resection margin [24]. International registry studies alongside ROLARR reported a rate of conversion from laparoscopic to open or transanal of 6.3%. We found significant differences between laparoscopic transanal that presented the highest rate of conversion (16.2%) and robotic transanal (0%). This is consistent with the findings

Table 3 Univariable and multilevel models for overall anastomotic leak (primary outcome measure).

		Anastomotic leak			
Factor	Level	No leak	Leak	OR (univariable)	OR (multilevel)
Approach	Laparoscopic – not transanal	806 (48.1)	72 (44.2)	– (Reference)	– (Reference)
	Laparoscopic – transanal	223 (13.3)	32 (19.6)	1.61 (1.02–2.48, $P = 0.036$)	1.23 (0.77–1.97, $P = 0.386$)
	Open	538 (32.1)	45 (27.6)	0.94 (0.63–1.37, $P = 0.740$)	0.93 (0.61–1.43, $P = 0.745$)
	Robotic – not transanal	86 (5.1)	8 (4.9)	1.04 (0.45–2.11, $P = 0.917$)	0.81 (0.36–1.78, $P = 0.594$)
	Robotic – transanal	22 (1.3)	6 (3.7)	3.05 (1.10–7.34, $P = 0.019$)	2.11 (0.79–5.62, $P = 0.135$)
Age	< 55	278 (16.6)	29 (17.8)	–	–
	55–70	775 (46.3)	77 (47.2)	0.95 (0.61–1.51, $P = 0.831$)	0.92 (0.58–1.47, $P = 0.729$)
	70–80	481 (28.7)	47 (28.8)	0.94 (0.58–1.54, $P = 0.792$)	0.87 (0.51–1.48, $P = 0.606$)
	> 80	141 (8.4)	10 (6.1)	0.68 (0.31–1.39, $P = 0.311$)	0.70 (0.31–1.58, $P = 0.394$)
Gender	Female	629 (37.6)	34 (20.9)	–	–
	Male	1046 (62.4)	129 (79.1)	2.28 (1.56–3.42, $P < 0.001$)	2.29 (1.52–3.44, $P < 0.001$)
ASA class	Low risk (ASA 1-2)	1150 (68.7)	114 (69.9)	–	–
	High risk (ASA 3-5)	525 (31.3)	49 (30.1)	0.94 (0.66–1.33, $P = 0.736$)	0.99 (0.66–1.49, $P = 0.969$)
BMI	Normal weight	515 (30.7)	53 (32.5)	–	–
	Underweight	30 (1.8)	4 (2.5)	1.30 (0.37–3.44, $P = 0.639$)	1.35 (0.45–4.10, $P = 0.594$)
	Overweight	741 (44.2)	71 (43.6)	0.93 (0.64–1.36, $P = 0.707$)	0.89 (0.60–1.33, $P = 0.577$)
	Obese	389 (23.2)	35 (21.5)	0.87 (0.56–1.36, $P = 0.556$)	0.86 (0.53–1.39, $P = 0.534$)
History of IHD/CVA	No	1420 (84.8)	138 (84.7)	–	–
	Yes	255 (15.2)	25 (15.3)	1.01 (0.63–1.55, $P = 0.969$)	1.16 (0.70–1.94, $P = 0.567$)
History of diabetes mellitus	No	1431 (85.4)	140 (85.9)	–	–
	Diabetes: any control	244 (14.6)	23 (14.1)	0.96 (0.59–1.50, $P = 0.874$)	0.87 (0.53–1.42, $P = 0.584$)
Smoking history	Non-smoker	1436 (85.7)	129 (79.1)	–	–
	Current	239 (14.3)	34 (20.9)	1.58 (1.05–2.34, $P = 0.025$)	1.46 (0.95–2.23, $P = 0.082$)
Operator type	Colorectal	1403 (83.8)	137 (84.0)	–	–
	General surgery	272 (16.2)	26 (16.0)	0.98 (0.62–1.49, $P = 0.924$)	1.11 (0.68–1.81, $P = 0.687$)
Neoadjuvant therapy	Chemotherapy only	69 (4.1)	3 (1.8)	–	–
	Long course CRTx	368 (22.0)	48 (29.4)	3.00 (1.06–12.58, $P = 0.071$)	1.75 (0.51–5.99, $P = 0.371$)
	Short course radiotherapy	80 (4.8)	14 (8.6)	4.02 (1.25–17.98, $P = 0.034$)	2.74 (0.73–10.30, $P = 0.136$)
	None	1158 (69.1)	98 (60.1)	1.95 (0.71–8.05, $P = 0.266$)	1.97 (0.59–6.55, $P = 0.271$)
Anastomosis height	High rectum	525 (31.3)	29 (17.8)	–	–
	Mid rectum	528 (31.5)	40 (24.5)	1.37 (0.84–2.26, $P = 0.209$)	1.33 (0.79–2.23, $P = 0.277$)
	Low rectum	622 (37.1)	94 (57.7)	2.74 (1.80–4.28, $P < 0.001$)	2.72 (1.55–4.77, $P < 0.001$)
Anastomotic configuration	End to End	1271 (75.9)	123 (75.5)	–	–
	Side to Side	83 (5.0)	2 (1.2)	0.25 (0.04–0.80, $P = 0.054$)	0.27 (0.06–1.16, $P = 0.079$)
	Side to End	321 (19.2)	38 (23.3)	1.22 (0.82–1.78, $P = 0.303$)	1.10 (0.73–1.65, $P = 0.662$)
Leak test performed	No	543 (32.4)	54 (33.1)	–	–
	Yes	1132 (67.6)	109 (66.9)	0.97 (0.69–1.37, $P = 0.853$)	1.11 (0.76–1.64, $P = 0.584$)
Defunctioning stoma	Yes	720 (43.0)	93 (57.1)	–	–
	No	955 (57.0)	70 (42.9)	0.57 (0.41–0.78, $P = 0.001$)	1.05 (0.68–1.63, $P = 0.813$)

AUROC:0.70, AIC: 1088.1

Overall anastomotic leak was pre-defined as either (i) gross anastomotic leakage proven radiologically or clinically, or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging. Patients with missing outcome or risk adjustment data have been excluded from this model. Odds ratio (OR) presented with 95% confidence intervals. % shown by column.

CRTx, Chemoradiotherapy; CVA, Cerebrovascular accident; IHD, Ischemic heart disease; IQR, Interquartile range; N/A, Not applicable; SD, Standard deviation.

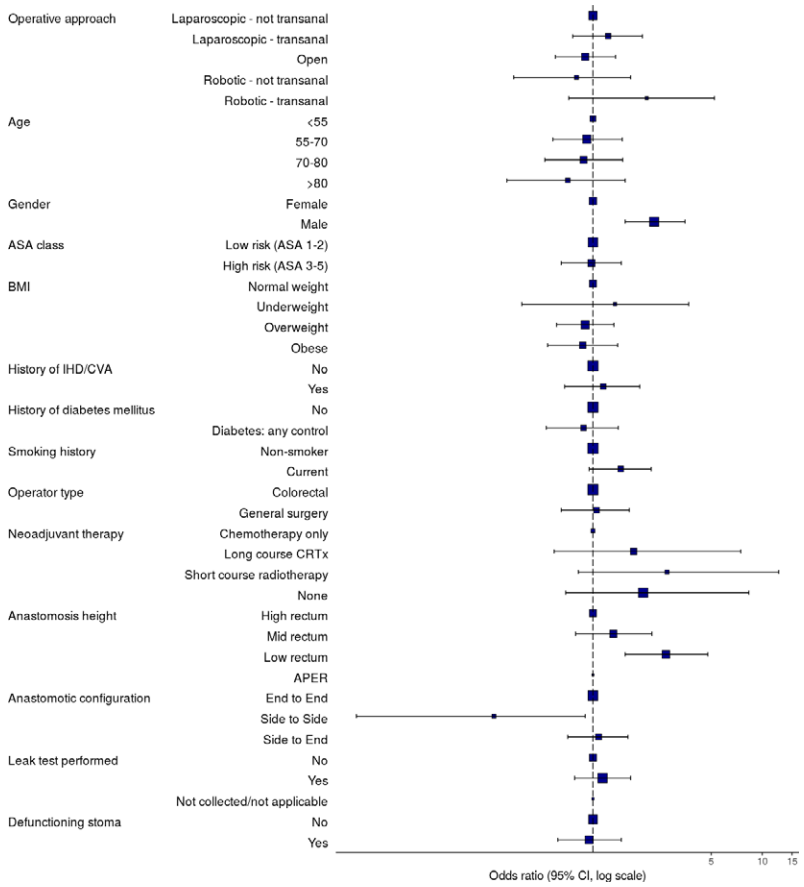

Figure 4 Forest plot for mixed effects model of factors associated with anastomotic leak in elective rectal cancer surgery with restorative anastomosis

Table 4 Balanced characteristics of propensity score matched groups.

		Perineal approach		
Factor	Level	Not transanal	Transanal	*P*-value
Age	< 55	108 (20.1)	56 (20.9)	0.979
	55–70	246 (45.9)	125 (46.6)	
	70–80	146 (27.2)	70 (26.1)	
	> 80	36 (6.7)	17 (6.3)	
Gender	Female	179 (33.4)	90 (33.6)	0.958
	Male	357 (66.6)	178 (66.4)	
Anastomosis height	High rectum	100 (18.7)	46 (17.2)	0.041
	Mid rectum	168 (31.3)	64 (23.9)	
	Low rectum	268 (50.0)	158 (59.0)	
MRI AJCC stage	Stage 1	167 (31.2)	72 (26.9)	0.553
	Stage 2	177 (33.0)	100 (37.3)	
	Stage 3	138 (25.7)	68 (25.4)	
	Stage 4	54 (10.1)	28 (10.4)	

P-value derived from χ^2 test for categorical variables. % shown by column.

Table 5 Summary of propensity score matched multivariable model for overall anastomotic leak.

Factor	Level	OR (multivariable)
Transanal component	No	–
	Yes	1.22 (0.75-1.96, $P = 0.420$)
Age	< 55	–
	55–70	0.92 (0.50-1.73, $P = 0.777$)
	70–80	0.68 (0.34-1.39, $P = 0.282$)
	> 80	0.47 (0.10-1.52, $P = 0.253$)
Gender	Female	–
	Male	2.94 (1.65-5.60, $P < 0.001$)
Anastomosis height	High rectum	–
	Mid rectum	1.81 (0.72-5.16, $P = 0.23$)
	Low rectum	3.75 (1.66-10.10, $P = 0.003$)
MRI AJCC stage	Stage 1	–
	Stage 2	1.18 (0.64-2.25, $P = 0.60$)
	Stage 3	1.55 (0.79-3.05, $P = 0.203$)
	Stage 4	1.03 (0.40-2.47, $P = 0.944$)

Overall anastomotic leak was pre-defined as either (i) gross anastomotic leakage proven radiologically or clinically, or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging. Odds ratio (OR) presented with 95% confidence intervals.

Table 6 Circumferential resection margin positive rates (pathological) by approach and height in rectum.

		Laparoscopic		Robotic	
	Open	Transanal	Not transanal	Transanal	Not transanal
Low rectum	19/236	9/163	16/198	0/19	1/27
	8.05%	5.52%	8.08%	0.00%	3.70%
Middle rectum	12/218	5/88	10/267	0/12	0/33
	5.50%	5.68%	3.75%	0.00%	0.00%
High Rectum	10/337	0/80	12/584	0/6	0/50
	2.96%	0.00%	2.05%	0.00%	0.00%

of ROLARR trial about the potential for robotic surgery to decrease the rate of conversion.

Finally the APER rate provides a contemporary permanent stoma rate across a variety of international sites for an operation with known variability between units [25]. Our group plans to produce a future report describing geographic variability in colorectal surgery, exploring differences in patient factors, disease presentations and techniques utilised internationally, across the last three international ESCP audits.

This study has limitations. Unadjusted outcomes showed higher major complication rates with robotic surgery and also transanal surgery, although without risk adjustment for confounding factors this must be interpreted with significant caution. Further research is needed to correctly risk-adjust for individual surgeon, or surgical team experience in TaTME, as well as unmeasured patient, tumour and operation-specific factors. Similarly, standardised definitions of anastomotic leakage and its detection remain uncommonly used between studies. Selection bias is an unavoidable factor in this type of observational research. We have attempted to minimize the effects of this by undertaking adjusted analyses using mixed-effects logistic regression models, but accept that this can never fully counteract the nuances involved in clinical decision-making. This said, the current study was designed to detect safety differences in current practice rather than test efficacy of treatments directly.

Results from randomised trials comparing outcomes after the variety of approaches available for rectal cancer surgery are now needed, particularly evaluating TaTME against laparoscopic TME without a transanal perineal component [26].

Table 7 Univariable and multilevel models for circumferential resection margin involvement.

		Resection margin			
Factor	Level	Negative	Positive	OR (univariable)	OR (multilevel)
Transanal component	No	1709 (79.3)	69 (76.7)	–	–
	Yes	445 (20.7)	21 (23.3)	1.17 (0.69–1.89, $P = 0.540$)	0.96 (0.56–1.65, $P = 0.889$)
Approach	Laparoscopic	1088 (50.5)	40 (44.4)	–	–
	Open	934 (43.4)	49 (54.4)	1.43 (0.93–2.20, $P = 0.102$)	1.50 (0.93–2.42, $P = 0.097$)
	Robotic	132 (6.1)	1 (1.1)	0.21 (0.01–0.96, $P = 0.120$)	0.17 (0.02–1.28, $P = 0.086$)
Age	< 55	335 (15.6)	19 (21.1)	–	–
	55–70	959 (44.5)	32 (35.6)	0.59 (0.33–1.07, $P = 0.074$)	0.57 (0.31–1.05, $P = 0.072$)
	70–80	626 (29.1)	25 (27.8)	0.70 (0.38–1.31, $P = 0.261$)	0.75 (0.39–1.45, $P = 0.393$)
	> 80	234 (10.9)	14 (15.6)	1.05 (0.51–2.14, $P = 0.883$)	1.37 (0.62–3.05, $P = 0.440$)
Gender	Female	781 (36.3)	32 (35.6)	–	–
	Male	1373 (63.7)	58 (64.4)	1.03 (0.67–1.62, $P = 0.892$)	1.08 (0.68–1.73, $P = 0.733$)
ASA class	Low risk (ASA 1-2)	1426 (66.2)	63 (70.0)	–	–
	High risk (ASA 3-5)	728 (33.8)	27 (30.0)	0.84 (0.52–1.31, $P = 0.456$)	0.64 (0.37–1.12, $P = 0.116$)
BMI	Normal weight	672 (31.2)	25 (27.8)	–	–
	Underweight	40 (1.9)	6 (6.7)	4.03 (1.43–9.82, $P = 0.004$)	4.71 (1.74–12.79, $P = 0.002$)
	Overweight	929 (43.1)	39 (43.3)	1.13 (0.68–1.90, $P = 0.644$)	1.32 (0.77–2.26, $P = 0.313$)
	Obese	513 (23.8)	20 (22.2)	1.05 (0.57–1.90, $P = 0.878$)	1.17 (0.62–2.23, $P = 0.626$)
History of IHD/CVA	No	1797 (83.4)	72 (80.0)	–	–
	Yes	357 (16.6)	18 (20.0)	1.26 (0.72–2.09, $P = 0.394$)	1.75 (0.93–3.26, $P = 0.081$)
History of diabetes mellitus	No	1818 (84.4)	80 (88.9)	–	–
	Diabetes: any control	336 (15.6)	10 (11.1)	0.68 (0.33–1.26, $P = 0.251$)	0.62 (0.31–1.25, $P = 0.180$)
Smoking history	Non-smoker	1824 (84.7)	74 (82.2)	–	–
	Current	330 (15.3)	16 (17.8)	1.20 (0.66–2.02, $P = 0.528$)	1.10 (0.61–2.00, $P = 0.756$)
Operator type	Colorectal	1836 (85.2)	79 (87.8)	–	–
	General surgery	318 (14.8)	11 (12.2)	0.80 (0.40–1.46, $P = 0.505$)	0.91 (0.45–1.85, $P = 0.791$)
Neoadjuvant therapy	Chemotherapy only	75 (3.5)	2 (2.2)	–	–
	Long course CRTx	555 (25.8)	40 (44.4)	2.70 (0.81–16.81, $P = 0.176$)	2.17 (0.49–9.60, $P = 0.307$)
	Short course radiotherapy	156 (7.2)	9 (10.0)	2.16 (0.54–14.42, $P = 0.331$)	1.76 (0.35–8.76, $P = 0.491$)
	None	1368 (63.5)	39 (43.3)	1.07 (0.32–6.65, $P = 0.928$)	1.11 (0.25–4.84, $P = 0.891$)
Anastomosis height	High rectum	540 (25.1)	13 (14.4)	–	–
	Mid rectum	574 (26.6)	16 (17.8)	1.16 (0.55–2.47, $P = 0.698$)	1.09 (0.51–2.31, $P = 0.831$)
	Low rectum	714 (33.1)	24 (26.7)	1.40 (0.72–2.85, $P = 0.339$)	1.08 (0.50–2.30, $P = 0.849$)
	APER	326 (15.1)	37 (41.1)	4.71 (2.53–9.33, $P < 0.001$)	3.55 (1.68–7.52, $P = 0.001$)

AUC:0.77, AIC: 731.5

Overall anastomotic leak was pre-defined as either (i) gross anastomotic leakage proven radiologically or clinically, or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on post-operative imaging. Odds ratio (OR) presented with 95% confidence intervals. % shown by column.

CRTx, Chemoradiotherapy; CVA, Cerebrovascular accident; IHD, Ischemic heart disease; IQR, Interquartile range; N/A, Not applicable; SD, Standard deviation.

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Conflicts of interests

None to declare.

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References

1 Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery–the clue to pelvic recurrence? *Br J Surg* 1982; **69:** 613–6.
2 Penna M, Knol JJ, Tuynman JB, Tekkis PP, Mortensen NJ, Hompes R. Four anastomotic techniques following transanal total mesorectal excision (TaTME). *Tech Coloproctol* 2016; **20:** 185–91.
3 Sylla P, Rattner DW, Delgado S, Lacy AM. NOTES transanal rectal cancer resection using transanal endoscopic microsurgery and laparoscopic assistance. *Surg Endosc* 2010; **24:** 1205–10.
4 Wolthuis AM, Cini C, Penninckx F, D'Hoore A. Transanal single port access to facilitate distal rectal mobilization in laparoscopic rectal sleeve resection with hand-sewn coloanal anastomosis. *Tech Coloproctol* 2012; **16:** 161–5.
5 Ito M, Sugito M, Kobayashi A, Nishizawa Y, Tsunoda Y, Saito N. Relationship between multiple numbers of stapler firings during rectal division and anastomotic leakage after laparoscopic rectal resection. *Int J Colorectal Dis* 2008; **23:** 703–7.
6 Emile SH, de Lacy FB, Keller DS *et al.* Evolution of transanal total mesorectal excision for rectal cancer: from top to bottom. *World J Gastrointest Surg* 2018; **10:** 28–39.
7 ACPGBI (2018) Pilot Training Initiative for TaTME. https://www.acpgbi.org.uk/education/tatme/ (accessed on 21st August 2018)
8 Koedam TWA, Veltcamp Helbach M, van de Ven PM *et al.* Transanal total mesorectal excision for rectal cancer: evaluation of the learning curve. *Tech Coloproctol* 2018; **22:** 279–87.
9 Mege D, Hain E, Lakkis Z, Maggiori L, Prost AlDJ, Panis Y. Is trans-anal total mesorectal excision really safe and better than laparoscopic total mesorectal excision with a perineal approach first in patients with low rectal cancer? A learning curve with case-matched study in 68 patients. *Colorectal Dis* 2018; **20:** O143–51.
10 Thomsen MH, Ovesen H, Eriksen JR. Combined laparoscopic and transanal total mesorectal excision for rectal cancer: initial experience and early results. *J Minim Access Surg* 2017; **13:** 113–7.
11 Deijen CL, Tsai A, Koedam TW *et al.* Clinical outcomes and case volume effect of transanal total mesorectal excision for rectal cancer: a systematic review. *Tech Coloproctol* 2016; **20:** 811–24.
12 Penna M, Hompes R, Arnold S *et al.* Incidence and risk factors for anastomotic failure in 1594 patients treated by transanal total mesorectal excision: results from the International TaTME Registry. *Ann Surg* 2018; [EPub Ahead of print] https://doi.org/10.1097/SLA.0000000000002653.
13 von Elm E, Altman DG, Egger M *et al.* Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007; **335:** 806–8.
14 Rasulov AO, Mamedli ZZ, Gordeyev SS, Kozlov NA, Dzhumabaev HE. Short-term outcomes after transanal and laparoscopic total mesorectal excision for rectal cancer. *Tech Coloproctol* 2016; **20:** 227–34.
15 Veltcamp Helbach M, Deijen CL, Velthuis S, Bonjer HJ, Tuynman JB, Sietses C. Transanal total mesorectal excision for rectal carcinoma: short-term outcomes and experience after 80 cases. *Surg Endosc* 2016; **30:** 464–70.
16 Adamina M, Buchs NC, Penna M, Hompes R, St.Gallen Colorectal Consensus Expert G. St.Gallen consensus on safe implementation of transanal total mesorectal excision. *Surg Endosc* 2018; **32:** 1091–103.
17 Penna M, Hompes R, Arnold S *et al.* Transanal total mesorectal excision: international registry results of the first 720 cases. *Ann Surg* 2017; **266:** 111–7.
18 van der Pas MH, Haglind E, Cuesta MA *et al.* Laparoscopic versus open surgery for rectal cancer (COLOR II): short-term outcomes of a randomised, phase 3 trial. *Lancet Oncol* 2013; **14:** 210–8.
19 Kang SB, Park JW, Jeong SY *et al.* Open versus laparoscopic surgery for mid or low rectal cancer after neoadjuvant chemoradiotherapy (COREAN trial): short-term outcomes of an open-label randomised controlled trial. *Lancet Oncol* 2010; **11:** 637–45.
20 Kang CY, Halabi WJ, Chaudhry OO *et al.* Risk factors for anastomotic leakage after anterior resection for rectal cancer. *JAMA Surg* 2013; **148:** 65–71.
21 Fleshman J, Branda M, Sargent DJ *et al.* Effect of laparoscopic-assisted resection vs open resection of stage II or III rectal cancer on pathologic outcomes: the ACOSOG Z6051 randomized clinical trial. *JAMA* 2015; **314:** 1346–55.
22 Stevenson AR, Solomon MJ, Lumley JW *et al.* Effect of laparoscopic-assisted resection vs open resection on pathological outcomes in rectal cancer: the ALaCaRT randomized clinical trial. *JAMA* 2015; **314:** 1356–63.
23 Guillou PJ, Quirke P, Thorpe H *et al.* Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. *Lancet* 2005; **365:** 1718–26.
24 Jayne D, Pigazzi A, Marshall H *et al.* Effect of robotic-assisted vs conventional laparoscopic surgery on risk of conversion to open laparotomy among patients undergoing resection for rectal cancer: the ROLARR randomized clinical trial. *JAMA* 2017; **318:** 1569–80.
25 Morris E, Quirke P, Thomas JD, Fairley L, Cottier B, Forman D. Unacceptable variation in abdominoperineal excision rates for rectal cancer: time to intervene? *Gut* 2008; **57:** 1690–7.
26 Perdawood SK, Al Khefagie GA. Transanal vs laparoscopic total mesorectal excision for rectal cancer: initial experience from Denmark. *Colorectal Dis* 2016; **18:** 51–8.

