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Kynurenine Metabolism and Organ Dysfunction in Human Acute Pancreatitis

by

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Dedicated to my wife Eva and my baby daughter Katerina

Πάντες ἄνθρωποι τοῦ εἰδέναι ὀρέγονται φύσει.

All men by nature desire to know.

– ARISTOTLE (384-322 BC)

Declaration

I declare that the present thesis has been composed by myself and that the work presented herein represents research that I have undertaken in The University of Edinburgh and the Department of Clinical Surgery, Royal Infirmary of Edinburgh between February 2013 and December 2016. My role was instrumental in all aspects of the work presented. Neither this thesis nor any part thereof has been submitted for any other university degree or professional qualification, except for the degree of Doctor of Medicine by The University of Edinburgh, as specified. All sources of information have been acknowledged.

In keeping with the collaborative nature of this work, assistance from others has been received and is gratefully acknowledged. Recruitment of participants to the IMOFAP study was supported by the Wellcome Trust Clinical Research Facility (WTCRF) – subsequently renamed Edinburgh Clinical Research Facility. Blood sample processing and analysis by liquid chromatography–tandem mass spectrometry was performed by a team of experts of The University of Edinburgh: Xiaozhong Zheng, Margaret Binnie, Dr. Natalie Z. M. Homer, and Dr. Scott P. Webster.

The bespoke diarized alert/reminder software application for the IMOFAP study was developed in collaboration with GlaxoSmithKline plc. (GSK) software developers Nikolay Nikolov and Nikolay Angelov. The bespoke data collection software application for the IMOFAP study was developed in collaboration with Dr. Andreas Tsoumanis, software engineer of the National Technical University of Athens. Lung ultrasound scans were performed and interpreted by Professor John T. Murchison, Dr. Joanne Sharkey, and Dr. Zoe A. Davis, NHS Lothian radiologists.

The following peer-reviewed publications arising from my work, are included in the present thesis, and respectively form chapters 2, 3 and 4:

1. Increased levels of 3-hydroxykynurenine parallel disease severity in

human acute pancreatitis. **Skouras C**, Zheng X, Binnie M, Homer NZM, Murray TBJ, Robertson D, Briody L, Paterson F, Spence H, Derr L, Hayes AJ, Tsoumanis A, Lyster D, Parks RW, Garden OJ, Iredale JP, Uings IJ, Liddle J, Wright WL, Dukes G, Webster SP, Mole DJ. *Sci Rep*. **2016** Sep 27;6:33951. doi: 10.1038/srep33951.

2. Lung ultrasonography as a direct measure of evolving respiratory dysfunction and disease severity in patients with acute pancreatitis. **Skouras C**, Davis ZA, Sharkey J, Parks RW, Garden OJ, Murchison JT, Mole DJ. *HPB (Oxford)*. **2016** Feb;18(2):159-69. doi: 10.1016/j.hpb.2015.10.002. Epub 2015 Nov 18. PMID: 26902135.
3. Early organ dysfunction affects long-term survival in acute pancreatitis patients. **Skouras C**, Hayes AJ, Williams L, Garden OJ, Parks RW, Mole DJ. *HPB (Oxford)*. **2014** Sep;16(9):789-96. doi: 10.1111/hpb.12259. Epub 2014 Apr 9. PMID: 24712663.

These publications have been appended on the appendix of the present thesis. Permissions to append copies of the publications on the thesis were deemed as not required, after communication with each respective publisher. Furthermore, my contribution of human interleukin-22 data arising from this thesis to the following publication (led by Dr. Rodger Duffin) was substantial:

- Prostaglandin E_2 constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. Duffin R, O'Connor RA, Crittenden S, Forster T, Yu C, Zheng X, Smyth D, Robb CT, Rossi F, **Skouras C**, Tang S, Richards J, Pellicoro A, Weller RB, Breyer RM, Mole DJ, Iredale JP, Anderton SM, Narumiya S, Maizels RM, Ghazal P, Howie SE, Rossi AG, Yao C. *Science*. **2016** Mar 18;351(6279):1333-8. doi: 10.1126/science.aad9903. PMID: 26989254.

The clinical studies were undertaken in accordance with the regulations of the Scotland A Research Ethics Committee, the NHS Lothian Research & Development committee, and the Declaration of Helsinki of the World Medical Association. Permission to access confidential medical records was granted by the NHS Lothian Caldicott Guardian. Written informed consent was

obtained from all participants or their legal representative. Adults without the capacity to give informed consent were recruited in accordance with the Adults with Incapacity (Scotland) Act 2000, Part 5.

This work was co-funded by The University of Edinburgh and GSK with a grant through the Discovery Partnerships in Academia (DPAc) initiative, and through a Spire Healthcare Clinical Research Fellowship. The University of Edinburgh receives milestone and royalty payments depending on the phase of the DPAc project, governed by The University of Edinburgh revenue sharing policy.

Christos Skouras – 20th of October 2017

Abstract

BACKGROUND: Acute pancreatitis (AP) is a sterile initiator of systemic inflammation that can trigger multiple organ dysfunction syndrome (MODS). In the acute phase of AP, the kynurenine pathway of tryptophan metabolism plays an important role in the genesis of AP-MODS in experimental animal models, but it is unknown whether the pathway is activated in human AP. Human data are required to support the rationale for kynurenine 3-monooxygenase (KMO) inhibition as a treatment for AP-MODS and reinforce the translational potential. Additionally, as respiratory dysfunction is frequent in severe AP, the role of lung ultrasonography in severity stratification deserves investigation. Furthermore, the effect of AP-MODS on long-term survival is unknown.

OBJECTIVES: My objectives were to: 1) Define the temporal and quantitative relationship of kynurenine metabolites with the onset and severity of AP-MODS, 2) Investigate the value of lung ultrasonography in the early diagnosis of respiratory dysfunction in human AP-MODS, and 3) Examine whether early AP-MODS impacts on long-term survival.

METHODS: 1) A prospective, observational, clinical experimental medicine study titled “Inflammation, Metabolism, and Organ Failure in Acute Pancreatitis” (IMOFAP) was performed. For 90 days, consecutive patients with a potential diagnosis of AP were recruited and venous blood was sampled at 0, 3, 6, 12, 24, 48, 72 and 168 hours post-recruitment. Kynurenine metabolite concentrations were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and analysed in the context of clinical data, disease severity indices, and cytokine profiles. 2) In a nested cohort within IMOFAP, 41 participants underwent lung ultrasonography to evaluate whether this imaging modality can detect respiratory dysfunction in AP. 3) Survival data for

a prospectively maintained database of patients with AP was analysed after accounting for in-hospital deaths.

RESULTS: 1) During the IMOFAP study, 79 patients were recruited with an elevated serum amylase, of which 57 patients met the diagnostic criteria for AP; 9 had severe disease. Temporal profiling revealed early tryptophan depletion and contemporaneous elevation of plasma concentrations of 3-hydroxykynurenine, which paralleled systemic inflammation and AP severity. 2) Lung ultrasonography findings correlated with respiratory dysfunction. 3) 694 patients were followed up for a median of 8.8 years. AP-MODS conferred a deleterious effect on overall survival which persisted after the exclusion of inhospital deaths (10.0 years, 95% C.I. = 9.4-10.6 years) compared to AP without MODS (11.6 years, 95% C.I. = 11.2-11.9 years; P = 0.001). This effect was independent of age.

CONCLUSIONS: In the acute phase of AP, metabolic flux through KMO is elevated and proportionate to AP severity. Lung ultrasonography may be a useful technique for evaluating AP-MODS. AP-MODS is an independent predictor of long-term mortality. Together, this work reinforces the rationale for investigating early phase KMO inhibition as a therapeutic strategy in humans.

Lay summary

The pancreas is an abdominal organ that is responsible for digestion. If the pancreas becomes acutely inflamed after drinking too much alcohol, or with an attack of gallstones, this leads to acute pancreatitis (AP). AP can be very serious and some people with AP need to be treated in intensive care. 1 in 5 people with AP who go to intensive care will die during that illness, and there is no specific treatment. AP may also have a lasting harmful effect on life expectancy for many years after the acute illness.

Recently, we have been measuring how the breakdown of the amino acid tryptophan may contribute to poor organ function in AP, and using laboratory experiments to see if medicines that block that metabolism may be a treatment for AP. I wanted to do research to see whether the metabolism of tryptophan is important during AP in humans. As part of this work, I measured tryptophan metabolism in patients who were admitted to hospital with AP, and researched life expectancy after AP.

I discovered that during AP, tryptophan metabolism increased, and these changes were more obvious when the disease was more severe. Additionally, I discovered that we could detect poor lung function during AP using ultrasound. Lastly, I found that in the long term, patients with severe AP died earlier than those with mild AP, irrespective of their age.

In conclusion, tryptophan metabolism is increased in severe AP. Furthermore, lung ultrasound scans can help to detect which patients with AP will become sicker. When patients' organs stop working properly because of AP, they not only have a serious risk of death during that attack, but also live less in the long-term. I hope that my research supports our overall strategy of making new medicines that block tryptophan metabolism as a treatment for AP.

Acknowledgements

This thesis has been conducted under the primary supervision of Mr. Damian J. Mole. It was great to work in the friendly and productive environment of Mr. Mole's team and I am most grateful to him for his enthusiastic support. His ceaseless encouragement was crucial in giving me the momentum to surmount the many hurdles encountered along the way. His experience in the field was crucial, and the network of scientists and his reputation in the unit opened many doors and facilitated my endeavor of conducting an acute clinical study. I am also enormously grateful to my second supervisor, Professor Rowan W. Parks, who guided and supported me throughout this project, and whose wisdom and insight were essential for its completion.

This work was by its nature highly collaborative and I have relied upon the cooperation of many people and departments. First and foremost, I would like to thank the team of research nurses and support staff of the Wellcome Trust Clinical Research Facility of the Royal Infirmary of Edinburgh, and especially Lisa Derr, Lesley Briody, Heather Spence, Sharon Cameron and Finny Patterson, for their tireless work and dedication during the challenging acute recruitment of the IMOFAP study.

In addition, I am thankful for the help, expert advice and support I received from my colleagues in the Department of Clinical Surgery of The University of Edinburgh, and particularly Professor O. James Garden and Mr. Alastair J. Hayes, and from Professor John P. Iredale, former Director of the Medical Research Council Centre for Inflammation Research at The University of Edinburgh; their insight has been crucial at all stages of my work. The welcoming and cooperative spirit of all staff in the Emergency Department of the Royal Infirmary of Edinburgh was impressive. The support from the EMERGE team, and especially Professor Alasdair Gray, Dr. Gareth Clegg, Judy Coyle, and Rachel O'Brien, was important to ensure a smooth recruitment in one of the busiest Emergency Departments in the country,

where several clinical studies ran simultaneously. Their help is gratefully acknowledged.

The experience and expertise of all members of the DPAc team and collaborators was critical in the design and realization of the IMOFAP study. Dr. Iain J. Uings, John Liddle, Wayne L. Wright, George Dukes, Darren Robertson, and Dr. Scott P. Webster have been enormously helpful and supportive from day one, and ensured the success of the project with their know-how and outstanding contributions. Additionally, the help from Toby B. J. Murray in managing the data arising from IMOFAP was very valuable.

I feel enormously grateful to Xiaozhong Zheng, Margaret Binnie, and Natalie Z. M. Homer, for their quintessential and intensive work with sample processing and analysis by liquid chromatography–tandem mass spectrometry. It is needless to say that without their contribution, this thesis would not have been materialised. Moreover, the expertise of Professor John T Murchison, Dr. Joanne Sharkey and Dr. Zoe A. Davis was crucial for the performance of lung ultrasound scans for the study reported in Chapter 3. Additionally, I am grateful for the help I received from Nikolay Nikolov and Nikolay Angelov in the development of the diarized alert software for the IMOFAP study, and to Dr. Andreas Tsoumanis for the challenging task of developing a sophisticated, bespoke data collection software, to reliably and efficiently gather acutely collected data at the participants' bedside. The expert guidance from statisticians Cat Graham (Edinburgh Clinical Research Facility) and Linda Williams (Usher Institute of Population Health Sciences and Informatics) is also gratefully acknowledged.

Furthermore, it is important to acknowledge the support I received from The University of Edinburgh/NHS Lothian Academic and Clinical Central Office for Research and Development (ACCORD); Dr. Diane Harbison, Marise Bucukoglu, Dr. Alex Bailey, Karen Maitland, and Dr. Raymond French lead me through the complex ethical and regulatory approval process, and ensured that no delays occurred. Lastly, I am indebted to the staff of the NHS biochemistry laboratory for their help, and particularly Dawn Lyster, and Lesley Harrower,

whose contribution in setting-up the IMOFAP study participant identification alert was important for the success of the study.

The IMOFAP study was co-funded by The University of Edinburgh and GSK, as part of the Discovery Partnerships in Academia (DPAc) initiative, and my work was supported through a Spire Healthcare Clinical Research Fellowship. This thesis would not have been possible without their generous support.

Importantly, this work would not have been possible without the patients who kindly agreed to take part in this study during a time of acute illness, and without the contribution of healthy volunteers.

My parents and my brother Thomas have been amazing throughout this endeavor, and I thank them for the endless encouragement and confidence in me. Most important of all, I am eternally grateful to my wonderful wife Eva for her patience and heartwarming support. I still wonder how she managed to tolerate me throughout this project, during a difficult pregnancy and later while looking after our baby daughter, who admittedly showed no understanding at all, as teething is certainly much more painful than data crunching! I hope that this thesis is a credit to her love and support.

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List of Abbreviations

3-HK	3-hydroxykynurenine
3-HAA	3-hydroxyanthranilic acid
95% C.I.	95% confidence interval
ABG	arterial blood gas
ACCORD	Academic and Clinical Central Office for Research and Development
AIS	alveolar-interstitial syndrome
AP	acute pancreatitis
AP-MODS	acute pancreatitis-associated multiple organ dysfunction syndrome
APACHE	Acute Physiology and Chronic Health Evaluation
ARDS	acute respiratory distress syndrome
AUC	area(s) under the curve
AUSS	Abdominal ultrasound scan
BSA	bovine serum albumin
CECT	contrast-enhanced computerized tomography
CTIMP	clinical trial of investigational medicinal product
CT	computerized tomography
CXR	chest X-ray
DPAc	Discovery Partnerships with Academia
ED	Emergency Department
EDTA	ethylenediaminetetraacetic acid
EUS	endoscopic ultrasound scan
ERCP	endoscopic retrograde cholangiopancreatography
FAD	flavin adenine dinucleotide
FiO_2	fraction of inspired oxygen
GSK	GlaxoSmithKline
HR	hazard ratio
HDU	High Dependency Unit
IDO	indoleamine 2,3-dioxygenase
IL	interleukin
IMOFAP	Inflammation, Metabolism, and Organ Failure in Acute Pancreatitis
InS:PIRE	Intensive care Syndrome: Promoting Independence and Return to Employment
IQR	interquartile range
IS	Information Services
ITU	Intensive Therapy Unit
KMO	kynurenine 3-monoxygenase
KYN	kynurenine

KYNA	kynurenic acid
LC-MS/MS	liquid chromatography–tandem mass spectrometry
LOS	length of stay
LPS	lipopolysaccharide
LUSS	lung ultrasonography
mL	millilitre(s)
MIRP	minimal invasive retroperitoneal pancreatic necrosectomy
MODS	multiple organ dysfunction syndrome
NA	not applicable
NAD	nicotinamide adenine dinucleotide
NHS	National Health Service
NO	nitric oxide
NPV	negative predictive value
QA	quinolinic acid
PaO_2	partial arterial pressure of oxygen
PG	prostaglandin
PPV	positive predictive value
R&D	Research and Development
REC	Research Ethics Committee
RIE	Royal Infirmary of Edinburgh
ROC	receiver operating characteristic
ROS	reactive oxygen species
SAP	severe acute pancreatitis
SD	standard deviation
SE	sensitivity
SP	specificity
SIRS	systemic inflammatory response syndrome
SpO_2	arterial oxygen saturation measured by pulse oximeter
Th	T helper lymphocyte
Treg	T regulatory lymphocyte
TRP	tryptophan
UoE	The University of Edinburgh
USS	ultrasound scan
WTCRF	Wellcome Trust Clinical Research Facility

Chapter 1 – Introduction

1.1 OUTLINE OF THESIS

The present thesis addresses previously unknown areas of knowledge in human acute pancreatitis (AP). An intensive, high resolution, prospective observational clinical study was undertaken to elucidate changes of the kynurenine pathway of tryptophan (TRP) metabolism in human AP. The aim was to acquire novel data which will inform the design of future trials of inhibitors in patients with AP. Next, the use of lung ultrasonography as a novel approach to the objective quantification of respiratory dysfunction in patients with AP was examined. Lastly, the long-term effects of AP-associated early organ dysfunction were examined under the hypothesis that life-expectancy of patients that survive an attack of AP with MODS is reduced.

1.2 ACUTE PANCREATITIS

Acute pancreatitis (AP) is defined as an inflammatory disorder of the pancreas, usually triggered by the passage of gallstones or excessive alcohol consumption (Goldacre and Roberts, 2004; Lankisch et al., 2015; Steinberg and Tenner, 1994). It is one of the common causes for emergency hospital admission in Scotland, with an incidence of 33.1 per 100000 per year (Mole et al., 2016a), and elsewhere (Appelros and Borgstrom, 1999; Halvorsen and Ritland, 1996), and as such it constitutes the leading gastrointestinal cause of hospital admission in many countries (Lankisch et al., 2015). Furthermore, the incidence of AP is rising steadily (Goldacre and Roberts, 2004; Yadav and Lowenfels, 2006). The reasons are unclear, but this fact is partly attributable to the increasing incidence of obesity, which promotes gallstone formation, and of alcohol misuse, although both explanations are speculative (Steinberg and Tenner, 1994; Yadav and Lowenfels, 2013).

AP has a highly variable course with a potential outcome that ranges from complete resolution to death (Johnson and Abu-Hilal, 2004; Lerch, 2013).

The overall reported mortality rate in the United Kingdom ranges between 5.0% and 9.1% (Johnson and Abu-Hilal, 2004; McKay et al., 1999; Mofidi et al., 2006; Mole et al., 2016a; Toh et al., 2000).

In up to 82% of patients, AP has a self-limiting course and resolves rapidly with simple measures that rarely include anything more than simple analgesia and a short period of fluid resuscitation (McKay et al., 2014; Mole et al., 2016a). For these patients, identification and treatment of the underlying cause is the main goal (McKay and Carter, 2012). However, approximately 20% of patients with AP will suffer a severe attack (Mitchell et al., 2003), with associated mortality rate that can exceed 30% (Lankisch et al., 2015). Importantly, up to 60% of all deaths occur during the first 7 days of hospital admission, as a result of progressive organ failure (McKay et al., 1999; Mole et al., 2009). Survivors who develop persistent extrapancreatic organ dysfunction will require critical care support and potentially a prolonged hospital stay, with substantial associated costs for the healthcare service (Fenton-Lee and Imrie, 1993; Neoptolemos et al., 1998).

1.2.1 AETIOLOGY

In the U.K., gallstones are identified in approximately 40% of patients who develop AP, whereas the proportion cases linked to alcohol consumption varies from approximately 30% in Scotland, to much higher figures in other parts of the world (McKay and Carter, 2012). The causes of AP (McKay and Carter, 2012; McKay et al., 2014) can be categorised as shown on **TABLE 1.1**.

1.2.2 CLINICAL FEATURES

AP usually manifests with constant, severe upper abdominal pain of sudden onset. The pain is usually severe, and accompanying symptoms include nausea, vomiting, and fever or diaphoresis (Banks et al., 2006; Wu and Banks, 2013). Physical examination usually reveals upper abdominal

tenderness which may be associated with guarding, as well as tachycardia, tachypnoea and hypotension, depending on the severity of the attack (Banks et al., 2006; McKay et al., 2014). The differential diagnosis is broad and includes mesenteric ischaemia, perforated viscus, acute cholecystitis, dissecting aortic aneurysm, intestinal obstruction, and myocardial infarction (Banks et al., 2006; Wu and Banks, 2013).

Class	**Specific cause**
Obstructive factors	Biliary disease
	Benign pancreatic duct stricture (choledochal cyst, duodenal duplication, anomalous pancreaticobiliary junction, pancreas divisum)
	Tumours of the ampulla or pancreas
	Infective (parasites, worms)
	Duodenal obstruction (duodenal diverticulum, afferent limb obstruction)
Toxic factors	Alcohol
	Viral infections (mumps, coxsackie B, viral hepatitis)
	Drugs (valproic acid, mesalazine, simvastatin, azathioprine, corticosteroids)
Metabolic factors	Hyperlipidaemia (Familial hypertriglyceridaemia)
	Hypercalcaemia (Hyperparathyroidism)
Genetic defects	Cystic fibrosis
	Hereditary pancreatitis (cationic trypsinogen gene mutation)
	SPINK1 mutation
Trauma	Blunt trauma
Iatrogenic causes	Endoscopic retrograde cholangiopancreatography (ERCP)
Inflammatory	Autoimmune pancreatitis
Physiological	Type 1 pancreatic sphincter dysfunction

TABLE 1.1 – Causes of AP.

1.2.3 DIAGNOSIS

According to the revised Atlanta classification of 2012 (Banks et al., 2013), the diagnosis of AP requires two of the following three features: 1) abdominal pain characteristic for AP, as described on section **1.2.2**, 2) serum concentration of amylase and/or lipase at least three times greater than the upper limit of normal, and 3) characteristic findings of AP on contrast enhanced

computerised tomography (CECT), and less commonly on magnetic resonance imaging (MRI) or transabdominal ultrasonography (AUSS) (Arvanitakis et al., 2004; Banks et al., 2006; Bollen et al., 2007; Uhl et al., 2002; UK Working Party on Acute Pancreatitis, 2005).

In general, both serum amylase and lipase are elevated during the course of the disease, although it is usually not necessary to measure both. Serum amylase levels rise sharply within the first 24 hours of an attack and decline steadily to a normal range over the subsequent few days, with a dramatic loss of diagnostic sensitivity over the first 48 hours. Consequently, the diagnostic accuracy of serum amylase may be influenced by the time interval between the onset of AP and presentation to hospital. In the study of Clavien et al, normal amylase activity was reported in 19% of 352 AP attacks diagnosed by computerized tomography (CT) or autopsy, and repeating serum amylase at 4-6 hours has been suggested as a helpful measure to establish the diagnosis in suspected cases (Clavien et al., 1989). Furthermore, the magnitude of elevation of serum amylase and lipase does not correlate with severity (Winslet et al., 1992).

Serum lipase is considered marginally more accurate than amylase in the diagnosis of AP, and may remain elevated for slightly longer. It remains normal in non-pancreatic conditions that may cause an increase in serum amylase, such as macroamylasemia, parotitis, and some types of carcinomata (Winslet et al., 1992). However, it is not commonly available in many hospital laboratories (McKay et al., 2014).

Where uncertainty exists, as for example in patients with delayed presentation and/or a non-diagnostic level of serum amylase, a CT scan can confirm the diagnosis of AP (Banks et al., 2006). The use of intravenous contrast can help to establish the diagnosis and extent of pancreatic necrosis, and may be valuable in identifying the aetiology of AP (Banks et al., 2006). CT is also used to identify local complications of AP, with thin section multidetector row contrast-enhanced CT (CECT) scan being the gold standard modality (Balthazar, 2002; Balthazar and Fisher, 2001; Balthazar et al., 1994). CT

findings that support the diagnosis include pancreatic enlargement with diffuse oedema, heterogeneity of the pancreatic parenchyma, peripancreatic stranding, and peripancreatic fluid collections.

AUSS should be performed in all patients with AP to investigate for the presence of gallstones as the aetiologic factor. In patients where the cause remains unidentified, endoscopic ultrasound (EUS) may detect microlithiasis or small pancreatic neoplasms. Additionally, EUS has emerged as an important tool for guided drainage of peripancreatic collections (McKay and Carter, 2012; McKay et al., 2014). The role of magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) in the diagnosis of AP and establishment of severity remains incompletely defined (Banks et al., 2006).

1.2.4 ASSESSMENT OF SEVERITY

The development of extrapancreatic organ dysfunction that persists beyond 48 hours is central to the definition of severe AP, and is associated with increased overall mortality (Johnson, 2015; McKay and Buter, 2003). Consequently, early identification of patients at risk of developing persistent organ dysfunction is critical, and has been the focus of extensive research over the last three decades (Forsmark et al., 2007; Mounzer et al., 2012). The aim has been to improve the triage of patients, to ensure prompt and comprehensive management in an appropriate critical care environment, to obtain timely specialist input and support, and to facilitate the design of clinical studies (Banks et al., 2013; McKay and Carter, 2012). Importantly, the majority of patients who eventually develop severe AP have evidence of early organ dysfunction, which is present upon admission to hospital or develops shortly afterwards. As a consequence of this, up to 60% of all AP deaths occur during the first 7 days of hospital admission (Buter et al., 2002; Johnson and Abu-Hilal, 2004; McKay et al., 1999; Mofidi et al., 2006; Mole et al., 2009; Renner

et al., 1985). Therefore, the group of patients who suffer from early organ dysfunction are at higher risk and merit close observation (McKay et al., 2014).

To achieve accurate severity stratification, multiple approaches have been proposed, such as using laboratory tests, clinical and radiographic features, as well as multifactorial scoring systems. The Glasgow Prognostic Score (Blamey et al., 1984), the APACHE II score (Wilson et al., 1990) and C-reactive protein (CRP) (Wilson et al., 1989) have been most widely studied. However, none of these have proved sufficiently accurate to influence clinical decision-making, particularly in the crucial initial 24-hour period after hospital admission (McKay and Carter, 2012). The composite multifactorial scoring systems identify established multisystem organ dysfunction more accurately, rather than predicting the subsequent development of organ failure, (Buter et al., 2002; Chauhan and Forsmark, 2010; Johnson, 2015; McKay et al., 2014). They have also been used extensively as methods of stratifying patients within clinical studies. Of these scores, APACHE II has been considered as the most accurate in prediction of mortality, however it is cumbersome for routine clinical use and suffers from its complexity (Chauhan and Forsmark, 2010). The mainstay of assessment remains repeated and thorough clinical evaluation (Gravante et al., 2009).

The original Atlanta classification of 1992 (Bradley, 1993) was deemed suboptimal as it failed to discriminate with accuracy between patient subgroups with different outcomes (Bollen et al., 2008). This realisation led to the revised Atlanta classification in 2012 (Banks et al., 2013), according to which three levels of severity are defined, based primarily on the presence and duration of organ dysfunction: mild, moderately severe, and severe AP (**TABLE 1.2**). Organ failure is classified as transient when present for less than 48 hours, or persistent when present for more than 48 hours. Local complications include peripancreatic fluid collections and acute necrotic collections, while systemic complications may be related to exacerbations of pre-existing co-morbidities.

Mortality in mild AP is very rare, and in moderately severe AP is far less common than that of severe AP. Conversely, severe AP patients who develop early persistent organ failure are at increased risk of death, with a reported mortality rate as great as 36-50% (Banks et al., 2013). Based on a retrospective cohort, Choi et al further proposed to examine severe AP patients with infected necrosis separately in order to more accurately assess clinical outcomes (Choi et al., 2014).

Additionally, the Determinant Based Classification has been developed, with similar insights (Dellinger et al., 2012). Comparison of the performance of the two systems has shown few differences (Acevedo-Piedra et al., 2014), with the revised Atlanta classification of 2012 being more relevant to the day-to-day clinical setting (Yadav, 2014), although the co-existence of two parallel classification systems seems conflicting and may limit their widespread implementation (Bakker et al., 2014).

Degrees of AP severity
Mild AP
No organ failure and no local or systemic complications
Moderately severe AP
Organ failure that resolves within 48 hours (transient organ failure), and/or
Local or systemic complications without persistent organ failure
Severe AP
Persistent organ failure (>48 hours) involving a single or multiple organ systems

TABLE 1.2 – Degrees of severity of acute pancreatitis according to the revised Atlanta classification of 2012.

In recent years, it has become apparent that the existing composite severity scoring systems may have reached their maximal prognostic efficacy. Combinations of prognostic rules only provide marginally greater accuracy, and are even more cumbersome and therefore of limited clinical applicability. As stated by Mounzer et al, our ability to predict AP severity is not expected to improve further unless new approaches are developed (Mounzer et al., 2012). This realisation has initially led to the development of simpler models of

outcome prediction, such as the BISAP score (Papachristou et al., 2010; Singh et al., 2009; Wu et al., 2008), with similar prognostic performance (Bollen et al., 2012; Cho et al., 2015). Moreover, individual parameters such as CRP, blood urea nitrogen, serum creatinine, blood glucose and chest radiographs have been highlighted as potential alternatives for the risk stratification and outcome prediction of patients with AP (Blum et al., 2001; Muddana et al., 2009; Talamini et al., 1999; Wu et al., 2011). More recently, novel approaches with the use of biomarkers, genetic polymorphisms and mutations, proteomic and metabolomic analyses, and new methods of analysis, such as artificial neural networks (Mofidi et al., 2007a) have been suggested as potential means to develop more accurate models of early prediction (Chauhan and Forsmark, 2010).

1.3 KYNURENINE PATHWAY METABOLISM

1.3.1 TRYPTOPHAN

Tryptophan (TRP) (**FIGURE 1.1**) is an essential amino acid with a principal role in protein synthesis. Its effects range widely, and it constitutes a critical component of multiple essential metabolic functions. (Richard et al., 2009). A clear association between TRP catabolism and inflammatory reactions has been observed in recent years, in a vast array of conditions. The exact roles of TRP and its metabolites in the immune system are as yet not fully known, and the biological significance of inflammation-related TRP breakdown remains incompletely understood (Moffett and Namboodiri, 2003).

TRP is primarily produced by bacteria, fungi and plants, which maintain TRP flux into the food chain. Animals and humans are unable to synthesize TRP from simpler molecules; hence it must be ingested in the form of proteins. These are hydrolysed and the resultant TRP – as a constituent amino acid – is delivered to the liver for protein synthesis, where it can play a rate-limiting

role, possibly because its concentration is the lowest of the 20 amino acids (Moffett and Namboodiri, 2003; Richard et al., 2009; Sainio et al., 1996). TRP is primarily transported bound to albumin (90%) or in free form (10%), and the two states exist in equilibrium (McMenamy, 1965).

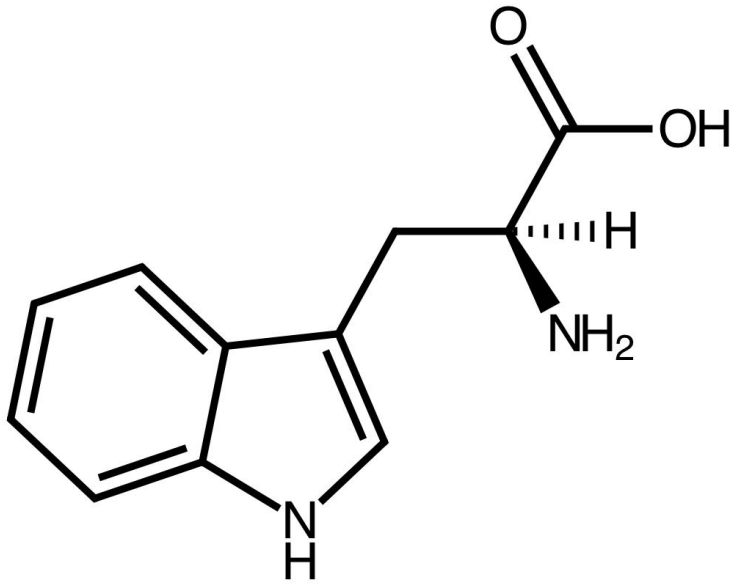

FIGURE 1.1 – L-Tryptophan.

As for all amino acids, only the L-isomer is used in protein synthesis, and unused TRP can be degraded in the liver through the kynurenine pathway, which accounts for approximately 90% of TRP catabolism (Moffett and Namboodiri, 2003; Sainio et al., 1996) (**FIGURE 1.2**). Ultimately, through the kynurenine pathway, TRP can act as the sole source for de novo synthesis of nicotinamide adenine dinucleotide (NAD^+) and NAD phosphate ($NADP^+$) – the cellular co-enzymes essential for electron transfer reactions – when dietary niacin is insufficient (Kurnasov et al., 2003; Richard et al., 2009; Sainio et al., 1996). NAD^+ synthesis in particular, has long been thought to take place exclusively in the liver (Moffett and Namboodiri, 2003). Additionally, TRP can also act as a substrate for niacin synthesis (Sainio et al., 1996).

The initial and rate-limiting step of the kynurenine pathway during which TRP is degraded to N-formylkynurenine is catalysed by three enzymes: indoleamine 2,3-dioxygenase (IDO), tryptophan 2,3-dioxygenase (TDO), and the recently discovered indoleamine 2,3-dioxygenase-2 enzyme (IDO2) (Ball et al., 2007; Ball et al., 2009; Salter and Pogson, 1985; Takikawa et al., 1986). TDO initiates TRP catalysis predominantly in the liver, and is induced by TRP and general regulators of metabolism, such as corticosteroids and insulin (Moffett and Namboodiri, 2003; Salter and Pogson, 1985).

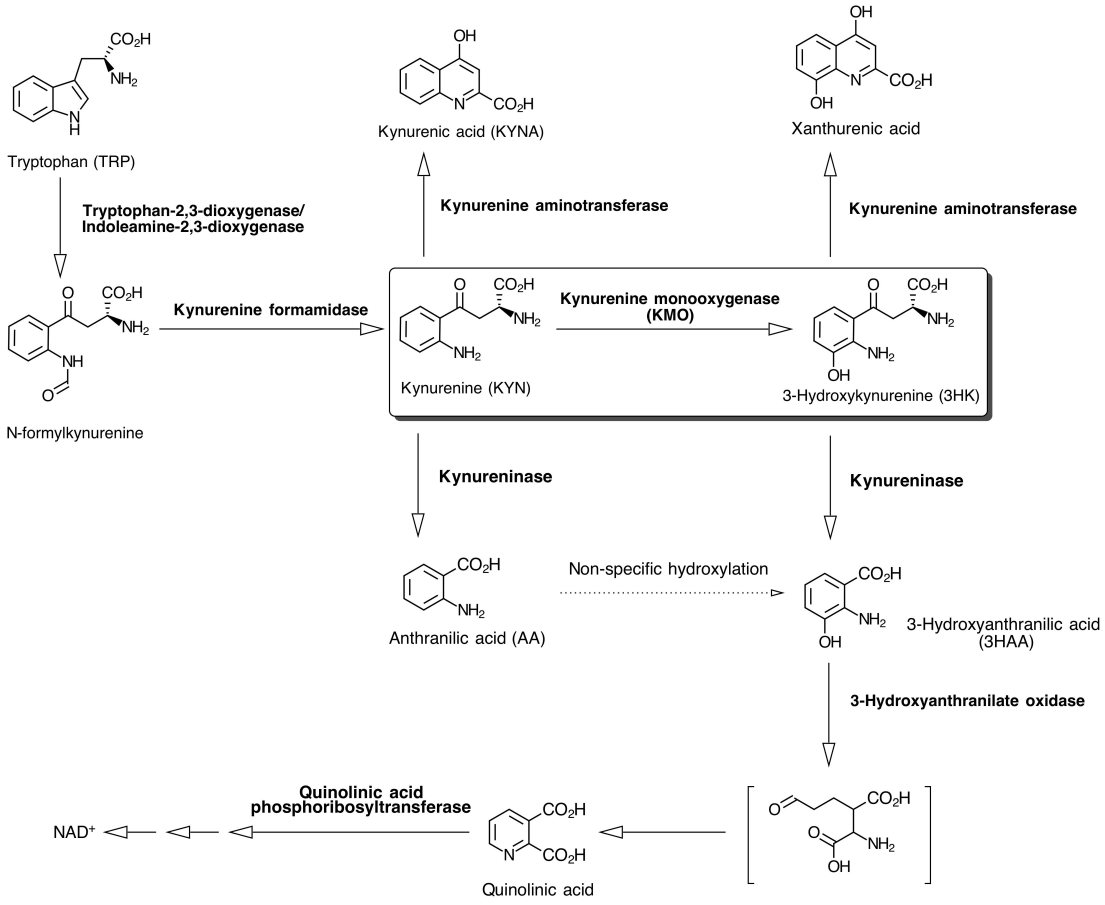

FIGURE 1.2 – The kynurenine pathway.

Conversely, IDO – the main extra-hepatic enzyme – and IDO-2 are expressed in a variety of cells. IDO is primarily induced by interferon-γ (IFN-γ) in response to inflammation, as well as by tumour necrosis factor alpha (TNF-

α), and other cytokines and lipopolysaccharides (LPS) (Sainio et al., 1996; Taylor and Feng, 1991; Werner et al., 1987). IFN-γ stimulates IDO activity potently by inducing its gene expression (Dai and Gupta, 1990; Hayaishi and Yoshida, 1978; Werner-Felmayer et al., 1989; Yasui et al., 1986). Increased IDO activity inhibits T cell function and proliferation (Boasso et al., 2007; Fallarino et al., 2006; Munn et al., 1999; Munn et al., 2002), contributes to T cell apoptosis (Fallarino et al., 2002), and inhibits nitric oxide (NO) synthase (Chiarugi et al., 2003; Samelson-Jones and Yeh, 2006; Sekkai et al., 1997).

