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The Usefulness of Inflammatory Biomarkers to Predict Anastomotic Leakage after Colorectal Surgery: Systematic Review and Meta-Analysis

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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.

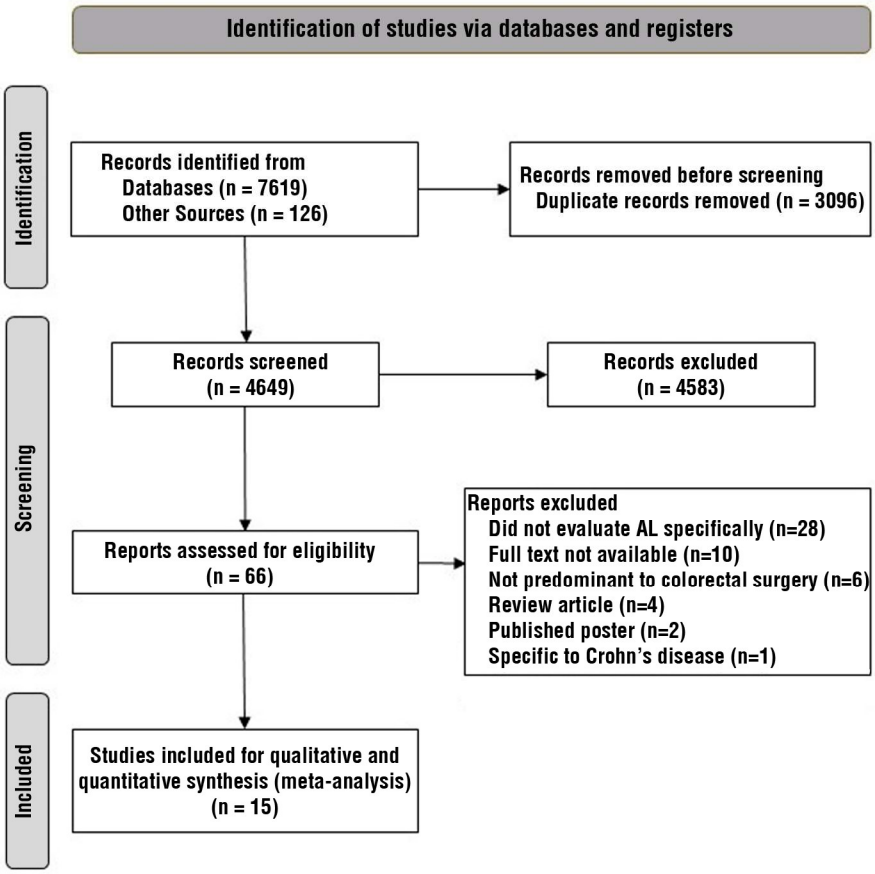
Study characteristics

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

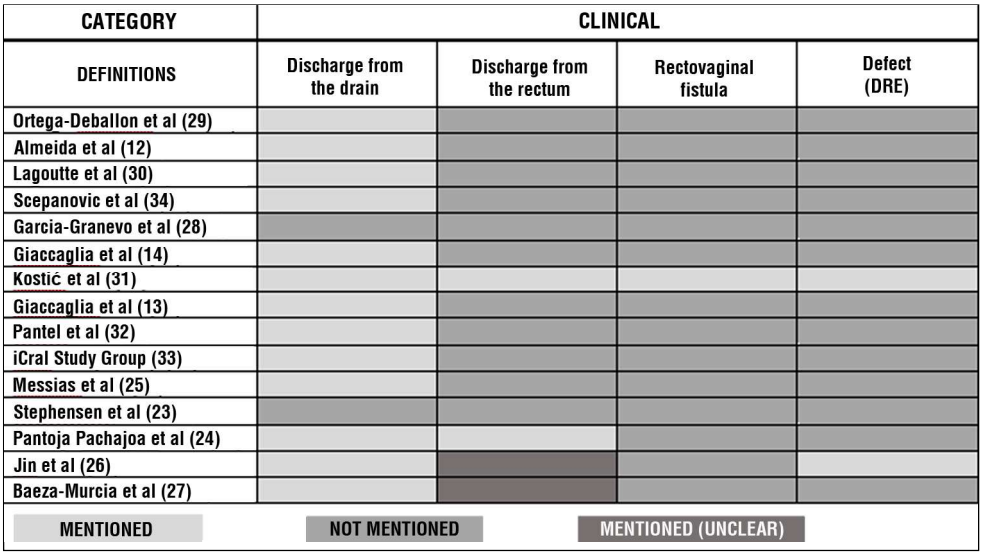


Table S2 - Qualitative analysis of AL definitions from the fifteen selected studies: clinical category. DRE, digital rectal examination