Authorship list

Writing group

Aneel Bhangu Ana María Minaya-Bravo, Gaetano Gallo, James C Glasbey, Sivesh Kamarajah, Dmitri Nepogodiev, Thomas Pinkney, Alaa El-Hussana (Chair).

APPENDIX 7

- The 2015 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** and al. from Portugal).

"Predictors for Anastomotic Leak, Post-operative Complications, and Mortality After Right Colectomy for Cancer: Results from an International Snapshot Audit."

in Diseases of Colon and Rectum · May 2020; DOI: 10.1097/DCR.0000000000001590.

Co-author (integrating ESCP collaborating group); Part 1 – Chapter 1.

ORIGINAL CONTRIBUTION

Predictors for Anastomotic Leak, Postoperative Complications, and Mortality After Right Colectomy for Cancer: Results From an International Snapshot Audit

2015 European Society of Coloproctology Collaborating Group*

BACKGROUND: A right hemicolectomy is among the most commonly performed operations for colon cancer, but modern high-quality, multination data addressing the morbidity and mortality rates are lacking.

OBJECTIVE: This study reports the morbidity and mortality rates for right-sided colon cancer and identifies predictors for unfavorable short-term outcome after right hemicolectomy.

DESIGN: This was a snapshot observational prospective study.

SETTING: The study was conducted as a multicenter international study.

PATIENTS: The 2015 European Society of Coloproctology snapshot study was a prospective multicenter international series that included all patients undergoing elective or emergency right hemicolectomy or ileocecal resection over a 2-month period in early 2015. This is a subanalysis of the colon cancer cohort of patients.

MAIN OUTCOME MEASURES: Predictors for anastomotic leak and 30-day postoperative morbidity and mortality were assessed using multivariable mixed-effect logistic regression models after variables selection with the Lasso method.

RESULTS: Of the 2515 included patients, an anastomosis was performed in 97.2% (n = 2444), handsewn in 38.5% (n = 940) and stapled in 61.5% (n = 1504) cases. The overall anastomotic leak rate was 7.4% (180/2444), 30-day morbidity was 38.0% (n = 956), and mortality was 2.6% (n = 66). Patients with anastomotic leak had a significantly increased mortality rate (10.6% vs 1.6% no-leak patients; $p > 0.001$). At multivariable analysis the following variables were associated with anastomotic leak: longer duration of surgery (OR = 1.007 per min; $p = 0.0037$), open approach (OR = 1.9; $p = 0.0037$), and stapled anastomosis (OR = 1.5; $p = 0.041$).

LIMITATIONS: This is an observational study, and therefore selection bias could be present. For this reason, a multivariable logistic regression model was performed, trying to correct possible confounding factors.

CONCLUSIONS: Anastomotic leak after oncologic right hemicolectomy is a frequent complication, and it is associated with increased mortality. The key contributing surgical factors for anastomotic leak were anastomotic technique, surgical approach, and duration of surgery. See **Video Abstract** at http://links.lww.com/DCR/B165.

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Correspondence: Matteo Frasson, Ph.D., E.B.S.Q.-c., M.D., Hospital Universitario y Politecnico La Fe, Avenida Fernando Abril Martorell, n.106, Piso 5, Torre G, 46026 Valencia, Spain. E-mail dr.frasson.matteo@gmail.com; Twitter: @frasson_matt

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PREDICTORES DE FUGA ANASTOMÓTICA, COMPLICACIONES POSTOPERATORIAS Y MORTALIDAD DESPUÉS DE LA COLECTOMÍA DERECHA POR CÁNCER: RESULTADOS DE UNA AUDITORÍA INTERNACIONAL DE CORTO PLAZO

ANTECEDENTES: La hemicolectomía derecha se encuentra entre las operaciones más frecuentemente realizadas para cáncer de colon, pero faltan datos

modernos multinacionales de alta calidad, que aborden las tasas de morbilidad y mortalidad.

OBJETIVO: Reportar la tasa de morbilidad y mortalidad para cáncer de colon del lado derecho, e identificar predictores de resultados desfavorables a corto plazo, después de la hemicolectomía derecha.

DISEÑO: Estudio prospectivo observacional de corto plazo.

LUGAR: Estudio multicéntrico internacional.

PACIENTES: El estudio de corto plazo de la Sociedad Europea de Coloproctología de 2015, fue una serie prospectiva multicéntrica internacional, que incluyó a todos los pacientes sometidos a hemicolectomía derecha electiva, de emergencia o resección ileocecal, por un período de dos meses y a principios de 2015. Este es un subanálisis, cohorte de pacientes con cáncer de colon.

PRINCIPALES MEDIDAS DE RESULTADO: Los predictores de fuga anastomótica, morbilidad y mortalidad postoperatorias a los 30 días, se evaluaron usando modelos de regresión logística de efectos multivariables mixtos, después de la selección de variables con el método Lasso.

RESULTADOS: De los 2,515 pacientes incluidos, se realizó una anastomosis en el 97,2% (n = 2,444); sutura manual en 38.5% (n = 940) y por engrapadora en 61.5% (n = 1504) casos. La tasa global de fuga anastomótica fue del 7,4% (180/2,444), morbilidad a los 30 días fue del 38,0% (n = 956) y la mortalidad fue del 2,6% (n = 66). Los pacientes con fuga anastomótica tuvieron una tasa de mortalidad significativamente mayor (10,6% frente al 1,6% de pacientes sin fuga, p> 0,001). En el análisis multivariable, las siguientes variables se asociaron con la fuga anastomótica: mayor duración de la cirugía (OR 1.007 por minuto, p = 0.0037), abordaje abierto (OR 1.9, p = 0.0037) y anastomosis por engrapadora (OR 1.5, p = 0.041).

LIMITACIONES: Este es un estudio observacional y por lo tanto podría estar presente el sesgo de selección. Por esta razón, se realizó un modelo de regresión logística multivariable, tratando de corregir posibles factores de confusión.

CONCLUSIONES: La fuga anastomótica después de la hemicolectomía derecha oncológica, es una complicación frecuente y asociada a mayor mortalidad. Los factores quirúrgicos clave que contribuyeron a la fuga anastomótica, fueron la técnica anastomótica, abordaje quirúrgico y duración de la cirugía. Consulte **Video Resumen** en http://links.lww.com/DCR/B165. *(Traducción—Dr. Fidel Ruiz Healy)*

KEY WORDS: Anastomotic leak; Colon cancer; Postoperative outcome; Right colectomy.

Right hemicolectomy is considered one of the simplest colorectal major procedures[1] and is often considered an appropriate first step for residents and young fellows. Despite this, complications after right hemicolectomy for cancer are common, at ≈30%, and postoperative mortality is reported to be ≈3%.[2–11] Anastomotic leak (AL) after right hemicolectomy for cancer is a major contributor to this short-term morbidity and mortality.[2–11] The document AL rate after right hemicolectomy ranges widely, from 1.3% to 8.4%.[2–11] This also has a significant impact on healthcare costs and major oncologic consequences, as demonstrated by the higher cancer recurrence rate after AL.[12,13]

Although predictors for AL after colon resection have been widely described, few prospective studies have specifically focused on predictors for AL in patients with colon cancer undergoing right hemicolectomy. Multicenter snapshot studies allow high-quality prospective data to be gathered on a large group of patients in a short period of time. This provides a more typical reflection of daily practice compared with a randomized clinical trial. They allow for exploration of differences among patients, techniques, and management across the cohort. This enables researchers to identify areas of practice variability that may result in differences in outcome. As such, this can provide hypothesis-generating areas that provide the foundation for future randomized controlled trials. Furthermore, this form of multicenter collaboration provides an opportunity for research-naïve units to participate in clinical studies, and it strengthens research networks nationally and internationally.

The aim of the present analysis was to develop a predictive model for AL in a large population of patients operated on for right colon cancer. Predictors for postoperative morbidity and mortality were also evaluated.

PATIENTS AND METHODS

This prospective, observational, multicenter study was performed according to a prespecified protocol (www.escp.eu.com/research/cohort-studies/2015-audit). The methodology for unit and patient recruitment, training, and data recording were detailed previously.[14]

Centers

All units performing GI surgery were eligible to register and recruit patients. The study was launched at the European Society of Coloproctology (ESCP) Scientific & Annual Meeting in Barcelona, Spain, in September 2014, and invitations to participate were subsequently distributed directly to all registered members of the ESCP. According to epidemiologic data, ≈85,000 right hemicolectomies are performed across Europe each year. Estimating 8% penetration of the study in European centers, with a 90% recruitment rate, a 2-month recruitment period was planned to include at least 1000 patients in the study. Par-

ticipating centers were responsible for completion of local governance and appropriate ethical approval before data collection began. Centers were required to submit details of named local investigators and needed to ensure that pathways that enabled consecutive identification of all eligible patients occurred during the study period. Data completeness of >95% was mandatory for each unit.

Patients

The present study is a large subset analysis of all patients with colon cancer from the 2015 ESCP snapshot study. The 2015 ESCP snapshot study recruited 3208 adult patients undergoing right hemicolectomy or ileocecal resection. This included all benign or malignant cases using any access technique in both the elective and emergency settings. Patients were excluded if their distal colonic transaction point beyond extended beyond the splenic flexure (eg, subtotal colectomy or panproctocolectomy) or a larger procedure (eg, cytoreductive surgery) occurred.[14]

Outcome Measures

The primary outcome for this study was AL, predefined as either type 1, a clinically suspected anastomotic leakage confirmed radiologically or intraoperatively, or type 2, the presence of an intraperitoneal (abdominal or pelvic) fluid collection on postoperative imaging.

Secondary outcome measures included mortality, overall morbidity, wound infection, reoperation, readmission, and length of hospital stay. Postoperative morbidity was classified according to the Clavien–Dindo system. *Intraoperative complications* were defined as unexpected surgical adverse events that occurred in the operating room during surgery. These included iatrogenic injury of bowel, other organs or blood vessels, bleeding (if clinically relevant), stapling device malfunction, redoing anastomosis because of technical problems, and "specified other." Patient fitness was graded by the ASA as I, normal healthy patient; II, mild systemic disease; III, severe systemic disease; and, IV, severe systemic disease that is a constant threat to life.

Data Collection

Sites were asked to include all consecutive eligible patients over an 8-week period during January 2015. The final permitted date of patient recruitment at any site was March 27, 2015.

Preoperative, operative, and postoperative data (30-day follow-up) were prospectively collected using an internationally accessible online database called REDCap (Research Electronic Data Capture), specifically designed for clinical research. *Emergency operations* were defined as interventions for acute onset or clinical deterioration of immediately or potentially life-threatening conditions. The extent of resection was classified as limited (proximal to hepatic flexure), complete (distal to hepatic flexure), or extended (middle or distal transverse colon; see Figure, Supplemental Digital Content 1, http://links.lww.com/DCR/B213). The *duration of surgery* was defined as the time interval from skin incision to closure in minutes.

Statistical Analysis

This report has been prepared in accordance with guidelines set by the Strengthening the Reporting of Observational Studies in Epidemiology statement for observational studies.[15] Categorical variables were described as number of patients and percentage, whereas continuous variables were described as median (25^{th}–75^{th} percentile). Association between categorical variables was assessed by χ^2 test. AL and/or abdominal abscess, postoperative morbidity, and postoperative mortality were considered as outcome variables.

The association of patient-related variables and intraoperative data with outcome variables (AL, postoperative morbidity, and postoperative mortality) was assessed using a mixed-effects logistic regression model. Because the patients from the same hospital are more likely to have a similar risk than those from other hospitals, the logistic regression model included the "hospital" variable as a random effect with random intercept to correct for the non-independence of the data.

Variables included in the models were selected using the L1-penalization (LASSO) technique.[16] Effect estimates were presented as ORs with 95% CIs and 2-sided p values. The 95% CIs were estimated using 2000 bootstrap replications. Because of the presence of multiple comparisons, p values were corrected using the false discovery rate method. Statistical significance was defined at the level of $p < 0.05$. SPSS (version 22.0.0; IBM SPSS Statistics, IBM Corp, Armonk, NY) was used for the descriptive analyses. The R software (version 3.4.1; www.r-project.org) was used for selection of variables with the LASSO model and logistic regression.

RESULTS

Patients, Centers, and Operative Data

The present analysis included 2515 patients who underwent right colon resection for cancer in 280 hospitals in 38 countries (5 outside Europe). The median (interquartile) number of patients included per hospital was 8 (5–11). The median (interquartile) age was 71 years (64–79 y), 52.1% were men, the majority were nonsmokers (63.1%), and 60.3% were classified as ASA I to II. Additional details are reported in Table 1. Overall, 88.1% (n = 2216) of patients underwent elective surgery, and 57.2% (n = 1440) were started laparoscopically, with a 15.2% conversion to open rate. The majority of operations (58.3%) were performed by a colorectal surgeon. Surgical details are reported in Table 2. An intraoperative complication occurred in 209 patients (8.3%), the most frequent

TABLE 1. Preoperative data

Variables	*Number*	*Data*
Age, y	71	64–79
Sex		
Men	1310	52.1%
Women	1205	47.9%
BMI		
Underweight (≤20)	151	6.0%
Normal (20–30)	1831	72.8%
Obese (≥30)	533	21.2%
BMI	26	23.0–29.0
Cardiac disease	548	21.8%
Diabetes mellitus		
No	2052	81.6%
Diet/tablet controlled	367	14.6%
Insulin	96	3.8%
Abnormal creatinine (>1.3 mg/dL)	294	11.6%
Tobacco		
No	1587	63.1%
Active	268	10.7%
Ex-smoker	486	19.3%
Missing	174	6.9%
Statin treatment	703	28.0%
Preoperative chemotherapy	51	2.0%
Previous abdominal surgery		
No	1908	75.9%
Appendicectomy	322	12.8%
Ileocecal resection	2	0.1%
Other	283	11.3%
ASA		
I	250	10.2%
II	1261	50.1%
III	903	35.9%
IV	98	3.9%
V	3	0.1%
Hemoglobin, g/dL	11.9	10.3–13.6
Disease		
Adenocarcinoma	2193	87.2%
Unresectable polyp	256	10.2%
Mass-stenosis	49	1.9%
Other cancer	16	0.6%

Data are expressed as number of patient and percentage or median and 25th–75th percentile.

being intraoperative bleeding (n = 157; 6.2%). In 11 cases an intraoperative complication related to anastomosis occurred (Table 3).

Anastomotic Technique

An anastomosis was performed in 97.2% (n = 2444) of the patients, with a defunctioning loop ileostomy 0.3% (7/2444) of cases. The anastomosis was handsewn in 37.4% (n = 940) and stapled in 59.8% (n = 1504) of the cases. Patients undergoing a handsewn anastomosis were significantly more likely to be emergency admissions (13.3% vs 8.4% stapled; $p < 0.001$) and more commonly underwent open surgery (53.2% vs 34.4%; $p < 0.001$). An intracorporeal anastomosis was performed in 211 (17.4%) of 1216 laparoscopic cases; the majority of these were stapled side-to-side anastomosis (n = 183; 86.7%). Additional details are reported in Table 2 and Table 4.

TABLE 2. Surgical details

Detail	Number	Data
Type of surgery		
Elective	2216	88.1%
Emergency	299	11.9%
Type of right colectomy[a]		
Limited (C1–C3)	247	9.8%
Complete (C4)	813	32.3%
Extended (C5–C7)	1455	57.8%
Operating surgeon		
Colorectal surgeon	1465	58.3%
Colorectal trainee	333	13.2%
General surgeon	467	18.6%
General surgery trainee	250	9.9%
Duration of surgery, min	130	105–170
Approach		
Laparoscopy	1221	48.5%
Laparoscopy converted	219	8.7%
Open	1075	42.7%
Anastomotic technique		
No anastomosis	71	2.8%
Handsewn	940	37.4%
Stapled	1504	59.8%
Anastomosis in laparoscopic approach (N = 1216)		
Intracorporeal	211	17.4%
Extracorporeal	1005	82.6%
Ileostomy		
Loop	7	0.3%
End	71	2.8%
Skin closure		
Subcuticular	842	33.5%
Staple	1450	57.7%
Other	223	8.9%

Data are expressed as number of patient and percentage or median and 25th–75th percentile.

[a]Please see Figure, Supplemental Digital Content 1, for definition of extent of resection.

AL and 30-Day Postoperative Outcome (Table 5)

An AL and/or intraperitoneal fluid collection was diagnosed in 7.4% (180/2444) of patients. The incidence was 4.6% (112/2444) when only considering a clinically suspected AL (type 1). Of the 180 patients with AL, 88 (48.9%) were reoperated, 25 (13.9%) needed percutaneous drainage, and 67 (37.2%) were treated only with antibiotics.

TABLE 3. Intraoperative Complications

Complication	Number	Percentage
None	2306	91.7
Bleeding	157	6.2
Enterotomy	20	0.8
Vascular injury	13	0.5
Anastomosis	11	0.4
Duodenal injury	4	0.2
Ureter injury	2	0.1
Liver injury	2	0.1
Gallbladder injury	1	0.1
Kidney injury	1	0.1
Other organs injury	4	0.2
Other	15	0.6

Some patients could have more than one complication. Data are expressed as number of patients and percentage.

TABLE 4. Details of anastomotic technique

Variable	*Number*	*Percentage*
Handsewn (N = 940)		
Technique		
Continuous	631	67.1
Interrupted	309	32.9
Size suture		
2/0	52	5.5
3/0	642	68.3
4/0	240	25.5
Other	6	0.6
Type suture		
Polyglactin (Vicryl/Polysorb)	366	38.9
Polydioxanone (PDS)	358	38.1
Polypropylene (Prolene/Surgipro)	26	2.8
Chromic gut	9	1.0
Other	181	19.3
Layers		
Single	522	55.5
Two	418	44.5
Layer sutured		
Full thickness	384	40.9
Seromuscular	556	59.1
Stapled (N = 1504)		
Technique		
Side-to-side	1320	87.8
Side-to-end	14	0.9
End-to-side	170	11.3

TABLE 5. Postoperative outcome

Variable	*Number*	*Data*
Critical care		
No	1742	69.3%
Planned	678	27.0%
Unplanned from theater	52	2.1%
Unplanned from ward	43	1.7%
Morbidity	956	38.0%
CD morbidity classification		
I	334	13.3%
II	290	11.5%
III	166	6.6%
IV	48	1.9%
V	66	2.6%
Missing	52	2.1%
Mortality	66	2.6%
Proven anastomotic leak (N = 2444)[a]	112	4.6%
Abdominal collection	125	5.0%
Anastomotic leak and/or abdominal collection in patients with anastomosis (N = 2444)	180	7.4%
Surgical site infection	246	9.8%
Reoperation	154	6.1%
Readmission	136	5.4%
Length of postoperative stay, d	7.0	5.0–11.0

Data are expressed as number of patients and percentage or median and 25th–75th percentile.
CD = Clavien-Dindo.
[a]Proven anastomotic leak indicates anastomotic leak diagnosed radiologically (with evidence of intraluminal contrast leak), clinically (with evidence of extravasation of bowel content or gas through a wound or drain), by endoscopy, or intraoperatively.

The overall 30-day mortality was 2.6% (66/2515); for those undergoing elective operations, this reduced to 1.6% versus 7.6% for emergency surgery ($p < 0.001$). Overall morbidity was 38.0%, and the median (interquartile) length of hospital stay was 7 days (5–11 d). An AL and/or intraperitoneal fluid collection was associated with a significant increase in the 30-day death rate (10.6% vs 1.6%; $p < 0.001$).

Predictors for AL

A fitted multivariable mixed-effects logistic regression model identified a statistically significant association (Table 6) between AL and duration of surgery (OR = 1.007 per min; $p = 0.0037$), open surgery (versus laparoscopic surgery: OR = 1.9; $p = 0.0037$), and stapled anastomosis (versus handsewn: OR = 1.5; $p = 0.04$). The variance between the participating hospitals was 0.82 on the logit scale.

Predictors for 30-Day Morbidity and Mortality

The following variables were found to be associated with 30-day postoperative complications (Table 7): duration of surgery (OR = 1.004 per min; $p = 0.0037$), urgent surgery (versus elective OR = 2.1; $p = 0.0037$), open approach versus laparoscopy (OR = 1.8; $p = 0.0037$), ASA score III to IV versus ASA score I to II (OR = 1.5; $p = 0.0037$), male sex (versus female OR = 1.5; $p = 0.0037$), age (OR = 1.012 per year; $p = 0.014$), and current use of tobacco (versus no smoker OR = 1.5; $p = 0.02$).

The most important predictor for postoperative mortality was age (OR = 1.05 per year; $p = 0.0037$). Other statistically significant predictors were urgent surgery ($p = 0.0037$), open versus laparoscopy approach (OR = 2.6; $p = 0.013$), ASA score III to IV versus ASA score I to II (OR = 2.4; $p = 0.014$), no anastomosis ($p = 0.024$), and intraoperative complications (OR = 1.2; $p = 0.041$; Table 8). The variance between the participating hospitals for postoperative morbidity and 30-day mortality was 0.54 and 0 on the logit scale.