The resultant TRP depletion may be an important factor in the suppression of lymphocyte proliferation, since TRP is essential for cell replication (Munn et al., 1999). As a consequence, a TRP-deficient state has been considered as a predisposing factor to poor clinical outcomes in critical illness, by altering host-immunity (Pellegrin et al., 2005). Furthermore, the increased production of L-kynurenine and other downstream metabolites, such as quinolinic acid (QA) and 3-hydroxykynurenine (3-HK), has been shown to cause direct damage to tissues and organs (Frumento et al., 2002; Molano et al., 2008).

1.3.2 KYNURENINE

The first stable intermediate metabolite of the kynurenine pathway is L-kynurenine (KYN) (Chen et al., 2010). KYN is derived from N-formylkynurenine by the enzymatic activity of arylformamidase. KYN is the substrate for several enzymes, and is a key precursor of a number of metabolites, most importantly kynurenic acid (KYNA) and QA (Kolodziej et al., 2011).

KYN metabolism may occur in the liver, kidneys or other tissues where the required enzymes are expressed and the substrate can access the relevant compartment. Due to the fact that KYN and its downstream metabolites are biologically active, its synthesis and metabolism must be tightly controlled (Kolodziej et al., 2011; Mehler and Knox, 1950; Oxenkrug, 2007; Richard et al., 2009).

1.3.3 3-HYDROXYKYNURENINE

3-HK is generated from KYN by the action of the flavin adenine dinucleotide (FAD)-dependent kynurenine 3-monooxygenase (KMO). 3-HK exhibits significant cellular toxicity primarily through production of endogenous free radicals and reactive oxygen species (ROS) which induce apoptotic cell death (Eastman and Guilarte, 1990; Grohmann et al., 2003; Okuda et al., 1998; Stone et al., 2013; Wang et al., 2014). Furthermore, the quinonimine product of 3-HK oxidation is highly reactive with the thiol- and amine- proteins groups (Giles et al., 2003). The destructive properties of 3-HK can also partly be attributed to its metabolism to 3-hydroxyanthranilic acid (3-HAA), which can undergo auto-oxidation and form superoxide anions (Stone and Darlington, 2002). According to Stone et al, this toxicity is probably produced at an intracellular site (Stone et al., 2013).

Fluctuations in 3-HK or 3-HAA concentrations play a potentially significant role in the regulation of tissue oxidative status, by showing pro- or anti-oxidant activity, depending on the local oxidative environment (Stone et al., 2013). Importantly, it has been demonstrated that 3-HK can inhibit T cell proliferation even in micromolar concentrations (Frumento et al., 2002; Terness et al., 2002).

1.3.4 KYNURENIC ACID

Kynurenic acid (KYNA) is the product of metabolism of KYN by kynurenine aminotransferases I and II. KYNA inhibits LPS-induced TNF-α release from human peripheral blood mononuclear cells by activating the orphan G protein–coupled receptor GP35 (Wang et al., 2006). It is also an agonist of the aryl hydrocarbon receptor, a nuclear protein involved in the regulation of gene transcription able to cause immunosuppression (Moroni et al., 2012).

The resultant elevation of KYNA plasma levels following KMO inhibition could be protective or harmful in the context of AP. It may contribute to the benefit seen in KMO^{null} mice with AP and GSK180-treated rats with AP by exerting an anti-inflammatory effect on innate immune cells (Mole et al., 2016b). In high concentrations KYNA is known to possess anti-convulsant and neuroprotective properties by potent antagonism at the glycine allosteric site of the N-methyl-D-aspartate (NMDA) receptor complex, therefore KMO inhibition may carry sedative effects (Foster et al., 1984a; Foster et al., 1984b; Mole et al., 2016b; Wang et al., 2006).

1.3.5 QUINOLINIC ACID

QA is another toxic intermediate metabolite along the synthetic route of nicotinic acid and NAD^+ (Wang et al., 2015). Its harmful effects can be attributed to producing mitochondrial dysfunction (Baran et al., 2001; Bordelon et al., 1997) or increasing generation of free radicals (Behan et al., 1999; Santamaria et al., 2001). Furthermore, it can selectively target immune cells undergoing activation, thus suppressing T cell proliferation, an ability shared with other metabolites of the pathway (Chen and Guillemin, 2009; Frumento et al., 2002).

1.3.6 3-HYDROXYANTHRANILIC ACID

3-hydroxyanthranilic acid (3-HAA) is the product of 3-HK metabolism by kynureninase. 3-HAA is a free-radical generator (Goldstein et al., 2000). It can act in concert with QA to produce an additive effect in suppressing T cell proliferation (Terness et al., 2002). 3-HAA also carries abilities which could compromise the biological functions of NO (Stone et al., 2013), as it inhibits NO synthase in macrophages (Sekkai et al., 1997), and can be readily nitrosylated to oxadiazole compounds, a property shared with 3-HK (Backhaus et al., 2008).

1.3.7 KYNURENINE 3-MONOOXYGENASE

Kynurenine 3-monooxygenase (KMO) is an outer mitochondrial membrane protein with a pivotal role in kynurenine metabolism (Uemura and Hirai, 1998; Zwilling et al., 2011). KMO converts KYN to 3-HK in the presence of the co-factor FAD. Under both physiological and pathological conditions, KMO possesses a high affinity for its substrate, suggesting that KMO metabolizes most of the available KYN to 3-HK (Lowe et al., 2014). It is expressed at high levels in human macrophages, and its expression increases in inflammatory conditions or following immune stimulation (Connor et al., 2008; Heyes et al., 1992).

Our group has shown that both lack of KMO activity in genetically-altered mice, and the use highly specific KMO inhibitors in rats result in the accumulation of KYN, and shift TRP metabolism toward increased KYNA production (Mole et al., 2016a), advancing the concept previously noted by others (Guidetti et al., 2007; Guidetti et al., 2004; Thevandavakkam, 2011; Uemura and Hirai, 1998; Zwilling et al., 2011).

1.4 EXISTING KNOWLEDGE AND CHALLENGES

1.4.1 THE ROLE OF 3-HK IN NEURODEGENERATIVE DISEASES

Numerous studies over the last few decades have confirmed the role of neuroactive metabolites of the kynurenine pathway in brain physiology and the pathogenesis of affective disorders, neuroinflammatory conditions and neurodegeneration (Beaumont et al., 2016; Maddison and Giorgini, 2015; Schwarcz et al., 2012). 3-HK has been identified as a biomarker in the cerebrospinal fluid of patients with Parkinson's and Alzheimer's disease, and has been found in high levels in various brain regions of patients with

Parkinson's and Huntington's disease (Lewitt et al., 2013; Ogawa et al., 1992; Pearson and Reynolds, 1992). In Alzheimer's disease, the oxidative stress caused by 3-HK and 3-HAA damages neuronal tissue and may potentially contribute to neurodegeneration (Gulaj et al., 2010).

The pharmacological manipulation of the pathway in experimental settings has proven beneficial, and constitutes a possible gateway for the treatment of such diseases (Maddison and Giorgini, 2015). In specific, KMO is considered a promising candidate for inhibition, aiming to achieve a pathway shift towards enhanced formation of KYNA, with a concurrent reduction of the synthesis of neurotoxic kynurenines (Majláth et al., 2015). Ro 61-8048, a widely used KMO inhibitor, demonstrated pronounced neuroprotective and antidyskinetic effects in animal models of focal or global ischaemia and L-dopa-induced dyskinesia (Cozzi et al., 1999; Gregoire et al., 2008; Schwarcz et al., 2012). Similarly, in a yeast model of Huntington's disease, KMO inhibition by a structural analog of Ro 61-8048 reduced toxicity (Giorgini et al., 2005), whereas in a Drosophila model, KMO inhibition diminished neurodegeneration (Campesan et al., 2011). In a mouse model of Alzheimer's disease, KMO inhibition with the small molecule JM6 – a pro-drug of Ro 61-8048 – prevented spatial memory deficits, anxiety-related behaviour and synaptic loss, whereas in Huntington's disease it prevented synaptic loss and CNS inflammation, resulting in significantly improved survival (Zwilling et al., 2011). More recently, the inhibitor CHDI-340246 was shown to restore several electrophysiological alterations in mouse models of Huntington's disease (Beaumont et al., 2016).

1.4.2 KYNURENINE METABOLISM IN CRITICAL ILLNESS

The hypothesis that TRP and its downstream metabolites along the kynurenine pathway are implicated in the pathophysiology of systemic inflammation and critical illness irrespective of aetiology is supported by the findings of several studies. The studies of Pellegrin et al and Logters et al

discovered a marked depression of plasma TRP in major trauma patients (Logters et al., 2009; Pellegrin et al., 2005). KYN and KYN/TRP ratio levels were significantly increased in the blood of major trauma patients who later developed sepsis and organ failure (Logters et al., 2009; Ploder et al., 2010). Furthermore, TRP depletion and/or elevated concentrations of kynurenine metabolites have been detected in patients following cardiac bypass surgery. (Forrest et al., 2011).

Implications in cardiovascular disease are also evident. Elevated KYN/TRP ratio was found in the blood (Wirleitner et al., 2003) and urine (Pedersen et al., 2013) of patients suffering from coronary artery disease and, in the latter study, this was found to be a strong predictor of major coronary events, acute myocardial infraction and mortality. Results from a Finnish prospective multicentre study showed that early activation of the kynurenine pathway predicts early death and poor long-term outcome in patients resuscitated from out-of-hospital cardiac arrest (Ristagno et al., 2014).

In recent years, TRP metabolism via the kynurenine pathway has also been the focus of research in human sepsis. In this context, IDO-mediated TRP catabolism has been associated with dysregulated immune responses and impaired microvascular reactivity (Darcy et al., 2011). Increased levels of plasma KYN and KYN/TRP ratio were associated with severe sepsis, and an increased activity of IDO was associated with higher mortality rates (Girgin et al., 2011; Tattevin et al., 2010). Excessive degradation of TRP was proposed as a potential risk factor for critically ill sepsis patients, with plasma concentrations of QA and QA/TRP ratio showing the best predictive value in the study of Zeden et al (Zeden et al., 2010).

In infected rodents, Wang et al showed that in systemic inflammation, TRP metabolism to KYN via the action of IDO – which is primarily expressed in vascular endothelial cells (Ball et al., 2002; Hansen et al., 2000) – contributes to vascular relaxation and blood pressure regulation (Wang et al., 2010). More recently TRP metabolism to KYN was confirmed as an important contributor to the regulation of vascular tone in human septic shock, as IDO

activity was found to change in parallel to inotrope requirements in septic shock patients (Changsirivathanathamrong et al., 2011). In addition, the lack of reduction of KYNA was presented as a potential predictor of fatal outcomes in septic shock patients with acute kidney injury (AKI), managed with continuous veno-venous haemofiltration (Dabrowski et al., 2014). IDO activity was found to be markedly increased in bacteraemic patients and has been established as an independent predictor of severe disease and mortality (Huttunen et al., 2010). In progressive HIV infection, IDO-dependent TRP catabolism was proposed as a potentially important link between immune activation and the observed gradual decline of immune function, and 3-HAA has been shown to invert the ratio of $CD4^+$ T helper17 (Th17) and T regulatory (Treg) cells (Favre et al., 2010).

From a slightly different perspective, plasma TRP has been identified as an independent risk factor for delirium in critically ill patients (Pandharipande et al., 2009a). The association of the kynurenine pathway with delirium was more recently supported by the findings of the prospective cohort study of Adams Wilson et al, who showed that increased baseline plasma KYN concentrations and KYN/TRP ratios were independently associated with acute brain dysfunction and fewer days free from delirium and coma in mechanically ventilated ITU patients (Adams Wilson et al., 2012).

In an inflammatory context, TRP metabolism through the kynurenine pathway is dramatically increased through induction of IDO expression by pro-inflammatory cytokines, particularly IFN-γ (Murakami and Saito, 2013). IDO upregulation increases substrate flux through downstream enzymes, in particular KMO, thereby generating biologically active metabolites that participate in multiple physiological and pathological processes (Bellac et al., 2010; Grohmann et al., 2002; Moffett and Namboodiri, 2003; Pellegrin et al., 2005; Schwarcz, 2004; Wang et al., 2010). Metabolites in the kynurenine pathway have been postulated to mediate immunological tolerance dependent on signalling via the aryl hydrocarbon receptor, whilst KYN specifically may

contribute to Th17 and T-reg lymphocyte differentiation (Mezrich et al., 2010; Nguyen et al., 2010).

The exact pathological mechanisms that drive AP-MODS remain incompletely understood, however the kynurenine pathway of TRP metabolism is emerging as a potentially important contributory mechanism. To date, the only human data of TRP catabolism in AP come from the study of Mole et al, who showed a greater increase of plasma KYN concentrations in AP patients with higher APACHE II or MODS score (Mole et al., 2008). In rodent models of AP, therapeutic blockade of KMO by genetic deletion in mice or pharmacological inhibition of KMO in rats reduced 3-HK formation and protected against lung and kidney injury in experimental models of AP (Mole et al., 2016b).

KMO inhibition may therefore offer a potential therapeutic strategy for reducing the incidence and severity of AP-MODS in humans, and although the exact effects of 3-HK activity in human AP are unclear, a translational study of KMO inhibition would be highly anticipated to support this novel concept (Mole et al., 2016b). As such, it is important to establish the precise timing and magnitude of activation of the kynurenine pathway in human patients with AP, in relation to the onset of the disease and the development of AP-MODS, the inflammatory burden and overall AP severity.

1.4.3 SPECIFIC THERAPIES FOR ACUTE PANCREATITIS

Effective specific therapies for AP and AP-associated organ dysfunction do not yet exist, with only few interventions offering benefit to patients. Despite several clinical trials, there is no licenced drug therapy available, and no treatment exists to prevent progression of AP to moderately severe and severe disease (Afghani et al., 2015). Management relies almost entirely on general supportive measures, and current optimal management may include fluid resuscitation, analgesia, nutritional support, prompt identification of severe

disease, support of organ function, and management of local and/or systemic complications (Wu and Banks, 2013).

The mechanisms that drive systemic inflammation and organ failure in severe AP share characteristics with those encountered in conditions such as sepsis, major surgery, trauma, or severe burns, thus research results from these conditions may be relevant (Kylanpaa et al., 2012; Wilson et al., 1998). Nevertheless, our understanding of AP pathophysiology remains limited, and we still lack the necessary knowledge into the mechanisms of inflammation and how to manipulate them. The inflammatory pathways involved in the genesis and evolution of AP-associated organ dysfunction are complex, therefore, it is thought that the development of effective treatments may require targeting multiple pathways, either with a versatile agent which will be potent against multiple receptors, or by combinations of agents active at different sites (Johnson, 2015). Notably, the window of opportunity for anti-inflammatory therapy to suppress excessive activation of the inflammatory response is thought to be very narrow due to the rapid progress of AP, making matters even more challenging (Kylanpaa et al., 2012).

An additional hurdle in the development of therapies for AP-associated organ failure is the relative lack of effective molecules to be tested in clinical trials. Several drugs tested in previous randomized controlled trials (RCT) failed to show a significant benefit (Afghani et al., 2015), including glucagon (Debas et al., 1980; Durr et al., 1978), gabexate mesilate (Buchler et al., 1993; Harada et al., 1991; Pezzilli and Miglioli, 2001; Valderrama et al., 1992; Yang et al., 1987), octreotide (Uhl et al., 1999; Wang et al., 2013), lexipafant (Johnson et al., 2001; Kingsnorth et al., 1995; McKay et al., 1997), and protease inhibitors (Piascik et al., 2010). Other agents recently reported to be effective in experimental AP models, have not been translated to the bedside due to the aforementioned inherent difficulties of clinical trials of investigational medicinal products (CTIMP) in AP (Afghani et al., 2015).

1.4.4 ACUTE CLINICAL RESEARCH IN ACUTE PANCREATITIS

The design of acute clinical research studies in AP is associated with challenges which relate to the rapid evolution of the disease. It is established that the majority of patients who eventually develop severe AP have evidence of early organ dysfunction upon presentation (McKay et al., 2014), a fact which necessitates rapid enrolment of participants in trials and clinical studies. In the multicentre study on lexipafant by Johnson et al (Johnson et al., 2001), over 40% of patients recruited within 72 hours had organ failure at the time of enrolment, and only a further 7% developed new organ failure during the first week, offering a valuable lesson in support of early recruitment (Johnson, 2015). Additionally, a paucity of early time-series data in human AP exists.

It would therefore be reasonable to focus enrolment of AP patients as early as their presentation to the Emergency Department (ED), or soon thereafter. This resource-intensive recruitment goal comes in addition to other well-known existing challenges encountered in single- and multi-center clinical research, as proven by acute research studies performed in different fields. These include difficulties pertaining to the timing of patient presentation, ED crowding, cooperation between clinical and research teams, patient acceptance, the method/strategy of obtaining consent, as well as the availability of infrastructure for early identification of participants, and ad-hoc intervention or sample and data collection, amongst others (Cofield et al., 2010; Johnson et al., 2016; Kendrick et al., 2007).

1.4.5 LUNG ULTRASONOGRAPY IN RESPIRATORY DYSFUNCTION

The respiratory system is the most frequent extra-pancreatic organ system to be affected in AP (Johnson and Abu-Hilal, 2004; Mole et al., 2009; Renner et al., 1985). Respiratory dysfunction often precedes renal,

cardiovascular and hepatic dysfunction and constitutes a major determinant of mortality in severe AP (Bhatia et al., 2000; Buter et al., 2002; Johnson and Abu-Hilal, 2004). Several hypotheses have been explored and several inflammatory mediators and signalling pathways have been implicated in the pathophysiology of AP-associated respiratory dysfunction, however the exact mechanisms remain incompletely understood (Chooklin, 2009; Shields et al., 2002; Zhou et al., 2010).

The sequelae of AP-associated respiratory dysfunction range from subclinical hypoxaemia to severe Acute Respiratory Distress Syndrome (ARDS) (Imrie et al., 1978; Johnson and Abu-Hilal, 2004; Pastor et al., 2003; Ranson et al., 1974b; Zhou et al., 2010). Radiologically evident complications such as atelectasis, pleural effusions, pulmonary infiltrates and/or oedema may frequently be present (Basran et al., 1987; Raghu et al., 2007). Notably, hypoxaemia has been described as an early indicator of pathological changes in the pulmonary parenchyma and may precede radiological findings (Polyzogopoulou et al., 2004). Consequently, a timely and accurate detection of evolving respiratory dysfunction may aid in the identification of patients at risk and may expedite the implementation of appropriate management.

Pulmonary conditions with diffuse involvement of the interstitium and impairment of the alveolo-capillary exchange capacity can be classified under the term Alveolar-Interstitial Syndrome (AIS) and are common in the critically ill (Stefanidis et al., 2012). This syndrome includes ARDS, acute pulmonary oedema, interstitial pneumonia, exacerbation of chronic interstitial lung disease, and miscellaneous other conditions (Lichtenstein et al., 1997; Pastor et al., 2003; Soldati et al., 2009; Volpicelli et al., 2006). ARDS in specific is defined as a type of acute diffuse inflammatory lung injury, characterised by increased permeability of the alveolo-capillary membrane with oedema, loss of aerated lung tissue, increased work of breathing and impaired gas exchange (Ferguson et al., 2012; Zompatori et al., 2014).

There has been an increasing interest in the application of lung ultrasonography (LUSS) in critical care, following the rapid improvement of its

diagnostic potential in recent years (Soldati et al., 2009). LUSS has been proposed as a useful non-invasive modality for diagnosing AIS in the critically ill, based on the detection and quantification of comet-tail artefacts, generated by the reverberation of the ultrasound beam (Lichtenstein et al., 1997; Stefanidis et al., 2012; Volpicelli et al., 2006). These are described as vertical artefacts fanning out from the lung-wall interface and spreading up to the edge of the screen, resulting from thickened interlobular septa and extravascular lung fluid (Lichtenstein et al., 1997). Several studies confirmed the value of LUSS in the diagnosis of AIS (Bouhemad et al., 2007; Lichtenstein et al., 1997; Stefanidis et al., 2012; Via et al., 2012; Volpicelli et al., 2006), and its high sensitivity and specificity (Lichtenstein et al., 2004; Lichtenstein et al., 1997; Stefanidis et al., 2012).

LUSS is a fast, economic, portable, radiation-free, highly reproducible modality with a short learning curve (Gardelli et al., 2012; Zompatori et al., 2014), and has advantages over other imaging modalities; bedside X-rays frequently pose diagnostic challenges (Bouhemad et al., 2007; Lichtenstein et al., 1997), whereas CT can be time-consuming and hazardous for critically ill patients (Beckmann et al., 2004; Stefanidis et al., 2012), and is associated with radiation exposure (Kalra et al., 2004).

The revised Atlanta classification of 2012 – similar to previous classification systems (Harrison et al., 2007; Imrie et al., 1978; Knaus et al., 1985; Ranson et al., 1974a) – use the long established PaO_2/FiO_2 ratio as a measure of respiratory dysfunction, which although undoubtedly invaluable, constitutes a relatively non-specific prognostic marker, a fact already noted by Ranson et al four decades ago (Ranson et al., 1973). The introduction of LUSS as a method supplementary to PaO_2/FiO_2 would aim to increase prognostic efficiency in AP. The hope is that it may potentially provide a valid and accurate measure of respiratory dysfunction and severity early in the course of the disease, by differentiating between established pathological pulmonary changes and transient physiological impairment. If validated, this non-invasive,

easily repeatable bedside method would allow for a more timely patient stratification in comparison to and/or complementary to existing scores.

1.4.6 LONG-TERM SURVIVAL FOLLOWING ACUTE PANCREATITIS

It is recognized that many survivors of critical illness have significantly higher long-term mortality compared to the general population. In the literature review of Brinkman et al which included 24 studies with more than 48 000 Intensive Therapy Unit (ITU) patients discharged alive between 2007 and 2011, the median mortality rate 1 year after ITU admission ranged between 16% and 44% (Brinkman et al., 2013). More recently, organ failure was amongst the important determinants of 1-year outcome in ITU survivors in the study of Ranzani et al, in which 1-year mortality was shown to be as high as 27% (Ranzani et al., 2015). Timmers et al achieved longer follow-up and demonstrated a mortality rate of 51% 11 years following discharge from surgical ITU (Timmers et al., 2011).

Lone and Walsh established that a higher burden of cardiovascular, respiratory and hepatic organ system failure during critical illness is strongly associated with higher 5-year mortality, and that this association remains strong even in patients who are alive 1 year after their episode of critical illness, suggesting an important residual effect of acute illness on long-term health and life-expectancy (Lone and Walsh, 2012).

Similarly, deleterious long-term outcomes have been documented following acute renal failure in several studies. Gallagher et al reported that the long-term mortality for patients that survived to 90 days following acute kidney injury requiring acute renal replacement therapy, was 31.9% in the 3.5 years of follow-up (Gallagher et al., 2014). Van Berendoncks et al stated that mortality during the 1st and 2nd year following discharge in a similar cohort was 23.0% and 7.6%, which was not related to disease severity or exact treatment modality (Van Berendoncks et al., 2010).

Although it is increasingly appreciated that long-term outcomes should be investigated following an episode of critical illness (Angus et al., 2003), the available data on the long-term survival of patients following an episode of AP are sparse. Few contemporary studies that have investigated long-term survival and the relevant prognostic factors in AP exist, and have for the most part examined morbidity outcomes, for example progression to chronic pancreatitis, or have focused upon a severe AP subgroup (Lankisch et al., 2009; Nøjgaard, 2010). Those that do report on survival suggest that hospital survivors after severe AP may exhibit reduced life expectancy (Halonen et al., 2003). After excluding inpatient mortality, death rates have been reported to be in the order of one death for every 4 to 8 AP patients , over 5 to 8 years (Halonen et al., 2003; Lankisch et al., 1996). Late deaths seem to be most commonly attributed to cardiovascular disease or malignancy (Lankisch et al., 2009; Nøjgaard, 2010), but the factors that reduce long-term survival are likely to be complex.

1.5 HYPOTHESES

The following hypotheses have been investigated in the respective chapters of the present thesis:

- **Hypothesis 1:** Peripheral plasma concentrations of kynurenine pathway metabolites are altered in AP in humans.
- **Hypothesis 2:** Transthoracic lung ultrasonography can detect respiratory dysfunction in AP-MODS.
- **Hypothesis 3:** AP-MODS confers a long-term survival disadvantage after hospital discharge compared to AP without MODS.

Chapter 2 – Kynurenine Metabolism in Acute Pancreatitis

2.1 SUMMARY OF CHAPTER

Therapeutic inhibition of KMO, a key enzyme in the kynurenine pathway of TRP metabolism, protects against MODS in experimental AP . In order to inform clinical trial design and assist the translation of KMO inhibitors into clinical practice, I undertook the “Inflammation, Metabolism, and Organ Failure in Acute Pancreatitis” (IMOFAP) study to define the activation state of the kynurenine pathway in relation to AP and AP-MODS in humans. This study comprises the major part of the thesis.

IMOFAP was a prospective, high-resolution, observational clinical study of all persons presenting to hospital with a potential diagnosis of AP at any time of day or night for 90 days. Clinical data and physiological measurements were recorded and peripheral venous blood was sampled at 0, 3, 6, 12, 24, 48, 72 and 168 hours post-recruitment. KYN metabolite concentrations were measured and analysed in the context of clinical disease severity indices, cytokine profiles and CRP concentrations.

Seventy-nine individuals were recruited (median age: 59.6 years; 47 males, 59.5%). Fifty-seven met the revised Atlanta definition of AP, and of those 25 suffered from mild, 23 from moderate, and 9 from severe AP. Median time to recruitment was 5.6 hours (95% C.I.: 4.1-6.7 hours). Plasma 3-HK concentrations correlated with contemporaneous APACHE II scores (R^2 = 0.273; Spearman rho = 0.581; $P < 0.001$) and CRP (R^2 = 0.132; Spearman rho = 0.455, $P < 0.001$). Temporal profiling showed early TRP depletion and contemporaneous 3-HK elevation. The magnitude of exposure to elevated 3-HK paralleled AP severity.

In conclusion, metabolic flux through KMO in human AP, and in particular 3-HK plasma concentrations, parallel systemic inflammation, MODS and overall disease severity. These findings reinforce the rationale for investigating early phase KMO inhibition as a therapeutic strategy to protect against AP-MODS in human AP.

2.2 AIM

The University of Edinburgh (UoE) is presently involved in a collaboration with GSK aiming to develop novel inhibitors of KMO through the DPAc initiative. The aim of the prospective, observational clinical research study titled "Inflammation, Metabolism, and Organ Failure in Acute Pancreatitis" (IMOFAP) was to define the precise temporal and quantitative relationship between the kynurenine pathway metabolism and the onset and severity of AP-MODS, in order to improve our understanding of the role of KYN metabolism in human AP. Ultimately, the IMOFAP study will inform the design of future studies aiming to address efficacy of KMO inhibition in AP-MODS (Skouras et al., 2016b).

2.3 METHODS

2.3.1 ETHICAL AND REGULATORY APPROVALS

The IMOFAP study received ethical approval by the Scotland A Research Ethics Committee (REC reference number: 13/SS/0136, dated 4th September 2013; amended REC REF AM01, dated 27th October 2013) and regulatory approval by the NHS Lothian Research & Development committee (Lothian R&D Project No: 2013/0098, dated 13th September 2013; SA1, dated 27th October 2013), as a non-CTIMP study. The study sponsor was the Academic and Clinical Central Office for Research and Development (ACCORD), a collaboration between UoE and NHS Lothian. Permission to access confidential medical records was granted by the NHS Lothian Caldicott Guardian.

Written informed consent was obtained from all participants or their legal representatives. Appropriate information sheets were provided to each participant or surrogate (**APPENDIX**). The General Practitioners (GPs) of

participants were informed in writing. Prior to commencing the study, a summary of the study protocol was registered in the public domain on the UK Clinical Trials Gateway (former UK Clinical Research Network – registration number: 16116). The study was conducted in accordance with the principles of the Declaration of Helsinki (World Medical Association, 2013).

2.3.2 RESEARCH SITES

Recruitment of participants was undertaken in the ED, and the General Surgery wards of the Royal Infirmary of Edinburgh (RIE). Patients who had originally presented to the ED of St. John's Hospital – a smaller District General Hospital within NHS Lothian serving a West Lothian community – and were transferred to the RIE for definitive management in accordance with the existing regional protocol, were also considered eligible for recruitment. In those circumstances, patients were enrolled and interventions were performed following their arrival at the RIE.

2.3.3 INCLUSION CRITERIA

All patients over the age of 16 years presenting to the ED of the RIE with a potential or confirmed new diagnosis of AP over the 90-day period between the 17th of September, and 16th of December 2013 were identified potential participants. For the diagnosis of AP, two of the following three criteria were required:

i. A clinical history of symptoms compatible with AP (i.e. abdominal pain, nausea, and/or vomiting);
ii. Serum amylase concentration greater than the upper limit of the reference range (> 100 IU/L). This threshold was decided upon in order to capture potential participants with an amylase level below the threshold of the revised Atlanta definition for AP, due to late or

atypical presentation. A serum amylase concentration in excess of 300 IU/L was required for the diagnosis of 'true' AP (tAP).

iii. Evidence of AP on CT and/or AUSS.

Furthermore, patients with AP transferred from other centres or existing inpatients with a new finding of elevated serum amylase and/or a radiological suspicion of AP were considered eligible for recruitment. Adults without the capacity to give informed consent were recruited in accordance with the Adults with Incapacity (AWI) (Scotland) Act 2000, Part 5 (Scotland, 2000). Informed consent was sought at a later stage for those who regained capacity after initial recruitment under the AWI act.

2.3.4 EXCLUSION CRITERIA

Patients from the following vulnerable groups were excluded:

i. Patients under the age of 16 years, and

ii. Prisoners & patients in police custody.

2.3.5 RECRUITMENT

The recruitment phase of the study lasted 90 calendar days – from 17th September to 16th December 2013 – and was carried out 24 hours per day, 7 days per week. The Wellcome Trust Clinical Research Facility (WTCRF) provided support for recruitment and consent, data collection, sample handling and storage, by staffing a 12-hour shift rota, with 30 research nurses, supported by two clinical support workers and one clinical technician. All participating research nurses and supporting staff attended two pre-recruitment meetings, led by the author and were given access to preparatory study material and to relevant study documents. Debriefing handover meetings of the on-call nurses, led by the author, were held between consecutive shifts and pending actions were highlighted. The author provided 24/7 on-call support whenever necessary.

A short time interval between each patient's presentation to hospital and recruitment to IMOFAP was of particular importance to ensure that participant capture and acquisition of samples were performed as early as possible in the course of AP. This point was quintessential in the design of IMOFAP, which was structured to maximize recruitment efficiency, a goal previously identified as challenging and resource-intensive in the context of clinical studies (Hunninghake et al., 1987).

To this effect, an automated alert was initiated by the biochemistry laboratory of the RIE and was transmitted to the on-call WTCRF research nurse on a dedicated study mobile telephone, for any patient with elevated serum amylase concentration (serum amylase > 100 IU/L). The dedicated study telephone number was also distributed to all ED and surgical team members. Upon each alert, the WTCRF nurse would review the clinical history of each potential participant via his or her electronic records on InterSystems® TrakCare®. If the clinical presentation was compatible with AP, the patient was visited and suitability for recruitment was confirmed. A member of the direct clinical care team made the initial approach to each potential participant prior to informed consent and recruitment by the WTCRF nurse team.

2.3.6 CONSENT

Upon identification of each potential participant – following a laboratory alert and review of the electronic clinical history – the on-call research nurse would liaise with a member of the direct clinical care team in order to obtain permission from the potential participant to be approached by a member of the research team. Each potential participant received a full explanation of the nature and purpose of the IMOFAP study, with the opportunity to ask questions. In addition, an information sheet and a consent form (**APPENDIX**) were provided. Recruitment was performed and informed consent was obtained by the on-call WTCRF nurse or the author. During the recruitment process, patients were being informed that they could withdraw from the study

at any point without justification and with no consequence to their care. Additionally, consent was being obtained to inform each participant's GP. The established procedures for consent agreed by the Lothian Ethics Committee, UoE and NHS Lothian have been adhered to at all times.

Patients willing to participate would sign and date the consent form, which would then be countersigned by the WTCRF nurse or the author. The signed form was photocopied; one copy was filed in the participant's case notes, and one was handed to the patient (or welfare guardian), while the original signed form was filed in a dedicated secure storage in the WTCRF at the RIE. The respective direct clinical care team was informed of each patient's participation in IMOFAP.

AWI were considered for participation in the study in accordance with the set of principles outlined in the relevant subsections of Part 5 of the AWI (Scotland) Act 2000 (Scotland, 2000). AWI consent was obtained from any guardian or welfare attorney with power to consent to participation in research, or from the nearest relative where no such guardian or welfare attorney existed.

Every effort was made to take into account prior wishes of the individual with incapacity about whom to consult, and to act in accordance with any relevant previous statement or wishes, however made, including non-verbal forms of communication. Adherence to the principles of the Act was ensured by applying the following measures, amongst others: advice was sought from the nursing staff caring for the patient regarding evidence or indications of unwillingness; each patient's multidisciplinary records were reviewed and any advance directive was noted; the advice of the nursing staff caring for AWI about any advance directive were taken into consideration. Critically ill individuals who had previously expressed a wish not to participate in research were not recruited.

For AWI, a senior member of the respective clinical care team obtained permission from the patient's guardian/welfare attorney to be approached by a member of the research team. The guardian/welfare attorney should

themselves have had capacity at the material time and should have been prepared to be consulted by a member of the research team about the possible involvement of the AWI in the project. An information sheet similar to the patient information leaflet for patients with capacity was given to the patient's guardian/welfare attorney and consent was sought (**APPENDIX**). Information and contact details about suitable independent sources of advice were provided. The guardian/welfare attorneys were able to advise the research team that the AWI would not want to continue participating at any stage of the study. In borderline cases, the recruiting member of the research team liaised with senior members of the respective direct clinical care team to decide whether a patient had capacity.

For participants with fluctuating capacity, every effort was made to establish their general wishes during a period that they had capacity. For participants that had provided consent and experienced a sudden or gradual decline of their cognitive status, a guardian/welfare attorney was sought and was provided with information about the research study and the nature of the consent given by the participant. Lastly, for the sub-group of participants who were originally recruited without capacity that gradually recovered and regained capacity to consent, informed consent was sought (**APPENDIX**), as described above.

Due to the potentially rapid onset of organ dysfunction in AP and especially as the aim of IMOFAP was to elucidate the mechanisms implicated in the genesis of AP-MODS, patients were given a maximum of 30 minutes to decide whether they would wish to participate. Nevertheless, deviations from this principle were allowed at the discretion of the WTCRF research nurse and/or the author based on individual patient circumstances.

2.3.7 WITHDRAWAL OF PARTICIPANTS

Participants were withdrawn from the study in the following circumstances:

i. In cases of withdrawal of informed consent, where capacity existed.

ii. In cases of withdrawal of consent by the patient's representative for AWI.

In the event of participants deciding to withdraw from the study, an option had been given in advance to permit ongoing use of data and samples which had already been collected, and/or future recording and usage of routinely collected clinical data and results. The decision was clearly documented on the original consent form.

When participants opted not to undergo venepuncture at any given time-point(s) for personal reasons, blood samples corresponding to the pre-defined time-point(s) were not obtained, without participants being withdrawn from the study.

2.3.8 STUDY PROCEDURES

For each patient, the duration of participation in the study was 7 days or until hospital discharge, whichever occurred sooner. A summary of all study procedures is presented on **TABLE 2.1**.

2.3.8.1 Calendar & software for time-point follow-up

A diarized alert/reminder software application was developed to generate laptop screen notifications and automated email alerts for each study intervention (i.e. blood sampling, etc.) to the research nurses and the author, as soon as each participant's identifier and recruitment details were recorded in the respective fields (**FIGURE 2.1**). This was particularly important to ensure all time-points were met when simultaneous and overlapping actions were due, as shown in **FIGURE 2.2**. In addition, a hand-written log was kept by the WTCRF research nurses to enter free text, notes and other information pertinent to the study for which no appropriate data field was available in the electronic study database, the details of which are described in section **2.3.9**. Cross-

referencing of the latter with the study database was performed at regular intervals.

2.3.8.2 Clinical history

For each participant, the clinical history obtained by the respective direct clinical care team was verified and any additional information necessary for the purposes of the IMOFAP study was collected ad-hoc by a member of the research team.