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)	MENTIONED	NOT MENTIONED	MENTIONED (UNCLEAR)	NOT MENTIONED
Almeida et al (12)	MENTIONED (UNCLEAR)	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED
Lagoutte et al (30)	NOT MENTIONED	MENTIONED	MENTIONED	NOT MENTIONED
Scepanovic et al (34)	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED
Garcia-Granevo et al (28)	MENTIONED	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED
Giaccaglia et al (14)	MENTIONED	MENTIONED	MENTIONED	NOT MENTIONED
Kostić et al (31)	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED
Giaccaglia et al (13)	MENTIONED	MENTIONED	MENTIONED	NOT MENTIONED
Pantel et al (32)	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED
iCral Study Group (33)	MENTIONED	MENTIONED	NOT MENTIONED	NOT MENTIONED
Messias et al (25)	NOT MENTIONED	MENTIONED	MENTIONED	NOT MENTIONED
Stephensen et al (23)	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED	NOT MENTIONED
Pantoja Pachajoa et al (24)	MENTIONED	MENTIONED	MENTIONED	NOT MENTIONED
Jin et al (26)	MENTIONED	MENTIONED	MENTIONED	NOT MENTIONED
Baeza-Murcia et al (27)	MENTIONED	NOT MENTIONED (UNCLEAR)	NOT MENTIONED (UNCLEAR)	NOT MENTIONED

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-

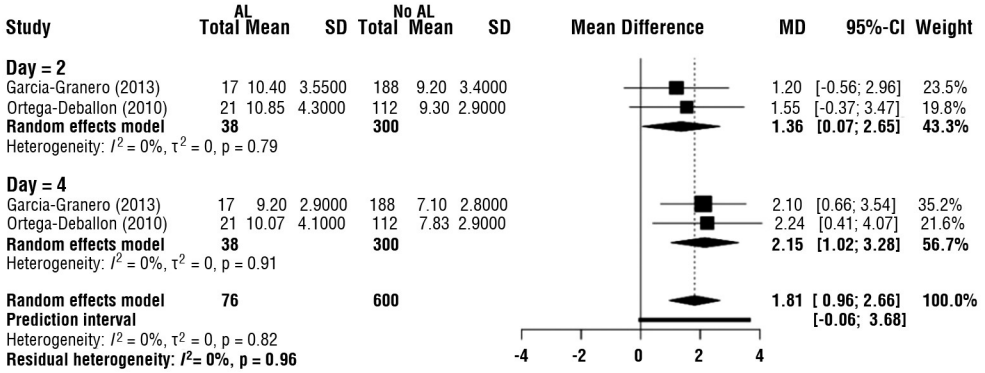


Figure S1 - Forest plot for WBC data showing the results of random-effects meta-analysis on different postoperative days