DISCUSSION

The present multicenter international snapshot audit has identified 3 surgeon-dependent variables significantly associated with AL: duration of surgery, surgical approach, and anastomotic technique. The knowledge of specific predictors for AL and postoperative morbidity and mortality is important for stratifying and personalizing the surgical risk. This can provide precise information to patients, enabling a more informed consent process. It can highlight high-risk patients who are likely to require intensive postoperative follow-up, and it may identify patients with modifiable risk factors who are likely to benefit from a period of preoperative optimization.

In this study, despite considerable discrepancies between outcomes for emergency and elective cases, the

TABLE 6. Predictors for anastomotic leak/intraabdominal collection at multivariate analysis

Variables	*Patients with AL (N = 180; 7.4%)*	*Patients without AL (N = 2264; 92.6%)*	*p (multivariate analysis)*	*OR*	*95% CI*	*Adjusted p*
Duration of surgery (per min)	150 (114–180)	130 (104–170)	<0.001	1.007	1.0–1.0	0.0037
Surgical approach			<0.001			0.0037
Intention laparoscopic	77 (5.4%)	1349 (94.6%)		1	–	
Intention open	103 (10.1%)	915 (89.9%)		1.9	1.3–2.8	
Anastomotic technique			0.02			0.041
Handsewn	58 (6.2%)	882 (93.8%)		1	–	
Stapled	122 (8.1%)	1382 (91.9%)		1.5	1.1–2.3	
Sex			0.048			0.088
Women	68 (5.8%)	1099 (94.2%)		1	–	
Men	112 (8.8%)	1165 (91.2%)		1.4	1.0–2.0	
Treatment with statins			0.09			0.149
No	140 (8.0%)	1616 (92.0%)		1	–	
Yes	40 (5.8%)	648 (94.2%)		0.7	0.5–1.05	
ASA score			0.12			0.18
I–II	101 (6.8%)	1390 (93.2%)		1	–	
III–IV	79 (8.3%)	874 (91.7%)		1.33	0.9–1.9	
Preoperative chemotherapy			0.13			0.187
No	170 (7.1%)	2224 (92.7%)		1	–	
Yes	10 (20.0%)	40 (80.0%)		1.9	0.8–4.4	
Type of surgery			0.17			0.224
Elective	149 (6.8%)	2044 (93.2%)		1	–	
Urgent	31 (12.4%)	220 (87.6%)		1.4	0.8–2.3	
Tobacco			0.2			0.224
No	102 (6.6%)	1445 (93.4%)		1	–	
Current smoker	26 (10.1%)	232 (89.9%)		1.4	0.8–2.2	
Ex-smoker	36 (7.6%)	438 (92.4%)		1	0.6–1.5	
Unknown	16 (9.7%)	149 (90.3%)		1.3	0.7–2.5	
Previous abdominal surgery			0.4			0.44
No	137 (7.4%)	1711 (92.6%)		1	–	
Appendicectomy	19 (6.0%)	298 (94.0%)		0.7	0.4–1.2	
Ileocecal resection	1 (50.0%)	1 (50.0%)		12.7	0.4–457.3	
Other	23 (8.3%)	254 (91.7%)		1.1	0.6–1.7	
Age (per year)	70 (63–77)	71 (64–79)	0.5	0.995	0.981–1.01	0.532

The variance between the participating hospitals was 0.82 on the logit scale. Descriptive data are expressed as number of patients (%) or median (25^{th}–75^{th} percentile).

operative decision-making did not appear to vary. An anastomosis was formed in 98.0% of patients, and only 0.3% received a defunctioning stoma. In high-risk patients, particularly selected emergency cases, different surgical strategies could be adopted, for example performing a double-barreled ileocolostomy or a diverting stoma to mitigate the consequences of an eventual AL.[17] Additional studies will be necessary to confirm the impact of these strategies on postoperative morbidity and mortality, along with the preoperative measures to optimize baseline function.

Right hemicolectomy is considered one of the most straightforward colorectal resections.[1] Despite that, this study showed that complications (38.0%), including AL (7.6%), are common after a right hemicolectomy, and mortality (2.6%) also needs to be discussed with patients. Our results are similar to the Spanish ANACO study, a recently published large (n = 1102) prospective series.[4] Their AL rate after oncologic right colectomy was 8.4%, and the 60-day morbidity and mortality were 29.0% and 2.6%.[4] A Dutch analysis of 15,667 patients undergoing anastomosis after colorectal cancer resection found an AL rate in the right hemicolectomy subgroup (n = 7788) of 6.4%.[8] However, the described AL rate after colon resection varies widely in the literature because of different methodologies of study and AL definition. Retrospective studies[2,3] usually report a lower AL rate, probably because of unreported ALs. Moreover, the AL definition used in the present study is the broadest because it includes both diagnosed AL and also abdominal collections.

In the present analysis, several surgical variables were associated with the greatest clinical risk of AL. Many studies have already highlighted the importance of the individual surgeon as a risk factor for AL and complications after colon resection.[18,19] In the present analysis, the open approach and the stapled technique were significantly associated with AL. Our findings are in contrast with the last Cochrane review,[20] which concluded that stapled ileocolic anastomosis was associated with fewer leaks than handsewn anastomosis. However, the conclusions of Choy et al[20] are based on only 4 randomized clinical trials (3 of them with the same author), the most recent being per-

TABLE 7. Predictors for postoperative complications at multivariate analysis

Variables	*Patients with postoperative complication (N = 956; 38.0%)*	*Patients without postoperative complication (N = 1559; 62.0%)*	*p (multivariate analysis)*	*OR*	*95% CI*	*Adjusted p*
Duration of surgery (per minute)	136.5 (109–180)	125 (101–165)	<0.001	1.004	1.002–1.006	0.0037
Type of surgery						0.0037
Elective	775 (35.0%)	1441 (65.0%)	<0.001	1	–	
Urgent	181 (60.5%)	118 (39.5%)		2.1	1.5–2.8	
Surgical approach						0.0037
Intention laparoscopic	453 (31.5%)	987 (68.5%)	<0.001	1	–	
Intention open	503 (46.8%)	572 (53.2%)		1.8	1.5–2.3	
ASA score						0.0037
I–II	1017 (67.3%)	494 (32.7%)	<0.001	1	–	
III–IV	542 (54.0%)	462 (46.0%)		1.5	1.2–1.9	
Sex						0.0037
Women	390 (32.4%)	815 (67.6%)	<0.001	1	–	
Men	566 (43.2%)	744 (56.8%)		1.5	1.2–1.8	
Age (per year)	73 (65-79)	70 (63-78)	0.005	1.012	1.003–1.021	0.014
Tobacco						0.020
No	546 (34.4%)	1041 (65.6%)	0.008	1	–	
Current smoker	120 (44.8%)	148 (55.2%)		1.5	1.1–2.0	
Ex-smoker	216 (44.4%)	270 (55.6%)		1.2	0.98–1.6	
Unknown	74 (42.5%)	100 (57.5%)		1.2	0.8–1.8	
Abnormal creatinine (>1.3 mg/dL)						0.078
No	810 (36.5%)	1412 (63.5%)	0.04	1	–	
Yes	146 (49.8%)	147 (50.2%)		1.3	1.01–1.8	
Intraoperative complications						0.18
No	855 (37.1%)	1451 (62.9%)	0.12	1	–	
Yes	101 (48.3%)	108 (51.7%)		1.3	0.9–1.8	
Anastomotic technique						0.206
No anastomosis	43 (60.6%)	28 (39.4%)	0.15	1	–	
Handsewn	329 (35.0%)	611 (65.0%)		0.66	0.4–1.2	
Stapled	584 (38.8%)	920 (61.2%)		0.93	0.5–1.6	
Type of resection						0.7
Limited	98 (39.7%)	149 (60.3%)	0.7	1	–	
Complete	307 (37.8%)	506 (62.2%)		1.05	0.7–1.4	
Extended	551 (37.9%)	904 (62.1%)		0.9	0.7–1.3	
Preoperative chemotherapy						0.244
No	932 (37.8%)	1532 (62.2%)	0.2	1	–	
Yes	24 (47.1%)	27 (52.9%)		1.5	0.8–2.8	
Treatment with statins						0.44
No	657 (36.3%)	1155 (63.7%)	0.4	1	–	
Yes	299 (42.5%)	404 (57.5%)		1.1	0.9–1.3	
Previous abdominal surgery						0.44
No	711 (37.3%)	1197 (62.7%)	0.4	1	–	
Appendicectomy	120 (37.3%)	202 (62.7%)		1.1	0.9–1.5	
Ileocecal resection	1 (50.0%)	1 (50.0%)		1.9	0.1–54.3	
Other	124 (43.8%)	159 (56.2%)		1.2	0.9–1.7	

The variance between the participating hospitals was 0.54 on the logit scale. Descriptive data are expressed as number of patients (%) or median (25^{th}–75^{th} percentile).

formed in 1995. On the other hand, all of the most recent large observational studies[2–4] have identified the stapled technique as an independent risk factor for ileocolic anastomosis leak. This emphasizes the need to perform a large, appropriately stratified, randomized trial to determine the best anastomotic technique after a colonic resection. We anticipate that early primary outcome measure would be AL along with other postoperative complications.

It has been repeatedly shown that a laparoscopic approach decreases morbidity and mortality after colorectal resection.[21–24] Similarly, in this study a laparoscopic approach is associated not only with decreased postoperative morbidity and mortality but also with a lower AL rate compared with an open approach. Laparoscopic colon resection has been related to a decreased postoperative stress and proinflammatory response in both oncologic and nononcologic patients.[25–27] Moreover, the reduction of postoperative complications contributes to an accelerated functional recovery in frail patients.[24,28] Despite these well-known advantages, in the present study (similar to other previous series[24]) the laparoscopic rate was as low as 50%. For all of these reasons a special effort in educa-

TABLE 8. Predictors for postoperative mortality at multivariate analysis

Variables	*Patients with postoperative mortality (N = 66; 2.6%)*	*Patients without postoperative mortality (N = 2449; 97.4%)*	*p (multivariate analysis)*	*OR*	*95% CI*	*Adjusted p*
Age (per year)	78.5 (71–85)	71 (64–78)	< 0.001	1.054	1.025–1.085	0.0037
Type of surgery						0.0037
Elective	37 (1.7%)	2179 (98.3%)	0.001	1	–	
Urgent	29 (9.7%)	270 (90.3%)		2.7	1.5–4.9	
Surgical approach						0.013
Intention laparoscopic	14 (1.0%)	1426 (99.0%)	0.004	1	–	
Intention open	52 (4.8%)	1023 (95.2%)		2.6	1.4–5.2	
ASA score						0.014
I–II	18 (1.2%)	1493 (98.8%)	0.005	1	–	
III–IV	48 (4.8%)	956 (95.2%)		2.4	1.3–4.4	
Anastomotic technique						0.024
No anastomosis	11 (15.5%)	60 (84.5%)	0.01	1	–	
Handsewn	22 (2.3%)	918 (97.7%)		0.3	0.14–0.80	
Stapled	33 (2.2%)	1471 (97.8%)		0.4	0.19–1.04	
Intraoperative complications						0.041
No	54 (2.3%)	2252 (97.7%)	0.02	1	–	
Yes	12 (5.7%)	197 (94.3%)		2.2	1.1–4.3	
Treatment with statins						0.139
No	52 (2.9%)	1760 (97.1%)	0.08	1	–	
Yes	14 (2.0%)	689 (98.0%)		1.2	0.6–2.2	
Abnormal creatinine (>1.3 mg/dL)						0.619
No	50 (2.3%)	2172 (97.7%)	0.6	1	–	
Yes	16 (5.5%)	277 (94.5%)		1.2	0.6–2.2	

The variance between the participating hospitals was 0 on the logit scale. Descriptive data are expressed as number of patients (%) or median (25^{th}–75^{th} percentile).

tion and diffusion of laparoscopic techniques should be undertaken with the aim of increasing the proportion of patients with colon cancer being offered a laparoscopic approach.

To the authors' knowledge, this is the first prospective, international, large study analysis identifying risk factors for AL after right colon resection for cancer. More than 1000 local researchers from 280 different centers simultaneously collected data adhering to a predefined protocol, bolstering the quality and homogeneity of collated data. This dramatically increases the generalizability of the findings, providing a valuable snapshot of the current management and outcomes for colon cancer in patients undergoing a right hemicolectomy. As a limitation of this study, because of its observational nature, selection bias needs to be carefully considered. For this reason, a multivariable logistic regression model applying advanced LASSO methodology was performed. This is a recognized approach for addressing and accounting for possible confounding factors. However, because no causal analysis was performed and not all confounding variables were included in this model, we believe this should only serve as a predictor, hypothesis-generating study, and additional randomized clinic trials are needed. Moreover, because of the reduced number of variables selected for the case report form, data on intraoperative bleeding, preoperative nutritional status, or blood transfusion were not collected.

CONCLUSION

Our data can be used to personalize surgical decision-making and to increase the surgeon's ability to undertake an informed discussion with the patient about the risks of the surgery. Surgical variables seem to be clearly associated with AL. Future studies to determine the best anastomotic technique are still required. In the meantime, continuous education related to decision-making and practical hands-on courses remains necessary.

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European Society of Coloproctology Collaborating Group Collaborators. See full list of names in Appendix.

REFERENCES

1. Jamali FR, Soweid AM, Dimassi H, Bailey C, Leroy J, Marescaux J. Evaluating the degree of difficulty of laparoscopic colorectal surgery. *Arch Surg.* 2008;143:762–768.
2. Jessen M, Nerstrøm M, Wilbek TE, Roepstorff S, Rasmussen MS, Krarup PM. Risk factors for clinical anastomotic leakage after right hemicolectomy. *Int J Colorectal Dis.* 2016;31:1619–1624.
3. Gustafsson P, Jestin P, Gunnarsson U, Lindforss U. Higher frequency of anastomotic leakage with stapled compared to handsewn ileocolic anastomosis in a large population-based study. *World J Surg.* 2015;39:1834–1839.
4. Frasson M, Granero-Castro P, Ramos Rodríguez JL, et al.; ANACO Study Group. Risk factors for anastomotic leak and post-

operative morbidity and mortality after elective right colectomy for cancer: results from a prospective, multicentric study of 1102 patients. *Int J Colorectal Dis.* 2016;31:105–114.
5. Veyrie N, Ata T, Muscari F, et al.; French Associations for Surgical Research. Anastomotic leakage after elective right versus left colectomy for cancer: prevalence and independent risk factors. *J Am Coll Surg.* 2007;205:785–793.
6. Schrock TR, Deveney CW, Dunphy JE. Factor contributing to leakage of colonic anastomoses. *Ann Surg.* 1973;177:513–518.
7. Braga M, Frasson M, Vignali A, Zuliani W, Di Carlo V. Open right colectomy is still effective compared to laparoscopy: results of a randomized trial. *Ann Surg.* 2007;246:1010–1014.
8. Bakker IS, Grossmann I, Henneman D, Havenga K, Wiggers T. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. *Br J Surg.* 2014;101:424–432.
9. Masoomi H, Buchberg B, Dang P, Carmichael JC, Mills S, Stamos MJ. Outcomes of right vs. left colectomy for colon cancer. *J Gastrointest Surg.* 2011;15:2023–2028.
10. Baker RP, Titu LV, Hartley JE, Lee PW, Monson JR. A case-control study of laparoscopic right hemicolectomy vs. open right hemicolectomy. *Dis Colon Rectum.* 2004;47:1675–1679.
11. Sancho-Muriel J, Frasson M, Hervás D, et al.; ANACO Study Group. Standard outcome indicators after colon cancer resection: creation of a nomogram for autoevaluation. *Cir Esp.* 2017;95:30–37.
12. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. *Ann Surg.* 2011;253:890–899.
13. Salvans S, Mayol X, Alonso S, et al. Postoperative peritoneal infection enhances migration and invasion capacities of tumor cells *in vitro*: an insight into the association between anastomotic leak and recurrence after surgery for colorectal cancer. *Ann Surg.* 2014;260:939–943.
14. 2015 European Society of Coloproctology Collaborating Group. The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit. *Colorectal Dis.* In press.
15. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol.* 2008;61:344–349.
16. Tibshirani R. Regression shrinkage and selection via the lasso. *J R Stat Soc B.* 1996;58:267–288.
17. Hüser N, Michalski CW, Erkan M, et al. Systematic review and meta-analysis of the role of defunctioning stoma in low rectal cancer surgery. *Ann Surg.* 2008;248:52–60.
18. Marinello FG, Baguena G, Lucas E, et al. Anastomotic leakage after colon cancer resection: does the individual surgeon matter? *Colorectal Dis.* 2016;18:562–569.
19. Pera M. The surgeon as a risk factor: the need for shared individual outcome reports and quality improvement strategies. *Colorectal Dis.* 2016;18:533–534.
20. Choy PY, Bissett IP, Docherty JG, Parry BR, Merrie A, Fitzgerald A. Stapled versus handsewn methods for ileocolic anastomoses. *Cochrane Database Syst Rev.* 2011;(9):CD004320.
21. Lacy AM, García-Valdecasas JC, Delgado S, et al. Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. *Lancet.* 2002;359:2224–2229.
22. Nelson H, Sargent DJ, Wieand HS, et al; Clinical Outcomes of Surgical Therapy Study Group. A comparison of laparoscopically assisted and open colectomy for colon cancer. *N Engl J Med.* 2004;350:2050–2059.
23. Veldkamp R, Kuhry E, Hop WC, et al.; COlon cancer Laparoscopic or Open Resection Study Group (COLOR). Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. *Lancet Oncol.* 2005;6:477–484.
24. Cerdán Santacruz C, Frasson M, Flor-Lorente B, et al.; ANACO Study Group. Laparoscopy may decrease morbidity and length of stay after elective colon cancer resection, especially in frail patients: results from an observational real-life study. *Surg Endosc.* 2017;31:5032–5042.
25. Nishiguchi K, Okuda J, Toyoda M, Tanaka K, Tanigawa N. Comparative evaluation of surgical stress of laparoscopic and open surgeries for colorectal carcinoma. *Dis Colon Rectum.* 2001;44:223–230.
26. Hildebrandt U, Kessler K, Plusczyk T, Pistorius G, Vollmar B, Menger MD. Comparison of surgical stress between laparoscopic and open colonic resections. *Surg Endosc.* 2003;17:242–246.
27. Janež J, Korać T, Kodre AR, Jelenc F, Ihan A. Laparoscopically assisted colorectal surgery provides better short-term clinical and inflammatory outcomes compared to open colorectal surgery. *Arch Med Sci.* 2015;11:1217–1226.
28. Tahiri M, Sikder T, Maimon G, et al. The impact of postoperative complications on the recovery of elderly surgical patients. *Surg Endosc.* 2016;30:1762–1770.

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APPENDIX

European Society of Coloproctology Collaborating Group Collaborators

Paper Writing Group: Matteo Frasson, Ph.D., E.B.S.Q.-c., M.D. (Coordinator), Nick J. Battersby, M.D., Aneel Bhangu, M.B.Ch.B., Ph.D. David Hervás, M.Sc., Alaa El-Hussuna, M.D., Ph.D., Gaetano Gallo, M.D., Francesco Pata, M.D., Thomas D. Pinkney, M.B.Ch.B., M.Med.Ed., M.D., Tomas Poskus, M.D., Ph.D., Baljit Singh, B.A., B.M., B.Ch., M.A., D.Phil.

***ESCP Cohort Studies Sub-Committee*:** T. Pinkney (Chair and Overall Guarantor), N. Battersby, A. Bhangu, S. Chaudhri, A. El-Hussuna, M. Frasson, D. Nepogodiev, B. Singh, S. Vennix, O. Zmora.

***ESCP Research Committee*:** W. Bemelman, P. Christensen, A. D'Hoore, S. Laurberg, D. Morton (Chair), T. Pinkney, M. Rubbini, C. Vaizey.

***Logistical Support and Data Collection*:** L. Magill, R. Perry, N. Sheward (Birmingham Surgical Trials Consortium, University of Birmingham)

APPENDIX 8

- The 2015 European Society of Coloproctology (ESCP) Collaborating Group (including **Nuno Rama** and al. from Portugal).

"The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit."

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Co-author (integrating ESCP collaborative group); Part 1 – Chapter 1.

Original article

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Relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit

The 2015 European Society of Coloproctology collaborating group[1]

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Abstract

Aim The anastomosis technique used following right-sided colonic resection is widely variable and may affect patient outcome. This study aimed to assess the association between leak and anastomosis technique (stapled *vs* handsewn).

Method This was a prospective, multicentre, international audit including patients undergoing elective or emergency right hemicolectomy or ileo-caecal resection operations over a 2-month period in early 2015. The primary outcome measure was the presence of anastomotic leak within 30 days of surgery, determined using a prespecified definition. Mixed effects logistic regression models were used to assess the association between leak and anastomosis method, adjusting for patient, disease and operative cofactors, with centre included as a random-effect variable.

Results This study included 3208 patients, of whom 78.4% (n = 2515) underwent surgery for malignancy and 11.7% (n = 375) underwent surgery for Crohn's disease. An anastomosis was performed in 94.8% (n = 3041) of patients, which was handsewn in 38.9% (n = 1183) and stapled in 61.1% (n = 1858). Patients undergoing handsewn anastomosis were more likely to be emergency admissions (20.5% handsewn *vs* 12.9% stapled) and to undergo open surgery (54.7% handsewn *vs* 36.6% stapled). The overall anastomotic leak rate was 8.1% (245/3041), which was similar following handsewn (7.4%) and stapled (8.5%) techniques (P = 0.3). After adjustment for cofactors, the odds of a leak were higher for stapled anastomosis (adjusted OR = 1.43; 95% CI: 1.04–1.95; P = 0.03).

Conclusion Despite being used in lower-risk patients, stapled anastomosis was associated with an increased anastomotic leak rate in this observational study. Further research is needed to define patient groups in whom a stapled anastomosis is safe.

Keywords Anastomotic leak, colorectal cancer, Crohn's disease, epidemiology, international

What does this paper add to the literature?
This study combined prospectively collected data from 284 centres across 39 countries. It explores differences in patients, techniques.