IMOFAP study procedures	Day 1					Day 2	Day 3-7		
	T_{minus}	T0	T3	T6	T12	T24	T48	T72	T168
Recruitment & informed consent		x							
Clinical history	x	x							
Vital signs	x	x	x	x	x	x	x	x	x
Venous blood sampling	x	x	x	x	x	x	x	x	x
Arterial blood sampling	x	x	x	x	x	x	x	x	x
Saliva sampling		x							
Urine sampling		x	x	x	x	x	x	x	x
Transthoracic USS							x		x

TABLE 2.1 – Summary and timing of study procedures for each participant. Routinely archived clinical samples (if available) were used to acquire venous blood samples at T_{minus}. Arterial blood samples were only obtained from participants with an A-line in situ. Urine samples were collected at the pre-defined time-points only from catheterised patients, and by convenience from non-catheterised patients. Performance of early transthoracic ultrasound scans (LUSS) varied from day 2 to day 4 and for late scans from day 6 to day 8.

2.3.8.3 Venous blood sampling

Peripheral venous blood was sampled at recruitment (T0) and 3 (T3), 6 (T6), 12 (T12), 24 (T24), 48 (T48), 72 (T72), and 168 (T168) hours (7 days) thereafter. An average maximum cumulative volume of approximately 80 mL of venous blood was retrieved from each patient over the course of their participation to IMOFAP. In addition, the respective original serum sample

obtained by the clinical care team upon each participant's presentation to hospital (T_{minus}), was also collected from the Biochemistry Laboratory of the RIE and was analysed.

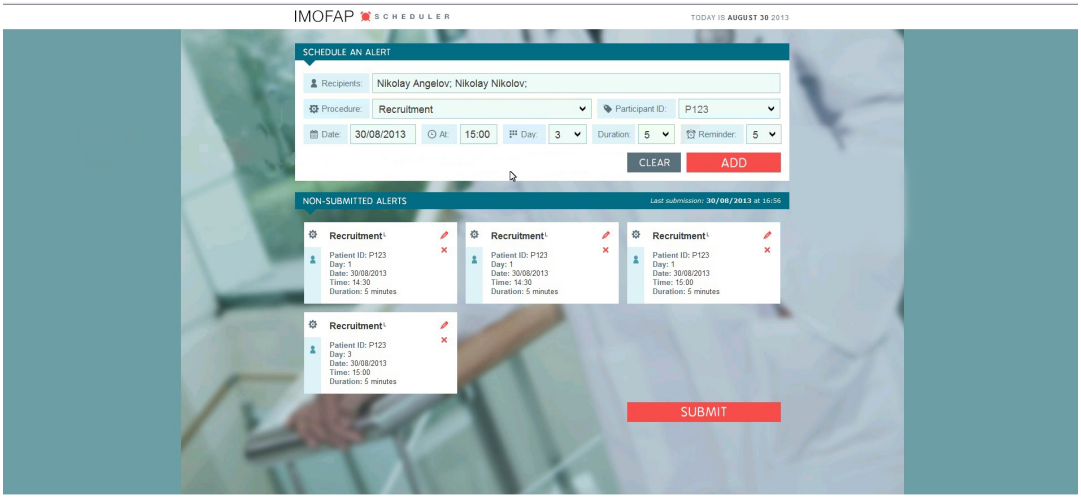

FIGURE 2.1 – Screenshot of the bespoke software application developed to ensure procedure follow-up for overlapping participant time-points. The application would create automated alerts and would email the research nurse on-call as soon as each participant's unique study identifier and details of recruitment were recorded in the respective field and the form was submitted.

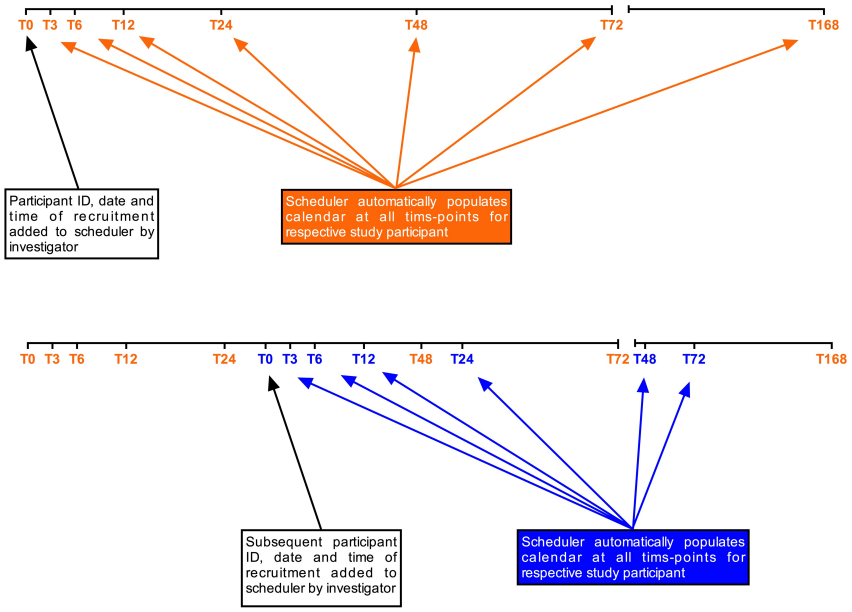

FIGURE 2.2 – Schematic representation of the progressively increasing complexity of study procedures with the recruitment of each consecutive participant.

Blood was sampled into gel clot-activator tubes for serum and into Ethylenediaminetetraacetic acid (EDTA) tubes for plasma, which were subsequently centrifuged at 3200 rpm for 8-10 minutes. All centrifuges had been serviced and calibrated prior to the start of the study. Serum and plasma were aliquoted and immediately frozen at -80°C, in freezers available in the WTCRF Unit of the RIE, until transfer on dry ice to long-term storage in a monitored, dedicated -80°C freezer in the Queen's Medical Research Institute of the UoE.

A sample history and tracking log was kept for all events. There were no breaches of the sample handling/storage protocol. Additionally, routine haematological and biochemical analyses were performed in accordance with standard RIE protocols used in clinical care.

2.3.8.4 Arterial blood sampling

For participants with an indwelling arterial cannula (A-line) in situ, arterial blood samples were collected at the same time-points as for venous blood, and these were processed as per routine local protocol. No arterial blood samples were taken from participants who did not already have an A-line in situ. When arterial blood samples were not available, the partial arterial pressure of oxygen (PaO_2) was extrapolated from the oxygen saturation values available from pulse oximetry measurements (SpO_2) at the corresponding time-points, by applying the method described by Severinghaus (Severinghaus, 1979), also used in different settings by other research groups (Dean et al., 2012). For this purpose, a conversion table was created by applying the Severinghaus formula and was used when necessary (**FIGURE 2.3**).

2.3.8.5 Saliva sampling

One salivary sample was obtained from each participant at T0, by using Sarstedt® Salivette®, as detailed on the respective section of the **APPENDIX**.

2.3.8.6 Urine sampling

A series of urine samples were obtained from each participant on the pre-defined time-points if an indwelling urinary catheter was in situ, or by convenience if the participant was not catheterised.

SpO_2	A	B	PaO_2	Air	Litres per minute					
					1-2	3-4	5-8	9-10	11-12	15
				FiO_2						
				0.21	0.25	0.3	0.4	0.5	0.6	0.9
				PaO_2 / FiO_2 ratio						
0.99	1158300.0	1158300.1	**131.9**	628	528	440	330	264	220	147
0.98	573300.0	573300.1	**104.2**	496	417	347	260	208	174	116
0.97	378300.0	378300.2	**90.6**	431	362	302	226	181	151	101
0.96	280800.0	280800.2	**81.9**	390	328	273	205	164	136	91
0.95	222300.0	222300.3	**75.7**	360	303	252	189	151	126	84
0.94	183300.0	183300.3	**70.9**	337	283	236	177	142	118	79
0.93	155442.9	155443.3	**67.0**	319	268	223	168	134	112	74
0.92	134550.0	134550.5	**63.8**	304	255	213	159	128	106	71
0.91	118300.0	118300.5	**61.0**	291	244	203	153	122	102	68
0.9	105300.0	105300.6	**58.7**	279	235	196	147	117	98	65
0.89	94663.6	94664.3	**56.6**	269	226	189	141	113	94	63
0.88	85800.0	85800.7	**54.7**	260	219	182	137	109	91	61
0.87	78300.0	78300.8	**53.0**	252	212	177	132	106	88	59
0.86	71871.4	71872.3	**51.4**	245	206	171	129	103	86	57
0.85	66300.0	66300.9	**50.0**	238	200	167	125	100	83	56
0.84	61425.0	61426.0	**48.7**	232	195	162	122	97	81	54
0.83	57123.5	57124.6	**47.5**	226	190	158	119	95	79	53
0.82	53300.0	53301.2	**46.4**	221	185	155	116	93	77	52
0.81	49878.9	49880.2	**45.3**	216	181	151	113	91	76	50
0.8	46800.0	46801.3	**44.3**	211	177	148	111	89	74	49
0.79	44014.3	44015.7	**43.4**	206	173	145	108	87	72	48
0.78	41481.8	41483.3	**42.5**	202	170	142	106	85	71	47
0.77	39169.6	39171.2	**41.6**	198	166	139	104	83	69	46
0.76	37050.0	37051.7	**40.8**	194	163	136	102	82	68	45
0.75	35100.0	35101.8	**40.0**	191	160	133	100	80	67	44
0.74	33300.0	33301.9	**39.3**	187	157	131	98	79	66	44
0.73	31633.3	31635.3	**38.6**	184	154	129	96	77	64	43
0.72	30085.7	30087.8	**37.9**	181	152	126	95	76	63	42
0.71	28644.8	28647.0	**37.3**	177	149	124	93	75	62	41
0.7	27300.0	27302.3	**36.6**	174	146	122	92	73	61	41

FIGURE 2.3 – Conversion table for the calculation of PaO_2/FiO_2 ratio of IMOFAP participants when an arterial blood sample was not available. The Severinghaus formula was used to calculate PaO_2 from SpO_2 at the corresponding time-point. PaO_2 was measured in kPa.

2.3.8.7 Lung ultrasonography

A nested cohort of participants in the IMOFAP study were recruited to additionally participate in the lung ultrasonography study, reported in **Chapter 3** of this thesis.

2.3.9 DATA COLLECTION & DATA MANAGEMENT

Apart from individual patient charts and clinical case notes, data were also collected from the following sources:

i. WardWatcher software (Critical Care Audit Ltd, Menston, United Kingdom)
ii. TrakCare® Patient Management System (InterSystems®, Massachusetts, USA)
iii. SCI Store data repository (Scottish Care Information – NHS National Services Scotland, Paisley, United Kingdom)
iv. Picture Archiving and Communication System (PACS) (AGFA-Gevaert group, Mortsel, Belgium)
v. Emergency Care Summary (ECS) database (National Information Systems Group, Paisley, United Kingdom)
vi. iLaboratory information system (iLab – APEX Healthware, San Antonio, USA)

Data for each participant were collected by the bedside on a dedicated, password-protected study laptop, after software encryption by the UoE Information Services (IS). A bespoke data collection software application was developed in Java™ with set limits to minimize the risk of mistyping of data entries and mandatory fields to ensure comprehensive recording (**FIGURE 2.4**). Input from the data collection software application was recorded automatically to a specifically developed Microsoft® Access® database (**FIGURE 2.5**).

Upon recruitment, each participant was delinked from the corresponding Unique Hospital Patient Identifier (UHPI), Community Health Index (CHI) and other identifiers such as name, date of birth and postcode,

and was assigned with a unique study number. The resulting link-anonymised data were stored separately from the identifiers and were regularly backed-up to minimize risk of loss, theft or unauthorized use, in accordance with the data protection principles of the UoE and the WTCRF. Back-up of the link-anonymised data was performed regularly to a secure network drive (file server). After back-up, all data was securely removed from the dedicated laptop with the use of the McAfee® Shredder™ application. Physical study records regardless of format, were stored securely in dedicated storage facilities of the WTCRF. Access to the data during all stages of the study was available only to the author and the Principal Investigator.

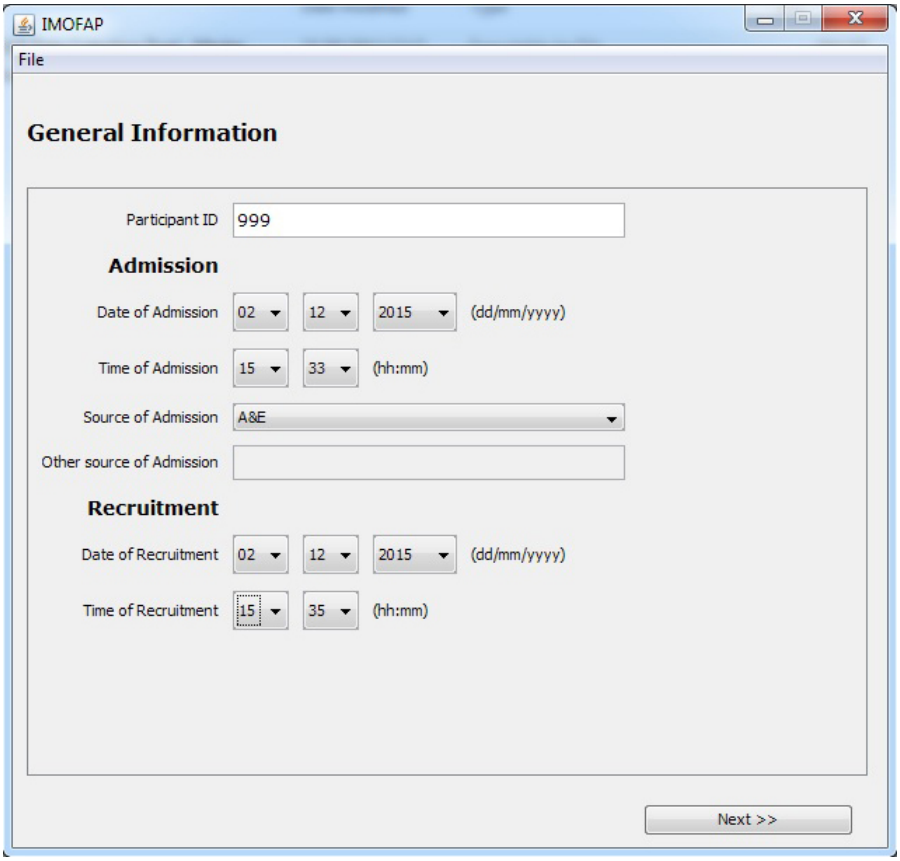

FIGURE 2.4 – Data collection software application screenshot. Set limits for all variables and mandatory fields were created to minimize mistyping of data entries and ensure comprehensive data input.

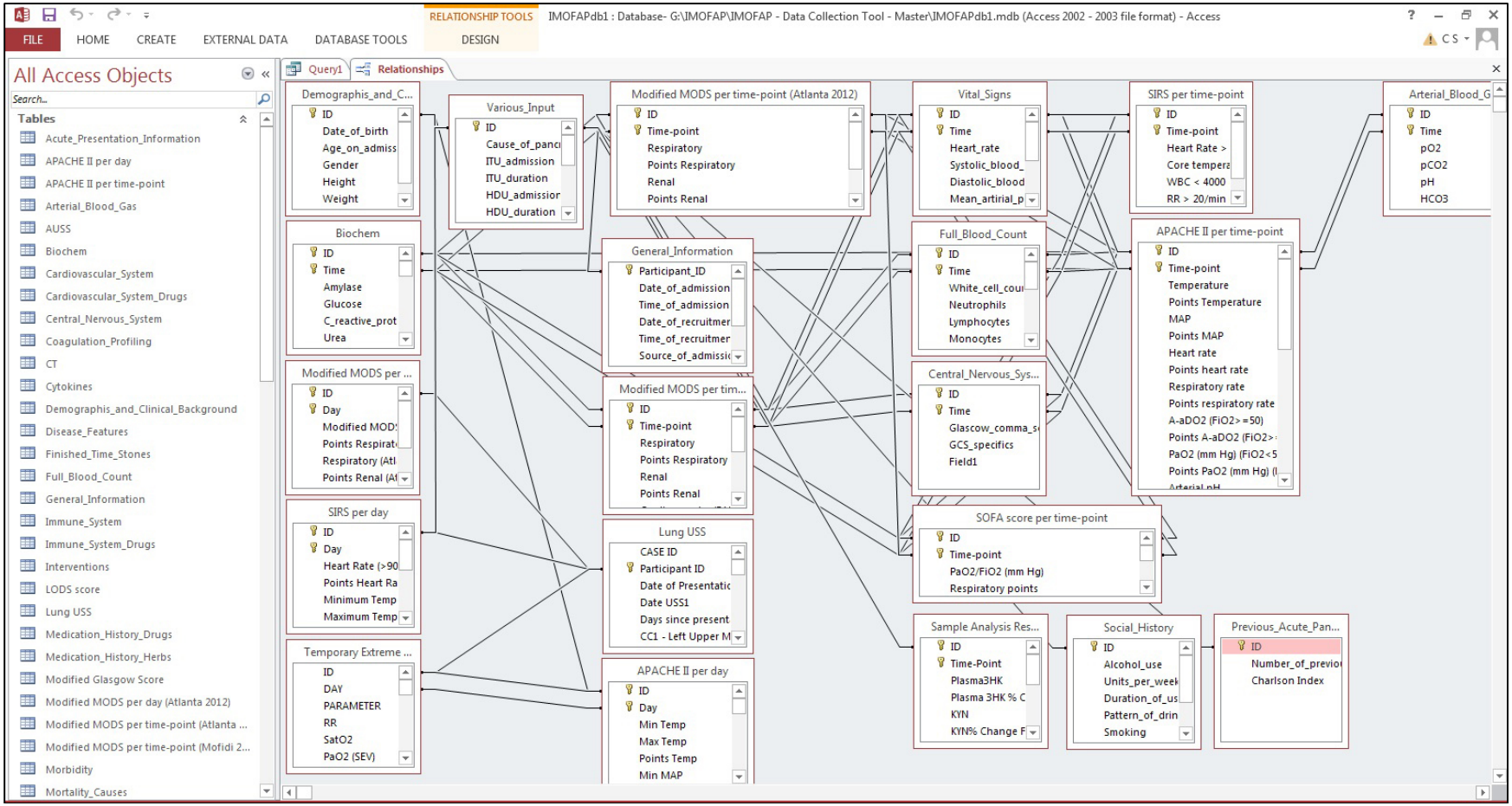

FIGURE 2.5 – Screenshot of the IMOFAP study Microsoft® Access® database. Input from the bespoke Java™ data collection software application was recorded in custom data tables of variables in the database. Relationships between data tables were formulated as shown and spreadsheets were exported for ad-hoc analyses.

2.3.10 COMPOSITE CLINICAL SCORES

For each participant, the APACHE II score (Knaus et al., 1985) was calculated on the day of admission, by using the most extreme values of the variables of interest (day 1 APACHE II) and on per time-point basis. Similarly, the MODS score, modified as detailed in the revised Atlanta Classification of AP, was calculated for each participant at each time-point and on a daily basis (Banks et al., 2013; Marshall et al., 1995).

2.3.11 TRYPTOPHAN METABOLITE MEASUREMENT

Kynurenine pathway metabolites were measured in plasma by two separate liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods. For the detection of TRP, KYN, KYNA and 3-HAA, plasma (5 µL) was initially diluted in phosphate-buffered saline (45 µL). The diluted plasma was added to 5mM ammonium formate containing 0.1% trifluoroacetic acid (130 µL). Protein was precipitated by the addition of ice-cold 100% trichloroacetic acid (20 µL), samples were vortexed briefly, incubated for 30 minutes at 4°C and centrifuged to obtain the supernatant. Separate standard curves were used to quantify levels of the individual metabolites.

For TRP, the standard curve consisted of serial dilutions of d5-TRP (as surrogate analyte of TRP) in 10% pooled human plasma. For KYN, 1% BSA in phosphate-buffered saline was used. For KYNA and 3-HAA, 0.2% BSA in phosphate-buffered saline was used. Samples (10 µL) were injected onto a Waters Select HSS XP column (3mm x 100 mm, 2.5 µm, Waters, Elstree, Herts) using a Waters Acquity UHPLC system, coupled to an AB Sciex™ QTRAP 5500 mass analyser. The flow rate was 0.35 mL/min at 25°C. Separation was carried out using a water:methanol gradient (both containing 0.1% formic acid). Chromatographic conditions were 50% methanol rising to 60% over 60 seconds, then to 65% over 180 seconds; held for 110 seconds, returned to 50% over 10 seconds and re-equilibrated for a further 200 seconds,

giving a total run time of 10.2 minutes. The mass spectrometer was operated in positive electrospray ionization mode. The multiple reaction monitoring (MRM) transitions for the protonated analytes were: TRP (m/z 205-188), KYN (m/z 209-146), KYNA (m/z 190-144) and 3-HAA (m/z 154-136). The transitions for d5-TRP standard were (m/z 210-122). Collision energies were 11, 29, 31, 33 and 37 eV respectively.

For the analysis of 3-HK, plasma (100 µL) was added to a 40:60 mixture of acetonitrile in water containing 0.1% formic acid (130 µL) and 50 ng/mL of internal standard. Protein was precipitated by the addition of trichloroacetic acid (33 µL), samples were vortexed briefly, incubated for 30 minutes at 4°C and centrifuged to obtain the supernatant. A standard curve containing serial dilutions of 3-HK in 10% BSA diluted with phosphate-buffered saline enriched with 50 ng/mL internal standard was used. 10 µL volumes of each sample were injected onto a Waters Select HSS XP column (30mm x 100 mm, 2.5 μm, Waters Corp, Elstree, Herts) using a Waters Acquity UHPLC system, coupled to an AB Sciex™ QTRAP 5500 mass analyser. The flow rate was 0.8 mL/min at 30°C. Separation was carried out using a water:methanol gradient (both containing 0.1% formic acid). Conditions were 5% methanol rising to 95% over 150 seconds, returning to 5% over 30 seconds, giving a total run time of 4 minutes. The mass spectrometer was operated in positive ion electrospray mode. The transitions for the protonated analytes were 3-HK (m/z 225-208) and internal standard (m/z 228-211). Collision energies were 20 and 12eV respectively.

Data were acquired and processed using Analyst® quantitation software version 1.3 (AB Sciex™, Framingham, USA). Results from retrieved from acute presentation samples (pre-recruitment samples) were excluded from the time-course analysis because serum-gel activator tube containers were routinely used by the clinical care teams, a fact that could have led to discrepancy upon comparison with results from the dedicated tripotasium-ethylenediaminetetraacetate containers that were used for the IMOFAP study.

Four samples obtained from two IMOFAP participants of the severe AP group returned values of KYN concentration that were greater than 20 standard deviations away from the remaining concentration values of the severe AP cohort, including temporally adjacent samples from the same patients. These samples were deemed to have resulted from contamination during sample analysis, were designated as extreme outliers and were removed from further analysis.

2.3.12 CYTOKINE ANALYSIS

Plasma cytokines were analysed using a custom-designed Human Magnetic Luminex Screening Assay according to the manufacturer's instructions (R&D Systems, Minneapolis, USA). Levels of insulin, interleukin-1 beta (IL-1B), insulin C-peptide and IFN-γ were measured using kit LXSAHM-4, cancer antigen 15-3 (CA 15-3) using kit LXSAHM-1 and TNF-α, B7 homolog 1 (B7-H1), chemokine (C-X-C motif) ligand 12 (CXCL 12), cluster of differentiation 163 (CD163), TNF superfamily member 10 (TNFS10), receptor for advanced glycation endproducts (RAGE), trefoil factor 3 (TFF3), cluster of differentiation 40 ligand (CD40 ligand), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 17A (IL-17A), cardiac troponin I and chemerin using kit LXSAHM-15, respectively. The concentrations of each cytokine were determined using Bio-Rad Bio-Plex 200 system (BioRad, California, USA).

2.3.13 HEALTHY VOLUNTEERS

Healthy volunteers over the age of 18 years were recruited with ethical approval (REC reference number: 08/S1103/38, United Kingdom). Volunteers were excluded from participation when any of the following conditions were present: renal dysfunction (eGFR < 30 mL/min); hepatic dysfunction (Child-Pugh score B or C); pregnancy or breast feeding; blood dyscrasia or anaemia

(haemoglobin < 12 g/dL); active malignancy; chronic inflammatory condition; any intercurrent illness and/or any recent surgical procedure.

2.3.14 STATISTICAL ANALYSIS

A data analysis plan was formulated prospectively and was adhered to. Quantitative data are presented as median and interquartile range (IQR) or mean and standard deviation (SD), as appropriate. Qualitative data are presented by utilising absolute and/or relative frequencies. Data conformity to the normal distribution was analysed by one-sample Kolmogorov-Smirnov testing, and comparisons between patient groups were performed by using one-way ANOVA or the Kruskal-Wallis H test, as appropriate. Spearman's rho was used to examine bivariate correlations of quantitative variables. Logarithmic transformations of non-normally distributed variables were used to establish the best linear regression fit during correlation. For the analysis of serial metabolite measurements, the method of summary measures was used (Matthews et al., 1990). The area under the curve (AUC) for each metabolite was calculated for each participant by using the trapezium rule, as described by Matthews et al (Matthews et al., 1990). These were standardized by duration of participation in the study, defined by the period between recruitment and the ultimate time-point of blood sampling for each participant. For the computation of each AUC, any uttermost missing values were omitted, whereas for any middle missing values AUCs were calculated by using the average of two immediately adjacent available values. The Kaplan-Meier method was used to compare time-to peak and time-to-minimum metabolite values between groups, and the log-rank test was applied to detect differences; only time-points between T0 up to and including T72 were considered in the latter analyses.

All statistical tests were based on a two-sided α-value of 0.05. Data were analysed using IBM SPSS® Statistics version 22.0 (IBM Corporation,

Armonk, New York, USA) and graphs were drawn using GraphPad Prism® version 6.0 (GraphPad Software, Inc., La Jolla, California, USA).

Achieved statistical power was calculated post-hoc using the G-Power algorithm: F tests – ANOVA: Fixed effects, omnibus, one-way (Faul et al., 2007). The effect size was computed from measured 3-HK concentrations in tAP as f = 5.78, with α error probability of 0.05. The noncentrality parameter, λ = 1902.25; Critical F = 3.17; numerator df = 2; denominator df = 54; computed power (1-β error probability) = 1.00.

2.4 RESULTS

2.4.1 RECRUITMENT PERFORMANCE

A total of 79 patients were recruited. Of those, 57 patients (72.2%) were diagnosed with AP according to the revised Atlanta guidelines ("true AP" – tAP), while the remaining 22 participants had a serum amylase level between 100 and 299 IU/L, and were categorised in the 'hyperamylasaemia' participant group. Of the 57 tAP patients, 10 (17.5%) had a serum amylase level below 300 IU/L upon presentation, and either a subsequent rise of the amylase concentration > 300 IU/L or an imaging confirmation of AP. The demographic characteristics of the study participants are summarized in **TABLE 2.2,** and a CONSORT diagram of the IMOFAP study is shown in **FIGURE 2.6**. No systematic bias was evident for the 29 potentially eligible patients that were not recruited due to no activation of the automated alert. Thirty patients declined to participate, of whom 12 had AP and 3 required admission to critical care. One male participant formally withdrew from the study within 12 hours from recruitment.

There was no effect of day of the week (P = 0.317) or time of day (P = 0.397) on recruitment and therefore no selection bias by time of day or by day of the week. The cumulative number of enrolled participants by calendar day

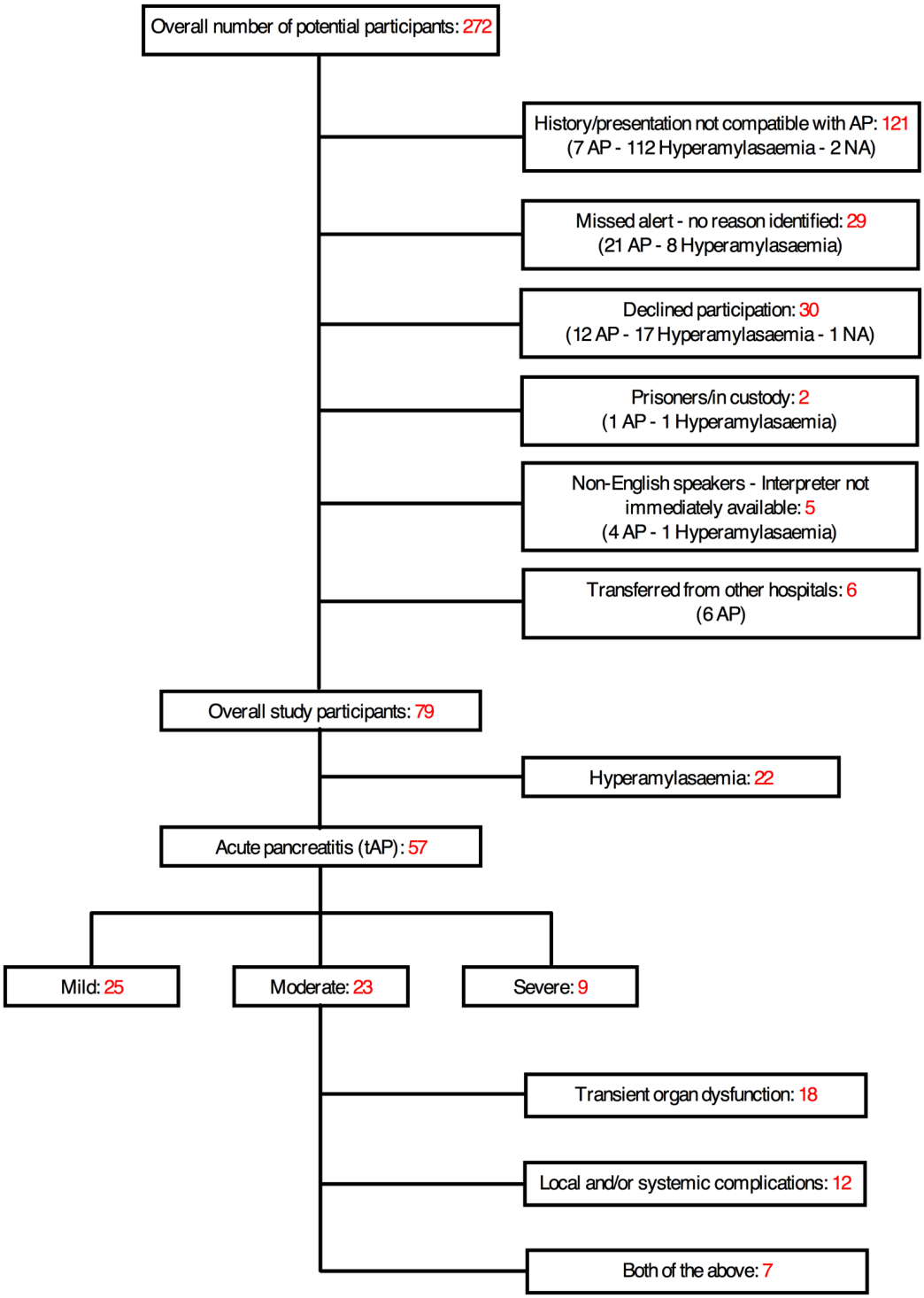

FIGURE 2.6 – CONSORT diagram of the IMOFAP study. Reasons for patient exclusion are summarised, with post-hoc diagnoses for each exclusion category included in brackets (NA = not available). Patients of the moderate AP severity group were divided to three further sub-groups according to the revised Atlanta guidelines of 2012 for explanatory purposes.

	Diagnosis			Modified MODS (Atlanta 2012)			APACHE II		In-hospital mortality	
	Overall	Non-AP	AP	Mild	Moderate	Severe	Mild	Severe	Survivors	Deaths
No of patients	79	22 (28%)	57 (72%)	25 (44%)	23 (40%)	9 (16%)	15 (26%)	42 (74%)	54 (95%)	3 (5%)
Gender										
Male	47 (60%)	15	32 (56.1%)	16	12	4	11	21	31/54	1/3
Age										
Median	59.6	60.7	57.0	62.3	54.8	64.8	45.9	64.9	57	81.6
IQR	47.7-73.9	50.1-70.0	47-77.1	46.4-79.0	47.3-67.5	49.4-80.6	37.7-52.3	51-80.6	47.3-75.7	45.8-88.0
BMI										
Mean	27.2	25.5	27.9	27	28	28	26	28	28	28
SD	6	4.7	6.3	5.6	7.4	5.8	7.3	5.9	6.4	4.5
Source of recruitment										
ED	70 (88.6%)	19	51 (89.5%)	21	22	8	11	40	48 (88.9%)	3
Other	9 (11.4%)	3	6 (10.5%)	4	1	1	4	2	6 (11.1%)	0
LOS										
Median	5	4	5	5	6	7	4	6	5	4
IQR	3-8	2-11	3-8	3-6	3-9	5-13	2-8	4-8	3-8	2-77
Aetiology										
Gallstones	NA	NA	27 (47.4%)	11	14	2	4	23	25 (46.3%)	2
Alcohol	NA	NA	18 (31.6%)	8	5	5	7	11	17 (31.5%)	1
Idiopathic	NA	NA	7 (12.3%)	2	3	2	2	5	7 (13.0%)	0
Other	NA	NA	5 (8.8%)	4	1	0	2	3	5 (9.3%)	0
Charlson Index										
Median	2	3	2	2	1	2	1	3	2	4
IQR	1-5	1-5	1-4	1-4	0-3	2-4	0-1	1-5	1-4	0-5
Mortality										
In-hospital	4 (5.1%)	1	3 (5.3%)	0	1	2	0	3	NA	NA
30-day	2 (2.5%)	0	2 (3.5%)	0	0	2	0	2	NA	2
Onset to presentation										
Median (hours)	21.4	46	15.3	9.8	23.4	21.3	24.2	21.3	15.3	15
IQR (hours)	8.5-73.3	10.6-135.7	8.3-50.1	5.3-37.8	9.2-75.5	9.2-73.0	8.5-75.5	9.2-73.0	8.1-48.3	10.8-73.0
Onset to recruitment										
Median (hours)	33	49.5	25.9	18.5	26.7	36.3	30.7	36.3	26.3	22.3
IQR (hours)	14.2-92.0	18.0-138.5	13.8-70.8	10-46.2	16.6-82.3	17.8-77.8	14.2-92.2	17.8-77.8	13.3-67.3	17.8-77.8
Critical Care admission										
ITU	2 (2.5%)	0	2 (3.5%)	0	0	2	0	2	0	2
HDU	5 (6.3%)	3	2 (3.5%)	0	0	2	0	2	2	0
Overall	7 (8.9%)	3	4 (7.0%)	0	0	4	0	4	2	2

TABLE 2.2 – Demographic characteristics of patients recruited in the IMOFAP study. For the computation of LOS, re-admission(s) with 10 days from discharge were taken into account.

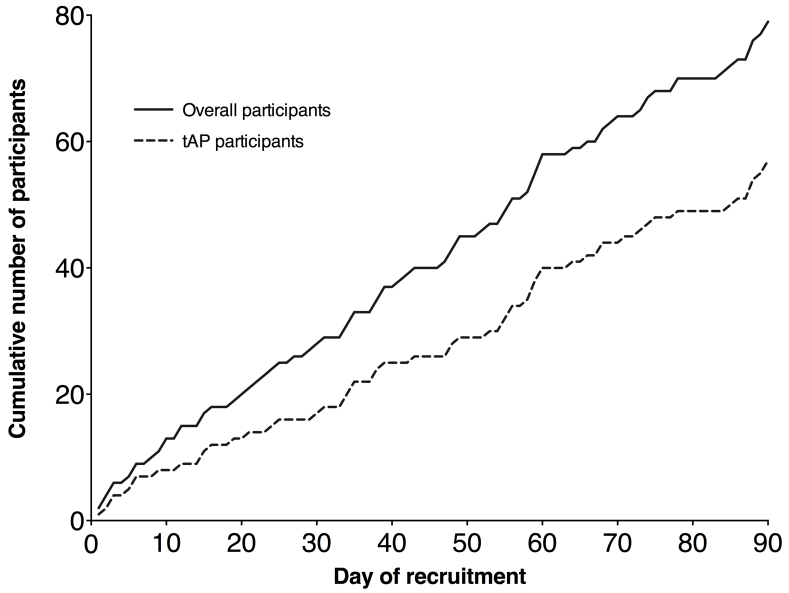

FIGURE 2.7 – Line graph demonstrating the cumulative number of participants enrolled during the recruitment phase of IMOFAP. The full line represents the number of overall participants and the dashed line the number of tAP participants.

	Overall		tAP		Non-tAP	
	N	%	N	%	N	%
Day of the week						
Monday	9	11	6	11	3	14
Tuesday	12	15	8	14	4	18
Wednesday	8	10	6	11	2	9
Thursday	8	23	14	25	4	18
Friday	9	11	7	12	2	9
Saturday	9	11	7	12	2	9
Sunday	14	18	9	16	5	23
WD vs. WE						
Weekday	56	71	41	72	15	68
Weekend	23	29	16	28	7	32
Time of day						
08:00-15:59	23	29	13	23	10	46
16:00-23:59	32	41	27	47	5	23
00:00-07:59	24	30	17	30	7	32
Overall	79	100	57	100	22	100

TABLE 2.3 – Timing of hospital presentation of study participants by day of the week and time of day. **WD**: weekday; **WE**: weekend.