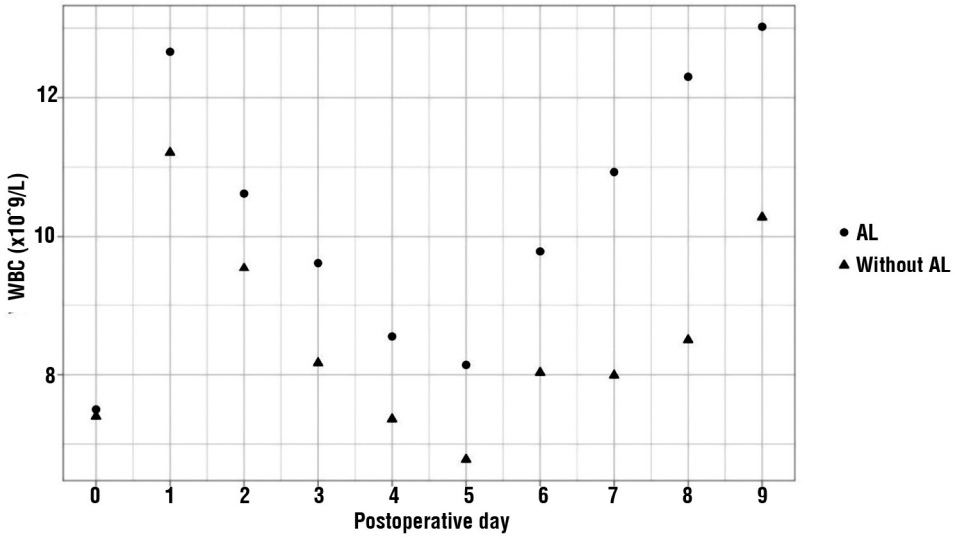


Figure S2 - WBC levels in the postoperative period in relation to AL. Values at each time point represent the pooled median/mean WBC level from the included studies [Ortega-Deballon (2010); Almeida (2012); Garcia-Granero (2013); Scepanovic (2013); Pantoja Pachajoa (2021)], with individual studies weighted by their sample size. AL, anastomotic leakage.