Introduction

Morbidity following colorectal resection is common. Up to 65.3% of patients experience a complication in the first 30 days after surgery, which is major in 17.1% (Clavien–Dindo Grade III–V) [1]. These complications impact upon both morbidity and mortality rates, and increase health-care costs [2–4]. Anastomotic leak is considered as one of the most devastating of these adverse events; it is associated with a reduction in both survival and quality of life and with an increased risk of disease recurrence in those patients with cancer [2].

Many factors are known to be associated with anastomotic leak, including patient comorbidity, underlying pathology and anastomotic technique. There is a wide variation in the use of handsewn anastomosis *vs* stapled anastomosis, illustrating the lack of high-quality evidence supporting either method [5]. More evidence is required to guide surgical practice. Right hemicolectomy (including ileo-caecal resection) is the most common colonic resection and is performed in both elective and emergency settings and for both neoplastic and non-neoplastic conditions. It therefore represents an

Correspondence to: Thomas Pinkney, Senior Lecturer in Colorectal Surgery, Academic Department of Surgery, Room 29, 4th Floor, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK.
E-mail: thomas.pinkney@uhb.nhs.uk
@pinkney_t

[1]Collaborating members are listed in Appendix 1

appropriate patient cohort in which to assess the relationship between method of anastomosis and outcome.

Multicentre snapshot audits have the ability to gather large patient numbers in short periods of time from many hospitals. They provide contemporaneous and population-based data that are representative of current practice and unconstrained by the confines often required in clinical trials. This first report from an international prospective cross-sectional cohort study of right hemicolectomy and ileo-caecal resections investigates the relationship between anastomosis method and subsequent anastomotic leak.

Method

This prospective, observational, multicentre study was performed according to a prespecified protocol (http://www.escp.eu.com/research/cohort-studies/2015-audit). The protocol and data-entry system were tested and modified following an external pilot conducted in eight centres across five countries before the start of the main project. Follow-up and data collected were restricted to routinely collected data fields.

Centres

Any unit performing gastrointestinal surgery was eligible to register and enter patients into the study. No unit size or case volume stipulations were made, and centres from any country were able to take part. The study was launched at the European Society of Coloproctology (ESCP) Scientific & Annual Meeting in Barcelona, September 2014, and invitations to participate were subsequently distributed directly to all registered members of the ESCP. Further dissemination was obtained via the national ESCP country representatives, including through national surgical or colorectal societies. In addition, the study was endorsed and disseminated by the surgical arm of the European Crohn's and Colitis Organisation.

Approvals

Participating centres were responsible for completion of local approvals before the start of the data-collection period. Regional or national ethics approval or indemnity was obtained where possible. Centres were asked to ensure that appropriate pathways and local investigators were in place to be able to include all consecutive eligible patients during the study period and provide > 95% completeness of data entry.

Patients

Adult patients undergoing right hemicolectomy or ileo-caecal resection for any pathological indication, via any operative approach in both elective and emergency settings, were included. Patients were excluded if their right-sided colonic resection was part of a larger procedure (e.g. subtotal colectomy or panproctocolectomy), as defined by a distal colonic transection point beyond the splenic flexure. In patients with Crohn's disease, those undergoing additional proximal strictureoplasty or resection/anastomosis of more proximal small bowel disease during the same operation were also excluded.

Outcome measures

The primary outcome for this study was overall anastomotic leak, predefined as either (i) gross anastomotic leakage proven radiologically or clinically and classified according to intervention necessary (Fig. 1); or (ii) the presence of an intraperitoneal (abdominal or pelvic) fluid collection on postoperative imaging. Secondary outcome measures included mortality, overall morbidity and length of hospital stay. An exploratory sensitivity analysis was also undertaken of those with only a 'proven' anastomotic leak (i.e. excluding those with an intraperitoneal fluid collection alone) for comparison.

Data collection

Sites were asked to include all consecutive eligible patients over an 8-week period, which could start at any time between 15 January 2015 and 30 January 2015. This flexible starting date was designed to maximise centre participation. The final date for any new patient inclusions at any site was 27 March 2015.

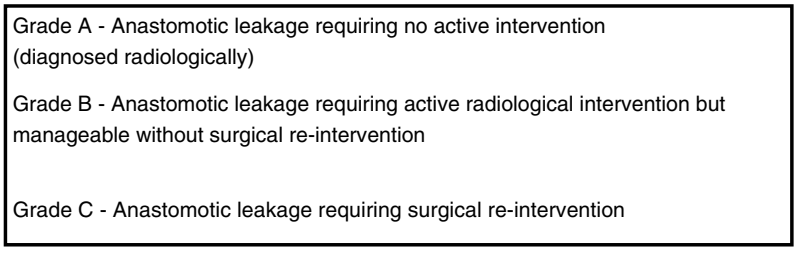

Figure 1 Classification of anastomotic leak. NB The highest score given during follow up (e.g. Grade C if percutaneous drainage is followed by laparotomy).

There were three main phases of data collection for each patient:

1 Preoperative: patient (e.g. age, gender, comorbidities) and disease demographics (e.g. indication, previous treatment).
2 Operative: technical details about the operation performed (e.g. handsewn or stapled anastomosis; laparoscopic or open approach; elective or emergency).
3 Follow-up: individual outcomes data (anastomotic leak, length of hospital stay, mortality); completed at 30 days postoperation.

Each of these phases had a separate clinical reporting form (CRF) that contained 10–12 main questions and was designed to fit in with data collected as part of normal clinical practice and be completed in 'real-time' with minimal extra work from the clinical team. Despite no changes being made to existing patients' pathways during this observational study, local investigators were asked to be proactive in identifying postoperative events. Methods included review of patient notes (paper and electronic) during admission and before discharge, reviewing hospital systems to check for re-attendances or re-admissions, and reviewing postoperative radiology reports. Some centres routinely reviewed patients 30 days after surgery or used a telephone review, both of which were used to identify adverse events. Data were recorded contemporaneously and stored on a dedicated, secure, Web-based platform without using patient identifiable information. Data were collected by a team of four or five people at each site, one of whom had to be a consultant surgeon who was responsible for the data quality at that centre.

Statistical analysis

This report has been prepared in accordance with guidelines set by the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for observational studies [6].

The primary aim of this study was to assess the association between the primary outcome measure (overall anastomotic leak) and the main explanatory variable of interest, anastomosis method (handsewn *vs* stapled). Univariate and multivariate mixed-effects logistic regression models (with centre included as a random effect) were fitted for overall anastomotic leak and the prespecified explanatory variables: anastomosis method (handsewn or stapled); age; gender (male or female); body mass index (normal, underweight, overweight or obese); smoking status (never, ex-smoker, current or not known); history of ischaemic heart disease or cerebrovascular disease (no or yes); history of diabetes (none, diet/tablet controlled or insulin controlled); indication for operation (malignancy, Crohn's disease or other); American Society of Anesthesiology (ASA) grade (low risk or high risk); surgery type (elective or emergency); operation type (laparoscopic or open) and extent of surgery (complete, extended or limited; Fig. 2). These factors were chosen based on clinical significance and were all prespecified in the statistical analysis plan. All the explanatory variables were included in the multivariate model, irrespective of statistical significance in the univariate model, as this allowed potential confounding factors relating to the patient, disease and operation to be taken into consideration in the multivariate model.

Effect estimates are presented as OR with 95% CI and two-sided *P*-values. An OR> 1 indicated increased likelihood of anastomotic leak with the relevant explanatory variable compared with the reference category for that variable. Statistical significance was defined at the level of $P < 0.05$. Data analysis was undertaken using STATA version 14 (StataCorp, College Station, Texas, USA).

Sensitivity analyses were undertaken, which included: (i) fitting a multivariate model that included anastomosis method and only those explanatory variables where $P \leq 0.1$ in the univariate analysis; (ii) fitting a multivariate model that included only those explanatory variables where $P \leq 0.1$ in the univariate analysis; and (iii) fitting a multivariate model as per the primary analysis, but only including those patients with a 'proven' anastomotic leak in the outcome variable.

Results

Data completeness

Overall, 97.4% of records had all data fields completed. Patient demographic details, basic operation details and 30-day outcome data were mandatory fields for records to be locked and as such had a 100% completion rate. The small levels of missing data predominantly related to patient smoking status and preoperative medical therapy (in the case of patients with Crohn's disease) subsections.

Patients and centres

This study included 3208 patients from 284 centres in 39 countries (Fig. 3). There were five participating centres outside Europe. The mean age of patients was 66 (range: 16–99) years, 50.8% were male and the majority were never-smokers (62%), did not have a history of ischaemic heart disease or cerebrovascular disease (80.5%) and were not diabetic (84.4%) (Table 1). Most patients underwent surgery for malignancy (78.4%;

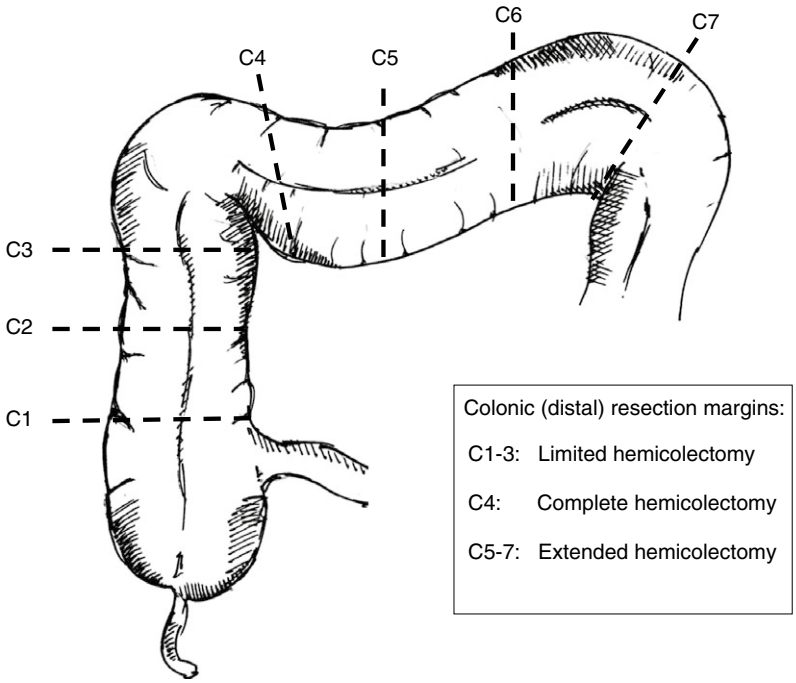

Figure 2 Extent of resection. The distal resection (colonic) margins are as allocated on the postoperative clinical reporting form (CRF).

$n = 2515$) or Crohn's disease (11.7%; $n = 375$). Overall, 81.3% ($n = 2609$) of patients underwent elective surgery, and 54.6% ($n = 1751$) of operations were started laparoscopically; 9.6% undergoing subsequent conversion to open. Further demographic details are shown in Table 1.

Anastomosis technique

An anastomosis was performed in 94.8% ($n = 3041$) of patients, which was handsewn in 38.9% ($n = 1183$) and stapled in 61.1% ($n = 1858$) (Table 1). There was no difference in stapled anastomosis rates in those undergoing surgery for malignancy (59.8%) and for Crohn's disease (58.7%). Patients undergoing handsewn anastomosis were more likely to be emergency admissions (20.5% *vs* 12.9% stapled) and to undergo open surgery (54.7% *vs* 36.6%).

Incidence of anastomotic leak

The primary outcome measure of anastomotic leak and/or intraperitoneal fluid collection was present in 8.1% (245/3041) of patients (Table 2).

Univariate analysis of anastomotic leak

The mixed effects logistic regression analysis included 3013 patients and 242 leaks [there were 28 (0.9%) patients with missing data on extent of surgery who were excluded from this analysis]. There was no evidence of an association between leak and anastomosis method (stapled *vs* handsewn: OR = 1.16, 95% CI: 0.86–1.57, $P = 0.3$) (Table 3). Female gender was significantly associated with a reduced risk of leak (OR = 0.70, 95% CI: 0.53–0.92, $P = 0.011$), whilst being a current smoker (*vs* never-smoker: OR = 1.68, 95% CI: 1.15–2.43, $P = 0.007$), other indication for surgery (*vs* malignant: OR = 2.39, 95% CI: 1.62–3.54, $P < 0.001$), emergency surgery (*vs* elective: OR = 2.33, 95% CI: 1.70–3.19, $P < 0.001$) and open incision (*vs* laparoscopic: OR = 2.32, 95% CI: 1.74–3.08, $P < 0.001$) were all associated with an increased risk of leak (Table 3). Weaker associations were found with age (OR = 0.99, 95% CI: 0.98–1.00, $P = 0.06$) and high ASA grade (*vs* low grade: OR = 1.30, 95% CI: 0.98–1.72, $P = 0.07$).

Multivariate analysis of anastomotic leak

When a multivariate mixed effects logistic regression model was fitted including all the prespecified variables, a significant association was found between leak and stapled anastomosis (*vs* handsewn: OR = 1.43, 95% CI: 1.04–1.95, $P = 0.03$). Other variables found to be significant under multivariate analysis were age (OR = 0.99, 95% CI: 0.98–1.00, $P = 0.04$), other indication for surgery (*vs* malignant: OR = 1.73, 95% CI: 1.05–2.85, $P = 0.03$) and open incision (*vs* laparoscopic OR = 2.09, 95% CI: 1.53–2.87, $P < 0.001$). Similar

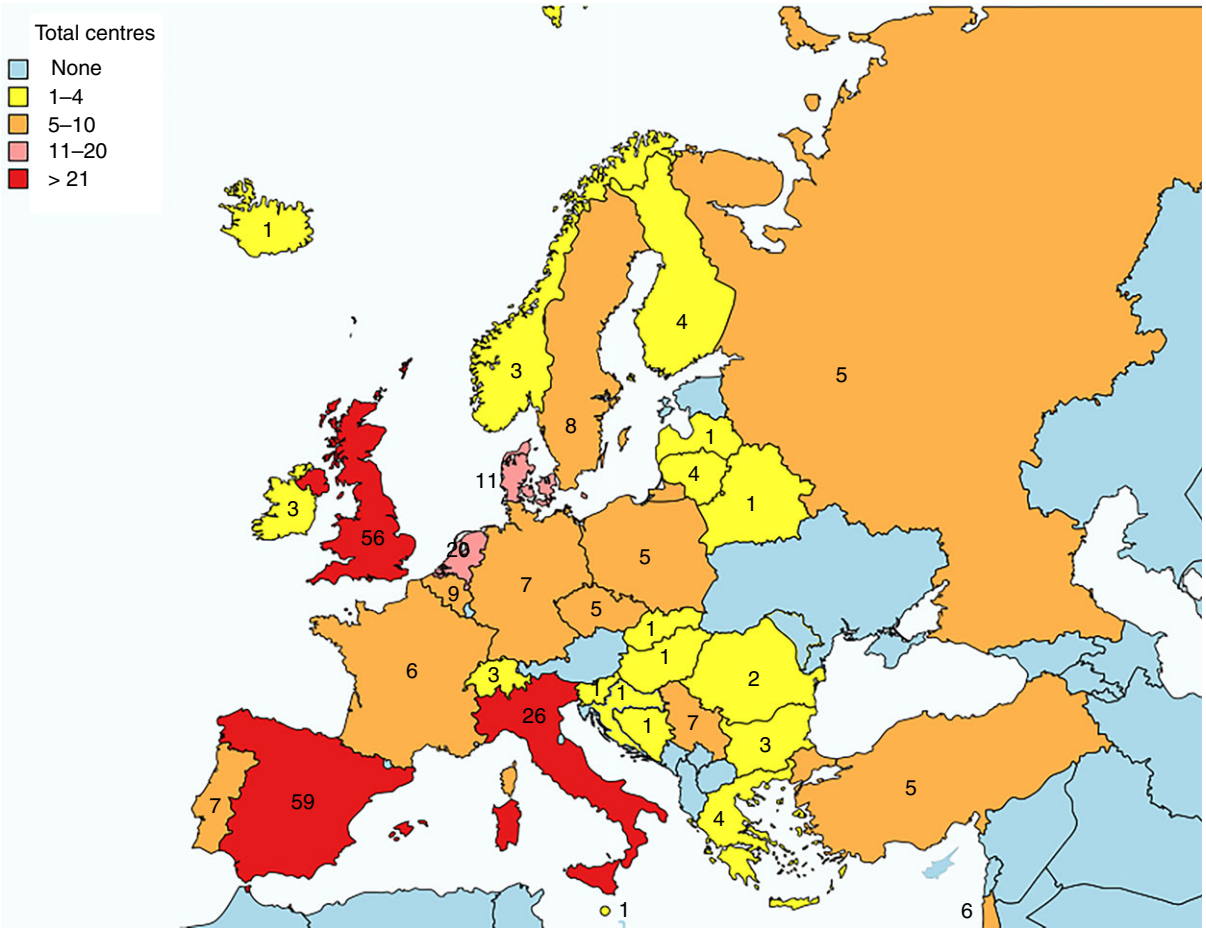

Figure 3 Total number of sites including patients in the audit, according to European country.

results were seen when the multivariate models were restricted only to those variables where $P \leq 0.1$ in the univariate analysis, with anastomosis method included and excluded as a cofactor. Another sensitivity analysis including only those patients with a 'proven' anastomotic leak (150/3041; 4.9%) also gave similar results (Tables S1 and S2).

Secondary outcomes

The overall 30-day death rate was 3.2% (103/3208) (Table 4); for those undergoing elective operations this reduced to 1.5% (38/2609). The median length of hospital stay was 7 (range: 1–30+) days, and the 30-day re-operation and re-admission rates were 6.6% and 5.7%, respectively. In those patients undergoing anastomosis who had an anastomotic leak and/or intraperitoneal fluid collection, the 30-day death rate increased to 9.8%, and the length of hospital stay was more than doubled to a median of 18 days (Table 4). When assessing only those patients with a 'proven' anastomotic leak, similar outcomes were seen: 30-day death rate, 11.3%; and length of hospital stay, median 21 days (Table 4).

Discussion

This multicentre international snapshot audit has identified a possible association between stapled anastomosis and anastomotic leak. This became apparent following multivariate analysis that adjusted for other patient and disease characteristics, and operative information (with centre included as a random effect). This finding was perhaps surprising given that stapling was used more frequently in the lower-risk groups, such as in elective and laparoscopic operations.

Multivariate analysis also found an association between operative approach and leak, with a greater risk of leak with open operations. This increased risk associated with open surgery was readily identifiable in both the emergency and elective settings and might be interpreted as

Table 1 Patient, disease and operative characteristics according to anastomosis type.

Variable	Handsewn (n = 1183)	Stapled (n = 1858)	No anastomosis (n = 167)	Total (n = 3208)
Patients' characteristics				
Age				
Mean ± SD	66.4 ± 16	66.1 ± 15.8	63.4 ± 18.6	66.0 ± 16.1
Median (IQR)	70 (59–78)	69 (59–77)	68 (54–77)	69 (59–77)
Min–Max	16–97	16–99	20–94	16–99
Gender				
Male	605 (51.1)	935 (50.3)	89 (53.3)	1629 (50.8)
Female	578 (48.9)	923 (49.7)	78 (46.7)	1579 (49.2)
Body mass index				
Normal	439 (37.1)	671 (36.1)	71 (42.5)	1181 (36.8)
Underweight	39 (3.3)	60 (3.2)	8 (4.8)	107 (3.3)
Overweight	384 (32.5)	631 (34)	39 (23.4)	1054 (32.9)
Obese	321 (27.1)	496 (26.7)	49 (29.3)	866 (27.0)
Smoking status				
Never	754 (63.7)	1141 (61.4)	94 (56.3)	1989 (62.0)
Ex-smoker	204 (17.2)	354 (19.1)	28 (16.8)	586 (18.3)
Current	160 (13.5)	224 (12.1)	24 (14.4)	408 (12.7)
Not known	65 (5.5)	139 (7.5)	21 (12.6)	225 (7.0)
History of ischaemic heart disease or cerebrovascular disease*				
No	918 (77.6)	1532 (82.5)	134 (80.2)	2584 (80.5)
Yes	265 (22.4)	326 (17.5)	33 (19.8)	624 (19.5)
History of diabetes				
None	1000 (84.5)	1564 (84.2)	142 (85)	2706 (84.4)
Diet/tablet controlled	141 (11.9)	239 (12.9)	18 (10.8)	398 (12.4)
Insulin controlled	42 (3.6)	55 (3)	7 (4.2)	104 (3.2)
Disease characteristics				
Indication				
Malignant	939 (79.4)	1503 (80.9)	73 (43.7)	2515 (78.4)
Crohn's disease	123 (10.4)	220 (11.8)	32 (19.2)	375 (11.7)
Other†	121 (10.2)	135 (7.3)	62 (37.1)	318 (9.9)
ASA grade				
Low risk	697 (58.9)	1250 (67.3)	60 (35.9)	2007 (62.6)
High risk	486 (41.1)	608 (32.7)	107 (64.1)	1201 (37.4)
Operative information				
Surgery type				
Elective	941 (79.5)	1618 (87.1)	50 (29.9)	2609 (81.3)
Emergency	242 (20.5)	240 (12.9)	117 (70.1)	599 (18.7)
Operation type				
Laparoscopic	536 (45.3)	1178 (63.4)	37 (22.2)	1751 (54.6)
Open	647 (54.7)	680 (36.6)	130 (77.8)	1457 (45.4)
Extent of surgery				
Complete (C4)	345 (29.2)	543 (29.2)	38 (22.8)	926 (28.9)
Extended (C5–7)	596 (50.4)	912 (49.1)	61 (36.5)	1569 (48.9)
Limited (C1–3)	232 (19.6)	385 (20.7)	66 (39.5)	683 (21.3)
Missing	10 (0.8)	18 (1.0)	2 (1.2)	30 (0.9)

ASA, American Society of Anesthesiology; IQR, interquartile range.
Values are given as n (%), except for age. Percentages are shown by column.
*Stroke or transient ischaemic attack (TIA).
†Includes appendix-related resections, ischaemia, volvulus, trauma and miscellaneous.

Table 2 Patient, disease and operative characteristics according to overall anastomotic leak* in patients for whom an anastomosis was performed.