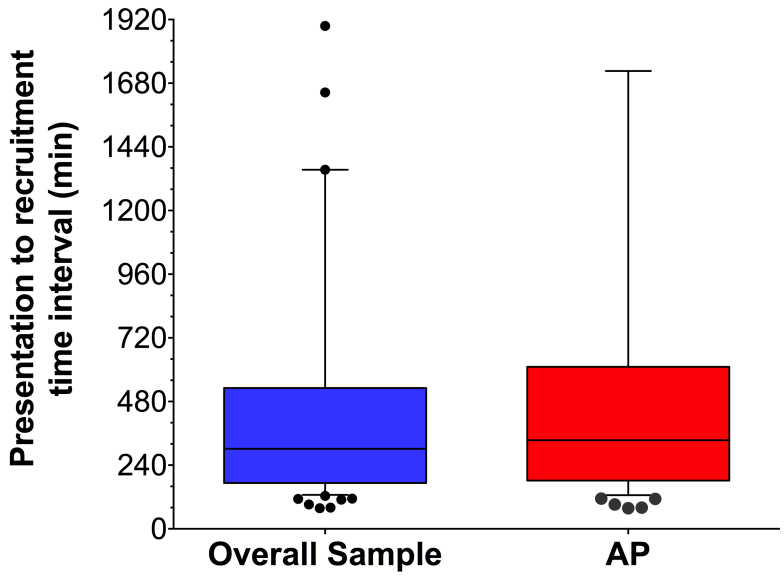

FIGURE 2.8 – Box plot showing time interval from acute hospital presentation to study recruitment (horizontal line: median value; boxes: 1st and 3rd quartile; error bars: 1st and 9th deciles).

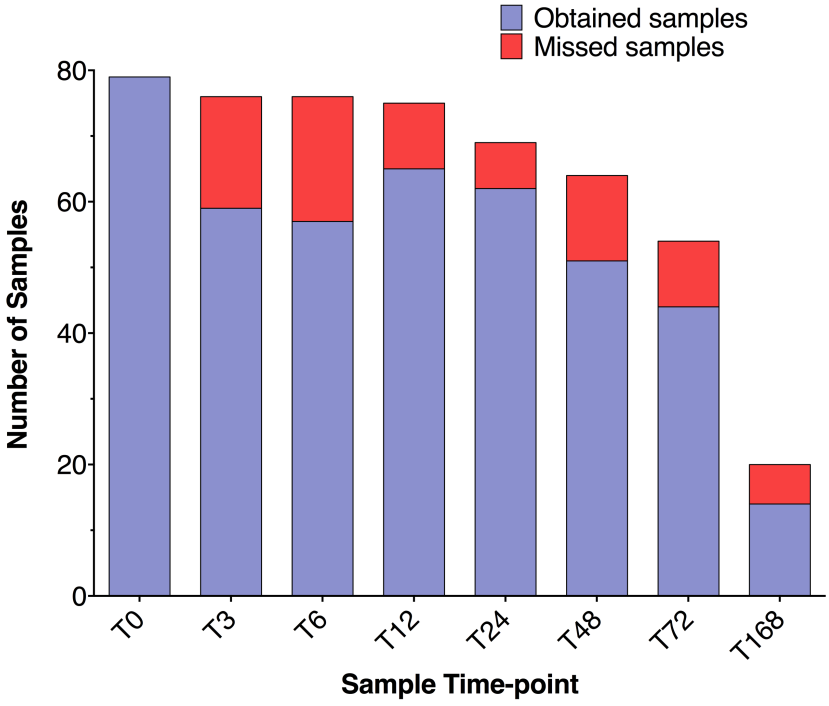

FIGURE 2.9 – Bar chart summarizing numbers of obtained (blue bars) and missed samples (red bars), per study time-point.

of the recruitment phase and the absolute and relative frequencies of participant enrolment by weekday and time of day are shown on **FIGURE 2.7** and **TABLE 2.3,** respectively.

Sampling performance was efficient, and a total of 508 samples were obtained an analysed. The median time interval between the acute presentation sample (pre-recruitment, T_{minus}) to recruitment was 5 hours and 34 minutes (IQR: 182-611 minutes) for tAP participants (**FIGURE 2.8**). This interval included examination and consultation by the direct clinical care team(s), routinely collected presentation sample processing and analysis, verification of the result by the hospital laboratory staff, communication with the study team for confirmation of the clinical history and informed consent. Due to the high temporal resolution of IMOFAP design, some sample time-points were missed, but no systematic bias was identified for the unobtained samples (**FIGURE 2.9**).

2.4.2 INFLAMMATORY INDICES, COMPOSITE SCORES & CYTOKINES

A clear rise in the level of CRP was evident for tAP participants (standardized AUC for CRP concentration, median (IQR): hyperamylasaemia group: 18.9 (5.8-45.4) mg/L; tAP participants 126.0 (49.9-201.1) mg/L; $P < 0.001$), the magnitude of which was proportionate to AP severity (**FIGURE 2.10a**) and in keeping with a substantial systemic inflammatory insult (standardized AUC for CRP concentration, median (IQR): mild: 58.9 (26.3-131.2) mg/L; moderate: 148.1 (74.5-243.9) mg/L; severe: 225.6 (174.9-260.2) mg/L; $P = 0.001$) (**FIGURE 2.10a & TABLE 2.4**). The acute presentation (pre-recruitment) mean CRP concentrations were higher in severe AP, but this difference was not statistically significant (pre-recruitment CRP concentration, median (IQR): mild: 11 (5-34) mg/L; moderate: 19 (9-39) mg/L; severe: 59 (30-65) mg/L; $P = 0.166$). Resolution of inflammation, indicated by a fall in CRP, occurred between 48 and 72 hours for the majority of participants. Conversely,

serum amylase was more than three times higher than the upper limit of normal range upon recruitment, and normalised within 24 hours for the majority of participants. There was no association between serum amylase level upon presentation and disease severity (**FIGURE 2.10b**). Furthermore, mean and minimum concentrations of albumin, as well as the corresponding mean standardized AUC for albumin were significantly lower in the severe AP group (**TABLE 2.4**).

Severe AP patients had a significantly higher modified MODS score upon presentation to hospital, when compared to the other two groups (mean MODS score, (SD; range): mild: 0.8 (0.7; 0-3); moderate: 0.8 (0.9, 0-4); severe: 2.8 (2.5; 0-8); P = 0.003), followed by a further rise during the initial 12-hour period after recruitment and apparent resolution (a reduction of MODS score to < 2) beyond the 48-hour mark (**FIGURE 2.10**). No association was discovered between the time interval from the onset of symptoms to study recruitment and modified MODS score upon presentation of severe AP patients (Spearman's rho = –0.037, P = 0.926). Both day 1 APACHE II and T_{minus} APACHE II scores were higher in the severe AP group (day 1 APACHE II score, mean (SD): mild: 8.2 (4.4); moderate: 11.4 (3.8); severe 19.3 (11.0); P = 0.001, and T_{minus} APACHE II score, mean (SD): mild: 6.0 (3.6); moderate: 7.6 (3.8); severe: 12.0 (5.6); P = 0.002). APACHE II scores declined after 48 hours, with the decrease being more prominent from T72 onwards (**FIGURE 2.10d**)**.** Importantly the decline of both modified MODS and APACHE II scores (calculated for each time-point) coincided with and is due to the deaths of two patients in the severe AP group, who died within 55 and 101 hours after recruitment. The respective scores for the surviving severe AP patients remained relatively unchanged for the duration of their participation in the study.

The inflammatory burden of the study cohort was further quantified by supplementary serial measurements of pro- and anti-inflammatory cytokines over the same time-points. The severe AP patient group was found to have significantly higher levels of peak and mean concentrations, as well as standardized AUC levels of IL-1B, IL-6, IL-10, TFF3, and CD163 (**TABLE 2.4**).

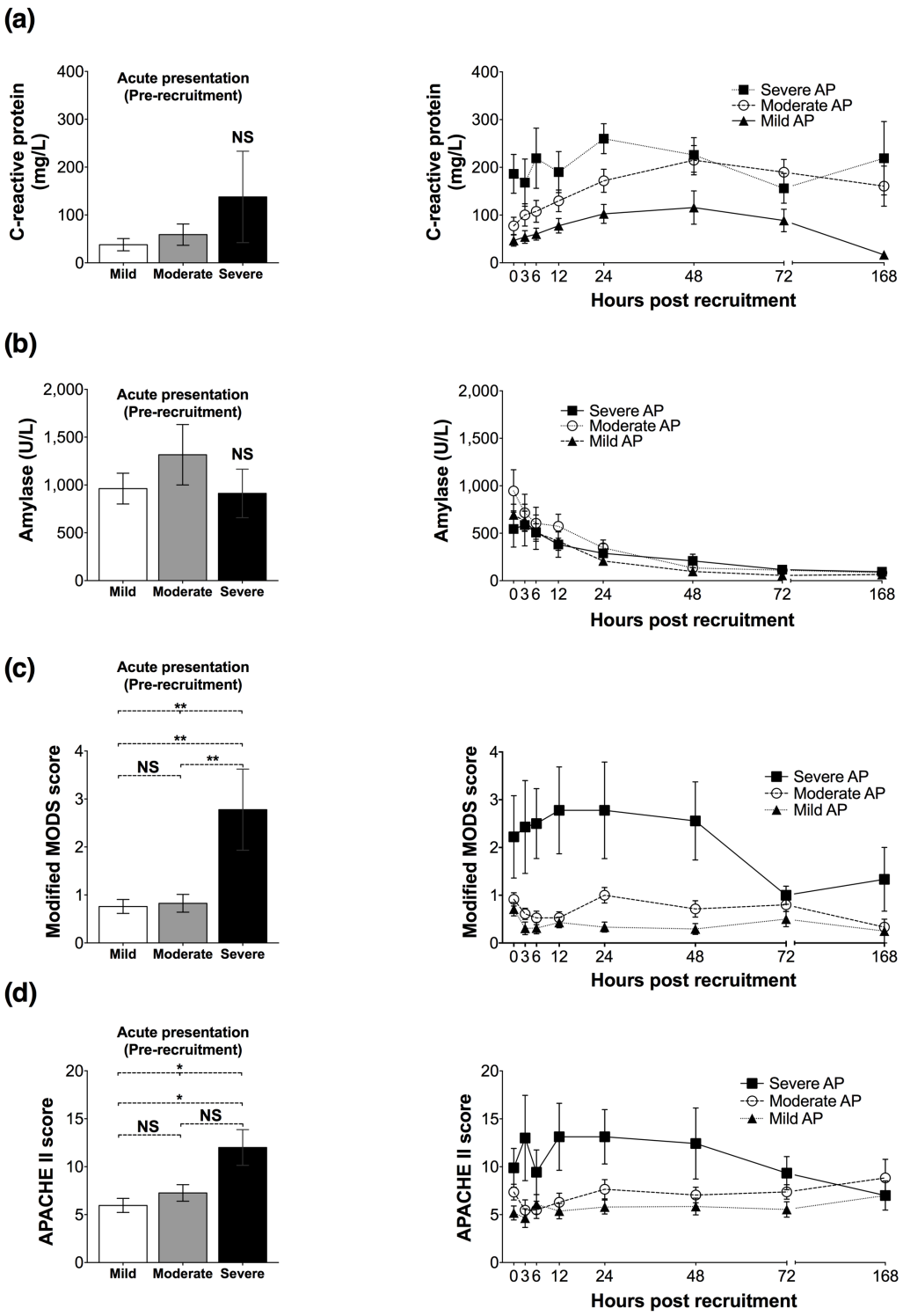

FIGURE 2.10 – Panel of comparisons between mild (n = 25), moderate (n = 23) and severe AP (n = 9) participant groups for **(a)** C-reactive protein; **(b)** Amylase; **(c)** Modified MODS score (as described in the revised Atlanta guidelines); **(d)** APACHE II score. Bar charts on the left hand side of the panel represent Tminus sample means (acute presentation – pre-recruitment samples), while right-hand side plots depict the time-course for each variable mean per participant group. NS: not statistically significant difference; *: $0.05 > P \geq 0.01$; **: $P < 0.01$; error bars represent standard errors of the mean.

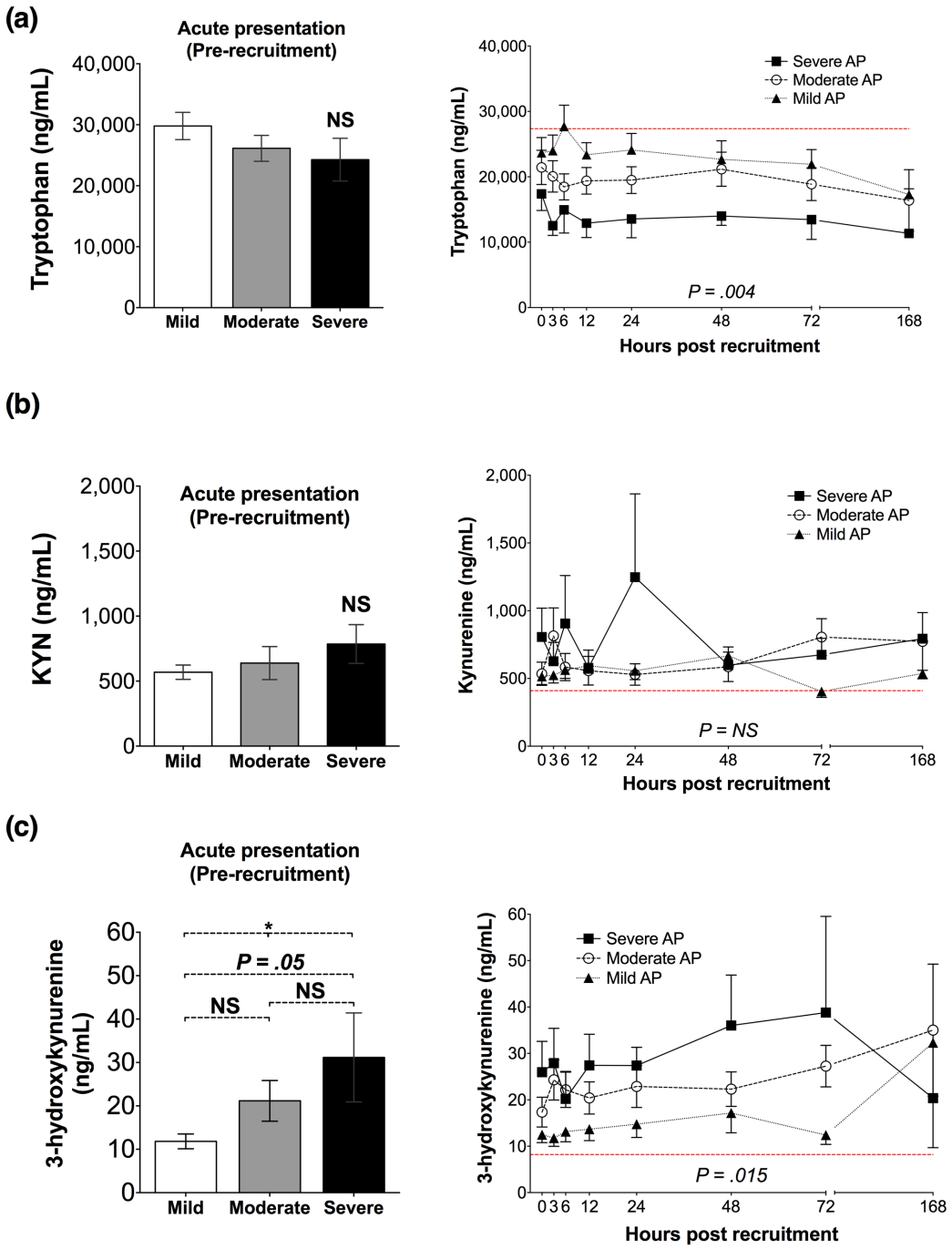

FIGURE 2.11 – Plasma concentrations of TRP and the kynurenine pathway metabolites in tAP participants over time, grouped by AP severity according to the revised Atlanta criteria (group sizes at recruitment: mild n = 25; moderate n = 23; severe n = 9 individuals). **(a)** TRP; **(b)** KYN; **(c)** 3-HK. For all panels, data points represent means and error bars represent standard errors of the mean. P-values for between-group comparisons of standardised AUC are appended. NS: not statistically significant. Dashed red lines represent healthy volunteer (n = 8) mean concentration of each metabolite. *: $0.05 > P \geq 0.01$; **: $P < 0.01$.

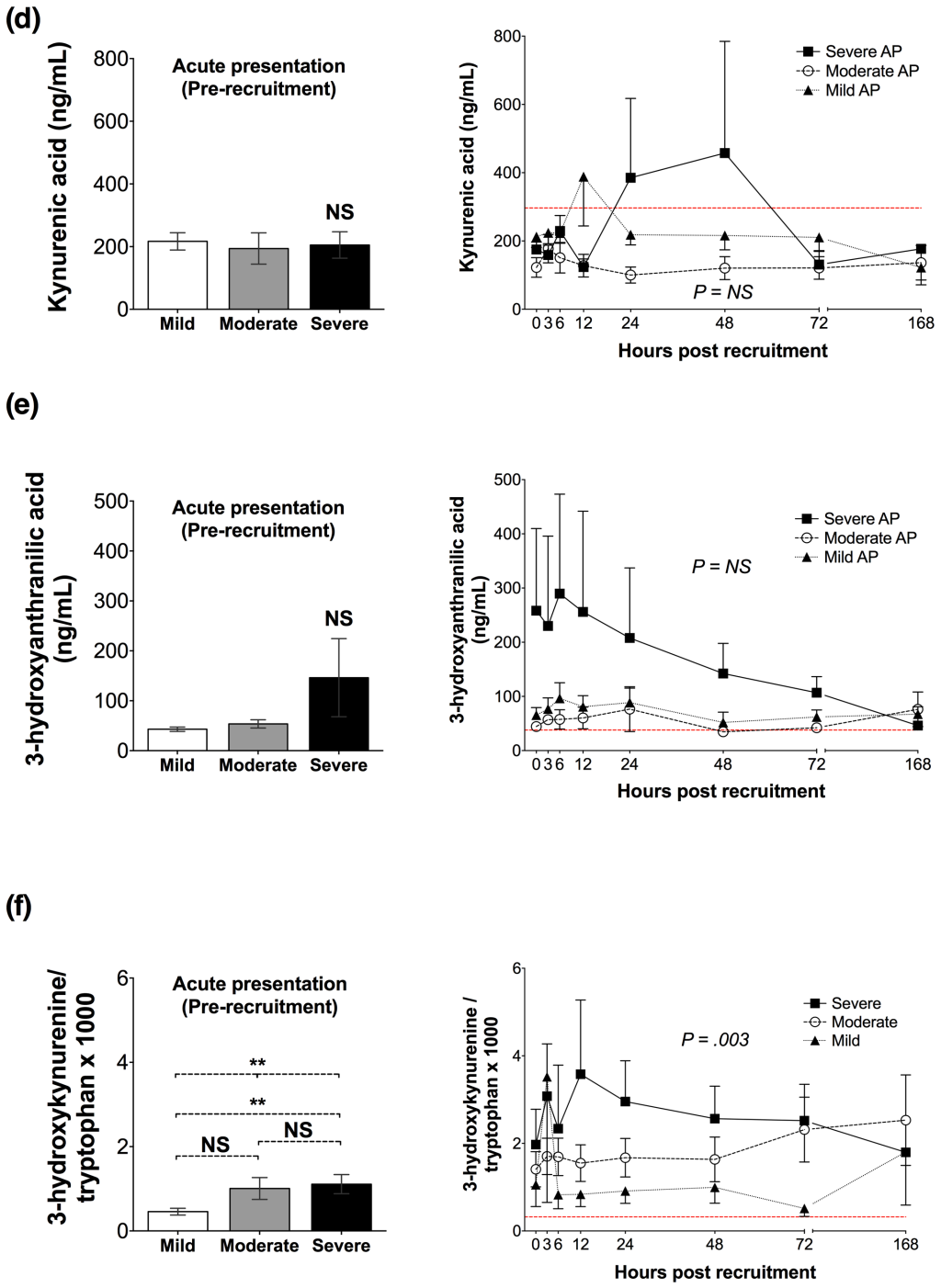

FIGURE 2.11 – Plasma concentrations of TRP and the kynurenine pathway metabolites in tAP participants over time, grouped by AP severity according to the revised Atlanta criteria (group sizes at recruitment: mild n = 25; moderate n = 23; severe n = 9 individuals). **(d)** KYNA; **(e)** 3-HAA; **(f)** 3-HK/TRP ratio multiplied by 1000. For all panels, data points represent means and error bars represent standard errors of the mean. P-values for between-group comparisons of standardised AUC are appended. NS: not statistically significant. Dashed red lines represent healthy volunteer (n = 8) mean concentration of each metabolite. *: $0.05 > P \geq 0.01$; **: $P < 0.01$

2.4.3 TRYPTOPHAN METABOLITES

Summary measures for the concentration of TRP and all studied TRP metabolites are presented in Error! Reference source not found.. TRP depletion was marked and proportionate to disease severity and especially profound in severe AP (standardised AUC for TRP concentration, mean (SD): mild: 24423.9 (8803.9) ng/mL; moderate: 19721.4 (8140.5) ng/mL; severe: 13782.4 (3789.8) ng/mL; P = 0.004). Importantly, upon presentation to hospital, no statistically significant difference in TRP concentration was evident among the three groups (acute presentation TRP concentration, mean (SD): mild 29796.6 (10939.7) ng/mL; moderate: 26133.0 (9973.8) ng/mL; severe: 24273.7 (9926.4) ng/mL; P = 0.281). Moreover, the observed effect on TRP levels was not a result of dilution due to resuscitation, as no statistically significant difference was observed in the standardised AUC of haematocrit between the three groups (standardized AUC for haematocrit, mean (SD): mild 0.361 (0.04); moderate: 0.350 (0.045); severe 0.330 (0.034); P = 0.161), a surrogate marker of haemodilution (Aggarwal et al., 2014).

No significant differences in peak level, mean level or standardized AUC of KYN, KYNA, and 3-HAA were detected amongst the three groups (Error! Reference source not found.). In specific, the observed difference of 3-HAA concentration on **Figure 2.11e**, although visually substantial, did not reflect a statistically significant difference, and was driven by the extreme values of 3-HAA in two individual participants of the severe AP group.

The severe AP group had a significantly higher peak 3-HK concentration across all study time-points (peak 3-HK concentration, median (IQR): mild: 13.5 (8.6-17.8) ng/mL; moderate: 20.0 (12.1-46.6) ng/mL; severe: 25.2 (14.3-35.6) ng/mL; P = 0.013). The standardized AUC for 3-HK was also different amongst the three groups, although only a marginal difference was observed between the moderate and the severe group, as shown on **Error! Reference source not found.** (standardized AUC for 3-HK concentration, median (IQR): mild: 10.8 (7.4-13.9) ng/mL; moderate: 18.2 (11.4-38.1) ng/mL;

severe: 21.6 (11.7-28.0) ng/mL; P = 0.015). The median time interval required for the peak 3-HK concentration to be reached was 24 hours (95% C.I.: 0.0-49.0 hours) in the severe AP group, compared to 12.0 hours (95% C.I.: 6.7-17.3 hours) in the mild AP group, while the mean time interval of the moderate group was 46.0 hours (95% C.I.: 32.9-59.0 hours; P = 0.019).

To provide an indication of flux through KMO we calculated the ratio 3-HK/TRP, multiplied by 1000 for practical purposes. A significant increase in the pathway flux through KMO was observed, which was proportionate to disease severity (standardized AUC for 3-HK/TRP ratio x 1000, median (IQR): mild: 0.45 (0.26-0.89); moderate: 0.73 (0.51-2.64); severe: 1.67 (0.93-1.84); P = 0.003). Time-plots for the concentration of each analysed metabolite and the 3-HK/TRP ratio are depicted on **FIGURE 2.11**.

When considering all samples obtained from T0 up to and including T48, logarithmic 3-HK levels demonstrated a weak to moderate correlation with contemporaneous CRP (R^2 = 0.132; rho = 0.455, P < 0.001) (**FIGURE 2.12a**) and APACHE II score (R^2 = 0.250; rho = 0.583; P < 0.001) (**FIGURE 2.12b**). Furthermore, a moderate degree of correlation was discovered between logarithmic 3-HK and contemporaneous levels of TFF3 (rho = 0.604, P < 0.001), albumin (rho = –0.568, P < 0.001), creatinine (rho = 0.531, P < 0.001), TNF-α (rho = 0.462, P < 0.001), and RAGE (rho = 0.455, P < 0.001), and a weaker correlation with IL-10 (rho = 0.357, P < 0.001), IL-6 (rho = 0.352, P < 0.001), IL-17A (rho = 0.349, P < 0.001), CD163 (rho = 0.298, P < 0.001), IL-8 (rho = 0.293, P < 0.001), TNFS10 (rho = –0.259, P < 0.001), chemerin (rho = 0.247, P < 0.001), IL-1 (rho = 0.220, P < 0.001), insulin C-peptide (rho = 0.230, P < 0.001), cardiac troponin I (rho = 0.217, P < 0.001), CXCL12 (rho = 0.195, P = 0.001), and CD40 ligand (rho = 0.181, P = 0.003). Conversely, no correlation was found with insulin (rho = 0.057, P = 0.352), CA 15-3 (rho = –0.079, P = 0.199), B7-H1 (rho = –0.062, P = 0.312), and IFN-γ (rho = –0.019, P = 0.758). Differences between participant groups for all studied cytokines are summarized in **TABLE 2.4**, and post-hoc pairwise comparisons of significantly different standardized AUC are depicted on **TABLE 2.7**.

	Summary Measure	Mild	Moderate	Severe	P-value
Albumin (g/L)	Peak values, median (IQR)	35 (32-37)	33 (30-37)	26 (24-31)	**0.006**
	Minimum values, median (IQR)	30 (27-32)	27 (23-31)	21 (19-26)	**0.001**
	Mean values, median (IQR)	32.9 (29.6-34.7)	29.8 (26.6-33.4)	22.6 (21.8-28.3)	**0.001**
	Time-to-Min, median hours (95% C.I.)	12.0 (7.8-16.2)	24.0 (16.6-31.4)	21.0 (0.0-47.3)	0.314
	Standardized AUC, median (IQR)	32.5 (29.6-34.0)	28.1 (25.9-31.5)	22.1 (21.3-27.4)	**<0.001**
C-reactive protein (mg/L)	Peak values, median (IQR)	97 (32-177)	191 (105-326)	283 (246-391)	**0.001**
	Minimum values, median (IQR)	15 (7-46)	38 (11-96)	103 (88-119)	**0.016**
	Mean values, median (IQR)	53 (22.7-122.2)	125.6 (56.2-190.7)	200.5 (180.6-255.0)	**0.001**
	Time-to-Peak, median hours (95% C.I.)	24.0 (13.7-34.3)	48.0 (40.4-55.6)	24.0 (0.0-76.6)	0.455
	Standardized AUC, median (IQR)	58.9 (26.3-131.2)	148.1 (74.5-243.9)	225.6 (174.9-260.2)	**0.001**
IL-1 Beta (pg/mL)	Peak values, median (IQR)	1.1 (0.0-4.5)	4.2 (0.0-7.3)	6.2 (4.2-12.2)	**0.007**
	Minimum values, median (IQR)	0.0 (0.0-2.6)	0.1 (0.0-3.3)	2.6 (0.1-3.5)	0.125
	Mean values, median (IQR)	0.6 (0.0-3.0)	1.9 (0.0-4.9)	4.6 (2.6-6.4)	**0.006**
	Time-to-Peak, median hours (95% C.I.)	3.5* (0.7-6.3)	6.0 (0.0-13.6)	14.7* (0.0-32.0)	0.057
	Standardized AUC, median (IQR)	0.2 (0.0-2.5)	2.6 (0.0-5.1)	4.0 (1.7-6.1)	**0.005**
IL-6 (pg/mL)	Peak values, median (IQR)	33.8 (13.4-80.5)	123.4 (44.2-406.1)	904.6 (202.4-1668.8)	**0.001**
	Minimum values, median (IQR)	9.75 (3.2-22.5)	22.5 (8.9-62.6)	40.3 (19.4-66.8)	**0.002**
	Mean values, median (IQR)	19.1 (10.5-56.6)	76.1 (32.8-185.5)	250.3 (116.2-587.7)	**0.001**
	Time-to-Peak, median hours (95% C.I.)	6.0 (0.2-11.8)	12.0 (6.4-17.6)	6.0 (3.6-8.5)	0.345
	Standardized AUC, median (IQR)	20.7 (9.9-53.5)	59.5 (26.8-188.1)	180.8 (83.9-373.2)	**0.003**
IL-8 (pg/mL)	Peak values, median (IQR)	16.2 (8.78-29.3)	16.2 (8.6-40.0)	81.8 (15.2-175.2)	0.061
	Minimum values, median (IQR)	4.0 (1.4-7.1)	3.5 (0.6-8.2)	9.4 (7.9-36.7)	**0.022**
	Mean values, median (IQR)	8.9 (5.3-21.1)	9.0 (4.7-22.3)	35.6 (12.3-109.2)	**0.032**
	Time-to-Peak, median hours (95% C.I.)	12.0 (7.3-16.7)	12.0 (6.8-17.2)	6.0 (1.6-10.4)	0.296
	Standardized AUC, median (IQR	9.4 (5.1-16.6)	8.8 (3.7-20.9)	29.6 (12.2-110.9)	**0.026**

IL-10 (pg/mL)	Peak values, median (IQR)	5.4 (2.3-11.4)	9.6 (4.2-18.4)	19.6 (10.1-43.0)	**0.006**
	Minimum values, median (IQR)	2.5 (0.0-4.5)	3.5 (0.0-6.4)	2.4 (0.0-7.0)	0.517
	Mean values, median (IQR)	3.2 (0.5-7.2)	5.6 (1.9-10.5)	14.7 (3.4-28.1)	0.073
	Time-to-Peak, median hours (95% C.I.)	3.0 (0.0-6.7)	12.0 (4.6-19.4)	8.0* (0.0-19.1)	0.369
	Standardized AUC, median (IQR)	4.1 (0.29-6.97)	4.6 (2.0-7.3)	9.2 (3.3-18.2)	0.209
IL-17A (pg/mL)	Peak values, median (IQR)	1.4 (0.0-3.8)	0.1 (0.0-2.2)	7.4 (3.0-12.8)	0.087
	Minimum values, median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	2.1 (0.9-3.5)	**<0.001**
	Mean values, median (IQR)	0.4 (0.0-1.0)	0.0 (0.0-0.9)	5.2 (1.6-8.2)	0.061
	Time-to-Peak, median hours (95% C.I.)	6.0 (0.4-11.6)	12.0 (5.6-18.4)	3.0 (0.0-6.9)	0.914
	Standardized AUC, median (IQR)	0.4 (0.0-1.0)	0.1 (0.0-1.2)	3.6 (1.4-7.6)	0.069
CA 15-3 (pg/mL)	Peak values, median (IQR)	20.2 (13.8-31.0)	15.0 (11.6-20.8)	18.5 (17.5-30.5)	0.083
	Minimum values, median (IQR)	12.4 (8.8-16.8)	8.0 (5.3-12.0)	11.0 (8.5-23.0)	0.072
	Mean values, median (IQR)	17.6 (13.0-21.8)	11.7 (6.8-16.3)	14.6 (11.8-26.7)	0.079
	Time-to-Min, median hours (95% C.I.)	6.0 (4.0-8.0)	24.0 (18.7-29.3)	12.0 (0.0-38.3)	**0.008**
	Standardized AUC, median (IQR)	16.7 (12.3-22.5)	10.9 (6.4-14.3)	15.2 (11.2-27.3)	**0.035**
Insulin (pg/mL)	Peak values, median (IQR)	1337.2 (953.7-1758.1)	1212.7 (600.6-2222.6)	764.2 (680.5-1630.3)	0.244
	Minimum values, median (IQR)	343.6 (230.1-641.6)	375.3 (198.9-619.3)	274.5 (197.3-330.7)	0.384
	Mean values, median (IQR)	742.1 (573.2-985.1)	780.2 (368.5-1350.6)	538.5 (506.3-650.3)	0.234
	Time-to-Min, median hours (95% C.I.)	NA	NA	NA	NA
	Standardized AUC, median (IQR)	760.5 (588.6-1123.7)	744.4 (478.0-1290.8)	440.0 (349.8-566.3)	**0.042**
Insulin C-Peptide (pg/mL)	Peak values, median (IQR)	3138.0 (2457.3-3817.9)	1898.8 (1135.7-3032.3)	1590.3 (863.4-4067.4)	0.205
	Minimum values, median (IQR)	976.0 (427.0-1636.4)	559.4 (119.1-1278.9)	421.2 (236.2-1294.4)	0.350
	Mean values, median (IQR)	1902.8 (1205.5-2725.3)	1193.2 (625.9-2040.3)	660.4 (515.5-2244.2)	0.311
	Time-to-Peak, median hours (95% C.I.)	24.0 (12.5-35.5)	24.0 (0.0-50.4)	6.0 (0.0-14.8)	0.548
	Standardized AUC, median (IQR)	1955.7 (1371.3-2715.2)	1320.5 (874.0-2096.0)	833.8 (501.7-2688.2)	0.324

CD40 ligand (ng/mL)	Peak values, median (IQR)	1.6 (0.9-2.6)	2.4 (1.5-3.9)	2.9 (1.2-3.1)	0.162
	Minimum values, median (IQR)	0.5 (0.4-0.7)	0.7 (0.4-1.1)	0.6 (0.3-0.7)	0.153
	Mean values, median (IQR)	0.9 (0.6-1.5)	1.3 (0.9-2.4)	1.1 (0.8-1.7)	0.092
	Time-to-Peak, median hours (95% C.I.)	6.0 (0.5-11.5)	3.0 (0.0-13.7)	12.0 (3.7-20.3)	0.947
	Standardized AUC, median (IQR)	0.8 (0.6-1.7)	1.5 (0.7-2.1)	1.0 (0.7-1.8)	0.199
Chemerin (ng/mL)	Peak values, median (IQR)	11.6 (8.9-13.6)	14.5 (11.8-17.0)	12.4 (10.3-17.0)	0.081
	Minimum values, median (IQR)	8.5 (6.7-10.7)	8.6 (6.6-12.7)	8.1 (5.7-9.2)	0.588
	Mean values, median (IQR)	9.9 (7.6-11.3)	10.7 (7.8-14.6)	9.6 (8.0-10.6)	0.345
	Time-to-Peak, median hours (95% C.I.)	24.0 (15.8-32.2)	48.0 (32.8-63.2)	48.0 (14.7-81.3)	0.293
	Standardized AUC, median (IQR)	9.9 (7.6-11.9)	11.3 (9.7-14.1)	9.5 (8.1-11.8)	0.127
TFF3 (ng/mL)	Peak values, median (IQR)	3.7 (3.1-4.6)	3.7 (3.1-5.6)	7.0 (5.4-8.4)	**0.008**
	Minimum values, median (IQR)	3.0 (2.4-3.9)	3.3 (2.4-4.2)	4.5 (4.0-6.4)	**0.025**
	Mean values, median (IQR)	3.3 (2.7-4.2)	3.5 (2.6-4.7)	5.4 (4.8-7.6)	**0.011**
	Time-to-Peak, median hours (95% C.I.)	6.0 (1.1-10.9)	12.0 (0.4-23.6)	6.0 (1.7-10.3)	0.719
	Standardized AUC, median (IQR)	3.33 (2.72-4.1)	3.5 (2.8-4.9)	5.2 (4.9-7.5)	**0.013**
RAGE (ng/mL)	Peak values, median (IQR)	2.3 (1.6-3.4)	2.1 (1.5-3.3)	2.8 (2.3-4.2)	0.269
	Minimum values, median (IQR)	1.7 (1.2-2.2)	1.5 (1.0-2.1)	1.9 (1.8-2.0)	0.374
	Mean values, median (IQR)	1.9 (1.3-2.8)	1.7 (1.3-2.6)	2.2 (2.1-3.8)	0.228
	Time-to-Peak, median hours (95% C.I.)	6.0 (3.6-8.4)	24.0 (15.6-32.5)	12.0 (3.7-20.3)	0.455
	Standardized AUC, median (IQR)	1.9 (1.4-2.7)	1.9 (1.6-2.6)	2.1 (2.0-3.3)	0.370
CD163 (ng/mL)	Peak values, median (IQR)	872.3 (585.3-1271.3)	1058.6 (827.2-1341.1)	1996.4 (1196.6-2640.0)	**0.017**
	Minimum values, median (IQR)	639.8 (449.7-996.6)	683.7 (543.95-897.8)	834.9 (699.8-1046.0)	0.480
	Mean values, median (IQR)	778.0 (531.9-1137.2)	861.9 (723.4-1171.5)	1636.0 (1003.7-1846.8)	**0.031**
	Time-to-Peak, median hours (95% C.I.)	3.0 (0.0-6.6)	3.0 (1.4-4.6)	6.0 (3.2-8.8)	0.112
	Standardized AUC, median (IQR)	743.74 (493.0-1143.4)	833.4 (664.4-1052.9)	1417.9 (952.8-1812.5)	**0.028**

FN-γ (pg/mL)	Peak values, median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.5)	0.0 (0.0-1.2)	0.362
	Minimum values, median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.162
	Mean values, median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.2)	0.0 (0.0-0.3)	0.295
	Time-to-Peak, median hours (95% C.I.)	3.0 (0.0-12.6)	12.0 (0.3-23.7)	36.0* (17.6-43.6)	0.990
	Standardized AUC, median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.2)	0.0 (0.0-0.6)	0.177
TNF-α (pg/mL)	Peak values, median (IQR)	1.3 (0.0-8.5)	3.1 (0.0-6.1)	9.9 (3.5-34.5)	**0.031**
	Minimum values, median (IQR)	0.0 (0.0-0.6)	0.0 (0.0-1.8)	1.3 (0.1-3.6)	0.079
	Mean values, median (IQR)	0.6 (0.0-3.8)	1.2 (0.0-3.2)	7.6 (2.6-15.5)	**0.010**
	Time-to-Min, median hours (95% C.I.)	3.0 (0.0-6.9)	6.0 (0.0-29.8)	12.0 (0.0-29.5)	0.561
	Standardized AUC, median (IQR)	0.4 (0.0-5.1)	1.1 (0.0-2.9)	7.2 (2.5-8.4)	**0.017**
TRAIL-TNFS10 (pg/mL)	Peak values, median (IQR)	30.4 (13.7-71.3)	20.26 (11.5-37.6)	31.5 (0.0-34.5)	0.382
	Minimum values, median (IQR)	10.8 (1.0-25.8)	10.1 (0.0-22.6)	14.1 (0.0-17.3)	0.639
	Mean values, median (IQR)	22.9 (7.9-36.9)	15.0 (4.3-33.9)	18.7 (0.0-25.9)	0.368
	Time-to-Peak, median hours (95% C.I.)	6.0 (3.2-8.8)	12.0 (7.2-16.8)	3.0 (0.0-17.4)	0.707
	Standardized AUC, median (IQR)	20.4 (7.6-45.2)	13.1 (5.1-30.7)	18.9 (0.0-24.4)	0.292
Cardiac Troponin I/c (pg/mL)	Peak values, median (IQR)	9.4 (0.0-18.3)	13.9 (0.0-38.3)	14.3 (7.6-26.1)	0.516
	Minimum values, median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.380
	Mean values, median (IQR)	1.9 (0.0-9.2)	4.64 (0.0-11.0)	4.8 (3.1-8.5)	0.868
	Time-to-Peak, median hours (95% C.I.)	6.0 (3.8-8.2)	48.0 (22.9-73.1)	3.0 (0.0-6.5)	**0.010**
	Standardized AUC, median (IQR)	1.5 (0.0-10.3)	5.4 (0.0-10.7)	4.3 (2.6-12.6)	0.772

TABLE 2.4 – Summary measures of serial CRP, albumin and cytokine measures, per patient group. Comparisons were performed by utilising one-way ANOVA or Kruskal-Wallis H testing, as appropriate, following normality testing (Kolmogorov-Smirnov). Time-to-Peak and Time-to-Min analyses were performed by applying the Kaplan-Meier method on concentration levels from T0 up to and including T72. *****: mean value. **95% C.I.**: 95% confidence interval

	Summary Measure	Mild	Moderate	Severe	P-value	HV
Tryptophan (ng/mL)	Minimum values, median (IQR)	16407.2 (13334.9-20463.6)	13993.6 (10714-20941.9)	8820.4 (7295.3-11355.6)	0.071	15876.3
	Mean values, mean (SD)	23840.4 (8970.0)	20346.2 (8144.3)	14421.2 (4317.7)	**0.014**	25215.8 (21675.9-33362.4)
	Time-to-Minimum, median hours (95% C.I.)	12 (3.5-20.5)	12 (0.0-27.7)	1 2 (3.2-20.8)	0.542	
	Standardized AUC, mean (SD)	24423.9 (8803.9)	19721.4 (8140.5)	13782.4 (3789.8)	**0.004**	
Kynurenine (ng/mL)	Peak values, median (IQR)	694.9 (429.4-857.9)	677.8 (408.2-900.8)	742.0 (626.6-1182.8)	0.680	622.3
	Mean values, median (IQR)	520.5 (338.8-708.0)	470.6 (302.1-874.4)	599.4 (547.1-912.4)	0.533	429.2 (353.0-463.1)
	Time-to-Peak, median hours (95% C.I.)	12 (0.0-29.6)	24 (6.3-41.7)	12 (0.0-25.2)	0.288	
	Standardized AUC, median (IQR)	554.8 (360.3-734.0)	522.7 (370.8-782.5)	634.3 (570.4-840.7)	0.584	
3- Hydroxykynurenine (ng/mL)	Peak values, median (IQR)	13.5 (8.6-17.8)	20.0 (12.1-46.6)	25.2 (14.3-35.6)	**0.013**	10.5
	Mean values, median (IQR)	10.7 (8.1-13.5)	15.6 (8.6-38.3)	22.3 (11.8-27.0)	**0.039**	8.1 (7.6-9.0)
	Time-to-Peak, median hours (95% C.I.)	12 (6.7-17.3)	46.0* (32.9-59.0)	24 (0.0-49.0)	**0.019**	
	Standardized AUC, median (IQR)	10.8 (7.4-13.9)	18.2 (11.4-38.1)	21.6 (11.7-28.0)	**0.015**	

TABLE 2.5 – Summary analysis measures of serial TRP metabolite values (T0 to T168). T168 values were excluded from Time-to-Peak/Minimum analysis. **HV**: Healthy volunteers. Significant P-values are highlighted in bold.