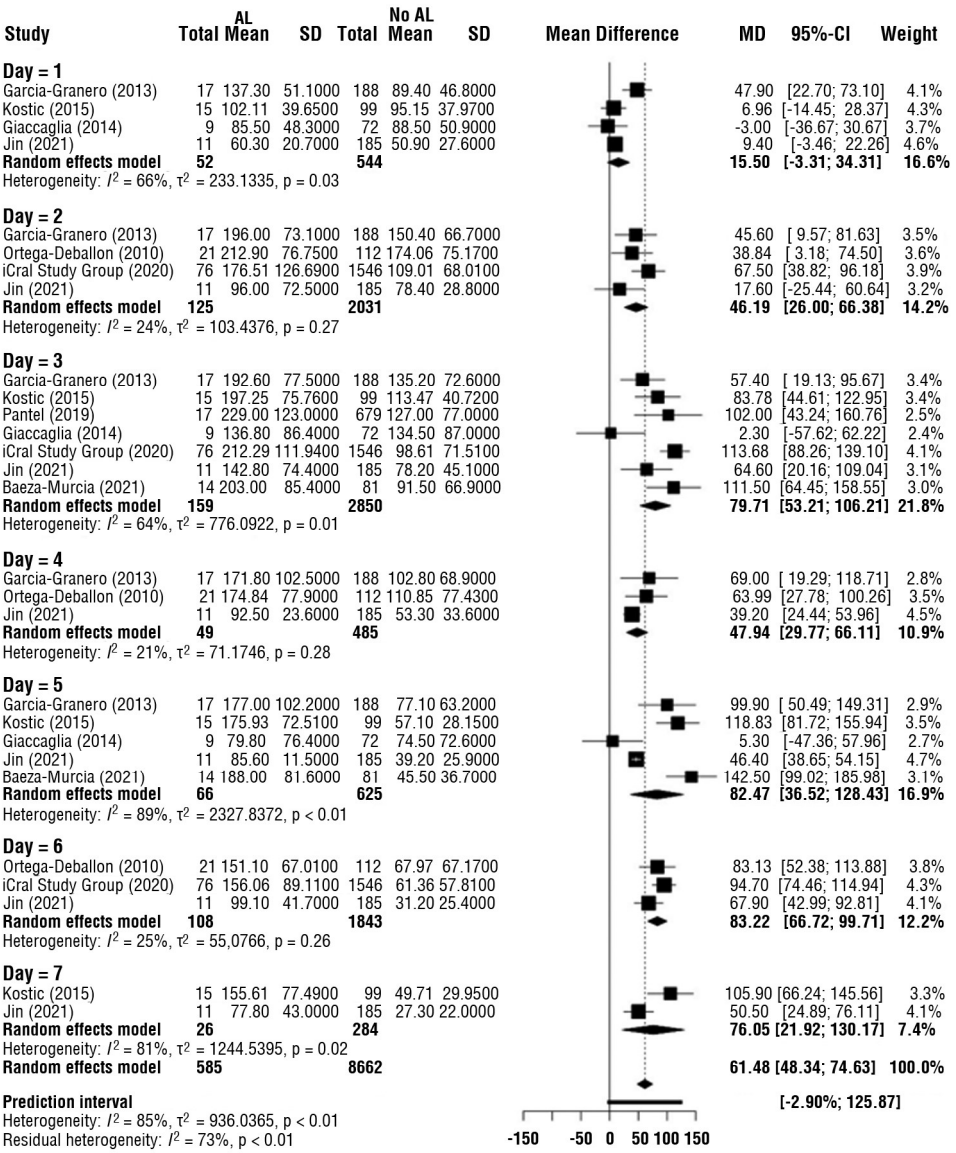


Figure 2 - Forest plot for CRP data showing the results of random-effects meta-analysis on different postoperative days

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

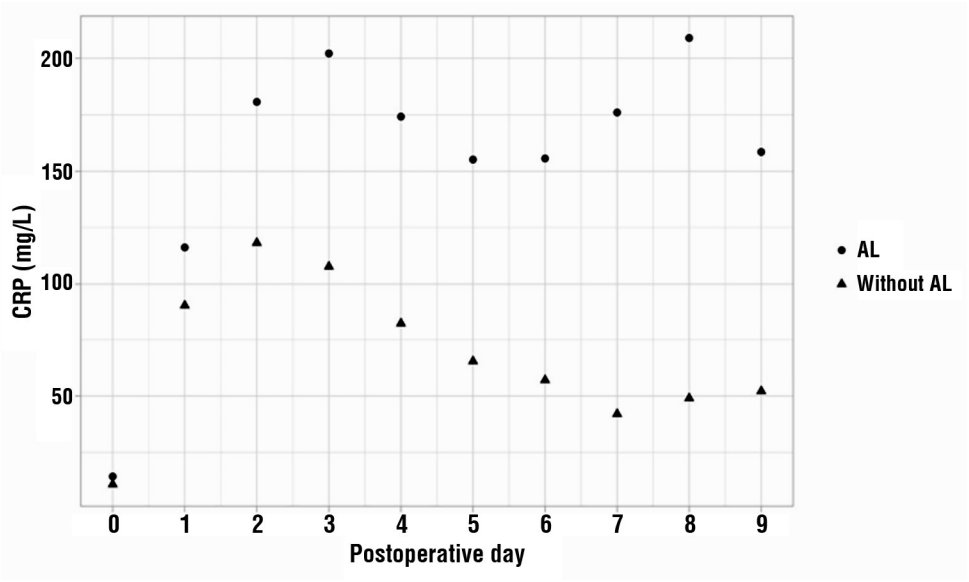


Figure 3 - C-reactive protein (CRP) levels in the postoperative period in relation to AL. Values at each time point represent the pooled median/mean CRP level from the included studies [Ortega-Deballon (2010); Almeida (2012); Lagoutte (2012); Garcia-Granero (2013); Scepanovic (2013); Giaccaglia (2014); 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