Variable	Overall anastomotic leak		
	No (n = 2796)	Yes (n = 245)	Total (n = 3041†)
Patient characteristics			
Age			
Mean ± SD	66.4 ± 15.9	64.1 ± 16	66.2 ± 15.9
Medium (IQR)	69 (59–78)	67 (57–75)	69 (59–77)
Min–Max	16–99	18–96	16–99
Gender			
Male	1396 (90.6)	144 (9.4)	1540 (50.6)
Female	1400 (93.3)	101 (6.7)	1501 (49.4)
Body mass index			
Normal	1023 (92.2)	87 (7.8)	1110 (36.5)
Underweight	88 (88.9)	11 (11.1)	99 (3.2)
Overweight	942 (92.8)	73 (7.2)	1015 (33.4)
Obese	743 (90.9)	74 (9.1)	817 (26.9)
Smoking status			
Never	1759 (92.8)	136 (7.2)	1895 (62.3)
Ex-smoker	513 (91.9)	45 (8.1)	558 (18.4)
Current	340 (88.5)	44 (11.5)	384 (12.6)
Not known	184 (90.2)	20 (9.8)	204 (6.7)
History of ischaemic heart disease or cerebrovascular disease‡			
No	2255 (92.0)	195 (8.0)	2450 (80.6)
Yes	541 (91.5)	50 (8.5)	591 (19.4)
History of diabetes			
None	2363 (92.2)	201 (7.8)	2564 (84.3)
Diet/tablet controlled	344 (90.5)	36 (9.5)	380 (12.5)
Insulin controlled	89 (91.8)	8 (8.2)	97 (3.2)
Disease characteristics			
Indication			
Malignant	2267 (92.8)	175 (7.2)	2442 (80.3)
Crohn's disease	312 (91.0)	31 (9.0)	343 (11.3)
Other	217 (84.8)	39 (15.2)	256 (8.4)
ASA grade			
Low risk	1802 (92.6)	145 (7.4)	1947 (64.0)
High risk	994 (90.9)	100 (9.1)	1094 (36.0)
Operative information			
Anastomosis method			
Handsewn	1096 (92.6)	87 (7.4)	1183 (38.9)
Stapled	1700 (91.5)	158 (8.5)	1858 (61.1)
Surgery type			
Elective	2383 (93.1)	176 (6.9)	2559 (84.1)
Emergency	413 (85.7)	69 (14.3)	482 (15.9)
Operation type			
Laparoscopic	1621 (94.6)	93 (5.4)	1714 (56.4)
Open	1175 (88.5)	152 (11.5)	1327 (43.6)
Extent of surgery			
Complete (C4)	819 (92.2)	69 (7.8)	888 (29.2)
Extended (C5–C7)	1383 (91.7)	125 (8.3)	1508 (49.6)
Limited (C1–C3)	569 (92.2)	48 (7.8)	617 (20.3)
Missing	25 (89.3)	3 (10.7)	28 (0.9)

Values are given as n (%), except for age, and are summed across rows.

*Includes those with clinically or radiologically proven leak or intraperitoneal (abdominal or pelvic) fluid collection on postoperative imaging.

†Excludes patients who are classed as anastomosis category 'none'.

‡Stroke or transient ischaemic attack (TIA).

ASA, American Society of Anesthesiology; IQR, interquartile range.

Table 3 Univariate and multivariate mixed-effects logistic regression analysis.

	Univariate analysis*				Multivariate analysis			
Outcome (anastomotic leak + abscess)	OR	95% CI	*P*	Overall *P*	OR	95% CI	*P*	Overall *P*
Anastomosis method								
Handsewn	–	–	–		–	–	–	
Stapled	1.16	(0.86–1.57)	0.342	0.342	1.43	(1.04–1.95)	0.026	0.026
Patient characteristics								
Age	0.99	(0.98–1.00)	0.064	0.064	0.99	(0.98–1.00)	0.037	0.037
Gender								
Male	–	–	–		–	–	–	
Female	0.70	(0.53–0.92)	0.011	0.011	0.76	(0.57–1.02)	0.066	0.066
Body mass index								
Normal	–	–	–		–	–	–	
Underweight	1.46	(0.73–2.91)	0.289		1.25	(0.61–2.56)	0.543	
Overweight	0.93	(0.66–1.30)	0.665		0.98	(0.69–1.38)	0.888	
Obese	1.23	(0.87–1.72)	0.241	0.315	1.14	(0.80–1.64)	0.463	0.768
Smoking status								
Never	–	–	–		–	–	–	
Ex–smoker	1.13	(0.79–1.63)	0.504		0.99	(0.67–1.46)	0.968	
Current smoker	1.68	(1.15–2.43)	0.007		1.38	(0.93–2.04)	0.106	
Not known	1.47	(0.86–2.49)	0.158	0.040	1.41	(0.81–2.44)	0.222	0.269
History of ischaemic heart disease or cerebrovascular disease								
No	–	–	–		–	–	–	
Yes	1.05	(0.75–1.47)	0.766	0.766	1.00	(0.69–1.47)	0.983	0.983
History of diabetes								
None	–	–	–		–	–	–	
Diet/tablet controlled	1.21	(0.82–1.78)	0.338		1.34	(0.89–2.02)	0.165	
Insulin controlled	1.10	(0.51–2.35)	0.811	0.624	1.16	(0.53–2.55)	0.717	0.375
Disease characteristics								
Indication								
Malignant	–	–	–		–	–	–	
Crohn's disease	1.27	(0.83–1.93)	0.270		1.29	(0.71–2.34)	0.398	
Other	2.39	(1.62–3.54)	< 0.001	< 0.001	1.73	(1.05–2.85)	0.031	0.095
ASA grade								
Low risk	–	–	–		–	–	–	
High risk	1.30	(0.98–1.72)	0.068	0.068	1.24	(0.89–1.72)	0.197	0.197
Operative information								
Surgery type								
Elective	–	–	–		–	–	–	
Emergency	2.33	(1.70–3.19)	< 0.001	< 0.001	1.40	(0.94–2.09)	0.101	0.101
Operation type								
Laparoscopy	–	–	–		–	–	–	
Open	2.32	(1.74–3.08)	< 0.001	< 0.001	2.09	(1.53–2.87)	< 0.001	< 0.001
Extent of surgery								
Complete (C4)	–	–	–		–	–	–	
Extended (C5–C7)	1.07	(0.77–1.48)	0.688		1.10	(0.79–1.53)	0.568	
Limited (C1–C3)	0.98	(0.66–1.47)	0.925	0.869	0.70	(0.44–1.11)	0.132	0.139

ASA, American Society of Anesthesiology.
*Univariate analysis included centre as a random effect to taken into account variation across centres.

suggesting that in modern surgical practice, the need for an operation to be undertaken using an open approach may be a surrogate marker of operative difficulty.

The association between anastomotic leakage and stapling only became apparent following multivariate analysis. There was a strong association between high-risk patients

Table 4 Impact of overall anastomotic leak (and the group with only a 'proven' leak) on clinical outcomes.

Group	*n*	30-day death rate *n* (%)	Length of stay (days) Median (IQR)
Full cohort	3208	103 (3.2)	7 (5–11)
No anastomosis made	167	30 (18.0)	11 (7–20)
In those undergoing anastomosis:	3041	73 (2.4)	7 (5–10)
No leak or collection evident	2796	49 (1.8)	7 (5–10)
Anastomotic leak and/or collection*	245	24 (9.8)	18 (10–27)
Proven anastomotic leak only	150	17 (11.3)	21 (13–30)

*Primary outcome of this study.
IQR, interquartile range.

and handsewn anastomosis, which may have influenced our results. It is impossible to assign causation to this association, but it is interesting to speculate on the possible explanations: the effects of operative approach (open *vs* laparoscopic), operation urgency (elective *vs* emergency) and anastomosis method (stapled *vs* handsewn) are all likely to have contributed to this effect. This situation, in which findings are nonsignificant in univariate analysis but significant in multivariate analysis, is well recognized in observational studies. Lo and colleagues identified various scenarios in which this situation may occur, one of which was indeed the presence of hidden interactions [7].

Strengths of this study

The prospective nature of data collection, using a standardized protocol and predesigned reporting system, ensured the quality and homogeneity of data returns. The wide variety of surgeons, sites and countries entering patients into this study increases the generalizability of the findings. Of the 39 countries involved, 34 were based in one continent (Europe), with other countries being spread across the world: Argentina, Brazil, China, Japan and USA. Bringing such a group together and coordinating over 1000 local researchers from 284 different centres to collect uniform data simultaneously and form a research network in this manner has been one of the most important successes of this first ESCP project. The number of sites involved, and patients entered, far exceeded our expectations when designing this project. Now the model has been shown to work, it is currently being used to undertake another prospective international audit [8] and the research network will also be perfectly poised to deliver future prospective interventional studies based on the areas of uncertainty identified in these audits.

Limitations

Selection bias will always be an issue in this type of observational research. We have attempted to minimize the effects of this by undertaking adjusted analyses using mixed effects logistic regression models, but we accept that this can never fully counteract the nuances involved in clinical decision-making. Nonetheless, one might have predicted that any major selection bias effect on the primary outcome would favour stapling being actually at a diminished risk, given the prevalence of its use within the lower-risk groups.

Reporting bias is also difficult to control for in this kind of study, where sites might have omitted uploading data for certain eligible patients within the study time period, either accidentally or deliberately, which could confer an impact on the results. We feel that this is unlikely given our study design, in which the first two phases of data collection were prospectively and contemporaneously uploaded onto the online system in the preoperative and immediate postoperative setting. This effectively 'locked' these patients into the audit and there was no case at any site where the follow-up data form was not completed for a patient whose data had already been entered into the first sections. Furthermore, our results showing a high overall anastomotic leak rate, an overall 30-day death rate of 3.2% and an elective 30-day death rate of 1.5% suggest that patients with poor early postoperative outcomes have not been omitted.

It is possible that some patients included in the study may have undergone additional procedures, such as simultaneous liver resection or extended resection, as a result of pathological involvement of other local organs, as these were not prespecified exclusion criteria. The numbers of such patients are likely to be very small and as such are unlikely to have conferred any major impact upon the main findings.

A potentially contentious decision was our inclusion of intra-abdominal and pelvic collections with the 'proven' anastomotic leak group in our primary outcome definition. There is no validated scoring system for anastomotic leak [9–11], and intraperitoneal fluid collections are considered by many surgeons as

representative of an anastomotic leak until proven otherwise. One recent study confirmed that isolated free intraperitoneal fluid was not a benign finding after anterior resection and another showed that many patients with ultimately proven anastomotic leakage did not have classical peri-anastomotic signs or extravasation of contrast on imaging [12,13]. It is our opinion that inclusion of patients with an intraperitoneal collection within the primary outcome group of anastomotic leak was justified given the similarities in adverse outcome rates between this group and others with a confirmed leak. Similarly, the sensitivity analysis that included only patients with a confirmed leak produced very similar results to those found in the main analysis We consider therefore that the majority of patients with isolated intraperitoneal collections had sustained an occult anastomotic leak.

Comparison with the literature

The anastomotic leak rate in this study compares closely with two other large-scale national audits utilizing prospective data collection. The Spanish ANACO group recently identified an overall leak rate of 8.4% in 1102 patients undergoing elective right hemicolectomy for cancer [5], and a Dutch analysis of 15,667 patients undergoing anastomosis after colorectal cancer resection found anastomotic leak rates in the right hemicolectomy ($n = 7788$) and ileo-caecal resection ($n = 240$) subgroups of 6.4% and 7.5%, respectively [14].

Our identification of stapling as a possible risk factor for anastomotic leak is contrary to a Cochrane review on the same topic [15]. In this review, data were pooled from 1125 patients undergoing an ileo-colic anastomosis within seven randomized trials and found fewer leaks after stapled anastomosis (2.5%; 11/441) compared with handsewn anastomosis (6.1%; 42/684), which was statistically significant: OR = 0.48, 95% CI: 0.24–0.95, $P = 0.03$. The authors rightly commented on the small patient numbers and the very low event rate. Whilst an apparently significant difference was found in leak rates, this did not correspond to a parallel impact upon re-operation rate, length of stay or mortality. Nevertheless this review concluded that 'stapled anastomoses are associated with fewer anastomotic leaks than handsewn, and should be considered the standard against which all other techniques should be compared'. It is likely that surgeons may have changed their practice based on the conclusions from this highly respected data source. Our conflicting message on stapled anastomoses could perhaps be written off as statistical anomaly, were it not for the very same finding being identified in the recent Spanish ANACO multicentre study [5]. This prospective observational study from 52 centres found major anastomotic leak (requiring intervention) rates of 3.4% in handsewn anastomoses and 7.8% in stapled anastomoses (OR = 2.1, 95% CI: 1.1–4.2, P = 0.007). Together with the current study, and accepting the potential shortfalls of observational research, this suggests that a more detailed investigation of stapled anastomosis *vs* handsewn anastomosis is certainly warranted.

Further research and analyses ongoing

We recognize that another limitation of this study relates to the fact that there are many different stapling techniques used in anastomosis and grouping them together may be inappropriate. These include bowel orientation (side-to-side, side-to-end, end-to-side), the type of stapler used (linear, circular), the stapler used for apical transection (linear cutting, linear noncutting) as well as other associated technical factors, such as the use of staple line oversewing and staple height selection. Similar, but less numerous, variabilities also exist within the handsewn group. These technical details were all collected prospectively during the project but will be analysed and reported in a subsequent paper. It is possible that certain technical aspects might account for a disproportionate number of leaks or be responsible for the apparent difference in leak rates compared with the patients undergoing handsewn anastomosis. Other subsequent reports from the study will explore the geographical variability in patients and techniques, and the impact of unit characteristics on outcome; and a detailed analysis of the perioperative management of patients with Crohn's disease against outcome is planned.

Despite being used in seemingly lower-risk patients, stapled anastomosis was associated with increased anastomotic leak in this observational study. These findings indicate the need for further high-quality, prospective and targeted research. It is likely that an updated large-scale randomized trial of anastomotic technique in patients undergoing right-sided bowel resection is needed.

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Conflict of interest

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References

1 STARSurg Collaborative. Impact of postoperative non-steroidal anti-inflammatory drugs on adverse events after gastrointestinal surgery. *Br J Surg* 2014; **101:** 1413–23.
2 Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. *Ann Surg* 2011; **253:** 890–9.
3 Bosma E, Pullens MJ, de Vries J, Roukema JA. The impact of complications on Quality of Life following colorectal surgery: a prospective cohort study to evaluate the Clavien-Dindo classification system. *Colorectal Dis* 2016; **18:** 594–602.
4 Lindsay JO, Bergman A, Patel AS, Alesso SM, Peyrin-Biroulet L. Systematic review: the financial burden of surgical complications in patients with ulcerative colitis. *Aliment Pharmacol Ther* 2015; **41:** 1066–78.
5 Frasson M, Granero-Castro P, Ramos Rodriguez JL *et al.* Risk factors for anastomotic leak and postoperative morbidity and mortality after elective right colectomy for cancer: results from a prospective, multicentric study of 1102 patients. *Int J Colorectal Dis* 2016; **31:** 105–14.
6 Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007; **370:** 1453–7.
7 Lo SK, Li IT, Tsou TS, See L. Non-significant in univariate but significant in multivariate analysis: a discussion with examples. *Changgeng Yi Xue Za Zhi* 1995; **18:** 95–101.
8 European Society of Coloproctology Cohort Study Committee. 2016 pan_European snapshot audit: closure of intestinal stoma. Available at http://www.escp.eu.com/research/cohort-studies/2016-audit (accessed August 2016).
9 Bruce J, Krukowski ZH, Al-Khairy G, Russell EM, Park KG. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. *Br J Surg* 2001; **88:** 1157–68.
10 McDermott FD, Arora S, Smith J, Steele RJC, Carlson GL, Winter DC. Issues in professional practice: Prevention, diagnosis and management of colorectal anastomotic leakage. London: Association of Surgeons of Great Britain and Ireland; 2016. http://www.acpgbi.org.uk/content/uploads/2016/03/management-of-colorectal-anastomtic-leakage.pdf (accessed August 2016).
11 Kulu Y, Ulrich A, Bruckner T *et al.* Validation of the International Study Group of Rectal Cancer definition and severity grading of anastomotic leakage. *Surgery* 2013; **153:** 753–61.
12 Caulfield H, Hyman NH. Anastomotic leak after low anterior resection: a spectrum of clinical entities. *JAMA Surg* 2013; **148:** 177–82.
13 Nicksa GA, Dring RV, Johnson KH, Sardella WV, Vignati PV, Cohen JL. Anastomotic leaks: what is the best diagnostic imaging study? *Dis Colon Rectum* 2007; **50:** 197–203.
14 Bakker IS, Grossmann I, Henneman D, Havenga K, Wiggers T. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. *Br J Surg* 2014; **101:** 424–32; discussion 32.
15 Choy PY, Bissett IP, Docherty JG, Parry BR, Merrie A, Fitzgerald A. Stapled versus handsewn methods for ileocolic anastomoses. *Cochrane Database Syst Rev* 2011: CD004320.

Appendix 1: Collaborators

Writing group and ESCP Cohort Studies + Audits Sub-Committee: T. Pinkney (chair and overall guarantor), N. Battersby, A. Bhangu, S. Chaudhri, A. El-Hussuna, M. Frasson, D. Nepogodiev, B. Singh, S. Vennix, O. Zmora.

ESCP Research Committee: D. Altomare, W. Bemelman, P. Christensen, A. D'Hoore, S. Laurberg, D. Morton (chair), T. Pinkney, M. Rubbini, C. Vaizey.

Logistical support and data collation: L. Magill, R. Perry, N. Sheward (Birmingham Surgical Trials Consortium, University of Birmingham).

Statistical analysis: N. Ives, S. Mehta (Birmingham Clinical Trials Unit, University of Birmingham).

Local Investigators:

Argentina: M. Cillo, D. Estefania, J. Patron Uriburu, H. Ruiz, M. Salomon (Hospital Britanico de Buenos Aires).

Belarus: A. Makhmudov, L. Selnyahina, A. Varabei, Y. Vizhynis (Surgical Department of the Belarusian Medical Academy of Postgraduate Education).

Belgium: D. Claeys, B. Defoort, F. Muysoms, P. Pletinckx, V. Vergucht (AZ Maria Middelares Gent); I. Debergh, T. Feryn, H. Reusens (AZ Sint-Jan); M. Nachtergaele (AZ St Jozef Malle); D. Francart, C. Jehaes, S. Markiewicz, B. Monami, J. Weerts (Clinique St Joseph, Liege); W. Bouckaert, B. Houben, J. Knol, G. Sergeant, G. Vangertruyden (Jessa Hospital Hasselt); L. Haeck, C. Lange, C. Sommeling, K. Vindevoghel (OLV van Lourdes Ziekenhuis); S. Castro, H. De Bruyn, M. Huyghe (St Augustinus General Hospital); E. De Wolf, D. Reynders (St Vincentius General Hospital); A. D'Hoore, A. de Buck van Overstraeten, A. Wolthuis (University Hospitals Leuven).

Bosnia and Herzegovina: S. Delibegovic (University Clinical Center Tuzla).

Brazil: A. Christiani, M. Marchiori Jr, C. Rocha de Moraes, V. Tercioti Jr (Centro Médico Campinas).

Bulgaria: E. Arabadjieva, D. Bulanov, D. Dardanov, V. Stoyanov, A. Yonkov (First Surgical Department, University Hospital Alexandrovska); K. Angelov, S.

APPENDIX 9

- CURRICULUM VITAE (SHORT FORM)

Curriculum Vitae

NUNO JOSÉ GOMES RAMA

Nuno José Gomes Rama é estudante de doutoramento em Ciências Médicas do ICBAS – Universidade do Porto. Licenciou-se em Medicina em 1999 (Faculdade de Medicina da Universidade de Coimbra - UC). Obteve o grau de especialista em Cirurgia Geral em 2008 (19,2 valores) e o de mestre em Gestão e Economia da Saúde em 2014, pela Faculdade de Economia da UC. Em maio de 2013, foi o primeiro português com a certificação da Division of Coloproctology – UEMS Section of Surgery & European Board of Surgery, em Maiorca. Realizou 3 Fellowship em cirurgia minimamente invasiva: 2006 (2 semanas) - São Paulo – Brasil (Dr. Miguel Pedroso); 2008-9 (6 meses) – Centro Hospitalar do Porto (Dra. Anabela Rocha); 2015(2 semanas) – HUMV – Santander (Marcos Gomez, PhD) -Cirurgia Robótica. Desde 2014 é Professor Auxiliar da Escola Superior de Saúde (Leiria), lecionando uma unidade curricular no Mestrado da Pessoa em Situação Crítica. Diferenciou-se desde 2010 em Cirurgia Colorectal e Minimamente invasiva, liderando a Unidade Funcional de patologia Colorectal desde 2015 3 o Centro de Referência de Cancro do recto desde 2017. Neste âmbito desenvolveu competências técnicas e científicas na abordagem de doentes com cancro do cólon (realizou mais de 600 colectomias) e recto (Ressecção Anterior / RA baixa - mais de 300 procedimentos; Resseção Abdominoperineal - cerca de 70 procedimentos). Participou e coordenou, regularmente, reuniões MDT (volume médio semanal de 10 doentes), introduzindo e desenvolvendo a abordagem “Watch and Wait” no tratamento de cancro do recto. Além disso, desenvolveu competências técnicas na abordagem transanal, com formação internacional específica (em Santander com o Prof. Marcos Gomez – 2015, e o Curso de "TATME" em Amesterdão -2018). No que diz respeito às perturbações colorectais funcionais, tem desenvolvido competências no tratamento cirúrgico do prolapso rectal, retocelo, inércia colónica/megacólon, entre outros. Realiza regularmente procedimentos por abordagem minimamente invasiva, como a retopexia ventral, ressecção- retopexia (operação Frykman-Golberg), STARR e abordagens perineais (operações de Délorme ou Altemeier). Além disso, tem uma experiência significativa no tratamento cirúrgico de incontinência anal (esfincteroplastia e aplicação de bulking agents). Finalmente, na área da proctologia, tem experiência relevante, especialmente em regime de ambulatório, na abordagem diagnóstica e terapêutica de doença hemorroidária, fístulas, fissuras, entre outras. Integrou o ciTechCare desde 2019 com membro colaborador / Join Steering Committe. Tem publicado 33 artigos em revistas com revisão por pares, 10 dos quais como primeiro autor, várias comunicações orais, poster e palestras (apenas apresentadas as realizadas após 2016). Desde 2016, organizou 17 eventos e participou em outros 40. Recebeu 9 prémios e participa e/ou participou como Investigador em 5 projetos sendo o Investigador responsável em quatro. Em 2018 obteve financiamento no âmbito do projeto PAIRAR no valor de 52,390,05€, essencial ao suporte do projeto de doutoramento: utilidade dos biomarcadores séricos na deteção precoce da deiscência anastomótica colorectal.