	Summary Measure	Mild	Moderate	Severe	P-value	HV
Kynurenic Acid (ng/mL)	Peak values, median (IQR)	211.5 (165.0-404.3)	184.8 (48.2-364.4)	198.4 (182.4-201.6)	0.303	372.1
	Mean values, median (IQR)	194.9 (146.0-364.4)	141.8 (41.8-184.8)	186.4 (172.1-197.5)	0.104	301.6 (267.7-327.5)
	Time-to-Peak, median hours (95% C.I.)	6 (3.2-8.9)	6 (2.7-9.3)	3 (0.0-7.2)	0.102	
	Standardized AUC, median (IQR)	296.7 (155.4-406.6)	74.1 (34.8-203.9)	208.3 (173.8-374.3)	0.110	
3-Hydroxyantranilic Acid (ng/mL)	Peak values, median (IQR)	48.8 (37.3-118.6)	48.8 (33.4-64.7)	65.0 (46.2-394.2)	0.236	54.3
	Mean values, median (IQR)	38.4 (25.9-85.3)	34.8 (26.0-62.8)	53.6 (41.9-215.1)	0.186	37.5 (28.7-48.8)
	Time-to-Peak, median hours (95% C.I.)	6 (0.0-12.1)	6 (0.0-15.1)	6 (0.0-14.8)	0.686	
	Standardized AUC, median (IQR)	45.7 (28.0-98.4)	34.9 (26.8-56.0)	51.2 (44.1-107.5)	0.165	
3-Hydroxykynurenine/ Tryptophan ratio x1000	Peak values, median (IQR)	0.673 (0.417-1.523)	0.878 (0.532-4.515)	1.876 (1.505-3.600)	0.052	0.46
	Minimum values, median (IQR)	0.341 (0.201-0.804)	0.428 (0.283-1.513)	0.872 (0.637-1.420)	**0.005**	0.18
	Mean value, median (IQR)	0.429 (0.321-1.255)	0.645 (0.409-2.902)	1.550 (0.831-1.769)	**0.022**	0.326 (0.248-0.384)
	Time-to-Peak, median hours (95% C.I.)	12.0 (0.0-26.6)	48 (21.6-74.4)	12 (0.0-24.5)	0.11	
	Standardized AUC, median (IQR)	0.447 (0.262-0.892)	0.734 (0.506-2.635)	1.667 (0.930-1.841)	**0.003**	

TABLE 2.6 – Summary analysis measures of serial TRP metabolite values (T0 to T168). T168 values were excluded from Time-to-Peak/Minimum analysis. **HV**: Healthy volunteers. Significant P-values are highlighted in bold.

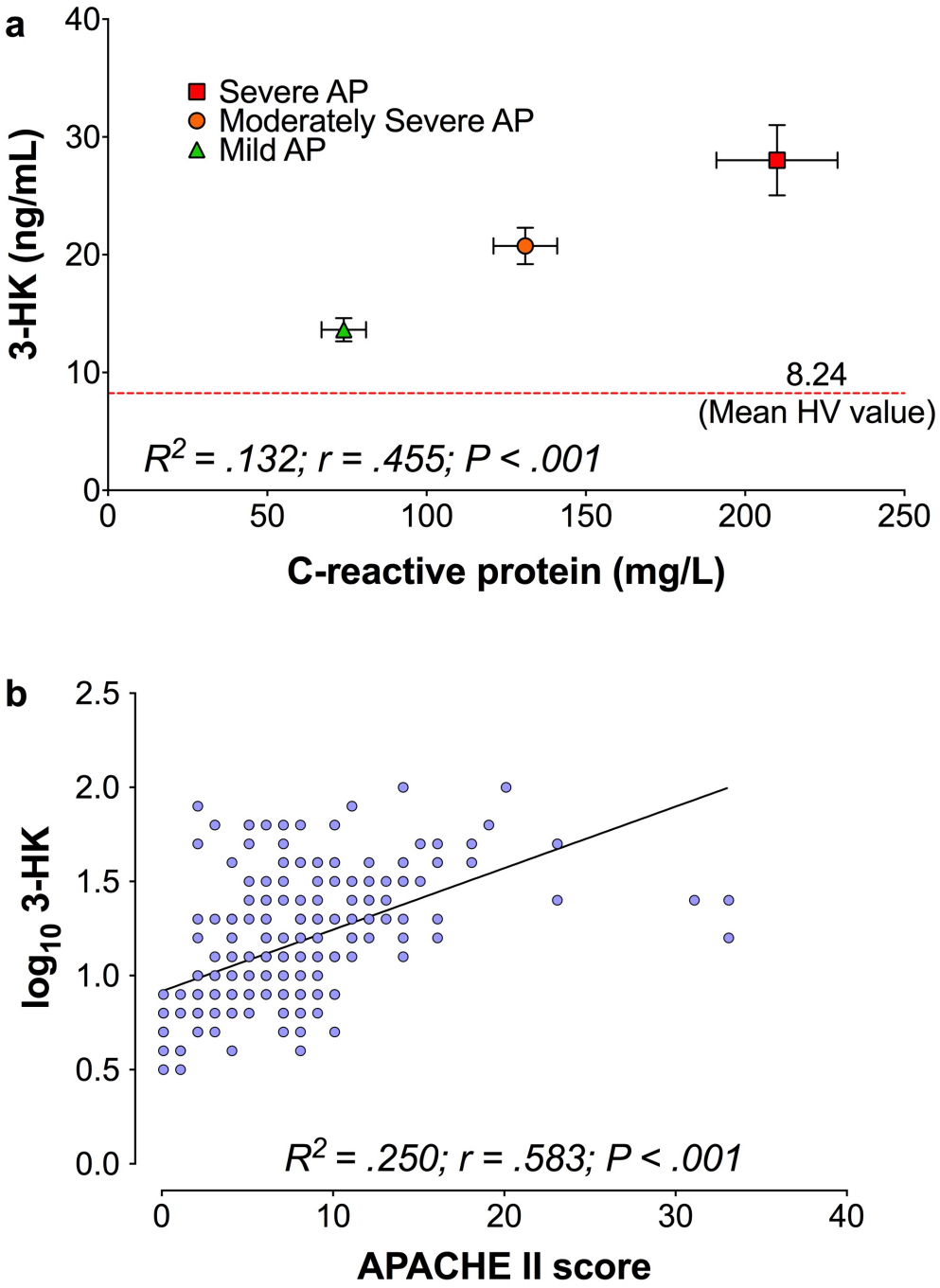

FIGURE 2.12 – Dot-plots of correlations between **(a)** Plasma concentrations of CRP and 3-HK, for the tAP cohort grouped by AP severity (mild AP n = 25; moderate AP n = 23; severe AP n = 9). Data points represent means; error bars show SD on both axes. The dashed red line represents mean 3-HK plasma concentration in healthy volunteers (n = 9). **(b)** APACHE II score and contemporaneous log10 3-HK plasma concentrations. Due to the non-normal distribution of plasma concentrations of 3-HK, a logarithmic transformation was used for the correlation to provide a better fit. For both correlations, results from samples obtained from T0 up to and including T48 were analysed. Results of respective Spearman correlations have been appended on each figure of the panel.

Metabolite	Pairwise Standardized AUC Comparison (Adjusted P-values)		
	Mild vs. Moderate	Mild vs. Severe	Moderate vs. Severe
TRP*	0.128	**0.003**	0.156
3-HK	**0.040**	0.064	1.000
3-HK/TRPx1000	0.057	**0.004**	0.493
Albumin	**0.028**	**<0.001**	0.171
CRP	**0.031**	**0.001**	0.367
IL-1 Beta	0.144	**0.005**	0.311
IL-6	0.090	**0.003**	0.304
IL-8	1.000	**0.043**	**0.032**
CA 15-3	0.058	1.000	0.158
Insulin	1.000	**0.044**	0.086
TFF3	1.000	**0.014**	**0.028**
CD163	1.000	**0.025**	0.083
TNF-α	1.000	**0.026**	**0.024**
CXCL12	1.000	**0.031**	**0.035**

TABLE 2.7 – Summary of P-values from post-hoc pairwise comparisons of statistically different standardized AUC of metabolites between patient groups, adjusted according to Dunn's test. Significant P-values are highlighted in bold. (*: Tukey's honestly significant difference test).

2.5 DISCUSSION

The kynurenine pathway of TRP metabolism and especially KMO, the enzyme that determines the metabolic fate of KYN, is increasingly recognized as a key contributor to the pathogenesis of AP-MODS. The IMOFAP study defined with precision the temporal profile of kynurenine pathway metabolite concentrations in peripheral blood in relation to the onset and severity of AP in humans (Skouras et al., 2016b). Data arising from IMOFAP show a noteworthy association between plasma concentrations of 3-HK and AP severity, as defined by standard classification systems. Furthermore, a weak to moderate correlation was discovered between 3-HK concentrations and the systemic inflammatory burden, measured by contemporaneous CRP levels and APACHE II score. Additionally, the classical paradigm pro-inflammatory cytokine profile in AP was observed, namely TNF-α and IL-6, as well as RAGE and TFF3, that correlated well with 3-HK in this cohort (Skouras et al., 2016b).

These novel findings add depth to the previously reported association between serum concentrations of KYN and the requirement for invasive renal

and respiratory support during AP-MODS (Mole et al., 2008). Moreover, these findings build on the recent discovery that genetic deletion of KMO and administration of a highly-specific KMO inhibitor protect against pulmonary and renal injury in experimental rodent models of AP (Mole et al., 2016b). Together, these data strongly support the translational potential of KMO inhibition as a therapeutic strategy to protect against MODS in human AP (Skouras et al., 2016b).

Strengths of the IMOFAP study include the efficiency of recruitment, sampling process, sampling frequency and high coverage, thus minimal sampling bias. Based on the knowledge that AP and the consequent systemic inflammatory response evolve rapidly, we hypothesise that KMO inhibition is likely to be most effective when delivered as early as possible in the course of the disease and therefore sought and achieved to define the evolving trajectory of the inflammatory response with high definition during the early phase.

The burden of systemic inflammation in the cohort – as measured by the magnitude of CRP rise – was substantial. The fact that the APACHE II score correlated well with disease severity as classified by the revised Atlanta criteria, and that the modified MODS score followed the same trajectory as CRP, provide additional reassurance that interpreting plasma concentrations of 3-HK and other KYN metabolites in this context is valid and appropriate (Skouras et al., 2016b).

In the present study, the magnitude of TRP depletion was proportional to AP severity and became more prominent over time, suggesting increased TRP metabolism. Although TRP levels can fluctuate diurnally (Fernstrom et al., 1979; Young et al., 1969), diurnal variation does not account for the difference in TRP metabolism between AP severity strata, because the time of day at which recruitment occurred was random. Furthermore, although it is possible that fasting due to hospital admission and/or critical illness may in theory account for part of the observed TRP depletion, Poesen et al demonstrated that plasma levels of TRP, KYN, and KYNA were not significantly different in healthy humans with low or high protein intake (Poesen

et al., 2015), hence a correlation with AP severity would not be expected if fasting was the cause. The extent of TRP depletion is too great to be due to haemodilution after intravenous fluid resuscitation, and indeed no statistically significant change was observed in haematocrit, and no dilution effect was recorded in other plasma analytes. It therefore seems reasonable to conclude that the observed decrease in TRP levels is likely to be the result of increased catabolism (Skouras et al., 2016b).

The increase in steady state 3-HK concentrations in plasma is consistent with increased flux through the kynurenine pathway. This change is proportionate to disease severity and augments with time. Importantly, elevated 3-HK was associated with increased inflammatory burden, as indicated by the correlation of plasma 3-HK levels with contemporaneous CRP concentrations. While it is curious that no significant changes were observed in other TRP metabolites, studies in rodents indicate that KMO represents the predominant route for this pathway. For this reason, the 3-HK/TRP ratio (multiplied by 1000 for practical purposes) was used in an attempt to express the pathway flux through KMO. Changes of this ratio were found to be time-dependent and proportionate to AP severity, with a lead-time of 12 hours in comparison to the timing of peak serum CRP, recorded at T24 for the severe AP group. Lastly, the elevation of 3-HK/TRP was greater in those few patients with specific respiratory, renal and cardiac dysfunction as defined by those specific components of the modified MODS score.

2.6 CONCLUSIONS OF CHAPTER

The IMOFAP study demonstrates that metabolic flux through KMO is elevated proportionately to disease severity in human AP. Plasma concentrations of 3-HK correlate with the burden of inflammation, incidence of organ dysfunction and AP severity. These findings reinforce the rationale for investigating early phase KMO inhibition as a therapeutic strategy to protect against AP-MODS in human AP (Skouras et al., 2016b).

Chapter 3 – Lung ultrasonography in acute pancreatitis

3.1 SUMMARY OF CHAPTER

The purpose of this prospective pilot study was to investigate the value of lung ultrasonography (LUSS) in the diagnosis of respiratory dysfunction and severity stratification in patients with AP. Over a 90-day period, 41 patients (median age: 59.1 years; 21 males) presenting with a diagnosis of potential AP were recruited through the IMOFAP study (**Chapter 2**). Each participant underwent LUSS and the number of comet tails was linked with contemporaneous clinical data. Group comparisons, AUC and respective measures of diagnostic accuracy were examined in relation to, 1) respiratory dysfunction, 2) disease severity, as defined by the revised Atlanta criteria for AP, 3) CRP contemporaneous with LUSS and, 4) maximum value of CRP during the first week of hospital admission.

A greater number of comet tails was evident in patients with respiratory dysfunction ($P = 0.021$), those with severe disease ($P < 0.001$) and when contemporaneous and maximum CRP exceeded 100 mg/L ($P = 0.048$ and $P = 0.003$ respectively). AUC of receiver operating characteristic (ROC) plots were greater when examining upper lung quadrants, using respiratory dysfunction (AUC = 0.783, 95% C.I. = 0.544-0.962) and AP severity (AUC = 0.996, 95% C.I. = 0.982-1.000) as the variables of interest. Examining all lung quadrants except for the lower lateral resulted in greater AUC for contemporaneous (AUC = 0.708, 95% C.I. = 0.510-0.883) and maximum CRP (AUC = 0.800, 95% C.I. = 0.640-0.929).

In conclusion, ultrasonography of non-dependent lung parenchyma was shown to have the potential to reliably detect evolving respiratory dysfunction in AP. This simple, easily repeatable, bedside technique shows promise as an adjunct to severity stratification.

3.2 AIM

The aim of the present study was to investigate the value of LUSS in the diagnosis of evolving respiratory dysfunction and severity stratification in patients with AP.

3.3 METHODS

The LUSS study was performed as part of the IMOFAP observational clinical research study, which received ethical approval by the Scotland A Research Ethics Committee and regulatory approval by the NHS Lothian Research & Development department, as detailed in section **2.3.1**. Written informed consent was obtained from all participants or in cases of AWI, from the named individual responsible for their welfare or their legal representative.

3.3.1 RECRUITMENT

Participants from the IMOFAP cohort were recruited to additionally participate in the LUSS study by convenience, with no conscious or deliberate selection bias. Data collection was planned in advance and was performed prospectively.

3.3.2 TRANSTHORACIC LUNG ULTRASOUND SCANS

All scans were performed by two consultant radiologists and a specialist registrar in radiology, in collaboration with the author. Bilateral intercostal LUSS was performed with participants in the supine position, after applying acoustic gel on the skin to provide an airless interface. In order to optimize the quality of imaging, the intercostal spaces were widened by raising each patient's ipsilateral arm up to or above the head level during the scan, and the

transducer was held perpendicular to the skin surface. For the purposes of the study, either a Micromaxx® portable ultrasound system fitted with a C60/5-2 MHz transducer (SonoSite, Inc., Washington, USA), an Acuson S2000™ system with a 4C1 transducer (Siemens Medical Solutions USA, Inc., California, USA) or an Acuson Antares™ Premium Edition ultrasound system with a CH4-1 transducer (Siemens Medical Solutions USA, Inc., California, USA) was used.

Each hemithorax was divided into anterior and lateral, upper and lower areas (**FIGURE 3.1.a & b**). For each hemithorax, the anterior area was delineated between the clavicle and the diaphragm and from the parasternal to the anterior axillary line. The lateral area was delineated between the axilla and the diaphragm and from the anterior to the posterior axillary line. The upper quadrants were demarcated from the 1st to the 3rd intercostal space and the lower quadrants from the 4th to the 6th intercostal space. Each of the 8 chest areas were visualised during normal respiration. The pattern analysed was the comet-tail artefact arising from the lung-wall interface (the hyperechogenic interface between the chest wall and the lung surface), which was defined as a hyperechogenic narrow-base reverberation artefact, spreading like a laser-ray up to the edge of the screen (Lichtenstein et al., 1997) (**FIGURE 3.2**). For each of the 8 quadrants, the number of identified comet-tail artefacts was recorded and was linked with contemporaneous, prospectively collected clinical data.

Based on findings from previous studies (Bouhemad et al., 2007; Lichtenstein et al., 1997; Wernecke et al., 1987), comet-tail artefacts may be present in dependent regions of normally aerated lungs, and can be observed in healthy patients. Therefore, in order to optimize the imaging protocol for sensitive detection of comet-tail artefacts, three scan zones for each participant were compared, namely: i) All lung quadrants, ii) All lung quadrants except for the lower lateral, and iii) Upper lung quadrants only, and the results were analysed, and compared.

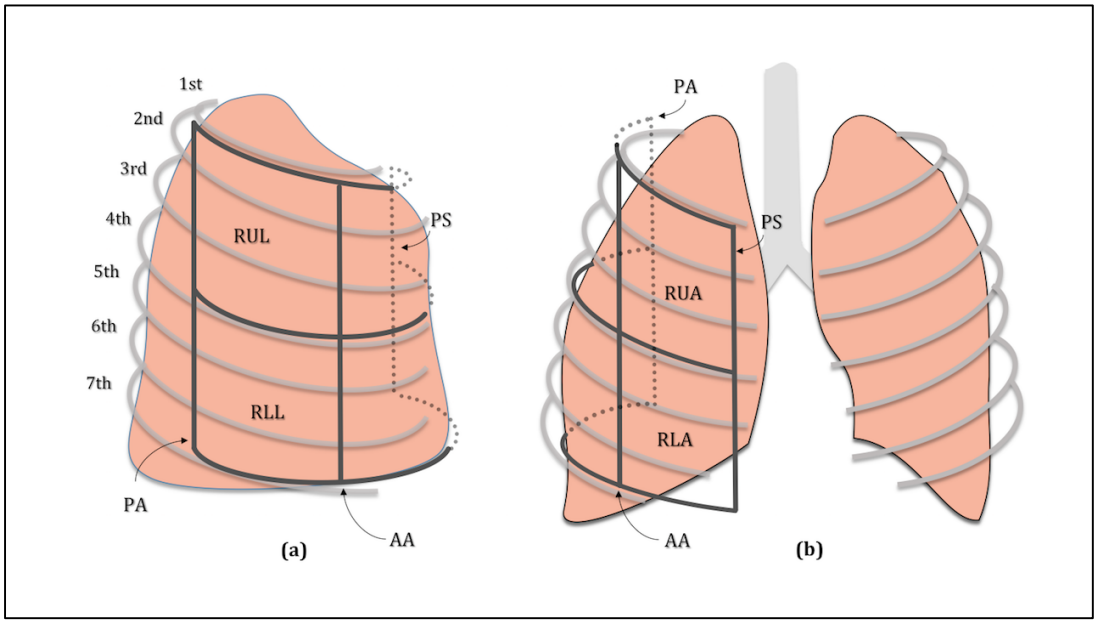

FIGURE 3.1.a – Lateral view of the right lung. Schematic representation of the areas scanned in relation to ribs (numbered) and intercostal spaces. **FIGURE 3.1.b** – Anterior view of the lungs. Schematic representation of the areas scanned (right hemithorax) in relation to ribs and intercostal spaces. (**PA**: posterior axillary line; **AA**: anterior axillary line; **PS**: parasternal line; **RUL**: right upper lateral area; **RLL**: right lower lateral area; **RUA**: right upper anterior area; **RLA**: right lower anterior area).

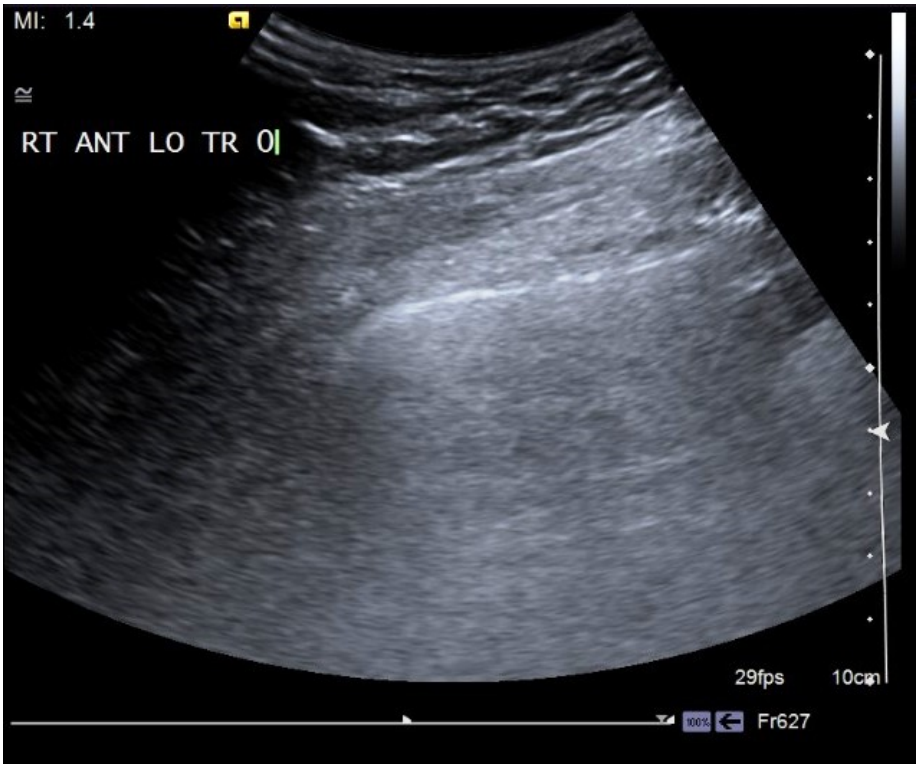

FIGURE 3.2.a – Sonographic image of normal lung

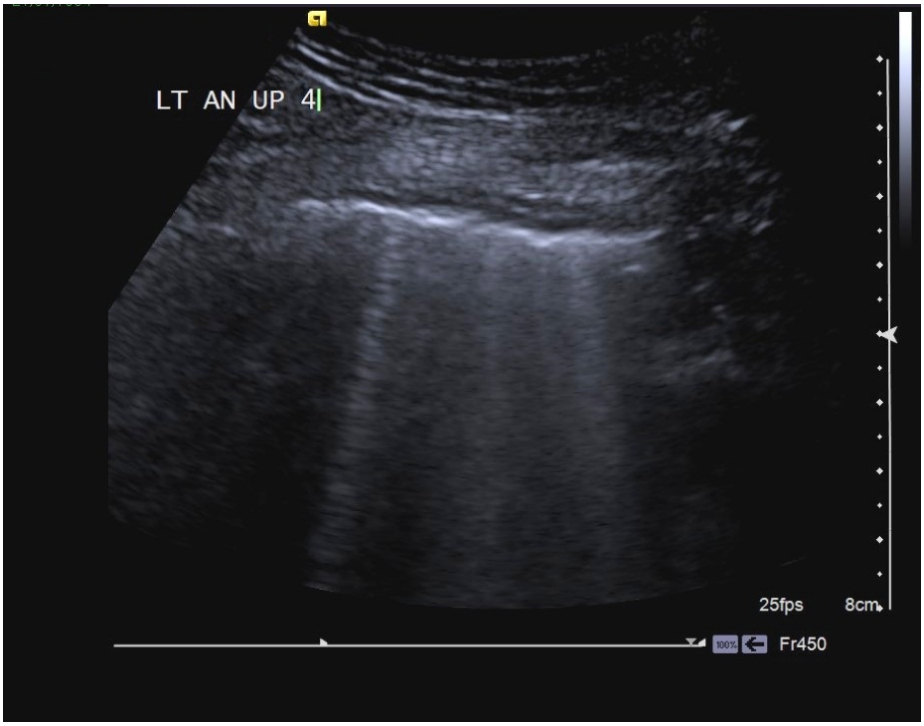

FIGURE 3.2.b – Anterior view of the lungs from a patient with AIS: Sonographic pattern of comet-tail artefacts fanning out vertically from the lung-wall interface and spreading up to the edge of the screen (B-lines).

With the exception of one patient who underwent an early scan on day 5 and a late scan on day 11 after recruitment, scans performed within 4 days from patient recruitment were defined as early scans and those performed later than 4 days were defined as late.

3.3.3 DEFINITIONS

3.3.3.1 Diagnosis of acute pancreatitis & severity stratification

The diagnosis of AP, the definition of organ failure and severity stratification were performed in accordance with the revised Atlanta guidelines for AP (Banks et al., 2013).

3.3.3.2 C-reactive protein

Plasma levels of CRP have been shown to correlate well with the presence of pancreatic necrosis and severity of AP. However due to the relatively late peak of CRP (36-72 hours after admission), CRP levels upon admission may not be useful in assessing severity (Banks et al., 2006). Therefore, both CRP contemporaneous with the LUSS (highest value within the same 24-hour period) and maximum CRP value during the first week of admission were used as additional surrogate markers of AP severity, by using 100 mg/L as the cut-off for the binary classification of the cohort (Mayer et al., 1984; Puolakkainen et al., 1987).

3.3.3.3 Alveolar-interstitial syndrome

Pulmonary diseases with involvement of the alveolar space and the interstitium are grouped under the term alveolar-interstitial syndrome (AIS) and include ARDS, pneumonia, acute cardiogenic pulmonary oedema, exacerbation of chronic interstitial lung disease and miscellaneous other pulmonary conditions (Lichtenstein et al., 1997; Stefanidis et al., 2012). The radiological diagnosis of AIS in the LUSS study was based on the presence of alveolar opacities (ill-defined shadowing, confluent opacities with air bronchograms) and/or interstitial opacities (septal lines, linear, reticular, or nodular opacities) on each participant's chest X-rays, as previously proposed by Lichtenstein et al (Lichtenstein et al., 1997).

3.3.3.4 Respiratory dysfunction

The lowest value of PaO_2/FiO_2 for the 24-hour period when each LUSS was performed was used as a metric of potential respiratory dysfunction. A threshold value of 300 was used to divide the cohort into two groups, since those with $PaO_2/FiO_2 < 300$ have a modified MODS score equal or greater than

2 on the modified MODS score, used by the revised Atlanta guidelines. When an arterial blood sample was not available, PaO_2 was extrapolated from SpO_2 by applying the method described by Severinghaus (Severinghaus, 1979). For non-ventilated patients receiving supplemental oxygen, the fraction of inspired oxygen (FiO_2) was estimated as described Banks et al (Banks et al., 2013), modified as shown in **TABLE 3.1**.

Supplemental oxygen (L/min)	FiO_2 (ratio)
Room air	0.21
1-2	0.25
3-4	0.30
5-8	0.40
9-10	0.50
11-12	0.60
15	0.90

Table 3.1 – FiO_2 for non-ventilated patients (modification of the values provided in the revised Atlanta guidelines for acute pancreatitis)

3.3.3.5 Statistical analysis

Data for continuous variables are presented as mean ± SD or as median and IQR. Categorical variables are presented as absolute and relative frequencies. Comparisons between independent groups were performed using the Mann-Whitney U-test. Spearman's rho was used to examine correlations between pairs of non-parametric variables. All statistical tests were based on a two-sided α-value of 0.05.

For early and late scans, independent samples analysis was performed with the cohort stratified by: i) respiratory dysfunction ($PaO_2/FiO_2 < 300$ vs. $PaO_2/FiO_2 \geq 300$), ii) disease severity (severe vs. non-severe), based on the revised Atlanta criteria for AP (modified MODS score < 2 vs. modified MODS score ≥ 2), iii) CRP value contemporaneous with LUSS (contemporaneous

CRP < 100 mg/L vs. CRP ≥ 100 mg/L), and, iv) maximum CRP value within the first week of admission (maximum CRP < 100 mg/L vs. CRP ≥ 100 mg/L).

ROC curves were plotted for each comparison. AUC and measures of diagnostic accuracy with respective confidence intervals were calculated, with the use of 'pROC' package (Robin et al., 2011) of RStudio version 0.98.1091 (RStudio, Inc., Massachusetts, USA), after identifying ROC curve thresholds by the use of Youden's index. AUC for paired ROC curves were compared with the DeLong method (DeLong et al., 1988). Additional statistical analyses were performed with IBM SPSS® Statistics Version 22.0 (IBM Corporation, Armonk, New York, USA). Figures were designed using GraphPad Prism® Version 6.0 (GraphPad Software, Inc., La Jolla, California, USA). Formal blinding of the LUSS assessors was not feasible due to the nature of the investigations. The principles of the STARD (STAndards for the Reporting of Diagnostic accuracy studies) statement have been adhered to (Bossuyt et al., 2003).

3.4 RESULTS

3.4.1 DEMOGRAPHICS

Forty-one patients were enrolled in the LUSS study. Based on the revised Atlanta guidelines, 32 of 41 participants had a diagnosis of 'true' AP (**2.3.3**), whereas the remaining 9 patients had hyperamylasaemia that did not satisfy the revised Atlanta definition of AP. All patients were included in the analysis. Of the tAP group, 12 participants had mild AP, 15 had moderately severe and 5 had severe disease. The demographic characteristics are summarized in **TABLE 3.2**.

Fifty-one LUSS were performed, of which 33 (64.7%) were performed early in the disease course, and 18 (35.3%) were performed at a late stage. Early scans were performed after a median of 3 days from the initial finding of

	Overall	Hyperamylasaemia	Mild AP	Moderate AP	Severe AP
n	41	9	12	15	5
Age (years)					
Median	59.1	59.1	69.7	57	49.4
IQR	49.2-67.5	50.7-64.6	50-81.6	49.2-67.5	45.8-63.9
Gender ratio, (%)					
Males	21/41 (51.2)	5	6	8	2
BMI					
Median	28	26	27	29	32
IQR	23-32	23-28	23-30	24-35	30-33
AP Aetiology ratio, (%)					
Gallstones	19 (59.4)	N/A	7	11	1
Alcohol	6 (18.8)	N/A	2	1	3
Idiopathic	5 (15.6)	N/A	2	2	1
Other	2 (6.3)	N/A	1	1	0
N/A	9 (22.0)	9	N/A	N/A	N/A
Amylase (IU/L)					
Median	507	145	1045	642	669
IQR	230-1143	138-197	434-1435	324-2314	654-1143
CRP (mg/L)					
Median	66	13	29	154	133
IQR	24-146	6-26	12-71	46-241	119-146
APACHE II score					
Median	10	12	9	10	17
IQR	8.5-16.5	9-17	6-12	9-15	10-31
SIRS					
Ratio (%)	23 (56.1%)	6	6	6	5
Modified MODS Score					
Median (IQR)	1 (1-2)	2 (1-3)	1 (1-2)	1 (1-2)	3 (2-8)
PaO_2/FiO_2 ratio					
Median	328	360	360	319	223
IQR	282-361	268-390	333-390	291-337	95-223

TABLE 3.2 – Demographic characteristics of study participants. (**BMI**: Body Mass Index; **LOS**: Length of hospital stay; **N/A**: Not applicable; **SIRS**: systemic inflammatory response syndrome). The maximum value of CRP and serum amylase during the first 24 hours of admission were used to calculate group medians and IQR. SIRS and APACHE II calculations were based on the most extreme values of the first 24 hours of admission.

elevated serum amylase (IQR = 1.5-4 days), and late scans after a median of 6 days (IQR = 5-9.3 days). Regarding the onset of symptoms, early scans were performed after a median of 5 days (IQR = 3-7 days), and late scans after a median of 8.5 days (IQR = 6-12.3 days). Twenty-three participants (53.7%) underwent an early scan only, 8 (22%) underwent a late scan and 10 (24.4%) underwent both. One late scan performed on day 4 after recruitment on a

participant who underwent an early scan on day 1 was excluded from further analysis.