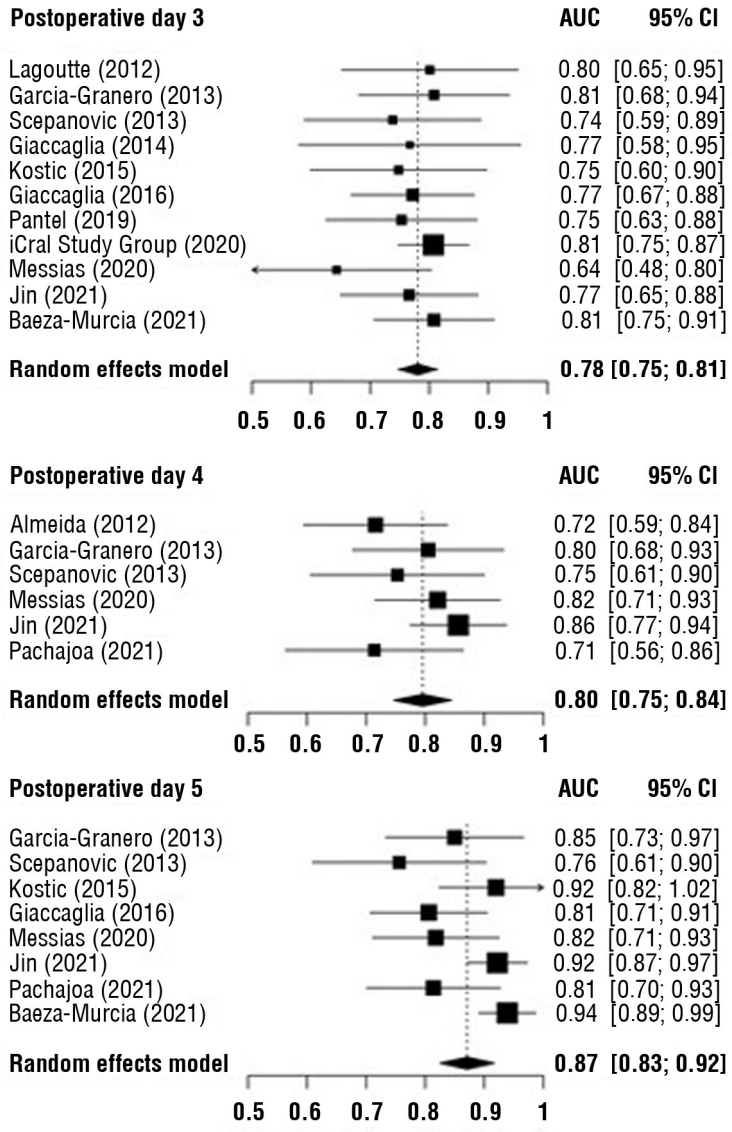


Figure S3 - Pooled area under the curve for anastomotic leakage at POD 3 (I^2 = 0.0%; Q = 4.87; p = 0.899), POD 4 (I^2 = 7.7%; Q = 5.42; p = 0.367) and POD 5 (I^2 = 55.1%; Q = 15.61; p = 0.029) for CRP. Values are shown with 95 per cent confidence intervals.

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: 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.