Identificação

Identificação pessoal

Nome completo
NUNO JOSÉ GOMES RAMA

Género
Masculino

Data de nascimento
1975/10/31

Nomes de citação

Rama, nuno

Identificadores de autor

Ciência ID
E012-FE6B-9A38

Endereços de correio eletrónico

ramanuno@gmail.com (Profissional)

Telefones

Telefone
244817061 (Profissional)

Telemóvel
967547698 (Pessoal)

Moradas

Rua das Olhalvas, 2410-197 L Leiria, Leiria, Portugal (Profissional)
Rua de Santo André – 66-68, Campus 5, Politécnico de Leiria, , 2410-541 Leiria, Leiria, Portugal (Profissional)

Websites

https://www.facebook.com/ramanuno (Rede social)
@NunoRama1 (Rede social)
https://www.researchgate.net/profile/Nuno_Rama (Académico)
https://citechcare.ipleiria.pt/people/collaborator-members/ (Académico)

Domínios de atuação

Ciências Médicas e da Saúde - Medicina Clínica - Cirurgia
Ciências Médicas e da Saúde - Medicina Clínica - Oncologia
Ciências Sociais - Economia e Gestão - Economia

Idiomas

Idioma	Conversação	Leitura	Escrita	Compreensão	Peer-review
Inglês	Utilizador independente (B2)	Utilizador independente (B2)	Utilizador independente (B2)	Utilizador independente (B2)	Utilizador independente (B1)
Português (Idioma materno)					
Espanhol; Castelhano	Utilizador independente (B1)	Utilizador independente (B2)	Utilizador independente (B1)	Utilizador independente (B1)	Utilizador independente (B1)
Francês	Utilizador elementar (A2)	Utilizador independente (B1)	Utilizador elementar (A2)	Utilizador independente (B1)	Utilizador elementar (A1)

Formação

	Grau	Classificação
2016/09 - 2022 Em curso	Ciências Médicas (Doutoramento) Universidade do Porto Instituto de Ciências Biomédicas Abel Salazar, Portugal *"EARLY DETECTION OF COLORECTAL ANASTOMOTIC*	

	LEAKAGE: USEFULNESS OF CLINICAL CRITERIA AND SERUM BIOMARKERS" (TESE/DISSERTAÇÃO)	
2012/10/03 - 2014/09/24 Concluído	Economia e Gestão em Organizações de Saúde (Especialização pós-licenciatura) Especialização em Gestão em Organizações de Saúde Universidade de Coimbra Faculdade de Economia, Portugal	18 (dezoito), Excelente / Escala Europeia de comparabilidade: A
2002/01/02 - 2008/02/22 Concluído	Cirurgia Geral (Título de especialista) Especialização em Cirurgia Geral Hospital de Santo André, Portugal	19.2 (Dezanove valores e dois décimos)
1993/01/02 - 1999/09/24 Concluído	Medicina (Licenciatura) Universidade de Coimbra Faculdade de Medicina, Portugal	16 (Dezasseis) valores

Percurso profissional

Outras Carreiras

	Categoria Profissional Instituição de acolhimento	Empregador
2018/01 - Atual	Assistente graduado (Médica) Hospital de Santo André, Portugal	Hospital de Santo André, Portugal
2008/03/01 - 2018/08/01	Assistente (Médica) Hospital de Santo André, Portugal	Hospital de Santo André, Portugal
2002/01 - 2008/02	Interno (Médica) Hospital de Santo André, Portugal	Hospital de Santo André, Portugal

Cargos e Funções

	Categoria Profissional Instituição de acolhimento	Empregador
2016/02 - Atual	Director de Unidade Orgânica	Hospital D. Manuel Aguiar, Portugal

Projetos

Projeto

	Designação	Financiadores
2019/01 - Atual	“PAIRAR - Joint Integrated Assistance Process” 000000 Investigador responsável	Ministério da Saúde, Portugal
2017 - 2022	“How can we early detect a colorectal anastomotic leakage: the usefulness of clinical criteria and biomarkers – Prospective Observational Single Centre Study". 0000 Investigador responsável	

Outro

	Designação	Financiadores
2020/04 - Atual	" COVID PANdemy and its Impact in Diagnosis of cOloRectal cAncer” (PANDORA STUDY)" 0000 Investigador responsável	
2019/01 - Atual	“Usefulness of inflammatory biomarkers to predict anastomotic leak after colorectal Surgery: Systematic review and meta-analysis " 161692 Investigador responsável	
2018/01 - Atual	Minimally Invasive Right Colectomy Anastomosis Study (MIRCAST)" 000000 Investigador	
2018 - Atual	“Surviving Rectal Cancer at the Cost of a Colostomy: Quality of Life, Socioeconomic Factors and Colostomy Impact in an International Perspective” 0000 Investigador	
2017 - Atual	"3rd ESCP Pan-European snapshot audit: Left colon, sigmoid and rectal resections”. 6 0000 Investigador European Society of Coloproctology, Reino Unido	

2016 - Atual	"2nd ESCP Pan-European snapshot audit: Stoma Closure Audit". 0000 Investigador European Society of Coloproctology, Reino Unido
2015 - 2016	"ESCP Pan-European snapshot audit" 0000 Investigador European Society of Coloproctology, Reino Unido

Produções

Publicações

Artigo em revista

1 "Development of a warning score for early detection of colorectal anastomotic leakage: Hype or Hope?". *British Journal of Surgery* (2022):
Submetido

2 "Usefulness of serum c-reactive protein and calprotectin for the early detection of colorectal anastomotic leakage: A prospective observational study". *World Journal of Gastroenterology* (2022):
Aceite para publicação

3 "The usefulness of inflammatory biomarkers to predict anastomotic leakage after colorectal surgery: systematic review and meta-analysis". *Gastroenterology Research and Practice* (2022):
Aceite para publicação

4 Zaborowski, Alexandra M; Abdile, Ahmed; Adamina, Michel; Aigner, Felix; d'Allens, Laura; Allmer, Caterina; Álvarez, Andrea; et al. "Microsatellite instability in young patients with rectal cancer: molecular findings and treatment response". *British Journal of Surgery* 109 3 (2022): 251-255. http://dx.doi.org/10.1093/bjs/znab437.
Publicado · 10.1093/bjs/znab437

5 Zaborowski, Alexandra M.; Abdile, Ahmed; Adamina, Michel; Aigner, Felix; d' Allens, Laura; Allmer, Caterina; Álvarez, Andrea; et al. "Characteristics of Early-Onset vs Late-Onset Colorectal Cancer". *JAMA Surgery* 156 9 (2021): 865. http://dx.doi.org/10.1001/jamasurg.2021.2380.
Publicado · 10.1001/jamasurg.2021.2380

6 "Anastomotic Leak in Colorectal Cancer Surgery: From Diagnosis to Management or Failure - A Retrospective Cohort Study.". *Surgery, Gastroenterology and Oncology · September 2021;* 26 3 (2021): 183-190.
Publicado · Acesso aberto

7 Nepogodiev, Dmitri; Simoes, Joana FF; Li, Elizabeth; Picciochi, Maria; Glasbey, James C; Baiocchi, Glauco; Blanco-Colino, Ruth; et al. "SARS-CoV-2 infection and venous thromboembolism after surgery: an international prospective cohort study". *Anaesthesia* 77 1 (2021): 28-39. http://dx.doi.org/10.1111/anae.15563.
Publicado · 10.1111/anae.15563

8 Nepogodiev, Dmitri; Simoes, Joana FF; Li, Elizabeth; Picciochi, Maria; Glasbey, James C; Baiocchi, Glauco; Blanco-Colino, Ruth; et al. "Effects of pre-operative isolation on postoperative pulmonary complications after elective surgery: an international prospective cohort study". *Anaesthesia* 76 11 (2021): 1454-1464. http://dx.doi.org/10.1111/anae.15560.

Publicado · 10.1111/anae.15560

9 Kristensen, Helle Ø; Thyø, Anne; Jøssing Emmertsen, Katrine; Smart, Neil J.; Pinkney, Thomas; Warwick, Andrea M.; Pang, Dong; et al. "Translation and international validation of the Colostomy Impact score". *Colorectal Disease* 23 7 (2021): 1866-1877. http://dx.doi.org/10.1111/codi.15635.

Publicado · 10.1111/codi.15635

10 "SARS-CoV-2 vaccination modelling for safe surgery to save lives: data from an international prospective cohort study". *British Journal of Surgery* 108 9 (2021): 1056-1063. http://dx.doi.org/10.1093/bjs/znab101.

Publicado · 10.1093/bjs/znab101

11 Nepogodiev, Dmitri; Simoes, Joana FF; Li, Elizabeth; Picciochi, Maria; Glasbey, James C; Baiocchi, Glauco; Blanco-Colino, Ruth; et al. "Timing of surgery following SARS-CoV-2 infection: an international prospective cohort study". *Anaesthesia* 76 6 (2021): 748-758. http://dx.doi.org/10.1111/anae.15458.

Publicado · 10.1111/anae.15458

12 "Predictors for Anastomotic Leak, Postoperative Complications, and Mortality After Right Colectomy for Cancer: Results From an International Snapshot Audit". *Diseases of the Colon & Rectum* 63 5 (2020): 606-618. http://dx.doi.org/10.1097/dcr.0000000000001590.

Publicado · 10.1097/dcr.0000000000001590

13 "Surgical treatment of pilonidal disease: A retrospective 4-year analysis". *Revista Portuguesa de Coloproctologia* 2 2 (2020): 27-32.

Publicado

14 Parente, Diana; Rama, Nuno; Sales, Inês; Neves, Miguel; Sousa, Inês; Gonçalves, Inês; Gil, Inês; et al. "Colorectal Cancer Surgical Management: Past, Present and Future". *European Journal of Surgical Oncology* 46 2 (2020): e93. http://dx.doi.org/10.1016/j.ejso.2019.11.225.

10.1016/j.ejso.2019.11.225

15 Parente, Diana Silva; Rama, Nuno; Sales, Inês; Neves, Miguel; Sousa, Inês; Gonçalves, Inês; Gil, Inês; et al. "Failure to Rescue in Colorectal Surgery: Why Looking Back?". *European Journal of Surgical Oncology* 46 2 (2020): e93. http://dx.doi.org/10.1016/j.ejso.2019.11.226.

10.1016/j.ejso.2019.11.226

16 "Hemorrhoidal Disease - Guidelines". *Revista Portuguesa de Coloproctologia* 2 1 (2020): 1-7.

Publicado · Acesso aberto

17 Rama, Nuno José; Ferreira, Pedro Lopes; Pimentel, João; Juul, Therese. "Validation of Portuguese version of the low anterior resection syndrome score". *Journal of Coloproctology* 39 01 (2019): 001-008. http://dx.doi.org/10.1016/j.jcol.2018.09.004.

Publicado · 10.1016/j.jcol.2018.09.004

18 "Safety of primary anastomosis following emergency left sided colorectal resection: an international, multi-centre prospective audit". *Colorectal Disease* 20 (2018): 47-57. http://dx.doi.org/10.1111/codi.14373.

Publicado · 10.1111/codi.14373

19 "An international multicentre prospective audit of elective rectal cancer surgery; operative approach versus outcome, including transanal total mesorectal excision (TaTME)". *Colorectal Disease* 20 (2018): 33-46. http://dx.doi.org/10.1111/codi.14376.

Publicado · 10.1111/codi.14376

20 "Evaluating the incidence of pathological complete response in current international rectal cancer practice: the barriers to widespread safe deferral of surgery". *Colorectal Disease* 20 (2018): 58-68. http://dx.doi.org/10.1111/codi.14361.

Publicado · 10.1111/codi.14361

21 "The impact of conversion on the risk of major complication following laparoscopic colonic surgery: an international, multicentre prospective audit". *Colorectal Disease* 20 (2018): 69-89. http://dx.doi.org/10.1111/codi.14371.

Publicado · 10.1111/codi.14371

22 Gil, Ines Campos; Parente, Diana; Rama, Nuno; Lopes, Branco; Paulino, Virginia; Amado, Cristina; Fernanda Cunha, M.; Sales, Ines; Faria, Vitor. "Pancreatic Adenocarcinoma Presenting as Acute Large Bowel Obstruction: Case Report". *Surgery, Gastroenterology and Oncology* 23 3 (2018): 204. http://dx.doi.org/10.21614/sgo-23-3-204.

10.21614/sgo-23-3-204

23 Gil, Ines Campos; Rama, Nuno; Parente, Diana; Sales, InÃªs; Alves, Paulo; Clara, Paulo; Amado, Sandra; Coelho, Miguel; Faria, VÃ-tor. "Intracorporeal versus Extracorporeal Anastomosis in Laparoscopic Right Colectomy: Short-Term Outcomes". *Surgery, Gastroenterology and Oncology* 23 1 (2018): 71. http://dx.doi.org/10.21614/sgo-23-1-71.

10.21614/sgo-23-1-71

24 Battersby, N.; Bhangu, A.; Chaudhri, S.; El-Hussuna, A.; Frasson, M.; Nepogodiev, D.; Singh, B.; et al. "Relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit". *Colorectal Disease* 19 8 (2017): http://dx.doi.org/10.1111/codi.13646.

Publicado · 10.1111/codi.13646

25 Barbeiro, Sandra; Brásio, Rita; Atalaia-Martins, Catarina; Marcos, Pedro; Gonçalves, Cláudia; Alves, Paulo; Rama, Nuno. "Klippel–Trenaunay syndrome: endoscopic findings". *Endoscopy* 48 S 01 (2016): E355-E356. http://dx.doi.org/10.1055/s-0042-119038.

Publicado · 10.1055/s-0042-119038

26 "The relevance of databases in health knowledge management". *Revista Portuguesa de Cirurgia* 36 (2016): 39-41.

Publicado · Acesso aberto

27 "Measuring the quality of life in patients with fecal incontinence". *Revista Portuguesa de Coloproctologia* 1 2 (2015): 26-32.

Publicado · Acesso aberto

28 Alves, P.; Rama, N.; Brásio, R.; Malaquias, R.; Gil, I.; Sales, I.; Jordão, D.; Andril, O.; Faria, V.. "60. Laparoscopic total mesorectal excision: Step-by-step technique". *European Journal of Surgical Oncology (EJSO)* 40 11 (2014): S31-S32. http://dx.doi.org/10.1016/j.ejso.2014.08.057.

10.1016/j.ejso.2014.08.057

29 Campos Gil, I.; Rama, N.; Malaquias, R.; Brásio, R.; Sales, I.; Garcia, R.; Pimentel, J.; et al. "391. Laparoscopic approach to retrorectal teratoma: Case report". *European Journal of Surgical Oncology (EJSO)* 40 11 (2014): S150-S151. http://dx.doi.org/10.1016/j.ejso.2014.08.381.

10.1016/j.ejso.2014.08.381

	30	Brásio, R.; Malaquias, A.R.; Gil, I.; Alves, P.F.; Rama, N.J.. "416. A rare cause of acute abdomen – Case report". *European Journal of Surgical Oncology (EJSO)* 40 11 (2014): S160. http://dx.doi.org/10.1016/j.ejso.2014.08.406. 10.1016/j.ejso.2014.08.406
	31	"Planning, valuing, preparing for the future". *Revista Portuguesa de Coloproctologia* 1 2 (2014): 1-2. Acesso aberto
	32	Rama, Nuno; Monteiro, Alexandre; Bernardo, João E.; Eugénio, Luís; Antunes, Manuel J.. "Lung metastases from colorectal cancer: surgical resection and prognostic factors¿". *European Journal of Cardio-Thoracic Surgery* 35 3 (2009): 444-449. http://dx.doi.org/10.1016/j.ejcts.2008.10.047. Publicado • 10.1016/j.ejcts.2008.10.047
	33	Rama, N.J.; Clara, P.J.; Ferraz, S.; Tavares, F.; Marques, U.; Lopes, B.; Paulino, V.; Baeta da Veiga, A.. "172 POSTER A rare cause of spontaneous haemoperitoneum". *European Journal of Surgical Oncology (EJSO)* 32 (2006): S51. http://dx.doi.org/10.1016/s0748-7983(06)70607-2. 10.1016/s0748-7983(06)70607-2
Relatório	1	2013. *Segurança do doente e Sistema de Gestão de Risco Clínico - Realidade Institucional*.
	2	2013. *A contratualização nos Cuidados de Saúde Primários Realidade Institucional – ACES Pinhal Litoral II*.
Tese / Dissertação	1	"Concepção de uma base de dados do cancro colorretal". Mestrado, Universidade de Coimbra Faculdade de Economia, 2014.

Atividades

Apresentação oral de trabalho

	Título da apresentação	Nome do evento Anfitrião (Local do evento)
2022/04/29	Incontinência Anal	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Vila Real, Portugal)
2022/04/29	Prolapso retal	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Vila Real, Portugal)

2022/04/28	Doença hemorroidária	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Vila Real, Portugal)
2022/04/23	DOENÇA DE CROHN DO ID/ILEOCECAL: Abordagem cirúrgica	8º CURSO COLOPROCTOLOGIA / MÓDULO I Sociedade Portuguesa de Cirurgia (Porto, Portugal)
2022/04/02	DIVERTICULITE AGUDA COMPLICADA	Reunião Regional da Sociedade Portuguesa de Coloproctologia - Sul Sociedade Portuguesa de Coloproctologia (Torres Novas, Portugal)
2021/10/07	Rectal Prolapse: What Is the Best Approach for Repair?	Primeira Reunião do Capítulo de Coloproctologia Sociedade Portuguesa de Cirurgia (Portimão, Portugal)
2021/10/01	Incontinência Anal	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Aveiro, Portugal)
2021/10/01	Prolapso Retal	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Aveiro, Portugal)
2021/09/30	Doença hemorroidária	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Aveiro, Portugal)
2021/07/02	Fluorescence Imaging – quantitative and qualitative evaluation	XXX CONGRESSO NACIONAL DE COLOPROCTOLOGIA Sociedade Portuguesa de Coloproctologia (Oeiras, Portugal)
2021/07/01	LIVING WITH AN OSTOMY	ESCP 9th Regional Masterclass: Complex Decisions in Coloproctology Sociedade Portuguesa de Coloproctologia + ESCP (Oeiras, Portugal)
2021/06/17	DIGESTIVE ANASTOMOTIC FAILURE	XLI CONGRESSO NACIONAL DE CIRURGIA Sociedade Portuguesa de Cirurgia (Figueira da Foz, Portugal)

2021/06/17	LOWER GI BLEEDING: From Knowledge to Practice	XLI CONGRESSO NACIONAL DE CIRURGIA Sociedade Portuguesa de Cirurgia (Figueira da Foz, Portugal)
2021/06/05	TaTME - ESTADO DA ARTE	Centro Hospitalar de Trás os Montes e Alto Douro (Vila Real, Portugal)
2021/05/14	Incontinência Anal	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2021/05/14	Prolapso Retal	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2021/05/13	Doença hemorroidária	Curso de Proctologia - Programa Formativo da SPCIR Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2021/03/16	Management of left-sided obstructing colonic cancer	Sessões cientificas 2021 Associação Gaúcha de Coloproctologia (Brasil)
2020/10/24	DOENÇA HEMORROIDÁRIA - Opções terapêuticas	Modulo IV - 7º Curso de Coloproctologia Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2020/10/24	Reparação esfincteriana - Em que casos?	Modulo IV - 7º Curso de Coloproctologia Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2020/08/17	A interdisciplinaridade na co construção de políticas públicas na área da saúde	Ciclo de Conferências Interdisciplinares | Associação Nacional Interdisciplinar da Economia Social
2020/01/24	Fistulas anais - Equilíbrio entre o sucesso e a continência	Primeiras Jornadas de Cirurgia do Centro Hospitalar Barreiro-Montijo Centro Hospitalar Barreiro Montijo (Barreiro, Portugal)
2020/01/24	Tratamento cirúrgico vs. conservador nas fissuras anais	Primeiras Jornadas de Cirurgia do Centro Hospitalar Barreiro-Montijo Centro Hospitalar Barreiro Montijo (Barreiro, Portugal)

2019/11/22	Cirurgia nas colites: quando e como	Sociedade Portuguesa de Coloproctologia (Figueira da Foz, Portugal)
2019/11/21	Doença Hemorroidária: tratamento cirúrgico convencional , 21 de novembro de 2019.	Curso de Patologia Ano-rectal: XXIX Congresso Nacional de Coloproctologia Sociedade Portuguesa de Coloproctologia (Figueira da Foz, Portugal)
2019/10/19	Doença hemorroidária: opções cirúrgicas	Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2019/10/19	Prolapso retal: opções cirúrgicas Leiria, 19 de outubro de 2019.	Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2019/10/19	- Reparação esfincteriana: Em que casos? Leiria, 19 de outubro de 2019.	Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2019/06/29	Doença de Crohn ID/Ileocecal – abordagem cirúrgica	Sociedade Portuguesa de Cirurgia (Porto, Portugal)
2019/06/14	Looking at functional results: validation of Portuguese version of the LARS Score.	European Association of Endoscopic Surgery (Sevilha, Espanha)
2019/06/12	Anastomotic leak in colorectal cancer surgery: from diagnosis to management or failure.	European Association of Endoscopic Surgery (Sevilha, Espanha)
2019/05/18	Diverticulite Aguda Complicada: Tratamento " - Sociedade Portuguesa de Cirurgia;	Reunião - Um dia, um tema: URGÊNCIAS CIRÚRGICAS EM PATOLOGIA COLORRETAL Sociedade Portuguesa de Cirurgia (Covilhã, Portugal)
2019/05/16	Early anastomotic leak diagnosis after colorectal surgery: Preliminary results from prospective observational study	AECP (Valhadolid, Espanha)
2019/05/11	Cancro do reto – abordagem laparoscópica	Sociedade Portuguesa de Cirurgia