3.4.2 EARLY SCANS

In patients with respiratory dysfunction (n = 7), a greater number of comet tails were evident on LUSS when compared to patients without respiratory dysfunction (n = 26), both when all lung quadrants except for the lower lateral were examined, and when upper quadrants alone were considered (P = 0.030 and P = 0.021 respectively). When all lung quadrants were taken into consideration this difference did not reach statistical significance (P = 0.067). When severity status was used as the defining parameter for the cohort, patients with severe AP (n = 5) had a greater number of comet tails than others (n = 28), by all three methods.

The number of comet tails was significantly different by all three methods when maximum CRP value of the first week of admission was examined. Conversely, for contemporaneous CRP a marginally significant difference was shown when scanning all lung quadrants and all quadrants except for the lower lateral (P = 0.048 for both methods), but only a non-significant difference when scanning upper quadrants alone (P = 0.074). The number of comet tails identified by each method is summarized in **TABLE 3.3** and group comparisons are depicted in **FIGURE 3.3.**

Of the three LUSS approaches, the AUC of the ROC curve was greater for the method examining upper lung quadrants alone when the cohort was categorised by respiratory dysfunction (AUC = 0.783, 95% C.I. = 0.544-0.962, P = 0.023) and disease severity (AUC = 0.996, 95% C.I. = 0.986-1.000, P < 0.001). Pairwise AUC comparisons between methods examining all quadrants, all quadrants except the lower lateral and upper quadrants alone, did not reveal a statistically significant difference (**FIGURE 3.4.a**).

	Respiratory Dysfunction							
	PaO2/FiO2 < 300			PaO2/FiO2 ≥ 300			Sub-group comparison P-value	Correlation* with PaO2/FiO2 (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	7	9	7-10	26	2	1-8	0.067	-0.456 (0.008)
All lung quadrants except lower lateral		7	5-9		2	0-6	0.030	-0.470 (0.006)
Upper lung quadrants		5	3-9		1	0-3	0.021	-0.409 (0.018)
	Disease Severity							
	Severe			Non-severe			Sub-group comparison P-value	Correlation* with Modified MODS score (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	5	11	10-20	28	2	1-8	0.001	0.388 (0.026)
All lung quadrants except lower lateral		10	9-13		2	0-6	0.001	0.426 (0.014)
Upper lung quadrants		8	7-9		1	0-3	< 0.001	0.396 (0.023)
	Contemporaneous CRP							
	≥ 100			< 100			Sub-group comparison P-value	Correlation* with CRP on the day of LUSS (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	13	9	6-10	20	2	1-8	0.048	0.406 (0.019)
All lung quadrants except lower lateral		6	2-9		2	0-5	0.048	0.354 (0.043)
Upper lung quadrants		4	1-6		1	0-3	0.074	0.306 (0.083)
	Maximum CRP							
	≥ 100			< 100			Sub-group comparison P-value	Correlation* with Maximum CRP (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	20	8	4-10	13	2	0-2	0.005	0.395 (0.023)
All lung quadrants except lower lateral		6	2-9		0	0-2	0.003	0.391 (0.024)
Upper lung quadrants		3	1-6		0	0-2	0.040	0.323 (0.066)

TABLE 3.3 – Descriptive summary of the number of comet tails identified by each method (early scans). *: Spearman correlation coefficient

		AUC (95 C.I.)	Threshold	Sensitivity (ratio; 95% C.I.)	Specificity (ratio; 95% C.I.)	PPV (ratio)	NPV (ratio)	Likelihood ratio
Respiratory dysfunction	All lung quadrants	0.728 (0.481-0.926)	7	0.857 (6/7; 0.571-1.000)	0.654 (17/26; 0.462-0.809)	0.400 (6/15)	0.944 (17/18)	2.5
	All lung quadrants except lower lateral	0.767 (0.544-0.948)	5	0.857 (6/7; 0.571-1.000)	0.692 (18/26; 0.500-0.846)	0.429 (6/14)	0.947 (18/19)	2.8
	Upper lung quadrants	0.783 (0.544-0.962)	3	0.857 (6/7; 0.571-1.000)	0.731 (19/26; 0.539-0.885)	0.462 (6/13)	0.950 (19/20)	3.2
Severity	All lung quadrants	0.968 (0.893-1.000)	9	1.000 (5/5; 1.000-1.000)	0.821 (23/28; 0.679-0.964)	0.714 (5/7)	1.000 (26/26)	5.6
	All lung quadrants except lower lateral	0.961 (0.868-1.000)	9	0.800 (4/5; 0.400-1.000)	0.964 (27/28; 0.893-1.000)	0.800 (4/5)	0.964 (27/28)	22.2
	Upper lung quadrants	0.996 (0.986-1.000)	6	1.000 (5/5; 1.000-1.000)	0.964 (27/28; 0.893-1.000)	0.833 (5/6)	1.000 (27/27)	27.8
CRP contemp. with early USS	All lung quadrants	0.706 (0.494-0.892)	8	0.692 (9/13; 0.462-0.923)	0.750 (15/20; 0.550-0.950)	0.643 (9/14)	0.789 (15/19)	2.8
	All lung quadrants except lower lateral	0.708 (0.510-0.883)	5	0.692 (9/13; 0.462-0.923)	0.750 (15/20; 0.550-0.900)	0.643 (9/14)	0.789 (15/19)	2.8
	Upper lung quadrants	0.689 (0.513-0.889)	3	0.615 (8/13; 0.308-0.846)	0.750 (15/20; 0.550-0.900)	0.615 (8/13)	0.750 (15/20)	2.5
Max CRP (first 7 days)	All lung quadrants	0.787 (0.610-0.937)	4	0.750 (15/20; 0.550-0.900)	0.846 (11/13; 0.615-1.000)	0.882 (15/17)	0.689 (11/16)	4.9
	All lung quadrants except lower lateral	0.800 (0.640-0.929)	3	0.650 (13/20; 0.450-0.850)	0.846 (11/13; 0.615-1.000)	0.867 (13/15)	0.611 (11/18)	4.2
	Upper lung quadrants	0.714 (0.529-0.864)	3	0.550 (11/20; 0.350-0.750)	0.846 (11/13; 0.615-1.000)	0.846 (11/13)	0.550 (11/20)	3.6
CXR diagnosed AIS	All lung quadrants	0.859 (0.705-0.980)	8	0.733 (11/15; 0.467-0.933)	0.923 (12/13; 0.769-1.000)	0.917 (11/12)	0.750 (12/16)	9.5
	All lung quadrants except lower lateral	0.818 (0.639-0.949)	3	0.733 (11/15; 0.467-0.933)	0.846 (11/13; 0.615-1.000)	0.846 (11/13)	0.733 (11/15)	4.8
	Upper lung quadrants	0.810 (0.631-0.946)	3	0.667 (10/15; 0.400-0.867)	0.923 (12/13; 0.769-1.000)	0.909 (10/11)	0.706 (12/17)	8.7

TABLE 3.4 – AUC, thresholds for the number of comet tails and parameters of diagnostic accuracy for each method, with regard to respiratory dysfunction, disease severity status and CRP. In order to examine the diagnostic association between the scan findings and each dichotomous variable, a value equal to or greater than the respective threshold of comet tails has been applied.

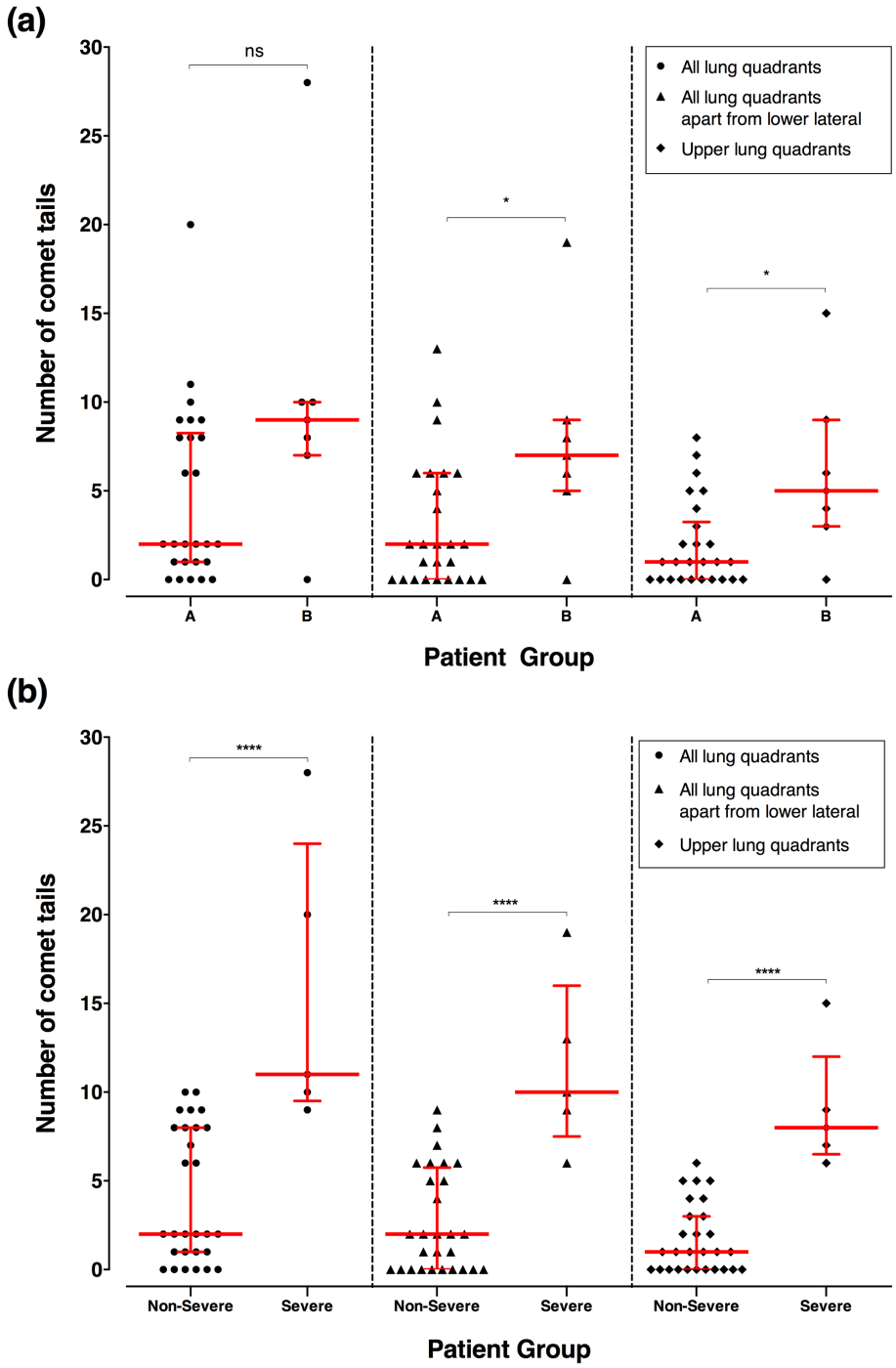

FIGURE 3.3 – Scatterplot of the total number of comet tails per patient group as determined by: **a.** Respiratory dysfunction status ($PaO_2/FiO_2 < 300$ vs. $PaO_2/FiO_2 \geq 300$). Red lines represent median and IQR for each patient group. (A: $PaO_2/FiO_2 \geq 300$; B: $PaO_2/FiO_2 < 300$) – **b.** AP severity status (Severe vs. Non-severe). Red lines represent median and interquartile range for each patient group. **ns**: $P \geq 0.05$; *: $P < 0.05$ ****: $P < 0.001$.

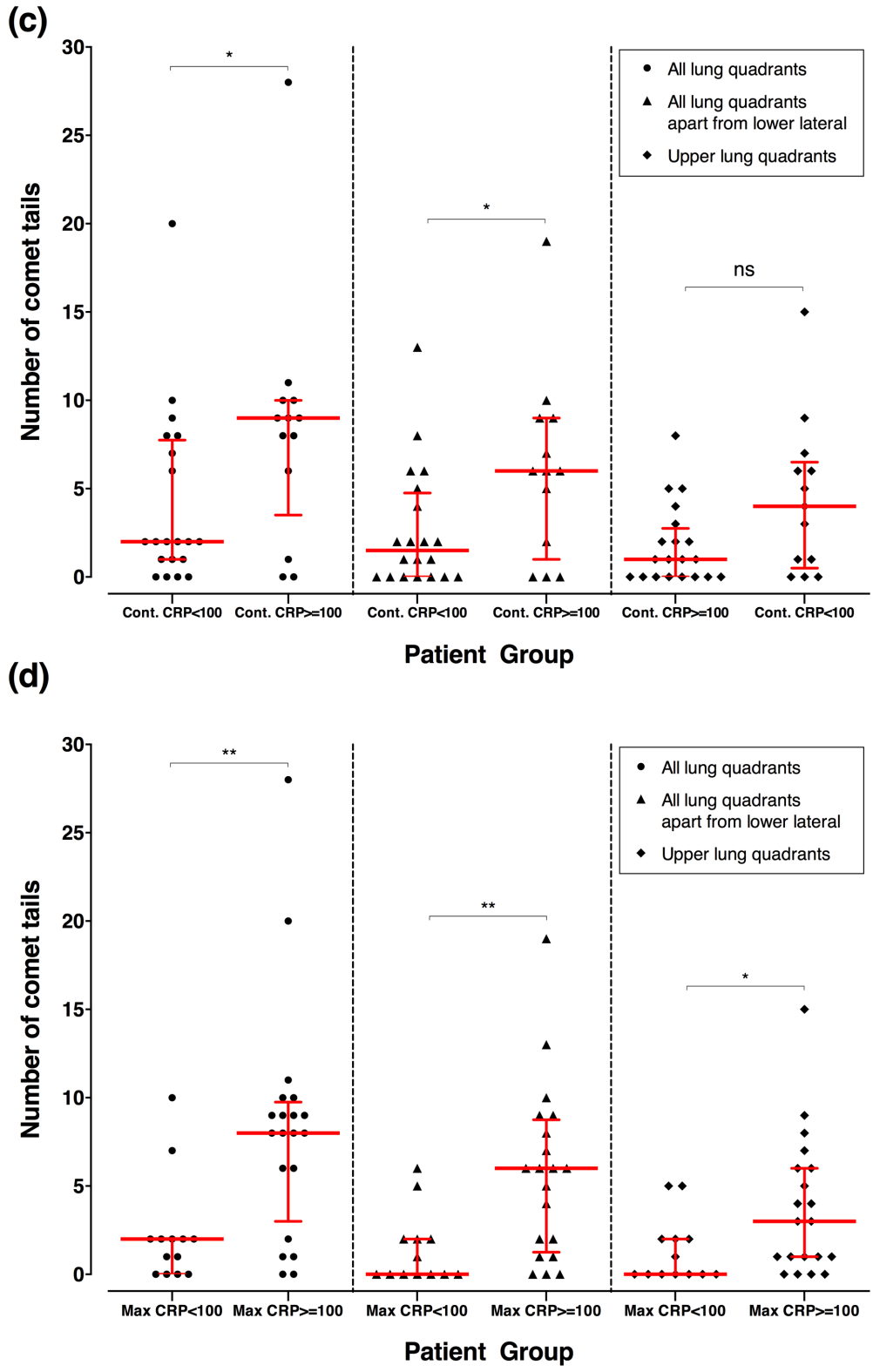

FIGURE 3.3 – Scatterplot of the total number of comet tails per patient group as determined by: **c.** Contemporaneous CRP – **d.** Maximum value of CRP. Red lines represent median and interquartile range for each patient group. **ns**: $P \geq 0.05$; *: $P < 0.05$ **: $P < 0.01$.

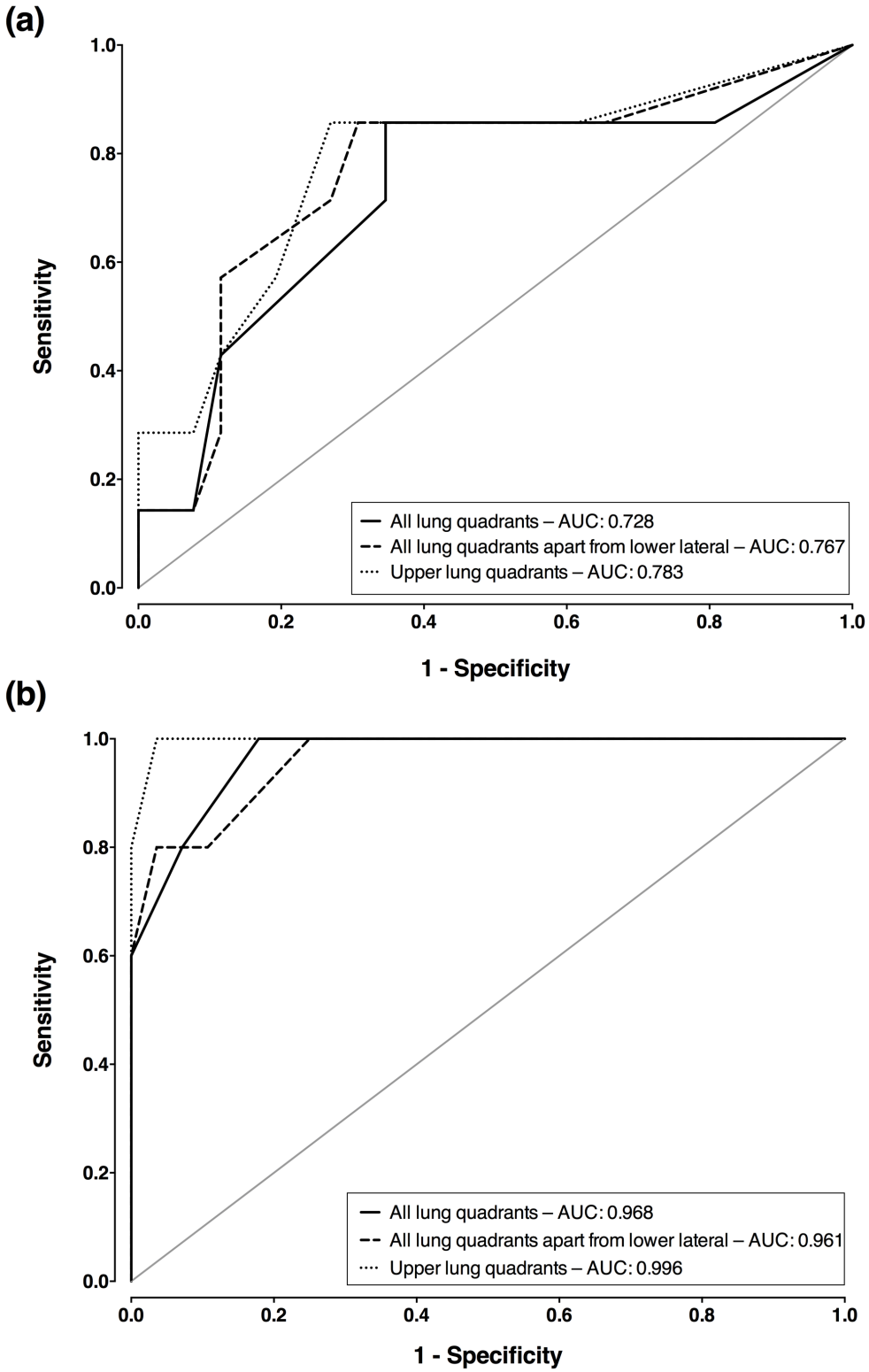

FIGURE 3.4 – ROC curves of the number of comet tails by: **a.** Respiratory dysfunction status – **b.** AP severity status.

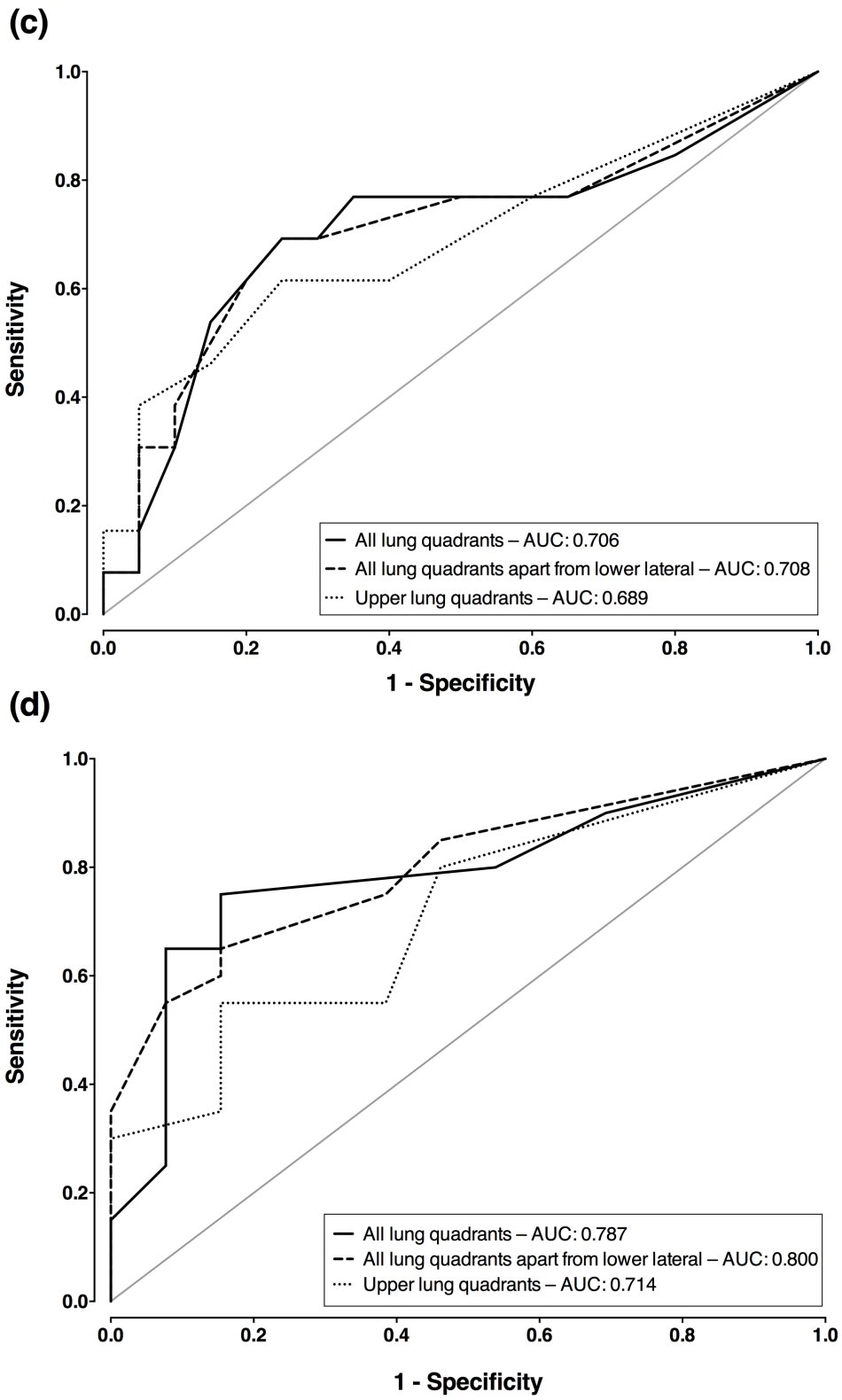

FIGURE 3.4 – ROC curves of the number of comet tails by: **c.** Contemporaneous CRP – **d.** Maximum value of CRP

The greatest AUC for contemporaneous and maximum CRP resulted from examining all lung quadrants except for the lower lateral (AUC = 0.708, 95% C.I. = 0.510-0.883, P = 0.047, and AUC = 0.800, 95% C.I. = 0.640-0.929, P = 0.004 respectively), as depicted on **FIGURE 3.4.b**. For contemporaneous CRP, pairwise comparisons did not reveal superiority of either of the three methods whereas for maximum CRP, examining all quadrants except for the lower lateral was superior to upper quadrants alone (P = 0.011).

For each LUSS method, the derived threshold for the number of comet tails, the corresponding sensitivity (SE), specificity (SP), positive (PPV) and negative predictive values (NPV), along with respective 95% C.I., likelihood ratios, as well as the correlation coefficients of the number of comet tails with PaO_2/FiO_2, modified MODS score, contemporaneous and maximum CRP are presented in ERROR! REFERENCE SOURCE NOT FOUND..

3.4.3 LATE SCANS

None of the 19 patients who underwent LUSS late in the course of the disease had concurrent respiratory dysfunction, therefore it was not feasible to examine the diagnostic accuracy of late scans in this context. No significant correlation between the number of late LUSS comet tails and either corresponding disease severity (contemporaneous modified MODS score), respiratory dysfunction (PaO_2/FiO_2), disease severity on admission (as defined by the revised Atlanta criteria), or contemporaneous/maximum CRP was discovered, regardless of the LUSS method used.

3.4.4 X-RAYS

Twenty-eight of the 33 participants who underwent an early LUSS, also had at least one prior CXR as part of their routine clinical management, and 15 of these showed AIS. Although LUSS were performed a median of 2 days

(IQR: 1-3 days) after the CXR, a satisfactory diagnostic association was demonstrated between CXR diagnosed AIS and LUSS findings (ERROR! REFERENCE SOURCE NOT FOUND.).

3.5 DISCUSSION

The findings of the LUSS study confirm a useful diagnostic association of early lung ultrasound scans with AP severity and respiratory dysfunction, for all three scanning methods applied, particularly when all lung quadrants excluding the lower lateral and when upper quadrants alone were examined. More comet tail artefacts are present in patients with respiratory dysfunction, severe AP and elevated CRP, and thresholds for the number of comet-tails have been discovered. The routine use of LUSS in the early stage of AP appears to be attractive as an initial proposition for further research, as a non-invasive, easily repeatable bedside adjunct that provides an accurate evaluation of disease severity and respiratory dysfunction (Skouras et al., 2016a).

Furthermore, in ITU, LUSS can support clinical decision making when patient pronation is under consideration, and offers the potential to monitor its effects in real time (Tsubo et al., 2004). It can be effective in the detection of derecruited pulmonary areas, allowing for optimization of ventilatory strategy, for example by means of postural therapy, and can assist in the diagnosis of several other pulmonary conditions which are common in critical illness, such as pulmonary atelectasis, pneumonia, pleural effusion, and/or pneumothorax, and to differentiate between cardiogenic pulmonary oedema and decompensated COPD (Via et al., 2012; Volpicelli et al., 2006). Importantly, as shown in the present pilot study, high patient BMI does not appear to affect LUSS accuracy (overall patient sample median BMI = 28; severe AP subgroup median BMI = 32).

The LUSS study has several potential limitations. The cohort size is relatively small, however as a pilot evaluation of this diagnostic approach it is

considered adequate. The number of late scans performed is insufficient to comment on its utility in the late phase of the AP and the small number of patients who underwent both early and late scans (n = 10) has not allowed for a valid comparison between these two groups. However, the fact that organ dysfunction and mortality are early events in AP does not obviate the utility of a late imaging approach. A potentially slower resolution of the pathological pulmonary changes in comparison to the anticipated clinical recovery of patients with non-severe AP may raise challenges on the potential application of late LUSS in monitoring disease progress.

Although the interobserver variability of LUSS findings has not been examined in the presented pilot study, evidence from previous studies reveal a satisfactory interobserver agreement for the detection of AIS, and offer an important appraisal for the quality of the findings arising from a method generally characterised by operator dependency. Volpicelli et al report interobserver variability of less than 5% (183 concordant and 9 discordant diagnoses) in a random set of 24 examinations performed by two independent investigators, whereas Lichtenstein et al report a kappa statistic of 0.74 from the assessment of 288 lung regions (Lichtenstein et al., 2004; Volpicelli et al., 2006). Accordingly, these reports support the robustness of the findings presented herein.

The most pertinent question regarding this novel method concerns its clinical relevance to the management of patients with AP. From the arising findings, it remains uncertain whether lung ultrasonography will offer an advantage in the prediction of severity or respiratory dysfunction when compared to the standard clinical assessment or use of existing composite scores. Although the present pilot study provides encouraging evidence about the value of LUSS as a diagnostic adjunct, additional evidence is required to support its role in the identification of AIS when it is clinically undiscernible, and further studies are required to clarify whether lung ultrasonography can pragmatically assist in stratifying AP patients that present with subtle or

established respiratory dysfunction to those who will develop persistent organ failure.

With regard to methodology, the convention used to extrapolate PaO_2/FiO_2 from $SatO_2$ may have introduced unknown error in the analysis, as the Severinghaus equation has not been validated for calculation of the modified MODS score in AP patient cohorts. Nevertheless, SpO_2 has previously been used for similar approximations, with reasonable accuracy (Pandharipande et al., 2009b; Serpa Neto et al., 2013). Additionally, the timing of chest X-rays for study participants was not altered from ordinary clinical care. LUSS were in general performed after X-rays, therefore the accuracy of the association between the findings of the two imaging methods may be confounded by this discrepancy.

The fact that early scans were performed a median of 3 days after the first finding of an elevated serum amylase, may have concealed an even greater prognostic association with early respiratory dysfunction. The exact prognostic features of early LUSS deserve further investigation and future studies are likely to benefit from standardizing the timing of scans within the first 48 hours. Nevertheless, the fact that scans performed very early may carry a high false negative ratio must be taken into consideration, as at that stage developing pulmonary involvement may not yet be identifiable with this method.

3.6 CONCLUSIONS OF CHAPTER

Ultrasonography of non-dependent lung parenchyma can reliably detect evolving respiratory dysfunction in AP, and shows promise as an adjunct to severity stratification. This simple, easily repeatable bedside test warrants further validation in a larger cohort (Skouras et al., 2016a).

Chapter 4 – Long-Term Survival in Acute Pancreatitis

4.1 SUMMARY OF CHAPTER

The effect of early organ dysfunction during AP on the long-term survival is unknown. A retrospective study was performed to ascertain whether the life expectancy of hospital survivors following AP-MODS is affected.

Data were collected on consecutive patients admitted to the Royal Infirmary of Edinburgh with AP during a 5-year period commencing January 2000. A MODS score ≥ 2 during the first week of admission was used to define early organ dysfunction. After accounting for in-hospital deaths, long-term survival probabilities were estimated using the Kaplan-Meier method. The prognostic significance of patient characteristics was assessed by univariate and multivariate analyses using Cox's proportional hazards methods.

694 patients were studied. Median follow-up was 8.8 years. Patients with early organ dysfunction died prematurely (mean survival = 8.6 years; 95% C.I. = 7.9-9.3 years), compared to those who did not develop organ dysfunction (mean survival = 11.5 years; 95% C.I. = 11.1-11.9 years, Wilcoxon $P < 0.001$, log-rank $P < 0.001$). Importantly, this effect was independent of age and persisted after the exclusion of in-hospital deaths. Multivariate analysis confirmed MODS as an independent negative predictor of long-term survival (Cox's Hazard Ratio = 1.528, 95% C.I. = 1.72-2.176, $P = 0.019$) along with age (HR = 1.062, $P < 0.001$), alcohol (HR = 2.027, $P = 0.001$) and idiopathic aetiology (HR = 1.548, $P = 0.048$).

Early organ dysfunction in AP is an independent negative predictor of long-term survival even when in-hospital deaths are accounted for. Negative predictors also include age, idiopathic and alcohol aetiology. This study demonstrates that the damaging effects of critical illness persist even after the initial organ dysfunction has resolved and adds a new dimension to the importance of predicting and preventing organ dysfunction in AP patients.

4.2 AIM

The aim of the AP long-term survival study was to determine whether early organ dysfunction during an episode of AP is associated with altered life expectancy of hospital survivors (Skouras et al., 2014).

4.3 METHODS

4.3.1 STUDY APPROVAL

Caldicott Guardian approval was obtained to allow the use of confidential patient data. The study was assessed by the UoE/NHS Lothian ACCORD Research and Development office and the South East Scotland Research Ethics Service and was declared exempted from formal Research Ethics Committee review as a quality improvement project.

4.3.2 DATA COLLECTION AND INCLUSION CRITERIA

In 2013, a retrospective analysis of long-term survival data was performed for a prospectively maintained database of patients with AP, admitted to the Royal Infirmary of Edinburgh (RIE) during the 5-year period between January 2000 and December 2004. This historical cohort was originally identified from the Lothian Surgical Audit database (Aitken et al., 1997), and the clinical details and immediate outcomes have been previously reported (Mofidi et al., 2007b). For this cohort, existing data on age, gender, organ dysfunction, aetiology of AP, necrosectomy and disease severity were supplemented with newly collected survival data. Patients for whom data on these characteristics were unavailable were excluded from subsequent analysis. For those patients with recurrent attacks of AP, the earliest episode

during the study period was taken as the index episode for the purposes of survival analysis.

Patient inclusion in the original cohort was based on clinical features compatible with AP, supported by the finding of elevated serum amylase (three times higher than the upper limit of the normal reference range). CT evidence of AP was required for the diagnosis in instances where a strong clinical suspicion of AP existed without a diagnostic level of serum amylase. Patients with chronic pancreatitis had been excluded.

4.3.3 DEFINITIONS

Severity stratification was performed according to the original version of the Atlanta consensus definition of 1992 (Bradley, 1993), which was the classification system in use at the RIE at the time when the patients of the study cohort had presented with AP. Patients were not retrospectively reclassified according to the revised Atlanta criteria of 2012 (Banks et al., 2013) to avoid introducing error. Patients with an APACHE II score of ≥ 8 were categorised in the severe AP group.

Gallstones were considered to be the precipitating cause of AP when gallbladder or bile duct calculi had been detected by any imaging modality. In the absence of gallstones and when excessive consumption of alcohol had been reported by the patient or the patient's family, AP was classified as alcohol induced. When no definite cause was identified, the disease was characterised as idiopathic. Rarer established causes were categorised collectively under the group 'other'.

Organ dysfunction scores were calculated for all patients at 24 hours, 48 hours and at 7 days, based on the most extreme laboratory values or clinical measurements during each 24-hour period, for five of six organ systems that constitute the MODS score reported by Marshall et al: respiratory, cardiovascular, renal, haematological, central nervous system (Marshall et al., 1995). Hepatic dysfunction – measured by the level of serum bilirubin – was

excluded to avoid the confounding effects of potential biliary obstruction. A MODS score of ≥ 2 (based on dysfunction of a single, or more than one organ system) at one or more time points was required for the definition of organ dysfunction. The duration and persistence of organ dysfunction could not be retrospectively ascertained from the existing database.

4.3.4 FOLLOW-UP & MORTALITY

In-hospital mortality, overall mortality and long-term survival were the main outcome measures of the long-term survival study. NHS Lothian electronic patient records were individually reviewed and survival data were collected. For surviving patients, the date of the most recent GP or hospital visit (outpatient clinic attendance or hospital discharge) was defined as the point of last known contact, whereas for non-survivors the date of death was recorded.

4.3.5 STATISTICAL ANALYSIS

Data for continuous variables are presented by using the mean and SD or median and IQR. Categorical variables are presented as absolute and relative frequencies. Comparisons between groups were performed by using Student's t-test, Mann-Whitney U-test, Kolmogorov-Smirnov test and χ^2 tests, as appropriate.

The Kaplan-Meier method was used to estimate survival probabilities. The generalized Wilcoxon test was used to detect early death rate differences between patient groups and the log-rank test was used to detect differences manifesting throughout the duration of the follow-up period. Survival times were calculated from the date of admission to the date of death from any cause, or date of last contact for survivors. Patients lost to follow-up were censored at the date of confirmed last contact.

The prognostic impact of patient characteristics on survival was assessed by univariate and multivariate Cox's proportional hazards regression. For univariate analyses, the proportional hazard assumption was examined graphically with the use of pairwise Cox log(-log) plots, revealing approximately parallel curves between groups for each of the categorical covariates: MODS, gender, severity and aetiology. Linear regression was implemented to test for goodness of fit of Schoenfeld partial residuals (Schoenfeld, 1982) against natural logarithmic survival times for each of the aforementioned covariates and no violation of the proportional hazards assumption was revealed. The linearity assumption for age was confirmed graphically by examining the respective Cox log(-log) plots, after transformation to a categorical variable with 6 strata. Similar procedures were used for the multivariate analyses.