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

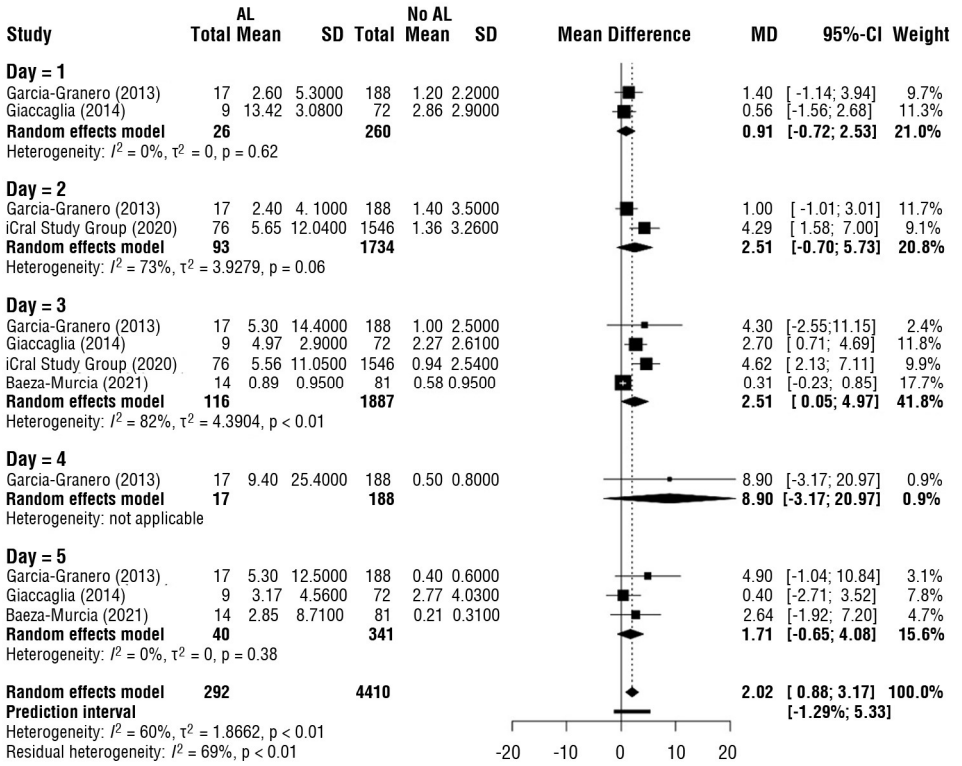


Figure 4 - Forest plot for PCT data showing the results of random-effects meta-analysis on different postoperative days. PCT, procalcitonin.

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.

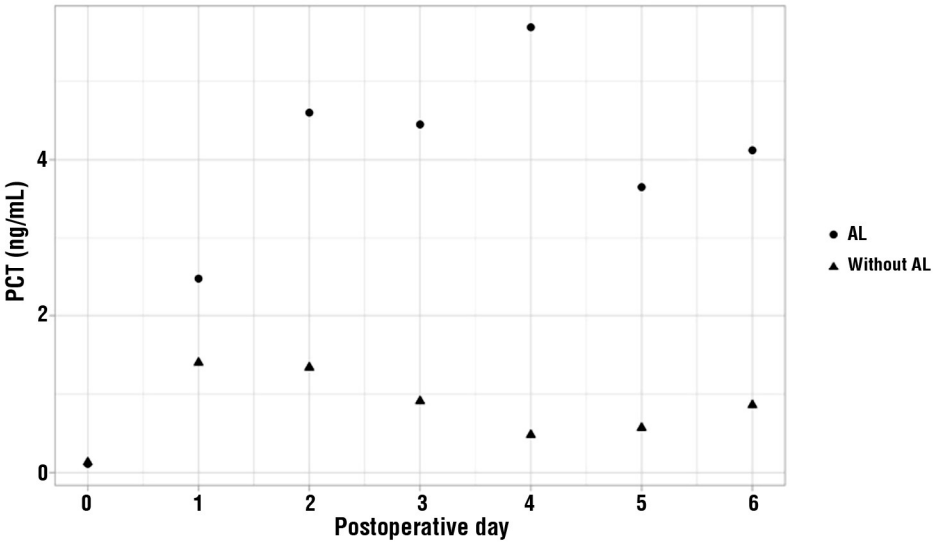


Figure S4 - Procalcitonin (PCT) levels in the postoperative period in relation to AL. Values at each time point represent the pooled median/ mean PCT level from the included studies [Lagoutte (2012); Garcia-Granero (2013); Giaccaglia (2014); Giaccaglia (2016); iCral Study Group (2020); Baeza-Murcia (2021)], with individual studies weighted by their sample size. AL, anastomotic leakage.

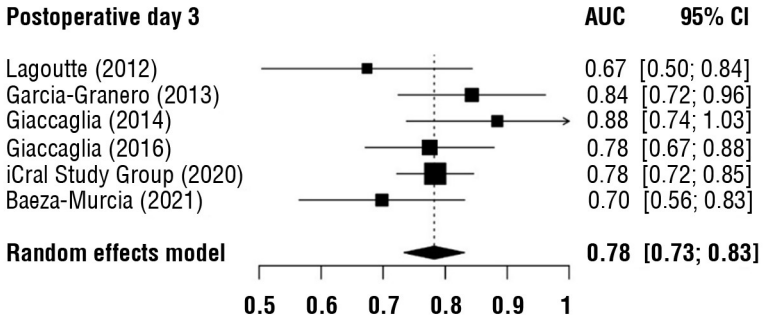


Figure S5 - Pooled area under the curve for anastomotic leakage at POD 3 for PCT (I2 = 16.4%; Q = 5.98; p = 0.308). Values are shown with 95 per cent confidence intervals. PCT, procalcitonin

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.

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