		(Lisboa, Portugal)
2019/04/25	Intracorporeal anastomosis in MIS right colectomy: The Multicentric Portuguese experience? Viena (Áustria), 25 de abril de 2019.	EFR - European Federation for coloRectal cancer (Viena, Áustria)
2019/04/06	Fistulas anais: Alternativas de tratamento poupadoras do esfíncter anal	Sociedade Portuguesa de Coloproctologia (Ponta Delgada, Portugal)
2019/03/21	Diverticulite Aguda Complicada: Do diagnóstico ao tratamento.	Sociedade Portuguesa de Cirurgia (Tomar, Portugal)
2019/02/02	Opções cirúrgicas nos prolapsos do compartimento posterior: Abordagem da coloproctologia	Simpósio APNUG 2019 (Évora, Portugal)
2019/01/17	Anastomose na hemicolectomia direita	Primeiras Jornadas de Cirurgia Colorectal do Algarve (Portimão, Portugal)
2018/11/22	Abcessos intra-abdominais na DII: Quando e como operar	Sociedade Portuguesa de Coloproctologia (Porto, Portugal)
2018/11/15	Intracorporeal anastomosis in MIS right colectomy: Technical aspects. , 15 de novembro de 2018.	Medtronic (Vila do Conde, Portugal)
2018/11/13	Descending Perineum Syndrome: What can we do?	(Porto, Portugal)
2018/10/20	Diverticulite Aguda Complicada: Do diagnóstico ao tratamento.	Sociedade Portuguesa de Cirurgia (Leiria, Portugal)
2018/09/09	Intracorporeal anastomosis in MIS right colectomy: The Multicentric Portuguese experience?	30th anniversary IASGO World Congress - 2018 International Association of Surgery, Gastroenterology and Oncology (Moscovo, Rússia)

2018/07/07	Carcinoma colorectal: Como estadiar?	Sociedade Portuguesa de Coloproctologia (Funchal, Portugal)
2018/06/15	Intracorporeal anastomosis in right colectomy: Outcomes	(Santarém, Portugal)
2018/05/17	External rectal prolapse and intussusception: How I do it!	Fundação Champalimaud (Lisboa, Portugal)
2018/04/28	Anastomotic leak after Anterior Resection: Diagnosis and criteria for non-operative management	(Porto, Portugal)
2018/04/21	Hemorroidopexia mecânica: o método preferido?	Sociedade Portuguesa de Cirurgia (Coimbra, Portugal)
2018/04/21	Desarterialização Hemorroidária por Doppler	Sociedade Portuguesa de Cirurgia (Coimbra, Portugal)
2018/04/21	Reparação esfincteriana: Em que casos? Coimbra, 21 de abril de 2018	Sociedade Portuguesa de Cirurgia (Coimbra)
2018/01/27	Papel do cirurgião na Doença de Crohn	Reunião Regional da Sociedade Portuguesa de Coloproctologia Sociedade Portuguesa de Coloproctologia (Covilhã, Portugal)
2018/01/13	Cirurgia na Colite Ulcerosa: Indicações e opções cirúrgicas	Sociedade Portuguesa de Cirurgia (Braga, Portugal)
2018/01/12	Haemorrhoidal Disease: Traditional Excisional Surgery	Masterclass: Doença Hemorroidária (Feira, Portugal)
2017/10/21	- Doença de Crohn intestinal: papel da cirurgia	Sociedade Portuguesa de Cirurgia (Porto, Portugal)
2017/06/16	- Intracorporal versus extracorporeal anastomosis during laparoscopic right hemicolectomy: An institutional experience – Poster presentation. , 16 de junho de 2017.	European Association of Endoscopic Surgery (Frankfurt, Alemanha)

2017/06/02	- Cancro do reto: novas abordagens cirúrgicas	Sociedade Portuguesa de Coloproctologia (Portimão, Portugal)
2017/05/05	- LARS: what can we do for our patients? , 16 de maio de 2017.	MIARC - Minimally Invasive Approach to Rectal Cancer 2017 Fundação Champalimaud (Lisboa, Portugal)
2017/04/29	- Rectal Cancer – MIS: Laparoscopy	Sociedade Portuguesa de Cirurgia (Lisboa, Portugal)
2017/04/22	- Fistulotomia /Fistulectomia: em que situações	(Porto, Portugal)
2017/04/08	- Papel do cirurgião na Doença de Crohn	Sociedade Portuguesa de Coloproctologia (Vila Real, Portugal)
2017/03/16	Abcesso perianal	Curso Pré-congresso – Coloproctologia - XXXVII Congresso Nacional de Cirurgia Sociedade Portuguesa de Cirurgia (Figueira da Foz, Portugal)
2017/03/16	- Diverticulite Aguda: Tratamento cirúrgico: qual, como e quando	Curso Pré-congresso – Coloproctologia - XXXVII Congresso Nacional de Cirurgia Sociedade Portuguesa de Cirurgia (Figueira da Foz)
2017/03/16	- Próteses cólicas autoexpansíveis: indicações	Curso Pré-congresso – Coloproctologia - XXXVII Congresso Nacional de Cirurgia Sociedade Portuguesa de Cirurgia (Figueira da Foz)
2016/11/26	- Seleção e preparação de doentes	(Vila do Conde, Portugal)
2016/10/29	- Cirurgia robótica: estado da arte.	Reunião Regional da Sociedade Portuguesa de Coloproctologia; Sociedade Portuguesa de Coloproctologia;

Organização de evento

	Nome do evento Tipo de evento (Tipo de participação)	Instituição / Organização
2020/10/24 - Atual	Atualização em Patologia Proctológica Benigna (2020/10/24 - 2020/10/24) Outro (Presidente da Comissão Organizadora)	Sociedade Portuguesa de Cirurgia, Portugal
2022/04/28 - 2022/04/29	Curso de Proctologia - Programa Formativo da SPCIR (2022/04/28 - 2022/04/29) Outro (Coorganizador)	Sociedade Portuguesa de Cirurgia, Portugal
2022/03/04 - 2022/03/04	I Masterclass de Desenvolvimento e melhoria de conhecimentos em cirurgia minimamente invasiva do colon (2022/03/04 - 2022/03/04) Oficina (workshop) (Presidente da Comissão Organizadora)	Medtronic Ibérica SA, Espanha
2021/09/30 - 2021/10/01	Curso de Proctologia - Programa Formativo da SPCIR (2021/09/30 - 2021/10/01) Outro (Coorganizador)	Sociedade Portuguesa de Cirurgia, Portugal
2021/07/01 - 2021/07/01	ESCP 9th Regional Masterclass: Complex Decisions in Coloproctology (2021/07/01 - 2021/07/01) Congresso (Presidente da Comissão Organizadora)	European Society of Coloproctology, Reino Unido Sociedade Portuguesa de Coloproctologia, Portugal
2021/06/17 - 2021/06/18	XLI CONGRESSO NACIONAL DE CIRURGIA Secretário - Geral (2021/06/17 - 2021/06/18) Congresso (Coorganizador)	Sociedade Portuguesa de Cirurgia, Portugal
2021/06/04 - 2021/06/04	TAMIS - Hands-on: tips and tricks - Sessão para IFE (2021/06/04 - 2021/06/04) Oficina (workshop) (Coorganizador)	Centro Hospitalar de Trás-os-montes e Alto Douro EPE, Portugal
2021/05/13 - 2021/05/14	Curso de Proctologia - Programa Formativo da SPCIR (2021/05/13 - 2021/05/14) Outro (Coorganizador)	Sociedade Portuguesa de Cirurgia, Portugal
2019/11/21 - 2019/11/22	Atualização em Coloproctologia XXIX Congresso Nacional de Coloproctologia (2019/11/21 - 2019/11/22) Congresso (Coorganizador)	Sociedade Portuguesa de Coloproctologia, Portugal
2019/11/21 - 2019/11/21	Atualização em Doença Hemorroidária (2019/11/21 - 2019/11/21)	Sociedade Portuguesa de Coloproctologia, Portugal

	Seminário (Coorganizador)	
2019/10/19 - 2019/10/19	Atualização em Proctologia Módulo IV – Capítulo de Coloproctologia da Sociedade Portuguesa de Cirurgia Leiria, 19 de outubro de 2019. (2019/10/19 - 2019/10/19) Outro	Sociedade Portuguesa de Cirurgia, Portugal
2019/06/07 - 2019/06/07	Atualização em coloproctologia III Jornadas de Cirurgia Colorectal entre o Lis e Tejo (2019/06/07 - 2019/06/07) Encontro (Presidente da Comissão Organizadora)	Hospital de Santo André, Portugal Hospital Distrital de Santarém EPE, Portugal
2018/11/15 - 2018/11/15	Formação em Coloproctologia - Laparoscopia avançada Colorectal (2018/11/15 - 2018/11/15) Outro (Presidente da Comissão Organizadora)	Medtronic Ibérica SA, Espanha
2018/10/20 - 2018/10/20	Atualização em Coloproctologia (2018/10/20 - 2018/10/20) Seminário (Presidente da Comissão Organizadora)	Sociedade Portuguesa de Cirurgia, Portugal
2018/06/15 - 2018/06/15	Atualização em Coloproctologia (2018/06/15 - 2018/06/15) Encontro (Coorganizador)	
2017/06/24 - 2017/06/24	Atualização em Coloproctologia (2017/06/24 - 2017/06/24) Encontro (Presidente da Comissão Organizadora)	
2016/11/24 - 2016/11/25	Atualização em coloproctologia - XXVI Congresso Nacional de Coloproctologia (2016/11/24 - 2016/11/25) Congresso (Membro da Comissão Organizadora)	Sociedade Portuguesa de Coloproctologia, Portugal

Participação em evento

	Descrição da atividade Tipo de evento	Nome do evento Instituição / Organização
2022/04/02 - 2022/04/02	Reunião Regional do Sul - Urgências em Coloproctologia Encontro	Reunião Regional da Sociedade Portuguesa de Coloproctologia - Sul Sociedade Portuguesa de Coloproctologia, Portugal

2021/10/07 - 2021/10/08	Atualização em Coloproctologia - Primeira RAC de Coloproctologia Encontro	Primeira Reunião do Capítulo de Coloproctologia Sociedade Portuguesa de Cirurgia, Portugal
2021/07/02 - 2021/07/02	XXX CONGRESSO NACIONAL DE COLOPROCTOLOGIA Atualização em Coloproctologia Moderador: Right hemicolectomy with d3 extended lymphadenectomy: indication and role of robotics Congresso	XXX CONGRESSO NACIONAL DE COLOPROCTOLOGIA Sociedade Portuguesa de Coloproctologia, Portugal
2021/06/18 - 2021/06/18	XLI CONGRESSO NACIONAL DE CIRURGIA Atualização em Cirurgia Moderador: Reduzir as complicações e as readmissões: a importância de valorizar a anemia Congresso	XLI CONGRESSO NACIONAL DE CIRURGIA Sociedade Portuguesa de Cirurgia, Portugal
2021/06/04 - 2021/06/05	Atualização em Coloproctologia - Reunião de Cirurgia - Douro Norte Moderador - Mesa redonda - 30 anos em Robótica Congresso	Reunião de Cirurgia Douro Norte Centro Hospitalar de Trás-os-montes e Alto Douro EPE, Portugal
2021/03/23 - 2021/03/23	Atualização em Coloproctologia - Sessão Cara ou Coroa (Moderador) Mesa-redonda	Sessão Cara ou Coroa - Neoplasia oclusiva do cólon Sociedade Portuguesa de Cirurgia, Portugal
2020/01/24 - 2020/01/25	Atualização em Cirurgia Congresso	Primeiras Jornadas de Cirurgia do Centro Hospitalar Barreiro-Montijo Centro Hospitalar Barreiro Montijo EPE, Portugal
2020/01/24 - 2020/01/24	O Presente e o Futuro no Centro de Investigação Moderador: A investigação no CHL Seminário	O Presente e o Futuro no Centro de Investigação Hospital de Santo André, Portugal
2019/11/21 - 2019/11/22	Atualização em Coloproctologia Congresso	XXIX Congresso Nacional de Coloproctologia Sociedade Portuguesa de Coloproctologia, Portugal
2019/11/21 - 2019/11/22	Atualização em coloproctologia XXIX Congresso Nacional de Coloproctologia Comissão Organizadora / Palestrante / Coordenador de Curso – Doença perianal benigna.	XXIX Congresso Nacional de Coloproctologia Sociedade Portuguesa de Coloproctologia, Portugal

	Congresso	
2019/11/11 - 2019/11/12	Atualização em Cirurgia XXXI Encontro Internacional de Cirurgia Moderador da mesa redonda: Cólon e Recto. Congresso	XXXI Encontro Internacional de Cirurgia Centro Hospitalar de Vila Nova de Gaia Espinho EPE, Portugal
2019/10/11 - 2019/10/12	Atualização em Cirurgia XXXII Jornadas Portuguesas de Cirurgia Moderador da mesa redonda: Prolapso retal Congresso	XXXII Jornadas Portuguesas de Cirurgia Centro Hospitalar Universitário do Porto EPE, Portugal
2019/09/25 - 2019/09/27	Atualização em coloproctologia ESCP 14th Scientific and Annual Meeting Participante e Representante Nacional da ESCP (reuniões paralelas). Congresso	ESCP 14th Scientific and Annual Meeting European Society of Coloproctology, Reino Unido
2019/06/14 - 2019/06/15	Atualização em Cirurgia Colorectal Spring colorectal meeting 2019 Braga, 14 e 15 de junho de 2019. Moderador da mesa redonda: “Panel III”. Encontro	Spring colorectal meeting 2019 Hospital de Braga, Portugal
2019/06/12 - 2019/06/14	Atualização em Cirurgia minimamente invasiva EAES Annual Meeting – 2019 Congresso	EAES Annual Meeting – 2019 The European Association for Endoscopic Surgery, Países Baixos
2019/06/07 - 2019/06/07	Atualização em coloproctologia III Jornadas de Cirurgia Colorectal entre o Lis e Tejo Leiria, 7 de Junho de 2019 Encontro	III Jornadas de Cirurgia Colorectal entre o Lis e Tejo Hospital de Santo André, Portugal Hospital Distrital de Santarém EPE, Portugal
2019/04/29 - 2019/05/21	Introdução à Bioestatística e suas aplicações em investigação clínica e epidemiológica: CURSO (24 horas) Porto Outro	Introdução à Bioestatística e suas aplicações em investigação clínica e epidemiológica: Universidade do Porto Instituto de Saúde Pública, Portugal

2019/05/15 - 2019/05/17	Atualização em coloproctologia XXIII REUNIÓN NACIONAL – FAECP Valhadolid, 15 a 17 de maio de 2019 Congresso	XXIII REUNIÓN NACIONAL – FAECP Asociación Española de Coloproctología, Espanha
2019/04/24 - 2019/04/27	Atualização em cancro colorectal EFR Congress 2019 Congresso	
2019/03/21 - 2019/03/23	Atualização em Cirurgia XXXIX Congresso Nacional de Cirurgia Tomar, 21 a 23 de março de 2019. Moderador da sessão: Comunicações Orais - 2 Coordenador e palestrante do Módulo I – Capítulo de Coloproctologia da Sociedade Portuguesa de Cirurgia. Congresso	XXXIX Congresso Nacional de Cirurgia Sociedade Portuguesa de Cirurgia, Portugal
2019/02/07 - 2019/02/08	Atualização em Cirurgia Colorectal Winter colorectal meeting 2019 Braga, 7 e 8 de fevereiro de 2019. Moderador da mesa redonda: “Mesa 2: Cirurgia NOSE do cólon esquerdo e recto - How I do it - vídeos”. Encontro	Winter colorectal meeting 2019 Hospital de Braga, Portugal
2019/02/01 - 2019/02/02	Atualização em Patologia Funcional do Pavimento Pélvico Simpósio APNUG 2019 Évora, 1 e 2 de fevereiro de 2019. Palestrante Encontro	Simpósio APNUG 2019
2019/01/17 - 2019/01/18	Atualização em Coloproctologia Primeiras Jornadas de Cirurgia Colorectal do Algarve Portimão, 17 e 18 de janeiro de 2019. Moderador da mesa redonda: “Sessão IV - ESTOMAS DE DERIVAÇÃO – CONTROVÉRSIAS” Encontro	Primeiras Jornadas de Cirurgia Colorectal do Algarve
2018/12/02 - 2018/12/06	Atualização em Cirurgia Colorectal Congresso	European Colorectal Congress & Masterclass Medkongress AG, Suiça

2018/09/26 - 2018/09/28	Atualização em coloproctologia Congresso	European Society of Coloproctology, Reino Unido
2018/09/12 - 2018/09/13	Atualização em TATME Curso Hands-on Outro	Amsterdam UMC Locatie VUmc Afdeling KNO-hoofd-halschirurgie, Países Baixos
2018/09/09 - 2018/09/11	Atualização em cirurgia colorectal Congresso	30th anniversary IASGO World Congress – 2018
2018/04/13 - 2018/04/13	Atualização em cirurgia colorectal Moderador Encontro	International Course of Surgery: Hepatobiliopancreatic & Colorectal Surgery
2018/03/08 - 2018/03/10	Atualização em Cirurgia Congresso	XXXVIII Congresso Nacional de Cirurgia Sociedade Portuguesa de Cirurgia, Portugal
2018/02/22 - 2018/02/23	Atualização em cancro do reto Encontro	Angels and Demons in Rectal Cancer: Challenging the Dogmas Fundação Champalimaud, Portugal
2018/02/14 - 2018/02/17	Atualização em Doença inflamatória intestinal 15 Unidades de Crédito (European CME credits) Viena (Austria) Congresso	European Crohn's and Colitis Organisation, Áustria
2017/10/05 - 2017/10/06	Atualização em Coloproctologia Encontro	
2017/09/20 - 2017/09/22	Atualização em Coloproctologia Congresso	European Society of Coloproctology, Reino Unido
2017/06/24 - 2017/06/24	Atualização em Coloproctologia Encontro	

2017/06/14 - 2017/06/17	Atualização em Coloproctologia Congresso	The European Association for Endoscopic Surgery, Países Baixos
2017/05/17 - 2017/05/19	Atualização em Coloproctologia ” , 17 a 19 de maio de 2017 Congresso	Anual Meeting of AECP - Almeria Asociación Española de Coloproctología, Espanha
2017/03/16 - 2017/03/18	Atualização em Coloproctologia Congresso	Sociedade Portuguesa de Cirurgia, Portugal
2017/01/09 - 2017/01/09	Atualização em coloproctologia TaTME - Transanal Total Mesorectal Excision Masterclass Congresso	TaTME - Transanal Total Mesorectal Excision Masterclass
2016/11/28 - 2016/12/02	Atualização em Coloproctologia Congresso	European Colorectal Congress - St. Gallen
2016/11/24 - 2016/11/25	Atualização em Coloproctologia Congresso	Sociedade Portuguesa de Coloproctologia, Portugal

Júri de grau académico

	Tema Tipo de participação	Nome do candidato (Tipo de grau) Instituição / Organização
2016/07/19	Qualidade da profilaxia da úlcera de stress: avaliação da eficácia de ações de formação e análise económica Arguente principal	Daniela Filipa de Sousa Viana (Mestrado) Universidade do Minho Escola de Medicina, Portugal
2016/07/19	Avaliação dos níveis de vitamina D numa população obesa, pré e pós cirurgia bariátrica. Arguente principal	Miguel Aires da Rocha Garcês (Mestrado) Universidade do Minho Escola de Medicina, Portugal

Arbitragem científica em conferência

	Nome da conferência	Local da conferência
2021/09/22 -	ESCP 2021 Virtual Conference	Virtual

2021/09/24		
2019/09/25 - 2019/09/27	ESCP 14th Scientific and Annual Meeting	Viena
2018/11/22 - 2018/11/23	XXVIII Congresso Nacional de Coloproctologia	Porto
2018/09/26 - 2018/09/28	ESCP 13rd Scientific and Annual Meeting	Nice (França)
2018/03/08 - 2018/03/10	XXXVIII Congresso Nacional de Cirurgia	Lisboa
2017/09/20 - 2017/09/22	ESCP Twelfth Scientific and Annual Meeting	Berlim
2016/11/24 - 2016/11/25	XXVI Congresso Nacional de Coloproctologia	Figueira da Foz

Comissão de avaliação

	Descrição da atividade Tipo de assessoria	Instituição / Organização	Entidade financiadora
2019/02/27 - 2019/11/30	Grupo de Trabalho para Avaliação da Situação dos Blocos Operatórios - Despacho n.º 2007/2019 Membro	Ministério da Saúde, Portugal	

Consultoria / Parecer

	Descrição da atividade	Instituição / Organização
2021/11/02 - Atual	Avaliador de Unidades de Saúde - Departamento de Qualidade em Saúde	Direcção-Geral da Saúde, Portugal

Curso / Disciplina lecionado

	Disciplina	Curso (Tipo)	Instituição / Organização
2014/01/02 - Atual	Processos Complexos de Doença Crítica	Enfermagem à Pessoa em Situação Crítica (Mestrado integrado)	Instituto Politécnico de Leiria Escola Superior de Saúde, Portugal

Expedição científica

	Descrição da atividade	Instituição / Organização
2017/09/21 - 2020/09/24	Representante Nacional da ESCP	European Society of Coloproctology, Reino Unido