Selection of variables for the multivariate Cox regression model was performed according to the 'Purposeful Selection' algorithm (Bursac et al., 2008; Heinze, 2008), originally described by Hosmer and Lemeshow (Hosmer et al., 2008.). Variables with a univariate P-value < 0.25 were originally selected as candidates for the multivariate analysis. Covariates were subsequently removed from the model if they were found to be non-significant at the $\alpha = 0.1$ level and not to be confounders. Confounding was defined as a change of > 15% in any of the remaining covariate estimates. Variables that were not selected for the original model was added back one at a time, and any that were significant at the $\alpha = 0.15$ level were retained (Bursac et al., 2008). This procedure resulted in a multivariate model containing age, MODS, gender, severity and aetiology. The necrosectomy variable did not satisfy the proportional hazards assumption and was examined with the use of Stratified Cox regression.

Results of the Cox models are presented as hazard ratios (HR) with 95% C.I., together with the P-values from Wald's tests. All statistical tests were based on a two-sided alpha level of 0.05. Statistical analysis was performed using IBM SPSS® Statistics version 19.0 (IBM Corporation, Armonk, New

York, USA) and figures were designed on GraphPad Prism® version 6 (GraphPad Software, Inc., La Jolla, California, USA). This double-arm cohort study followed the principles of the STROBE (strengthening the reporting of observational studies in epidemiology statement (von Elm et al., 2007).

4.4 RESULTS

4.4.1 DEMOGRAPHICS

Unique patient identifiers with corresponding survival data and variables of interest were available for 694 of 759 (91.4%) AP patients in the database and were included in the study. With regard to classification of disease severity based on APACHE II score, 256 (36.9%) patients were predicted to have a severe attack, although ultimately 268 (38.6%) suffered from some degree of organ dysfunction during the first week of admission. A total of 235 (33.9%) patients had a MODS score of ≥ 2 at one or more time-points during the first week of their hospital stay and were classified as having organ dysfunction (denoted as 'MODS group'). Sixty-nine (9.9%) patients underwent one or more necrosectomy procedures during the study interval. These were preformed predominantly as open procedures since minimally invasive retroperitoneal pancreatic (MIRP) necrosectomy was not routinely performed in the RIE during the study period. The demographic characteristics are summarized in ERROR! REFERENCE SOURCE NOT FOUND..

4.4.2 STUDY FOLLOW-UP

The median follow-up for the whole patient cohort was 8.8 years. After excluding in-hospital deaths, median follow-up was 9.0 years. This period was marginally comparable between the MODS group and non-MODS group, after exclusion of in-hospital deaths (MODS group median follow-up: 8.7 years, IQR

Characteristics	Overall sample	Non-MODS group	MODS group	In-hospital deaths	Survivors	Non-survivors
	N (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Sample size	694 (100)	455 (69.1)	203 (30.9)	36 (5.2)	479 (72.8)	179 (27.2)
Median age (IQR)	57.1 (43.2-71.2)	54.3 (40.3-69.3)	60.0 (47.7-74.0)	70.9 (57.4-79.9)	52.1 (39.0-65.0)	66.7 (54.2-79.5)
Gender						
Male	355 (51.2)	228 (50.1)	106 (52.2)	21 (58.3)	236 (49.3)	98 (54.7)
Female	339 (48.8)	227 (49.9)	97 (47.8)	15 (41.7)	243 (50.7)	81 (45.3)
Aetiology of AP						
Gallstones	337 (48.6)	223 (49)	104 (51.2)	10 (27.8)	237 (49.5)	90 (50.3)
Alcohol	223 (32.1)	159 (34.9)	54 (26.6)	10 (27.8)	158 (33.0)	55 (30.7)
Idiopathic	92 (13.3)	54 (11.9)	27 (13.3)	11 (30.6)	54 (11.3)	27 (15.1)
Other	42 (6.1)	19 (4.2)	18 (8.9)	5 (13.9)	30 (6.3)	7 (3.9)
Severity						
Mild	438 (63.1)	380 (83.5)	56 (27.6)	2 (5.6)	338 (70.6)	98 (54.7)
Severe	256 (36.9)	75 (16.5)	147 (72.4)	34 (94.4)	141 (29.4)	81 (45.3)
Necrosectomy	69 (9.9)	17 (3.7)	42 (20.7)	10 (27.8)	43 (9.0)	16 (8.9)
In-hospital deaths	36 (5.2)	n/a	n/a	36 (100)	n/a	n/a
Post-discharge deaths	179 (25.8)	107 (23.5)	72 (35.5)	n/a	n/a	179 (100)
MODS	235 (33.9)	n/a	n/a	32 (88.9)	131 (27.3)	72 (40.2)

TABLE 4.1 – Demographic characteristics. Non-MODS/MODS and Survivor/Non-Survivor groups include all patients after exclusion of in-hospital deaths.

= 4.7-10.5 years vs. non-MODS median follow-up: 9.1 years, IQR = 6.6-10.6 years; Mann-Whitney U-test: P = 0.046, Kolmogorov-Smirnov test: P = 0.105).

4.4.3 OVERALL SURVIVAL

In total, there were 36 (5.2%) in-hospital deaths, 35 of which occurred within 2.2 months following admission to hospital. During the follow-up period, 179 patients died, resulting in an overall mortality rate of 31.0% (215 of 694 patients). Median survival in the overall cohort could not be defined, because more than 50% of patients were still alive at the end of the follow-up period. The overall mean survival was 10.6 years (95% C.I. = 10.2-10.9 years), which after exclusion of the in-hospital deaths increased to 11.1 years (95% C.I. = 10.8-11.5 years) (**FIGURE 4.1**).

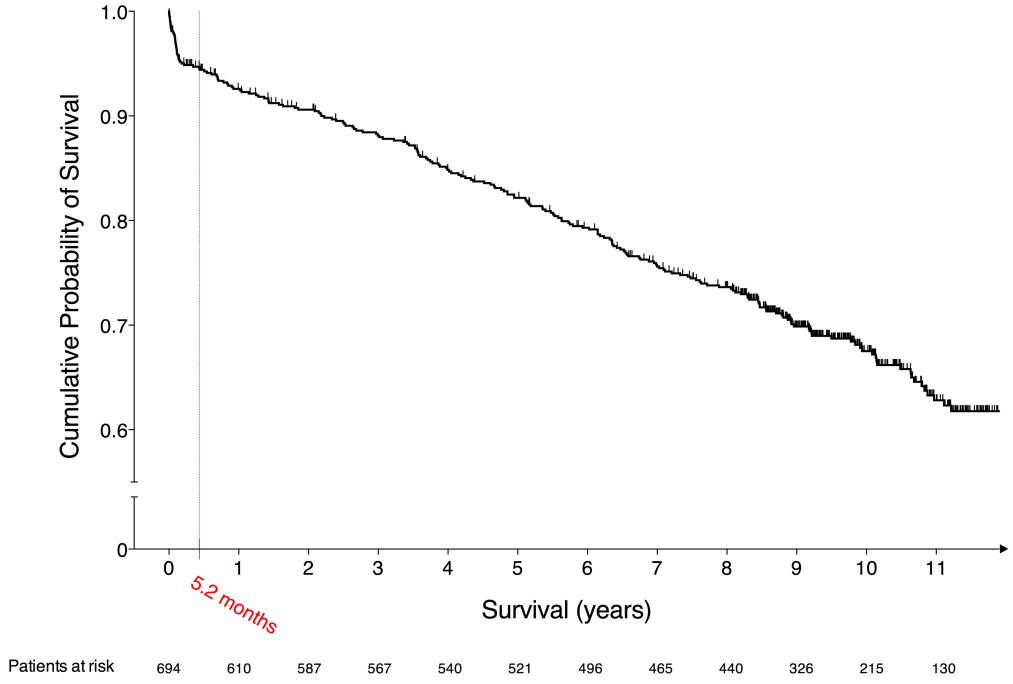

FIGURE 4.1 – Kaplan-Meier survival curve for the overall sample of patients (N = 694), representing percentage of surviving patients per time in years. A distinct, steep decrease is evident during the first 2.2 months of the study, corresponding to in-hospital deaths. The dashed red line represents the time-point of the last observed in-hospital death. Vertical tick-marks represent right-censored cases. The survival curve has been truncated when the number of patients at risk reached 1/10th of the original denominator.

4.4.4 ORGAN FAILURE AND SURVIVAL

Analysis of the overall patient sample demonstrated a clear difference in survival between the MODS and non-MODS groups (**FIGURE 4.2**). Mean survival in the MODS group (8.6 years, 95% C.I. = 7.9-9.3years) was significantly lower than that of the non-MODS group (11.5 years, 95% C.I. = 11.1-11.9 years) (Wilcoxon test: $P < 0.001$, log-rank test: $P < 0.001$). The highest frequency of death occurred during the in-hospital period; the majority of these patients had a MODS score of ≥ 2. Four of the 36 patients who died during their hospital stay had a MODS score of < 2.

Next, to investigate long-term mortality, in-patient deaths were removed from the analysis. Importantly, with this exclusion, mean survival of the MODS group hospital survivors (10.0 years, 95% C.I. = 9.4-10.6 years) remained significantly shorter than that in non-MODS group hospital survivors (11.6 years, 95% C.I. = 11.2-11.9 years) (Wilcoxon test: $P = 0.002$, log-rank test: $P = 0.001$). Furthermore, the survival curves continued to diverge for several years after the index episode of AP (**FIGURE 4.3**). Again, median survival was not defined; however the 75th percentiles of survival were 6.5 years and 9.9 years for the MODS and non-MODS groups, respectively. The survival rates for each group at 1, 3, 5 and 10 years are shown in **TABLE 4.2**.

To account for the mean age difference between the MODS (59.9 ± 17.1 years) and the non-MODS (54.3 ± 17.9 years) groups ($P < 0.001$), and for the impact other AP characteristics may have on long-term survival, a multivariate analysis was performed. The MODS score was shown to be an independent predictor of patient survival, with the MODS group carrying a higher level of risk for post-discharge death than the non-MODS group, in both univariate and multivariate analyses. Age and cause of AP were also established as individual predictors, whereas severity and gender were identified as confounding factors (**TABLE 4.3**).

Notably, apart from alcohol, idiopathic aetiology was associated with an increased risk of reduced long-term survival in the multivariate analysis. As

shown in ERROR! REFERENCE SOURCE NOT FOUND., a relatively high proportion of patients (13.1%) in the overall cohort suffered from idiopathic AP, which was even higher for patients who died following their discharge from hospital (15.1%). The idiopathic subgroup is likely to include patients with undiagnosed causes, in particular microlithiasis, who are likely to suffer from recurrent attacks of AP. This fact may potentially explain why idiopathic aetiology predisposes to reduced life expectancy, although unfortunately these data were not available for confirmation in the present study. When necrosectomy was added on the multivariate Cox regression model by stratification, a negligible effect was observed. Specifically, the resulting HR for the MODS group was 1.573 (95% C.I. = 1.102-2.246, P = 0.013).

A supplementary univariate Cox regression analysis was performed on the MODS group alone (after exclusion of in-hospital deaths) in order to examine whether the magnitude of MODS score (worst MODS score value of days 1, 2 and 7 of admission) or APACHE II score on the day of admission had any effect on long-term survival. No significant effects were revealed for either the MODS score (HR = 0.876, 95% C.I. = 0.748-1.026; P = 0.102) or the APACHE II score (HR = 1.019, 95% C.I. = 0.954-1.088; P = 0.573).

4.5 DISCUSSION

The long-term survival study demonstrated that organ dysfunction has a lasting negative impact on life expectancy after an attack of AP. Importantly, this effect is seen after excluding in-hospital deaths and is independent of age, aetiology and gender. In addition, age and an alcohol or idiopathic aetiology were also confirmed as factors with deleterious impact (Skouras et al., 2014).

The extent to which the consequences of critical illness contribute to post-discharge mortality is poorly understood (Angus et al., 2003; Kaplan and Angus, 2002; Keenan et al., 2002; Williams et al., 2005), especially in AP. No previous study has specifically investigated the impact of early organ dysfunction as a predictive marker for long-term mortality following an episode

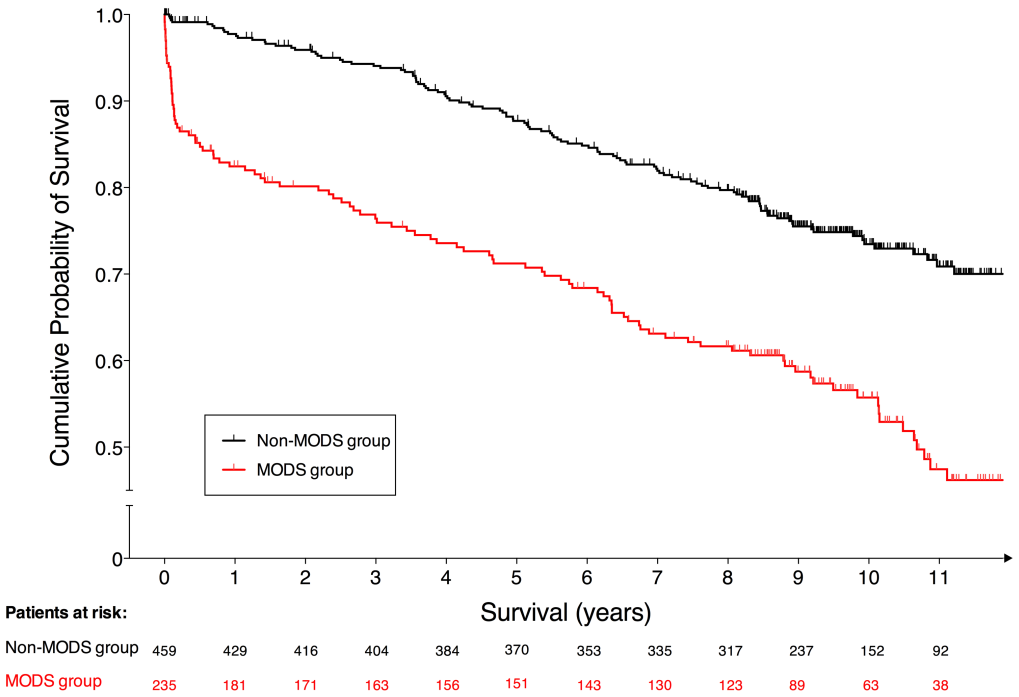

FIGURE 4.2 – Kaplan-Meier survival plot by group of patients (black line: non-MODS group, n = 459 – red line: MODS group, n = 235), representing cumulative percentage of surviving patients per time in years. A distinct, steep decrease is evident on the MODS curve during the first months of the study, corresponding to in-hospital deaths. A clear difference in survival is observed between the two groups. Vertical tick-marks represent right-censored cases. Survival curves have been truncated when the number of patients at risk reached 1/10th of the original denominator.

Followh-up	MODS group			Non-MODS group		
	Patients at risk	Proportion of patients surviving	Standard Error	Patients at risk	Proportion of patients surviving	Standard Error
1 year	178.5	0.92	0.02	426.5	0.97	0.01
3 years	162.5	0.85	0.03	401.5	0.91	0.01
5 years	151	0.79	0.03	367.5	0.86	0.02
10 years	54.5	0.55	0.04	123.5	0.72	0.02

TABLE 4.2 – Number of patients at risk and survival rates for each group at 1, 3, 5 and 10 years of follow-up.

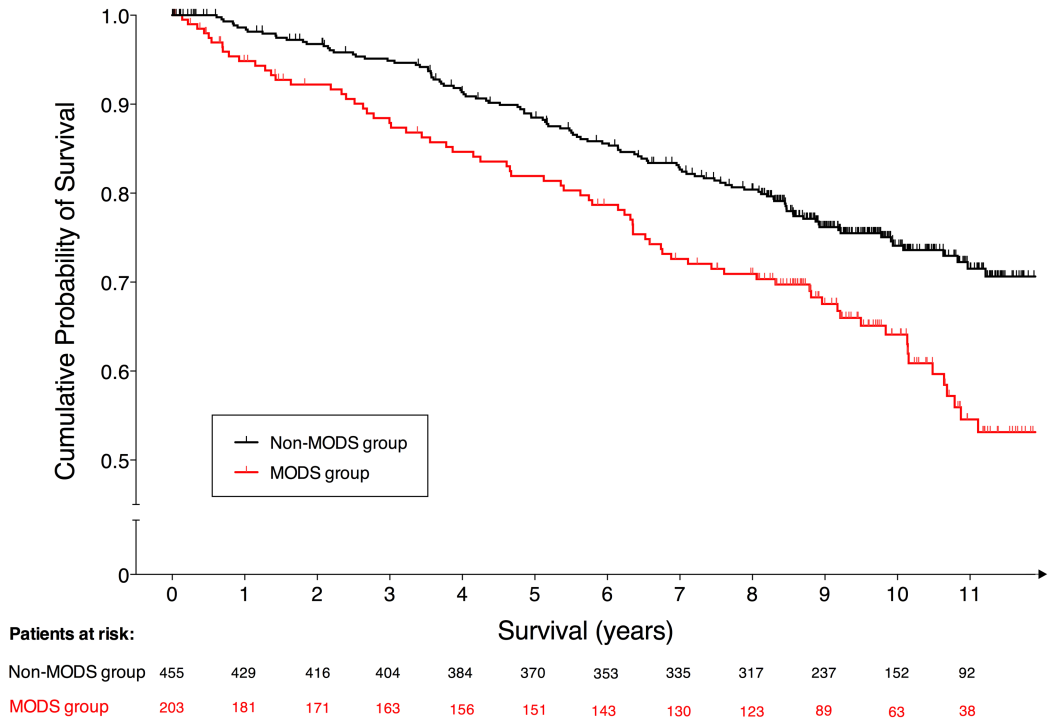

FIGURE 4.3 – Kaplan-Meier survival curves for each group of patients, after exclusion of in-hospital deaths (black line: non-MODS group, n = 455 – red line: MODS group, n = 203), representing percentage of surviving patients per time in years. A clear, moderately increasing difference in survival is observed between the two groups. Vertical tick-marks represent right-censored cases. Survival curves have been truncated when the number of patients at risk reached 1/10th of the original denominator.

Factors	Overall Survival					
	Univariate Analyses			Multivariate Analysis		
	Crude HR	95% C.I.	P-value	Adjusted HR	95% C.I.	P-value
MODS						
Yes	1.661	1.232-2.240	0.001	1.528	1.072-2.176	**0.019**
Gender						
Male	1.181	0.879-1.585	0.269	1.302	0.946-1.793	0.106
Age	1.050	1.040-1.061	<0.001	1.062	1.049-1.075	**<0.001**
Severity						
Severe	1.862	1.387-2.499	<0.001	0.850	0.587-1.231	0.390
Cause						
Gallstones	1			1		
Alcohol	0.927	0.662-1.296	0.656	2.027	1.359-3.024	**0.001**
Idiopathic	1.350	0.878-2.076	0.171	1.548	1.004-2.387	**0.048**
Other	0.788	0.365-1.701	0.544	1.488	0.679-3.263	0.321

TABLE 4.3 – Association of patient characteristics with overall survival, after exclusion of in-hospital deaths.

of AP. Findings of the available long-term studies in AP, have been equivocal in associating multiple organ failure or various surrogate markers of organ dysfunction with long-term survival. Lund et al, found that an increased Ranson's score and prolonged length of stay in ITU were associated with an increased long-term incidence of mortality in univariate, but not multivariate analyses (Lund et al., 2006). Conversely, Halonen et al reported that a smaller proportion of late-death patients had suffered multiple organ failure than had long-term survivors (Halonen et al., 2003). There was no statistical analysis supporting this finding as the study had different objectives pertaining to quality of life outcomes, but this surprising result is difficult to rationalise. However, median follow-up in this study was limited to 61 months, the rate of capture rate of events (deaths) was low, and the number of participants relatively small (Halonen et al., 2003). Furthermore, acute organ dysfunction has been shown to be associated with reduced life expectancy amongst survivors of critical illness compared with matched controls in a number of other conditions (Ulvik et al., 2007; Wright et al., 2003).

In addition to early organ dysfunction, increasing age was found to be an independent predictor of reduced survival. It is important not to dismiss this finding as obvious, albeit expected, because this observation refers to more than just the fact that older people die sooner, from all causes. Although age has been established as an independent predictor in the present and previous studies, (Dragsted, 1991; Niskanen et al., 1996; Thoner, 1987) it has been suggested that poorer outcomes in elderly patients may reflect residual functional disability and dysfunction of organ systems caused by critical illness (Ridley et al., 1990). This may be supported by the findings of Lankisch et al who reported that the most common causes of death were cardiovascular and cerebrovascular diseases (Lankisch et al., 2009). This finding was echoed by Nøjgaard et al. with reference to cardiovascular disease (Nøjgaard et al., 2011).

The exact pathophysiological mechanisms and long-term confounding effects that predispose to reduced long-term survival in survivors of critical

illness are yet unknown and deserve further investigation in AP and in general (Skouras et al., 2014). One of the proposed processes implicated in the reduction of life expectancy after an episode of critical illness is accelerated telomere shortening (Blasco, 2005). A potential decrease in telomerase activity levels is accompanied by shorter telomeres, premature loss of fitness and decreased lifespan. In addition, many other proteins that are important in regulating telomere length and function may also have an important role as they can regulate the action of telomerase at telomeres (Blasco, 2005). Telomere shortening may lead to premature senescence and has been implicated in numerous metabolic and inflammatory diseases, where external factors that promote telomere attrition – such as inflammatory and oxidative stress – play a key role (Kong et al., 2013). In the future, the aforementioned hypothesis warrants validation in the context of AP and critical illness.

With regard to AP-specific long-term sequelae, progression to chronic pancreatitis can predispose to reduced life expectancy of hospital survivors. In the study of Nøjgaard et al, patients who developed chronic pancreatitis carried 2.7 times higher long-term mortality compared to those who did not, and up to 6.5 times higher long-term mortality compared to the general population (Nojgaard et al., 2011). Furthermore, the incidence of chronic pancreatitis is higher following episodes of severe AP and it is more common when alcohol is the cause (Ammann and Muellhaupt, 1994). The resultant development of endocrine (type 3c diabetes mellitus) or exocrine insufficiency (maldigestion and malabsorption), as well as the associated predisposition to cardiovascular, infectious and malignant complications constitute additional long-term risk factors (Gudipaty and Rickels, 2015; Spanier et al., 2008).

It is known that the aetiology of AP can influence morbidity of hospital survivors, but the influence of aetiology on long-term mortality has been less well researched. Nøjgaard et al reported a significantly higher rate of long-term mortality in patients with AP related to high alcohol consumption (Nøjgaard et al., 2011), in keeping with the results of the present study. Compared to biliary AP, alcohol seems to have additional deleterious effects by precipitating

exocrine insufficiency and morphologic changes to the pancreas (Lankisch et al., 2009), thus it may be unsurprising that patients with alcohol-related AP have a shorter life expectancy than that reported for gallstone AP.

The longitudinal design of the present study, its long follow-up period with a median of approximately 9 years, and the fact that it was conducted in a single centre by including consecutive AP patients regardless of disease severity are amongst its strengths. Potential weaknesses include the retrospective design, although a prospective long-term survival study of this duration would be a major undertaking. Secondly, there is little information regarding the mode of death or any subsequent co-morbidities of survivors, which would certainly merit further investigation. Furthermore, it was not feasible to retrieve and include in the analysis data on the comorbid status of the presented cohort. It is therefore possible, and indeed one might argue reasonable, that patients with comorbidities may have been at higher risk of organ dysfunction during AP, which as a consequence resulted in shorter survival. Future studies would benefit from including details on important confounders such as detailed patient co-morbid status, presence of pancreatic necrosis, incidence of pancreatic insufficiency and other long-term sequelae, as well as specific duration and severity of organ system dysfunction. Lastly, the duration of follow-up for survivors was determined by the date of last known contact (GP or hospital visit) via their electronic records, as detailed in the methods section. More accurate information would have been obtained by contacting patients directly or via their GPs, with an aim to achieve longer and possibly more objective follow-up.

Notwithstanding these weaknesses, the long-term survival study in AP identifies an important negative long-term impact of organ dysfunction in AP, which extends beyond the injury seen in the critical care unit during the index episode. This work reinforces the importance of early identification, and appropriate management or prevention of extra-pancreatic organ dysfunction in AP.

4.6 CONCLUSIONS OF CHAPTER

Early organ dysfunction in AP is associated with a shortened lifespan. It is hypothesised that patients who sustain organ dysfunction during their index episode, and who survive, accrue systemic changes which impact negatively on life expectancy in comparison to hospital survivors without organ dysfunction. In AP, the effects of critical illness do not cease after the initial insult has subsided. This study reinforces the cardinal need to identify and pre-empt organ dysfunction, and introduces a need to consider interventions that impact on long-term outcomes in high-risk individuals (Skouras et al., 2014).

Chapter 5 – Reflections, Future Directions and Conclusions

5.1 PHARMACOLOGICAL THERAPY IN ACUTE PANCREATITIS

Until now, a great part of research in pancreatitis has focused on improving accuracy and timeliness of identification of severe disease. Despite extensive efforts for the last several years, a reliable method to predict AP severity with high accuracy is not yet available. This fact may indicate a need to shift focus from improving prediction of severity, towards discovering novel methods to halt progression to organ dysfunction.

The onset of AP-MODS is an early event during the course of AP which may evolve within hours from the onset of symptoms (Mole et al., 2016a). As a consequence of this, and based on my work in the present thesis, I propose that future trials and clinical studies in AP should aim to examine the effects of treatment administered as soon as possible after symptom onset, aiming to maximize the potential of any novel agent to interrupt the inflammatory cascade (Park, 2015). Although the goal would be to investigate any reduction of incidence and severity of AP-MODS which occurs only in a subset of patients with AP, it is possible that the way forward may be to recruit and treat 'all-comers' immediately after presentation to hospital, irrespective of AP severity and aetiology, in an effort to address the lack of a robust and highly accurate predictive system. In order to support this concept, the candidate drug should be safe, cheap and easily administered.

In recent years, advances in the understanding of basic science and pathophysiology of AP have led to the development of novel pharmacological agents. Although encouraging findings are available for some drug targets, the vast majority of the proposed novel drugs have failed to demonstrate consistent benefits in large validation studies (Park, 2015). Due to this lack of firm evidence, no therapeutic agent is yet recognised for the treatment of AP in clinical practice (Bi and Vege, 2015). Discrepancy exists between findings from animal studies and human clinical trials, a fact which can in part be

attributed to methodological flaws in human clinical study design. This makes the highly accurate and robust, unbiased approach that I have taken in this thesis all the more translationally relevant.

The role of the kynurenine pathway of TRP metabolism as a key contributory mechanism of remote organ injury in AP has recently been shown in the preclinical rodent study by Mole et al (Mole et al., 2016b). Furthermore, KMO was found to be susceptible to therapeutic blockade, and KMO inhibition was shown to protect against extrapancreatic organ injury, irrespective of the gravity of the initiating pancreatic tissue injury, findings that strongly support a potential therapeutic role for KMO inhibitors in human AP (Mole et al., 2016b). The next logical step would be to evaluate the efficacy of KMO inhibition in human AP, and the novel data arising from IMOFAP constitute an invaluable resource for the design and execution of such studies. Currently, our group is working on the development of KMO inhibitors and candidate drugs are already being progressed into clinical research.

Despite past shortcomings, there remains promise in the discovery of an effective pharmacological therapy for AP. Better designed RCTs – based on lessons learned from the past – along with recent knowledge advances will open the way for such a discovery which may fundamentally change the current approach of supportive management to one of directed therapy.

5.2 LESSONS IN THE DESIGN OF CLINICAL STUDIES IN ACUTE PANCREATITIS

It is well established that the factors and underlying mechanisms that contribute or predispose to organ dysfunction during an episode of AP are complex; hence, accurate early prediction of AP severity is challenging. Apart from its significance in clinical practice, this fact has had considerable implications for clinical research in AP thus far. Several previous clinical studies investigating management strategies in AP have been deemed

suboptimal due to poorly standardized patient classification, or due to the fact that only few participants went on to develop severe disease or the complication of interest, contrary to prognoses, while in others, patients were recruited late in the course of AP (Ebbehoj et al., 1985; Eckerwall et al., 2006; Isenmann et al., 2004; Johnson et al., 2001). Consequently, many previous drug studies in AP were not adequately powered, a fact which may have led to inappropriate conclusions and the dismissal of potentially beneficial treatment strategies (De Waele, 2007).

Disease trajectories clearly indicate that AP evolves extremely rapidly, and this can be additionally supported by studies on early and pre-hospital mortality by our group and others (Andersson et al., 2004; Andersson and Andren-Sandberg, 2003; Johnson and Abu-Hilal, 2004; McKay et al., 1999; Mole et al., 2016a). Based on the nature and rapidity of AP evolution, a rationale for prompt patient enrolment in clinical research can be supported. Findings from the IMOFAP study presented herein, reinforce the argument for expedited recruitment, which should be aimed at the time of patient presentation to the ED. Notably, most interventions designed to inhibit the mechanisms implicated in the genesis of organ dysfunction may work better if administered at an early stage, with an aim to prevent progression to organ dysfunction rather than to reverse established failure (Park, 2015). Thus, intervention at this early pathophysiological stage of the disease process will be critical.

Furthermore, a rapid approach allows for timely enrolment of patients presenting to hospital with already established organ dysfunction, who constitute a substantial proportion of the AP population, that cannot afford to be missed from any clinical study or trial. In addition, expedited recruitment offers the potential to minimize participant selection bias and recruitment discrepancies. Nevertheless, subjectivity in severity stratification of participants remains an issue, which can be minimized by adopting universally accepted guidelines. To this aim, the revised Atlanta classification (Banks et

al., 2013), although imperfect, is a valuable tool that provides a simple, objective and replicable approach.

As shown by the findings of IMOFAP, expedited recruitment in AP studies is realistic and feasible, but is associated with additional challenges, that would not be present in routine clinical research. Our endeavour was expected to be resource intensive on many levels. Firstly, a full roster – consisting of more than 30 dedicated research nurses and support staff – was required to succeed in minimizing the time interval between patient presentation to hospital and recruitment. To this effect, a recruitment rota was formulated with an aim to screen patients, recruit participants, and obtain samples around the clock for 90 days. Furthermore, recruiting patients from the ED and critical care, as well as out-of-hours recruitment, is associated with considerably more challenges than usual elective participant enrolment in an outpatient or ward setting, and as such necessitates appropriate training of the research staff. For example, special 'lone working' training sessions were required for all research nurses participating in the IMOFAP study, and two preparatory lectures were held, apart from providing all the necessary paperwork and associated preparatory material. Arrangements for appropriate supervision and coordination of research staff were also made, particularly during out-of-hours recruitment, and the author acted as the point of contact to provide support when necessary.

Moreover, expedited recruitment requires appropriate infrastructure; sampling and sample handling equipment, centrifuges and processing equipment, freezers and storage facilities, should be available in close proximity and easily accessible at any time from the ED and critical care. Importantly, data collection in this acute setting poses another significant challenge, as data input may need to be performed ad-hoc at the bedside, either in the ED, critical care or the ward, without the luxury of time, particularly when time-point sampling is expected. For this reason, a bespoke data collection software application was built for IMOFAP, and it is recommended that this approach is considered in future studies.

Next, a close collaboration between research and clinical teams is particularly important. Although clinical care always comes first, it is imperative that clinical departments are research-aware to facilitate the actions of research staff without compromising the provision of clinical care. Apart from obtaining appropriate permissions from all senior clinical staff of the departments involved, as well as the medical director, much can be done by the investigator and research staff during an acute AP study to cultivate a good spirit of collaboration. Open discussion boards can be held prior to the commencement of the study thus providing an opportunity to clinical team members to ask questions, raise concerns and/or make practical suggestions. Information sheets can be distributed to ED and critical care staff, posters can be appended to key clinical areas, and frequent updates of the recruitment phase progress can be provided by means of email newsletters. It is also considered important to provide regular feedback during the course of the recruitment. These measures are particularly useful in busy centres which have the potential to offer a greater volume of participants, but have a heavy workload, with involvement of multiple teams and healthcare professionals.

Importantly, to achieve an expedited recruitment with a short time interval from presentation, the means for identification of potential participants should be disconnected from the duties of associated clinical teams, otherwise considerable delays may be caused in recruitment. Therefore, we devised a strategy of participant identification, which would be rapid, objective and automated. In IMOFAP, the biochemistry laboratory staff was trusted with this duty, alerting researchers for any new finding of elevate serum amylase, with an acceptable ratio of missed potential participants, rapid identification, and relative protection from selection bias. The choice of serum amylase as the identifying parameter appears to be the most attractive approach due to its widespread availability, low cost, and high diagnostic value.

Lastly, the fact that a significant proportion of patients with severe AP often lie in the spectrum of incapacity due to their critical illness, should alert research teams to prepare for recruiting patients with incapacity. This would

require appropriate ethical approval, information and consent form authoring, as well as staff training in this field. Ultimately, there is an argument about approaching acute clinical research in AP similar to research in other hyper-acute clinical conditions, such as stroke, trauma, or cardiac arrest, where rapid enrolment and sample acquisition is crucial and may necessitate a waiver of informed consent (Luce, 2003). As this remains a matter of debate, the currently established ethical principles should be adhered to, but further discussion is deemed necessary.

5.3 ACUTE PANCREATITIS IN CLINICAL RESEARCH OF INFLAMMATORY DISEASES

Systemic inflammation is almost invariably present in critical illness, regardless of the underlying cause (Wunsch et al., 2011). It is induced by infectious and non-infectious insults and is characterized by dysregulation of the innate immune system and overproduction of pro-inflammatory cytokines (Duffin et al., 2016). The complications that develop in the majority of critically ill patients are uniform, and existing evidence suggests that the activation of the pro-inflammatory cascade follows common routes (Wilson et al., 1998), while the inflammatory reaction is driven by common mediators and shared signalling pathways (Dulhunty et al., 2008; Fullerton et al., 2014; Xu et al., 2009; Zhang et al., 2010). Despite copious research on this field, our understanding of the precise underlying mechanisms remains incomplete (Duffin et al., 2016).

AP constitutes a sterile initiator of systemic inflammation that may result in multiple organ dysfunction, and although it is not yet fully understood how the initial insult to the pancreas escalates to systemic response, AP can be used as a clinical research model on inflammatory conditions. Patients with AP offer certain advantages over other patient groups on the study of SIRS and related conditions (Wilson et al., 1998). Firstly, SIRS is common in AP and is

frequently severe enough to precipitate organ failure (Winslet et al., 1992). In addition, a lot more is already known about the inflammatory cascade in AP, in comparison to existing knowledge for other inflammatory conditions. Conversely, many challenges may be associated with other conditions that result in systemic inflammation, such as sepsis, as patients of this group are often suffering from widely disparate pathology and severity can be even more difficult to be defined at presentation (Wilson et al., 1998).

In the foreseeable future, the application of clinical data from AP patients in the study of inflammation is expected to increase and play an important role. As an example, interleukin 22 data from participants of the IMOFAP study were used in the study by Duffin et al (Duffin et al., 2016), exploring the mechanistic involvement of prostaglandin E_2 in the suppression of systemic inflammation.

5.4 LUNG ULTRASONOGRAPHY IN ACUTE PANCREATITIS

Current composite severity scores appear to have reached their maximum efficacy, therefore novel approaches need to be introduced to expedite and further improve accuracy of disease severity stratification. The respiratory system is the organ system that most commonly fails in severe AP, secondary to accumulation of fluid between the alveolar membrane and the pulmonary capillaries, leading to reduced gas exchange and low partial arterial pressure of oxygen (Johnson, 2015). Hence, lung ultrasonography constitutes an attractive field of research in AP, since its application in monitoring critically ill patients is increasing. As shown in the pilot study presented in **Chapter 3**, LUSS can reliably detect evolving respiratory dysfunction in AP, and deserves further validation in a larger cohort.

LUSS has specific limitations. It provides less information than CT; visualisation of lung parenchyma may be difficult in obesity, and adequate

interpretation of sonographic findings requires formal training and related experience. However, it does not involve radiation exposure, intravenous contrast administration is not required, it does not require patient transportation from ITU (which in the case of patients with respiratory failure can be challenging), it is less costly, easily repeatable, and can be performed rapidly (Lichtenstein et al., 2004). Furthermore, the operator learning curve is estimated to be less than six weeks (Bouhemad et al., 2007). The hope is that this technique will progress further to a rapid, easily applicable test for patients at risk of developing AP-MODS, and may evolve into an important component of severity prediction in the future. Validation of this method in larger AP studies is deemed necessary (Skouras et al., 2016a).

5.5 SHIFTING THE RATIONALE OF ACUTE PANCREATITIS MANAGEMENT

Long-term survival following critical illness is reduced when compared to age- and gender-matched general populations (Williams et al., 2008). The finding that life expectancy of survivors may be substantially reduced after an episode of AP associated with early organ dysfunction constitutes a novel and significant reason for supporting change in the rationale of AP management. Based on these data, the existing follow-up policy for severe AP survivors may require to be re-examined, and long-term follow-up may be deemed appropriate for high risk patient subgroups. Additionally, survival until discharge should no longer be considered a complete success, as functional recovery and quality of life are increasingly becoming important patient-centred goals (Williams and Leslie, 2011). Therefore, care should focus not only on the management of established organ failure and the prevention of its deterioration, but should also aim to maximise quality of survival and post-discharge function of critically ill AP patients.