Membro de associação

	Nome da associação	Tipo de participação
2022/03/18 - Atual	Sociedade Portuguesa de Cirurgia	Secretário - Geral
2019/01/03 - Atual	Secção Sub-Regional de Leiria da Ordem dos Médicos	Presidente da Assembleia Sub-regional
2019/01/02 - Atual	ciTechCare	Membro - Colaborador; Membro do Join Steering Committe (2020)
2018/12/11 - Atual	Educational Union - Medical Association (EU-MÂO)	Presidente da Direção
2017/02/02 - Atual	· European Crohn`s and Colitis Organization (ECCO);	Sócio
2016/02/02 - Atual	International Association of Surgeons, Gastroenterologists and Oncologists (IASGO).	Sócio
2014/11/24 - Atual	Sociedade Portuguesa de Coloproctologia	Vogal
2014/04/02 - Atual	Sociedade Portuguesa de Cirurgia Minimamente Invasiva	Sócio
2014/02/02 - Atual	European Association for Endoscopic Surgery (EAES);	Sócio
2012/03/03 - Atual	European Society of Coloproctology - ESCP	Sócio
2010/02/02 - Atual	International Society of Surgery (ISS) /Société Internationale de Chirurgie (SIC), and of the International Association for Trauma Surgery and Intensive Care (IATSIC), society integrated in ISS/SIC.	Sócio
1999/11/23 - Atual	Ordem dos Médicos Portugueses - (Cédula Profissional nº 39269)	Membro
2020/03/16 - 2022/03/18	Sociedade Portuguesa de Cirurgia	Secretário - Geral
2017/09/22 - 2020/09/24	European Society of Coloproctology - ESCP	National Representative
2015/03/15 - 2019/03/18	Capitulo De Coloproctologia da Sociedade Portuguesa de Cirurgia	Vogal
2011/01/02 - 2019/01/02	Secção Sub-Regional de Leiria da Ordem dos Médicos	Vogal

Membro de comissão

	Descrição da atividade Tipo de participação	Instituição / Organização
2018/01 - Atual	Centro de Referência - Cancro Recto / Unidade Funcional de Patologia colorectal Coordenador	Hospital de Santo André, Portugal
2010/01 - 2022/04	Grupo de Feridas Membro	Hospital de Santo André, Portugal
2008/02 - 2022/04	Grupo MMU Membro	Hospital de Santo André, Portugal
2010/01 - 2018/03	Coordenação Hospitalar de Doação Coordenador	Hospital de Santo André, Portugal
2009/01 - 2018/03	Grupo de Gestão do Risco Membro	Hospital de Santo André, Portugal
2008/08 - 2016/02	Comissão de Farmácia e Terapêutica; Membro	Hospital de Santo André, Portugal

Outro júri / avaliação

	Descrição da atividade	Instituição / Organização
2012 - Atual	Exame Final de Cirurgia Geral	Ordem dos Médicos, Portugal

Tutoria

	Tópico	Nome do aluno
2021/04/01 - Atual	Internato Médico em Cirurgia Geral	Inês Aranha Sousa
2015/01/02 - 2020/11/25	Internato Médico em Cirurgia Geral	Inês Ferrer Sales

Distinções

Prémio

2021	Melhor Vídeo - Hemicolectomia Direita Laparoscópica com uso de Verde de Indocianina Sociedade Portuguesa de Cirurgia, Portugal
2018	Melhor Poster - Ta-TME: Short term outcomes Sociedade Portuguesa de Coloproctologia, Portugal

2015	Melhor Artigo Científico publicado em 2015 na Revista Portuguesa de Coloproctologia Sociedade Portuguesa de Coloproctologia, Portugal
2015	Melhor Aluno 2014 - Mestrado em Gestão e Economia da Saúde Universidade de Coimbra Faculdade de Economia, Portugal
2014	Menção Honrosa - Póster - HEMORRAGIA DIGESTIVA BAIXA – SÍNDROME DE KLIPPEL-TRENAUNAY Sociedade Portuguesa de Coloproctologia, Portugal
2013	Melhor Vídeo - Hemicolectomia Direita com Acesso por Porta Única Sociedade Portuguesa de Coloproctologia, Portugal

Título

2014	Competência em emergência médica Ordem dos Médicos, Portugal
2014	Mestre em Gestão e Economia da Saúde Universidade de Coimbra Faculdade de Economia, Portugal
2013	Fellow of the Division of Coloproctology, of the UEMS Section of Surgery & European Board of Surgery. European Union of Medical Specialists, Bélgica

APPENDIX 10

- PORTUGUESE DATA PROTECTION AUTHORITY DECLARATION

Autorização n.º 9930/ 2016

Nuno José Gomes Rama notificou à Comissão Nacional de Protecção de Dados (CNPD) um tratamento de dados pessoais com a finalidade de realizar um Estudo Clínico sem Intervenção, denominado COMO DETETAR PRECOCEMENTE UMA DEISCÊNCIA ANASTOMÓTICA COLORECTAL: UTILIDADE DE CRITERIOS CLINICOS E BIOMARCADORES. .

O participante é identificado por um código especificamente criado para este estudo, constituído de modo a não permitir a imediata identificação do titular dos dados; designadamente, não são utilizados códigos que coincidam com os números de identificação, iniciais do nome, data de nascimento, número de telefone, ou resultem de uma composição simples desse tipo de dados. A chave da codificação só é conhecida do(s) investigador(es).

É recolhido o consentimento expresso do participante ou do seu representante legal.

A informação é recolhida diretamente do titular e indiretamente do processo clínico.

As eventuais transmissões de informação são efetuadas por referência ao código do participante, sendo, nessa medida, anónimas para o destinatário.

A CNPD já se pronunciou na Deliberação n.º 1704/2015 sobre o enquadramento legal, os fundamentos de legitimidade, os princípios aplicáveis para o correto cumprimento da Lei n.º 67/98, de 26 de outubro, alterada pela Lei n.º 103/2015, de 24 de agosto, doravante LPD, bem como sobre as condições e limites aplicáveis ao tratamento de dados efetuados para a finalidade de investigação clínica.

No caso em apreço, o tratamento objeto da notificação enquadra-se no âmbito daquela deliberação e o responsável declara expressamente que cumpre os limites e condições aplicáveis por força da LPD e da Lei n.º 21/2014, de 16 de abril, alterada pela Lei n.º 73/2015, de 27 de junho – Lei da Investigação Clínica –, explicitados na Deliberação n.º 1704/2015.

O fundamento de legitimidade é o consentimento do titular.

A informação tratada é recolhida de forma lícita, para finalidade determinada, explícita e legitima e não é excessiva – cf. alíneas a), b) e c) do n.º 1 do artigo 5.º da LPD.

Assim, nos termos das disposições conjugadas do n.º 2 do artigo 7.º, da alínea a) do n.º 1 do artigo 28.º e do artigo 30.º da LPD, bem como do n.º 3 do artigo 1.º e do n.º 9 do artigo 16.º ambos da Lei de Investigação Clínica, com as condições e limites explicitados na Deliberação da CNPD n.º 1704/2015, que aqui se dão por reproduzidos, autoriza-se o presente tratamento de dados pessoais nos seguintes termos:

Responsável – Nuno José Gomes Rama

Finalidade – Estudo Clínico sem Intervenção, denominado COMO DETETAR PRECOCEMENTE UMA DEISCÊNCIA ANASTOMÓTICA COLORECTAL: UTILIDADE DE CRITERIOS CLINICOS E BIOMARCADORES.

Categoria de dados pessoais tratados – Código do participante; idade/data de nascimento; género; dados antropométricos; sinais vitais; dados da história clínica; dados dados de exame físico; dados de meios complementares de diagnóstico; medicação prévia concomitante; dados de qualidade de vida/efeitos psicológicos; eventos adversos

Exercício do direito de acesso – Através dos investigadores, presencialmente

Comunicações, interconexões e fluxos transfronteiriços de dados pessoais identificáveis no destinatário – Não existem

Prazo máximo de conservação dos dados – A chave que produziu o código que permite a identificação indireta do titular dos dados deve ser eliminada 5 anos após o fim do estudo.

Da LPD e da Lei de Investigação Clínica, nos termos e condições fixados na presente Autorização e desenvolvidos na Deliberação da CNPD n.º 1704/2015, resultam obrigações que o responsável tem de cumprir. Destas deve dar conhecimento a todos os que intervenham no tratamento de dados pessoais.

Lisboa, 14-09-2016

A Presidente

Filipa Calvão

APPENDIX 11

- INFORMED CONSENT DOCUMENT

APÊNDICE 1:

CONSENTIMENTO INFORMADO

COMO DETETAR PRECOCEMENTE UMA DEISCÊNCIA ANASTOMÓTICA COLORECTAL: UTILIDADE DE CRITERIOS CLINICOS E BIOMARCADORES.

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Informação ao doente e consentimento informado

"COMO DETETAR PRECOCEMENTE UMA DEISCÊNCIA ANASTOMÓTICA COLORECTAL: UTILIDADE DE CRITERIOS CLINICOS E BIOMARCADORES."

Informação ao doente / representante legal

Introdução

Está a ser convidado(a) a participar num estudo clínico, como doente ou seu representante legal. Antes de decidir se quer participar, é importante que compreenda porque está a ser feito este estudo. Por favor, leve o tempo que precisar a ler esta informação atentamente e discuta-a com a família e amigos se assim o desejar. O seu médico utilizará o tempo necessário para lhe explicar o estudo. Por favor, pergunte ao seu médico a um elemento da equipa médica do estudo se necessitar de mais informações ou esclarecimentos.

Qual o objetivo do estudo?

O objetivo do estudo é determinar qual a melhor forma de detetar o mais cedo possível uma deiscência de uma anastomose colorectal (adiante explicado), nos doentes submetidos a cirurgia colorectal (operações ao intestino grosso) que permitam a junção no mesmo ato do intestino (anastomose). O estudo inclui os doentes operados no Centro Hospitalar de Leiria.

Porque fui escolhido?

Foi convidado a participar porque lhe foi diagnosticada uma doença no intestino que necessita de cirurgia colorectal para o seu tratamento. No período de recuperação (pós-operatório) uma das complicações que pode surgir é a deiscência de anastomose, de modo que se pretende detetá-la o mais cedo possível, no sentido de a tratar com mais rapidez e assim tentar diminuir os riscos associados.

O que é uma deiscência de uma anastomose?

Quando tratamos uma doença do intestino com a resseção (remoção) de um segmento de intestino, podemos quando possível voltar a anastomosar (unir ou juntar) os topos com o objetivo de manter o trânsito intestinal natural. Porém as anastomoses (junções do intestino) podem não cicatrizar adequadamente e sofrer a chamada deiscência. Esta deiscência causa a infeção da cavidade abdominal que pode determinar uma infeção grave local e depois disseminar-se, sendo potencialmente ameaçadora da vida.

Detetar (diagnosticar) precocemente a infeção que resulta da deiscência é muito importante para se fazer o tratamento adequado o mais cedo possível. O diagnóstico nem sempre é fácil e implica uma avaliação clínica minuciosa, análises de sangue e exames de imagem (radiografias).

Tenho de participar?

A sua participação neste estudo é inteiramente voluntária. Cabe a si decidir se quer participar ou não. Se decidir não participar o seu cuidado médico futuro não será afetado de nenhuma maneira.

Se decidir participar, ser-lhe-á pedido que assine uma folha chamada consentimento informado. Uma cópia do folheto informativo ao doente e do consentimento informado assinado ser-lhe-á entregue. Se mudar de ideias mais tarde, basta informar o seu médico ou a equipa do estudo que já não deseja participar.

O que me vai acontecer ou ao meu representado?

Se aceitar participar neste estudo, ser-lhe-á solicitado a si ou ao seu representado amostras de sangue diárias (a maioria das quais já são rotina) para detetar sinais de deiscência de anastomose intestinal.

O que vai ser feito com as informações pessoais colhidas durante o estudo?

A equipa de médico e enfermeiros que participa neste estudo irá analisar mais tarde todas apenas este grupo é que sabe o seu nome ou do seu representado, que nunca será revelado a outras pessoas. Assim, embora as informações sobre a sua doença sejam investigadas, apenas o seu médico tem acesso a informações sobre a sua identificação.

Vou ser informado sobre o resultado das análises para diagnóstico da deiscência da anastomose?

A equipa médica vai dar-lhe a informação que desejar sobre o resultado das análises. Em caso de deteção de deiscência de anastomose, ser-lhe-á informado se as análises contribuíram para a identificação do problema, e o tratamento proposto de acordo com os protocolos em uso na instituição.

Quem reviu este estudo?

Uma comissão do Centro Hospitalar de Leiria (Comissão de Ética) avaliou os objetivos e os procedimentos deste estudo e deu a sua aprovação e opinião favorável.

P´LA EQUIPA DO ESTUDO

Nuno Rama, MD, FEBS-C; MBA (H&E) – Investigador Principal

CONSENTIMENTO INFORMADO

"COMO DETETAR PRECOCEMENTE UMA DEISCÊNCIA ANASTOMÓTICA COLORECTAL: UTILIDADE DE CRITERIOS CLINICOS E BIOMARCADORES."

Compreendi a informação que me foi prestada pelo Dr. _____ respeitante ao estudo de "Como detetar precocemente uma deiscência anastomótica colorectal: utilidade de critérios clínicos e biomarcadores."

Confirmo que fui informado de que:

a. A minha participação ou do meu representado é voluntária e a minha recusa não terá influência na forma de tratamento futura.
b. Ao participar serão recolhidos dados sobre mim ou sobre o meu representado, nomeadamente sobre doenças no passado e estado de saúde atual.
c. Será necessário colher amostras de sangue para ajuda no despiste da deiscência da anastomose intestinal.
d. Os resultados, incluindo entre outros dados, a idade, o sexo, e as minhas doenças atuais ou passadas, serão utilizados de forma completamente anónima e sem fazerem referência aos meus dados pessoais (ou do meu representado).
e. Na eventualidade da apresentação e da publicação dos resultados deste projeto, será garantida a confidencialidade da minha identidade (ou do meu representado).

Assim, declaro a minha vontade em participar voluntariamente neste estudo. Concordo com a utilização dos meus registos clínicos, ou do meu representado tal como acima descrito.

Nome do Doente (em letra de imprensa)

Nome do Representante Legal (se aplicável, em letra de imprensa)

_____ **___/____/20___**

Assinatura do Doente **Data**

_____ **___/____/20___**

Assinatura do Representante Legal **Data**
(se aplicável)

O doente foi por mim informado sobre a natureza e objetivo deste estudo.

Nome do Médico (em letra de imprensa)

_____ **___/____/20___**

Assinatura do Médico **Data**

CONSENTIMENTO INFORMADO
(CÓPIA DO UTENTE)

"COMO DETETAR PRECOCEMENTE UMA DEISCÊNCIA ANASTOMÓTICA COLORECTAL: UTILIDADE DE CRITERIOS CLINICOS E BIOMARCADORES."

Compreendi a informação que me foi prestada pelo Dr. _____ respeitante ao estudo de "Como detetar precocemente uma deiscência anastomótica colorectal: utilidade de critérios clínicos e biomarcadores."

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CONSENTIMENTO INFORMADO
(CÓPIA DO UTENTE)

Assim, declaro a minha vontade em participar voluntariamente neste estudo. Concordo com a utilização dos meus registos clínicos, ou do meu representado tal como acima descrito.

Nome do Doente (em letra de imprensa)

Nome do Representante Legal (se aplicável, em letra de imprensa)

_____	**___/____/20___**
Assinatura do Doente	**Data**
_____	**___/____/20___**
Assinatura do Representante Legal (se aplicável)	**Data**

O doente foi por mim informado sobre a natureza e objetivo deste estudo.

Nome do Médico (em letra de imprensa)

_____	**___/____/20___**
Assinatura do Médico	**Data**

APPENDIX 12

- STUDY PROTOCOL DOCUMENT

STUDY PROTOCOL

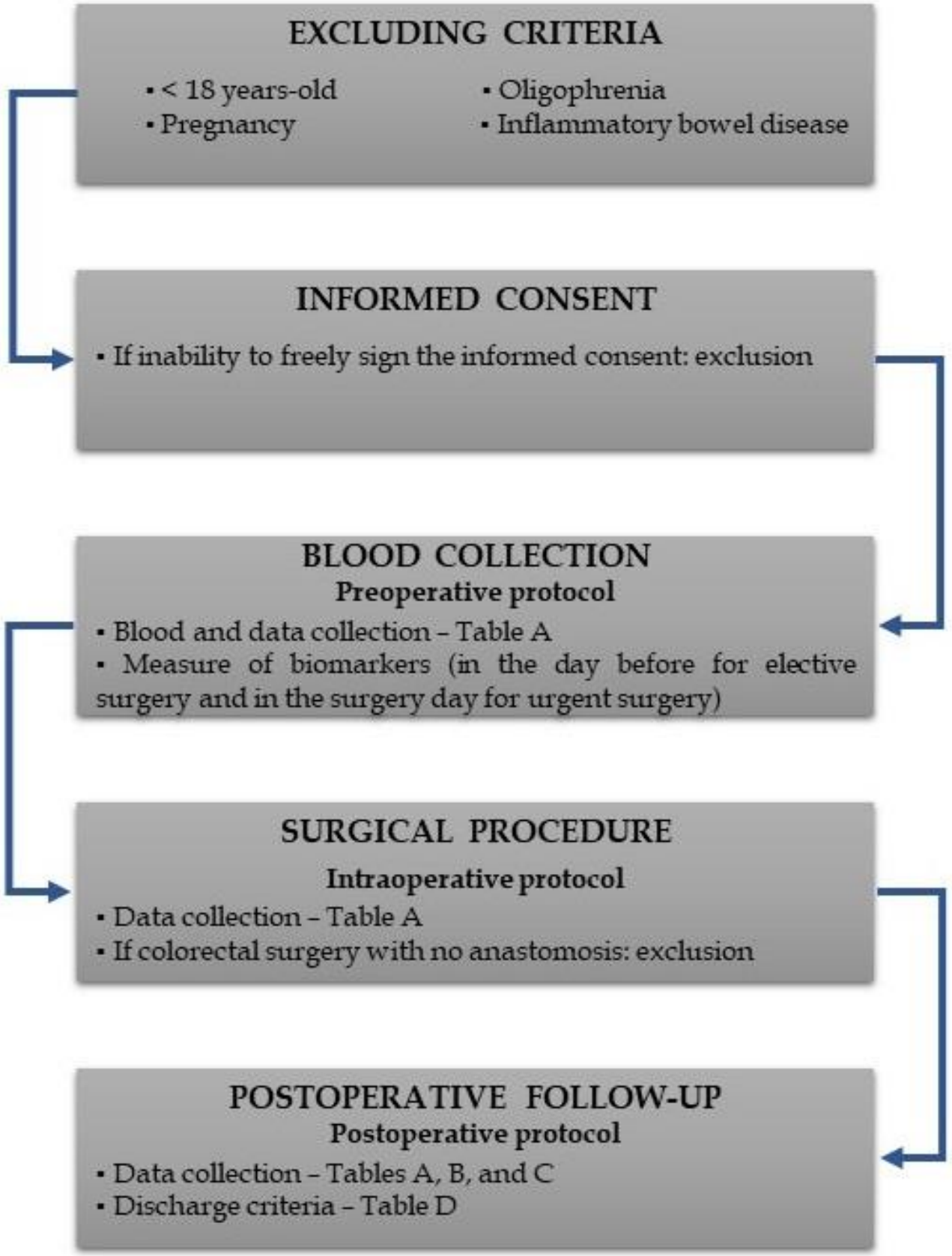


Table A Protocol variables

Variables	
Demographic	Age
	Sex
Preoperative	Health-related quality of life – EQ5D5L
	Nutritional status
	Comorbidities
	Charlson Comorbidity Index score
	Smoking and alcohol habits
	Allergies
	Previous abdominal surgery
	Steroids or immunosuppression in the last 6 mo
	Preoperative diagnosis
	Preoperative staging
	Bowel preparation
	American Society of Anesthesiologists grade
Intraoperative	Type of anesthesia
	Anastomosis technique
	Blood loss
	Blood transfusion
	Surgical complications

	Level of surgical contamination
	Duration of surgical procedure
	Surgical specimen
	Surgical approach
Postoperative	Morbidity
	Mortality
	Time of follow-up
	Intensive care unit stay

Table B Postoperative follow-up: clinical findings

Signs/Symptoms	DBS	POD1	POD2	POD3	POD4	POD5
Temperature						
Heart rate						
Respiratory rate						
Urinary debit						
Mental status						
Clinical status						
Gastric emptying						
Bowel movements						
Abdominal pain						
Surgical wound infection						
Pain (VAS)						
Complications						

Intensive care unit

DBS: Day before surgery; POD: Postoperative day; VAS: Visual analogue scale.

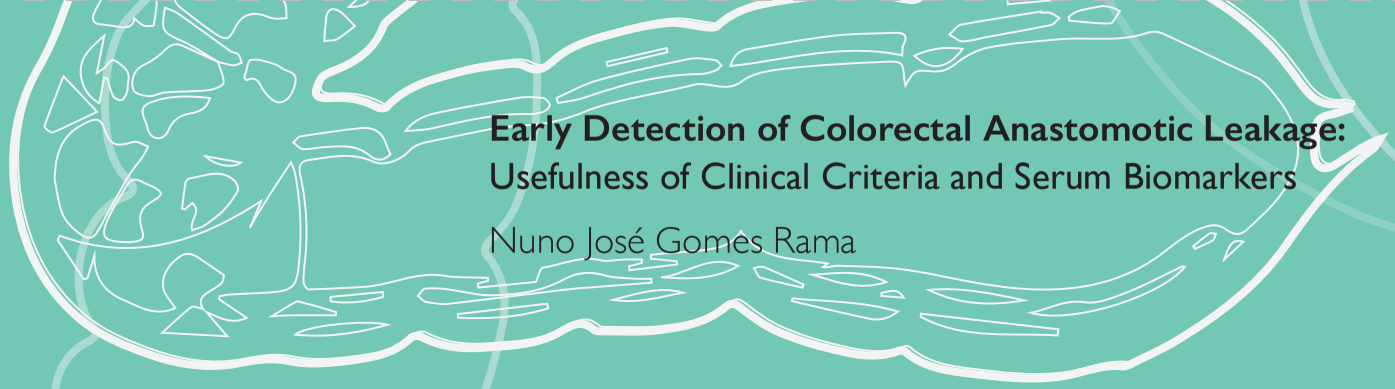
Table C Postoperative follow-up: laboratory findings

Biomarkers	DBS	POD1	POD2	POD3	POD4	POD5
White blood cell count						
Eosinophil cell count						
Urea						
Creatinine						
C-reactive protein						
Procalcitonin						
Calprotectin						
Albumin						

DBS: Day before surgery; POD: Postoperative day.

Table D Discharge clinical criteria

Oral tolerance
Bowel movements
Pain control with oral analgesic
No signs of sepsis
Institutional social criteria for discharge fulfilled



Early Detection of Colorectal Anastomotic Leakage:
Usefulness of Clinical Criteria and Serum Biomarkers

Nuno José Gomes Rama

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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