The InS:PIRE scheme (Intensive care Syndrome: Promoting Independence and Return to Employment), led by the NHS Greater Glasgow and Clyde Health Board in partnership with the University of Glasgow constitutes a paradigm of change in the post-discharge management of critically ill patients (Quasim and McPeake, 2016). This five-week peer-supported programme focuses on patient education and aims to facilitate and empower patients and relatives to improve their health and well-being following discharge from ITU. It entails interventions from a multidisciplinary team, including weekly physiotherapy sessions and individualised sessions with healthcare professionals, such as pharmacists, psychologists, and dieticians, amongst others. The preliminary effects of InS:PIRE are encouraging, and the scheme is being expanded to five further centres across four Health Boards in Scotland(Quasim and McPeake, 2016). Similar schemes have been adopted elsewhere (Lasiter et al., 2016; Modrykamien, 2012; Wood, 2015).

Nevertheless, despite the increasing acknowledgement that follow-up of survivors of critical illness is important, the ideal follow-up scheme has yet to be established (Williams and Leslie, 2011), and this field warrants investigation in future AP studies (Lasiter et al., 2016). Importantly, these findings offer further justification for the need to shift the focus of AP research towards prevention of organ dysfunction, in those patients where the opportunity still exists.

5.6 CONCLUSIONS

In human AP, plasma concentrations of kynurenine metabolites are altered proportionately to disease severity. Transthoracic ultrasonography shows promise in the detection of respiratory dysfunction in AP-MODS. Lastly, life expectancy of hospital survivors following an episode of AP-MODS is reduced.

References

Acevedo-Piedra, N.G., Moya-Hoyo, N., Rey-Riveiro, M., Gil, S., Sempere, L., Martinez, J., Lluis, F., Sanchez-Paya, J., and de-Madaria, E. (2014). Validation of the determinant-based classification and revision of the Atlanta classification systems for acute pancreatitis. Clin Gastroenterol Hepatol *12*, 311-316.

Adams Wilson, J.R., Morandi, A., Girard, T.D., Thompson, J.L., Boomershine, C.S., Shintani, A.K., Ely, E.W., and Pandharipande, P.P. (2012). The association of the kynurenine pathway of tryptophan metabolism with acute brain dysfunction during critical illness*. Crit Care Med *40*, 835-841.

Afghani, E., Pandol, S.J., Shimosegawa, T., Sutton, R., Wu, B.U., Vege, S.S., Gorelick, F., Hirota, M., Windsor, J., Lo, S.K.*, et al.* (2015). Acute Pancreatitis-Progress and Challenges: A Report on an International Symposium. Pancreas *44*, 1195-1210.

Aggarwal, A., Manrai, M., and Kochhar, R. (2014). Fluid resuscitation in acute pancreatitis. World J Gastroenterol *20*, 18092-18103.

Aitken, R.J., Nixon, S.J., and Ruckley, C.V. (1997). Lothian surgical audit: a 15-year experience of improvement in surgical practice through regional computerised audit. Lancet *350*, 800-804.

Ammann, R.W., and Muellhaupt, B. (1994). Progression of alcoholic acute to chronic pancreatitis. Gut *35*, 552-556.

Andersson, R., Andersson, B., Haraldsen, P., Drewsen, G., and Eckerwall, G. (2004). Incidence, management and recurrence rate of acute pancreatitis. Scand J Gastroenterol *39*, 891-894.

Andersson, R., and Andren-Sandberg, A. (2003). Fatal acute pancreatitis. Characteristics of patients never reaching hospital. Pancreatology *3*, 64-66.

Angus, D.C., Carlet, J., and Brussels Roundtable Participants (2003). Surviving intensive care: a report from the 2002 Brussels Roundtable. Intensive Care Med *29*, 368-377.

Appelros, S., and Borgstrom, A. (1999). Incidence, aetiology and mortality rate of acute pancreatitis over 10 years in a defined urban population in Sweden. Br J Surg *86*, 465-470.

Arvanitakis, M., Delhaye, M., De Maertelaere, V., Bali, M., Winant, C., Coppens, E., Jeanmart, J., Zalcman, M., Van Gansbeke, D., Deviere, J.*, et al.* (2004). Computed tomography and magnetic resonance imaging in the assessment of acute pancreatitis. Gastroenterology *126*, 715-723.

Backhaus, C., Rahman, H., Scheffler, S., Laatsch, H., and Hardeland, R. (2008). NO scavenging by 3-hydroxyanthranilic acid and 3-hydroxykynurenine: N-nitrosation leads via oxadiazoles to o-quinone diazides. Nitric Oxide *19*, 237-244.

Bakker, O.J., Issa, Y., van Santvoort, H.C., Besselink, M.G., Schepers, N.J., Bruno, M.J., Boermeester, M.A., and Gooszen, H.G. (2014). Treatment options for acute pancreatitis. Nat Rev Gastroenterol Hepatol *11*, 462-469.

Ball, H.J., McParland, B., Driussi, C., and Hunt, N.H. (2002). Isolating vessels from the mouse brain for gene expression analysis using laser capture microdissection. Brain Res Brain Res Protoc *9*, 206-213.

Ball, H.J., Sanchez-Perez, A., Weiser, S., Austin, C.J., Astelbauer, F., Miu, J., McQuillan, J.A., Stocker, R., Jermiin, L.S., and Hunt, N.H. (2007). Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. Gene *396*, 203-213.

Ball, H.J., Yuasa, H.J., Austin, C.J., Weiser, S., and Hunt, N.H. (2009). Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. Int J Biochem Cell Biol *41*, 467-471.

Balthazar, E.J. (2002). Acute pancreatitis: assessment of severity with clinical and CT evaluation. Radiology *223*, 603-613.

Balthazar, E.J., and Fisher, L.A. (2001). Hemorrhagic complications of pancreatitis: radiologic evaluation with emphasis on CT imaging. Pancreatology *1*, 306-313.

Balthazar, E.J., Freeny, P.C., and vanSonnenberg, E. (1994). Imaging and intervention in acute pancreatitis. Radiology *193*, 297-306.

Banks, P.A., Bollen, T.L., Dervenis, C., Gooszen, H.G., Johnson, C.D., Sarr, M.G., Tsiotos, G.G., Vege, S.S., and Acute Pancreatitis Classification Working Group (2013). Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut *62*, 102-111.

Banks, P.A., Freeman, M.L., and Practice Parameters Committee of the American College of, G. (2006). Practice guidelines in acute pancreatitis. Am J Gastroenterol *101*, 2379-2400.

Baran, H., Staniek, K., Kepplinger, B., Gille, L., Stolze, K., and Nohl, H. (2001). Kynurenic acid influences the respiratory parameters of rat heart mitochondria. Pharmacology *62*, 119-123.

Basran, G.S., Ramasubramanian, R., and Verma, R. (1987). Intrathoracic complications of acute pancreatitis. Br J Dis Chest *81*, 326-331.

Beaumont, V., Mrzljak, L., Dijkman, U., Freije, R., Heins, M., Rassoulpour, A., Tombaugh, G., Gelman, S., Bradaia, A., Steidl, E.*, et al.* (2016). The novel KMO inhibitor CHDI-340246 leads to a restoration of electrophysiological alterations in mouse models of Huntington's disease. Exp Neurol *282*, 99-118.

Beckmann, U., Gillies, D.M., Berenholtz, S.M., Wu, A.W., and Pronovost, P. (2004). Incidents relating to the intra-hospital transfer of critically ill patients. An analysis of the reports submitted to the Australian Incident Monitoring Study in Intensive Care. Intensive Care Med *30*, 1579-1585.

Behan, W.M., McDonald, M., Darlington, L.G., and Stone, T.W. (1999). Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl. Br J Pharmacol *128*, 1754-1760.

Bellac, C.L., Coimbra, R.S., Christen, S., and Leib, S.L. (2010). Inhibition of the kynurenine-NAD+ pathway leads to energy failure and exacerbates apoptosis in pneumococcal meningitis. J Neuropathol Exp Neurol *69*, 1096-1104.

Bhatia, M., Brady, M., Shokuhi, S., Christmas, S., Neoptolemos, J.P., and Slavin, J. (2000). Inflammatory mediators in acute pancreatitis. J Pathol *190*, 117-125.

Bi, Y., and Vege, S.S. (2015). Is There a Drug on the Horizon for Acute Pancreatitis? (In the American Gastroenterological Association. http://www.gastro.org/news_items/is-there-a-drug-on-the-horizon-for-acute-pancreatitis).

Blamey, S.L., Imrie, C.W., O'Neill, J., Gilmour, W.H., and Carter, D.C. (1984). Prognostic factors in acute pancreatitis. Gut *25*, 1340-1346.

Blasco, M.A. (2005). Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet *6*, 611-622.

Blum, T., Maisonneuve, P., Lowenfels, A.B., and Lankisch, P.G. (2001). Fatal outcome in acute pancreatitis: its occurrence and early prediction. Pancreatology *1*, 237-241.

Boasso, A., Herbeuval, J.P., Hardy, A.W., Anderson, S.A., Dolan, M.J., Fuchs, D., and Shearer, G.M. (2007). HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells. Blood *109*, 3351-3359.

Bollen, T.L., Singh, V.K., Maurer, R., Repas, K., van Es, H.W., Banks, P.A., and Mortele, K.J. (2012). A comparative evaluation of radiologic and clinical scoring systems in the early prediction of severity in acute pancreatitis. Am J Gastroenterol *107*, 612-619.

Bollen, T.L., van Santvoort, H.C., Besselink, M.G., van Es, W.H., Gooszen, H.G., and van Leeuwen, M.S. (2007). Update on acute pancreatitis: ultrasound, computed tomography, and magnetic resonance imaging features. Semin Ultrasound CT MR *28*, 371-383.

Bollen, T.L., van Santvoort, H.C., Besselink, M.G., van Leeuwen, M.S., Horvath, K.D., Freeny, P.C., Gooszen, H.G., and Dutch Acute Pancreatitis Study, G. (2008). The Atlanta Classification of acute pancreatitis revisited. Br J Surg *95*, 6-21.

Bordelon, Y.M., Chesselet, M.F., Nelson, D., Welsh, F., and Erecinska, M. (1997). Energetic dysfunction in quinolinic acid-lesioned rat striatum. J Neurochem *69*, 1629-1639.

Bossuyt, P.M., Reitsma, J.B., Bruns, D.E., Gatsonis, C.A., Glasziou, P.P., Irwig, L.M., Lijmer, J.G., Moher, D., Rennie, D., de Vet, H.C.*, et al.* (2003). Towards complete and accurate reporting of studies of diagnostic accuracy: The STARD Initiative. Radiology *226*, 24-28.

Bouhemad, B., Zhang, M., Lu, Q., and Rouby, J.J. (2007). Clinical review: Bedside lung ultrasound in critical care practice. Crit Care *11*, 205.

Bradley, E.L., 3rd (1993). A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg *128*, 586-590.

Brinkman, S., Bakhshi-Raiez, F., Abu-Hanna, A., de Jonge, E., and de Keizer, N.F. (2013). Determinants of mortality after hospital discharge in ICU patients: literature review and Dutch cohort study. Crit Care Med *41*, 1237-1251.

Buchler, M., Malfertheiner, P., Uhl, W., Scholmerich, J., Stockmann, F., Adler, G., Gaus, W., Rolle, K., and Beger, H.G. (1993). Gabexate mesilate in human acute pancreatitis. German Pancreatitis Study Group. Gastroenterology *104*, 1165-1170.

Bursac, Z., Gauss, C.H., Williams, D.K., and Hosmer, D.W. (2008). Purposeful selection of variables in logistic regression. Source Code Biol Med *3*, 17.

Buter, A., Imrie, C.W., Carter, C.R., Evans, S., and McKay, C.J. (2002). Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. Br J Surg *89*, 298-302.

Campesan, S., Green, E.W., Breda, C., Sathyasaikumar, K.V., Muchowski, P.J., Schwarcz, R., Kyriacou, C.P., and Giorgini, F. (2011). The kynurenine pathway modulates neurodegeneration in a Drosophila model of Huntington's disease. Current biology : CB *21*, 961-966.

Changsirivathanathamrong, D., Wang, Y., Rajbhandari, D., Maghzal, G.J., Mak, W.M., Woolfe, C., Duflou, J., Gebski, V., dos Remedios, C.G., Celermajer, D.S.*, et al.* (2011). Tryptophan metabolism to kynurenine is a potential novel contributor to hypotension in human sepsis. Crit Care Med *39*, 2678-2683.

Chauhan, S., and Forsmark, C.E. (2010). The difficulty in predicting outcome in acute pancreatitis. Am J Gastroenterol *105*, 443-445.

Chen, Y., and Guillemin, G.J. (2009). Kynurenine pathway metabolites in humans: disease and healthy States. Int J Tryptophan Res *2*, 1-19.

Chen, Y., Stankovic, R., Cullen, K.M., Meininger, V., Garner, B., Coggan, S., Grant, R., Brew, B.J., and Guillemin, G.J. (2010). The kynurenine pathway and inflammation in amyotrophic lateral sclerosis. Neurotox Res *18*, 132-142.

Chiarugi, A., Rovida, E., Dello Sbarba, P., and Moroni, F. (2003). Tryptophan availability selectively limits NO-synthase induction in macrophages. J Leukoc Biol *73*, 172-177.

Cho, J.H., Kim, T.N., Chung, H.H., and Kim, K.H. (2015). Comparison of scoring systems in predicting the severity of acute pancreatitis. World J Gastroenterol *21*, 2387-2394.

Choi, J.H., Kim, M.H., Oh, D., Paik, W.H., Park, D.H., Lee, S.S., Seo, D.W., and Lee, S.K. (2014). Clinical relevance of the revised Atlanta classification focusing on severity stratification system. Pancreatology *14*, 324-329.

Chooklin, S. (2009). Pathogenic aspects of pulmonary complications in acute pancreatitis patients. Hepatobiliary Pancreat Dis Int *8*, 186-192.

Clavien, P.A., Robert, J., Meyer, P., Borst, F., Hauser, H., Herrmann, F., Dunand, V., and Rohner, A. (1989). Acute pancreatitis and normoamylasemia. Not an uncommon combination. Ann Surg *210*, 614-620.

Cofield, S.S., Conwit, R., Barsan, W., and Quinn, J. (2010). Recruitment and retention of patients into emergency medicine clinical trials. Acad Emerg Med *17*, 1104-1112.

Connor, T.J., Starr, N., O'Sullivan, J.B., and Harkin, A. (2008). Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? Neurosci Lett *441*, 29-34.

Cozzi, A., Carpenedo, R., and Moroni, F. (1999). Kynurenine hydroxylase inhibitors reduce ischemic brain damage: studies with (m-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[-N-4-(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61-8048) in models of focal or global brain ischemia. J Cereb Blood Flow Metab *19*, 771-777.

Dabrowski, W., Kocki, T., Pilat, J., Parada-Turska, J., and Malbrain, M.L. (2014). Changes in plasma kynurenic acid concentration in septic shock patients undergoing continuous veno-venous haemofiltration. Inflammation *37*, 223-234.

Dai, W., and Gupta, S.L. (1990). Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-gamma. Upstream control region discriminates between interferon-gamma and interferon-alpha. J Biol Chem *265*, 19871-19877.

Darcy, C.J., Davis, J.S., Woodberry, T., McNeil, Y.R., Stephens, D.P., Yeo, T.W., and Anstey, N.M. (2011). An observational cohort study of the kynurenine to tryptophan ratio in sepsis: association with impaired immune and microvascular function. PLoS One *6*, e21185.

De Waele, J.J. (2007). Clinical research in acute pancreatitis and the failure to predict severe disease. Ann Surg *246*, 689; author reply 689-690.

Dean, N.C., Jones, J.P., Aronsky, D., Brown, S., Vines, C.G., Jones, B.E., and Allen, T. (2012). Hospital admission decision for patients with community-acquired pneumonia: variability among physicians in an emergency department. Ann Emerg Med *59*, 35-41.

Debas, H.T., Hancock, R.J., Soon-Shiong, P., Smythe, H.A., and Cassim, M.M. (1980). Glucagon therapy in acute pancreatitis: prospective randomized double-blind study. Can J Surg *23*, 578-580.

Dellinger, E.P., Forsmark, C.E., Layer, P., Levy, P., Maravi-Poma, E., Petrov, M.S., Shimosegawa, T., Siriwardena, A.K., Uomo, G., Whitcomb, D.C*., et al.* (2012). Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. Ann Surg *256*, 875-880.

DeLong, E.R., DeLong, D.M., and Clarke-Pearson, D.L. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics, 837-845.

Dragsted, L. (1991). Outcome from intensive care. A five year study of 1,308 patients. Dan Med Bull *38*, 365-374.

Duffin, R., O'Connor, R.A., Crittenden, S., Forster, T., Yu, C., Zheng, X., Smyth, D., Robb, C.T., Rossi, F., Skouras, C*., et al.* (2016). Prostaglandin E(2) constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. Science *351*, 1333-1338.

Dulhunty, J.M., Lipman, J., Finfer, S., and Sepsis Study Investigators for the Anzics Clinical Trials Group (2008). Does severe non-infectious SIRS differ from severe sepsis? Results from a multi-centre Australian and New Zealand intensive care unit study. Intensive Care Med *34*, 1654-1661.

Durr, H.K., Maroske, D., Zelder, O., and Bode, J.C. (1978). Glucagon therapy in acute pancreatitis. Report of a double-blind trial. Gut *19*, 175-179.

Eastman, C.L., and Guilarte, T.R. (1990). The role of hydrogen peroxide in the in vitro cytotoxicity of 3-hydroxykynurenine. Neurochem Res *15*, 1101-1107.

Ebbehoj, N., Friis, J., Svendsen, L.B., Bulow, S., and Madsen, P. (1985). Indomethacin treatment of acute pancreatitis. A controlled double-blind trial. Scand J Gastroenterol *20*, 798-800.

Eckerwall, G.E., Axelsson, J.B., and Andersson, R.G. (2006). Early nasogastric feeding in predicted severe acute pancreatitis: A clinical, randomized study. Ann Surg *244*, 959-965; discussion 965-957.

Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M.C., and Puccetti, P. (2002). T cell apoptosis by tryptophan catabolism. Cell Death Differ *9*, 1069-1077.

Fallarino, F., Grohmann, U., You, S., McGrath, B.C., Cavener, D.R., Vacca, C., Orabona, C., Bianchi, R., Belladonna, M.L., Volpi, C*., et al.* (2006). The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J Immunol *176*, 6752-6761.

Faul, F., Erdfelder, E., Lang, A.G., and Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods *39*, 175-191.

Favre, D., Mold, J., Hunt, P.W., Kanwar, B., Loke, P., Seu, L., Barbour, J.D., Lowe, M.M., Jayawardene, A., Aweeka, F*., et al.* (2010). Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. Sci Transl Med *2*, 32ra36.

Fenton-Lee, D., and Imrie, C.W. (1993). Pancreatic necrosis: assessment of outcome related to quality of life and cost of management. Br J Surg *80*, 1579-1582.

Ferguson, N.D., Fan, E., Camporota, L., Antonelli, M., Anzueto, A., Beale, R., Brochard, L., Brower, R., Esteban, A., Gattinoni, L*., et al.* (2012). The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. Intensive Care Med *38*, 1573-1582.

Fernstrom, J.D., Wurtman, R.J., Hammarstrom-Wiklund, B., Rand, W.M., Munro, H.N., and Davidson, C.S. (1979). Diurnal variations in plasma concentrations of tryptophan, tryosine, and other neutral amino acids: effect of dietary protein intake. Am J Clin Nutr *32*, 1912-1922.

Forrest, C.M., Mackay, G.M., Oxford, L., Millar, K., Darlington, L.G., Higgins, M.J., and Stone, T.W. (2011). Kynurenine metabolism predicts cognitive function in patients following cardiac bypass and thoracic surgery. J Neurochem *119*, 136-152.

Forsmark, C.E., Baillie, J., AGA Institute Clinical Practice and Economics Committee, and AGA Institute Governing Board (2007). AGA Institute technical review on acute pancreatitis. Gastroenterology *132*, 2022-2044.

Foster, A.C., Miller, L.P., Oldendorf, W.H., and Schwarcz, R. (1984a). Studies on the disposition of quinolinic acid after intracerebral or systemic administration in the rat. Exp Neurol *84*, 428-440.

Foster, A.C., Vezzani, A., French, E.D., and Schwarcz, R. (1984b). Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett *48*, 273-278.

Frumento, G., Rotondo, R., Tonetti, M., Damonte, G., Benatti, U., and Ferrara, G.B. (2002). Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med *196*, 459-468.

Fullerton, J.N., O'Brien, A.J., and Gilroy, D.W. (2014). Lipid mediators in immune dysfunction after severe inflammation. Trends Immunol *35*, 12-21.

Gallagher, M., Cass, A., Bellomo, R., Finfer, S., Gattas, D., Lee, J., Lo, S., McGuinness, S., Myburgh, J., Parke, R.*, et al.* (2014). Long-term survival and dialysis dependency following acute kidney injury in intensive care: extended follow-up of a randomized controlled trial. PLoS Med *11*, e1001601.

Gardelli, G., Feletti, F., Nanni, A., Mughetti, M., Piraccini, A., and Zompatori, M. (2012). Chest ultrasonography in the ICU. Respir Care *57*, 773-781.

Giles, G.I., Collins, C.A., Stone, T.W., and Jacob, C. (2003). Electrochemical and in vitro evaluation of the redox-properties of kynurenine species. Biochem Biophys Res Commun *300*, 719-724.

Giorgini, F., Guidetti, P., Nguyen, Q., Bennett, S.C., and Muchowski, P.J. (2005). A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. Nat Genet *37*, 526-531.

Girgin, G., Sahin, T.T., Fuchs, D., Yuksel, O., Kurukahvecioglu, O., Sare, M., and Baydar, T. (2011). Tryptophan degradation and serum neopterin concentrations in intensive care unit patients. Toxicol Mech Methods *21*, 231-235.

Goldacre, M.J., and Roberts, S.E. (2004). Hospital admission for acute pancreatitis in an English population, 1963-98: database study of incidence and mortality. BMJ *328*, 1466-1469.

Goldstein, L.E., Leopold, M.C., Huang, X., Atwood, C.S., Saunders, A.J., Hartshorn, M., Lim, J.T., Faget, K.Y., Muffat, J.A., Scarpa, R.C.*, et al.* (2000). 3-Hydroxykynurenine and 3-hydroxyanthranilic acid generate hydrogen

peroxide and promote alpha-crystallin cross-linking by metal ion reduction. Biochemistry *39*, 7266-7275.

Gravante, G., Garcea, G., Ong, S.L., Metcalfe, M.S., Berry, D.P., Lloyd, D.M., and Dennison, A.R. (2009). Prediction of mortality in acute pancreatitis: a systematic review of the published evidence. Pancreatology *9*, 601-614.

Gregoire, L., Rassoulpour, A., Guidetti, P., Samadi, P., Bedard, P.J., Izzo, E., Schwarcz, R., and Di Paolo, T. (2008). Prolonged kynurenine 3-hydroxylase inhibition reduces development of levodopa-induced dyskinesias in parkinsonian monkeys. Behav Brain Res *186*, 161-167.

Grohmann, U., Fallarino, F., and Puccetti, P. (2003). Tolerance, DCs and tryptophan: much ado about IDO. Trends Immunol *24*, 242-248.

Grohmann, U., Orabona, C., Fallarino, F., Vacca, C., Calcinaro, F., Falorni, A., Candeloro, P., Belladonna, M.L., Bianchi, R., Fioretti, M.C*., et al.* (2002). CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol *3*, 1097-1101.

Gudipaty, L., and Rickels, M.R. (2015). Pancreatogenic (Type 3c) Diabetes (The Pancreapedia: Exocrine Pancreas Knowledge Base: American Pancreatic Association).

Guidetti, P., Amori, L., Sapko, M.T., Okuno, E., and Schwarcz, R. (2007). Mitochondrial aspartate aminotransferase: a third kynurenate-producing enzyme in the mammalian brain. J Neurochem *102*, 103-111.

Guidetti, P., Luthi-Carter, R.E., Augood, S.J., and Schwarcz, R. (2004). Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. Neurobiol Dis *17*, 455-461.

Gulaj, E., Pawlak, K., Bien, B., and Pawlak, D. (2010). Kynurenine and its metabolites in Alzheimer's disease patients. Adv Med Sci *55*, 204-211.

Halonen, K.I., Pettila, V., Leppaniemi, A.K., Kemppainen, E.A., Puolakkainen, P.A., and Haapiainen, R.K. (2003). Long-term health-related quality of life in survivors of severe acute pancreatitis. Intensive Care Med *29*, 782-786.

Halvorsen, F.A., and Ritland, S. (1996). Acute pancreatitis in Buskerud County, Norway. Incidence and etiology. Scand J Gastroenterol *31*, 411-414.

Hansen, A.M., Driussi, C., Turner, V., Takikawa, O., and Hunt, N.H. (2000). Tissue distribution of indoleamine 2,3-dioxygenase in normal and malaria-infected tissue. Redox Rep *5*, 112-115.

Harada, H., Miyake, H., Ochi, K., Tanaka, J., and Kimura, I. (1991). Clinical trial with a protease inhibitor gabexate mesilate in acute pancreatitis. Int J Pancreatol *9*, 75-79.

Harrison, D.A., D'Amico, G., and Singer, M. (2007). The Pancreatitis Outcome Prediction (POP) Score: a new prognostic index for patients with severe acute pancreatitis. Crit Care Med *35*, 1703-1708.

Hayaishi, O., and Yoshida, R. (1978). Specific induction of pulmonary indoleamine 2,3-dioxygenase by bacterial lipopolysaccharide. Ciba Found Symp, 199-203.

Heinze, G. (2008). Medical Biostatistics 2 (Medical University of Vienna).

Heyes, M.P., Saito, K., and Markey, S.P. (1992). Human macrophages convert L-tryptophan into the neurotoxin quinolinic acid. Biochem J *283 (Pt 3)*, 633-635.

Hosmer, J.D., Lemeshow, S., and May, S. (2008.). Applied survival analysis: regression modeling of time to event data (New York: Wiley).

Hunninghake, D.B., Darby, C.A., and Probstfield, J.L. (1987). Recruitment experience in clinical trials: literature summary and annotated bibliography. Control Clin Trials *8*, 6S-30S.

Huttunen, R., Syrjanen, J., Aittoniemi, J., Oja, S.S., Raitala, A., Laine, J., Pertovaara, M., Vuento, R., Huhtala, H., and Hurme, M. (2010). High activity of indoleamine 2,3 dioxygenase enzyme predicts disease severity and case fatality in bacteremic patients. Shock *33*, 149-154.

Imrie, C.W., Benjamin, I.S., Ferguson, J.C., McKay, A.J., Mackenzie, I., O'Neill, J., and Blumgart, L.H. (1978). A single-centre double-blind trial of Trasylol therapy in primary acute pancreatitis. Br J Surg *65*, 337-341.

Isenmann, R., Runzi, M., Kron, M., Kahl, S., Kraus, D., Jung, N., Maier, L., Malfertheiner, P., Goebell, H., Beger, H.G.*, et al.* (2004). Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo-controlled, double-blind trial. Gastroenterology *126*, 997-1004.

Johnson, C.D. (2015). Organ Failure and Acute Pancreatitis. In Prediction and Management of Severe Acute Pancreatitis, C.E. Forsmark, and T.B. Gardner, eds. (New York: Springer Science+Business Media), pp. 15-28.

Johnson, C.D., and Abu-Hilal, M. (2004). Persistent organ failure during the first week as a marker of fatal outcome in acute pancreatitis. Gut *53*, 1340-1344.

Johnson, C.D., Kingsnorth, A.N., Imrie, C.W., McMahon, M.J., Neoptolemos, J.P., McKay, C., Toh, S.K., Skaife, P., Leeder, P.C., Wilson, P.*, et al.* (2001). Double blind, randomised, placebo controlled study of a platelet activating factor antagonist, lexipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis. Gut *48*, 62-69.

Johnson, R., Kuczawski, M., and Mason, S. (2016). Why is it so difficult to recruit patients to research in emergency care? Lessons from the AHEAD study. Emerg Med J *33*, 52-56.

Kalra, M.K., Maher, M.M., Toth, T.L., Hamberg, L.M., Blake, M.A., Shepard, J.A., and Saini, S. (2004). Strategies for CT radiation dose optimization. Radiology *230*, 619-628.

Kaplan, V., and Angus, D.C. (2002). Surviving intensive care. Crit Care Med *30*, 703-705.

Keenan, S.P., Dodek, P., Chan, K., Hogg, R.S., Craib, K.J., Anis, A.H., and Spinelli, J.J. (2002). Intensive care unit admission has minimal impact on long-term mortality. Crit Care Med *30*, 501-507.

Kendrick, D., Lyons, R., Christie, N., Towner, E., Benger, J., Groom, L., Coffey, F., Miller, P., Murphy, R., and UK Burden of Injury Study Group (2007). Recruiting participants for injury studies in emergency departments. Inj Prev *13*, 75-77.

Kingsnorth, A.N., Galloway, S.W., and Formela, L.J. (1995). Randomized, double-blind phase II trial of Lexipafant, a platelet-activating factor antagonist, in human acute pancreatitis. Br J Surg *82*, 1414-1420.

Knaus, W.A., Draper, E.A., Wagner, D.P., and Zimmerman, J.E. (1985). APACHE II: a severity of disease classification system. Crit Care Med *13*, 818-829.

Kolodziej, L.R., Paleolog, E.M., and Williams, R.O. (2011). Kynurenine metabolism in health and disease. Amino Acids *41*, 1173-1183.

Kong, C.M., Lee, X.W., and Wang, X. (2013). Telomere shortening in human diseases. FEBS J *280*, 3180-3193.

Kurnasov, O., Goral, V., Colabroy, K., Gerdes, S., Anantha, S., Osterman, A., and Begley, T.P. (2003). NAD biosynthesis: identification of the tryptophan to quinolinate pathway in bacteria. Chem Biol *10*, 1195-1204.

Kylanpaa, L., Rakonczay, Z., Jr., and O'Reilly, D.A. (2012). The clinical course of acute pancreatitis and the inflammatory mediators that drive it. Int J Inflam *2012*, 360685.

Lankisch, P.G., Apte, M., and Banks, P.A. (2015). Acute pancreatitis. Lancet *386*, 85-96.

Lankisch, P.G., Breuer, N., Bruns, A., Weber-Dany, B., Lowenfels, A.B., and Maisonneuve, P. (2009). Natural history of acute pancreatitis: a long-term population-based study. Am J Gastroenterol *104*, 2797-2805; quiz 2806.

Lankisch, P.G., Burchard-Reckert, S., Petersen, M., Lehnick, D., Schirren, C.A., Stockmann, F., and Kohler, H. (1996). Etiology and age have only a limited influence on the course of acute pancreatitis. Pancreas *13*, 344-349.

Lasiter, S., Oles, S.K., Mundell, J., London, S., and Khan, B. (2016). Critical Care Follow-up Clinics: A Scoping Review of Interventions and Outcomes. Clin Nurse Spec *30*, 227-237.

Lerch, M.M. (2013). Classifying an unpredictable disease: the revised Atlanta classification of acute pancreatitis. Gut *62*, 2-3.

Lewitt, P.A., Li, J., Lu, M., Beach, T.G., Adler, C.H., Guo, L., and Arizona Parkinson's Disease, C. (2013). 3-hydroxykynurenine and other Parkinson's disease biomarkers discovered by metabolomic analysis. Mov Disord *28*, 1653-1660.

Lichtenstein, D., Goldstein, I., Mourgeon, E., Cluzel, P., Grenier, P., and Rouby, J.J. (2004). Comparative diagnostic performances of auscultation, chest radiography, and lung ultrasonography in acute respiratory distress syndrome. Anesthesiology *100*, 9-15.

Lichtenstein, D., Meziere, G., Biderman, P., Gepner, A., and Barre, O. (1997). The comet-tail artifact. An ultrasound sign of alveolar-interstitial syndrome. Am J Respir Crit Care Med *156*, 1640-1646.

Logters, T.T., Laryea, M.D., Altrichter, J., Sokolowski, J., Cinatl, J., Reipen, J., Linhart, W., Windolf, J., Scholz, M., and Wild, M. (2009). Increased plasma kynurenine values and kynurenine-tryptophan ratios after major trauma are early indicators for the development of sepsis. Shock *32*, 29-34.

Lone, N.I., and Walsh, T.S. (2012). Impact of intensive care unit organ failures on mortality during the five years after a critical illness. Am J Respir Crit Care Med *186*, 640-647.

Lowe, D.M., Gee, M., Haslam, C., Leavens, B., Christodoulou, E., Hissey, P., Hardwicke, P., Argyrou, A., Webster, S.P., Mole, D.J*., et al.* (2014). Lead discovery for human kynurenine 3-monooxygenase by high-throughput RapidFire mass spectrometry. J Biomol Screen *19*, 508-515.

Luce, J.M. (2003). Is the concept of informed consent applicable to clinical research involving critically ill patients? Crit Care Med *31*, S153-160.

Lund, H., Tonnesen, H., Tonnesen, M.H., and Olsen, O. (2006). Long-term recurrence and death rates after acute pancreatitis. Scand J Gastroenterol *41*, 234-238.

Maddison, D.C., and Giorgini, F. (2015). The kynurenine pathway and neurodegenerative disease. Semin Cell Dev Biol *40*, 134-141.

Majláth, Z., Toldi, J., and Vécsei, L. (2015). The Role of the Kynurenine Pathway in Neurodegenerative Diseases. In Targeting the Broadly Pathogenic Kynurenine Pathway, S. Mittal, ed. (Switzerland 2015: Springer International Publishing), pp. 193-204.

Marshall, J.C., Cook, D.J., Christou, N.V., Bernard, G.R., Sprung, C.L., and Sibbald, W.J. (1995). Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. Crit Care Med *23*, 1638-1652.

Matthews, J.N., Altman, D.G., Campbell, M.J., and Royston, P. (1990). Analysis of serial measurements in medical research. BMJ *300*, 230-235.

Mayer, A.D., McMahon, M.J., Bowen, M., and Cooper, E.H. (1984). C reactive protein: an aid to assessment and monitoring of acute pancreatitis. J Clin Pathol *37*, 207-211.

McKay, C.J., and Buter, A. (2003). Natural history of organ failure in acute pancreatitis. Pancreatology *3*, 111-114.

McKay, C.J., and Carter, C.R. (2012). The pancreas and spleen. In Principles and Practice of Surgery, O.J. Garden, A.W. Bradbury, J.L.R. Forsythe, and R.W. Parks, eds. (Edinburgh: Churchill Livingstone Elsevier), pp. 215-232.

McKay, C.J., Curran, F., Sharples, C., Baxter, J.N., and Imrie, C.W. (1997). Prospective placebo-controlled randomized trial of lexipafant in predicted severe acute pancreatitis. Br J Surg *84*, 1239-1243.

McKay, C.J., Dickson, E.J., and Carter, C.R. (2014). Acute pancreatitis. In Hepatobiliary and Pancreatic Surgery, O.J. Garden, and R.W. Parks, eds. (Edinburgh: Saunders Elsevier), pp. 244-259.

McKay, C.J., Evans, S., Sinclair, M., Carter, C.R., and Imrie, C.W. (1999). High early mortality rate from acute pancreatitis in Scotland, 1984-1995. Br J Surg *86*, 1302-1305.

McMenamy, R.H. (1965). Binding of indole analogues to human serum albumin. Effects of fatty acids. J Biol Chem *240*, 4235-4243.

Mehler, A.H., and Knox, W.E. (1950). The conversion of tryptophan to kynurenine in liver. II. The enzymatic hydrolysis of formylkynurenine. J Biol Chem *187*, 431-438.

Mezrich, J.D., Fechner, J.H., Zhang, X., Johnson, B.P., Burlingham, W.J., and Bradfield, C.A. (2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol *185*, 3190-3198.

Mitchell, R.M., Byrne, M.F., and Baillie, J. (2003). Pancreatitis. Lancet *361*, 1447-1455.

Modrykamien, A.M. (2012). The ICU follow-up clinic: a new paradigm for intensivists. Respir Care *57*, 764-772.

Moffett, J.R., and Namboodiri, M.A. (2003). Tryptophan and the immune response. Immunol Cell Biol *81*, 247-265.

Mofidi, R., Duff, M.D., Madhavan, K.K., Garden, O.J., and Parks, R.W. (2007a). Identification of severe acute pancreatitis using an artificial neural network. Surgery *141*, 59-66.

Mofidi, R., Duff, M.D., Wigmore, S.J., Madhavan, K.K., Garden, O.J., and Parks, R.W. (2006). Association between early systemic inflammatory

response, severity of multiorgan dysfunction and death in acute pancreatitis. Br J Surg *93*, 738-744.

Mofidi, R., Madhavan, K.K., Garden, O.J., and Parks, R.W. (2007b). An audit of the management of patients with acute pancreatitis against national standards of practice. Br J Surg *94*, 844-848.

Molano, A., Illarionov, P.A., Besra, G.S., Putterman, C., and Porcelli, S.A. (2008). Modulation of invariant natural killer T cell cytokine responses by indoleamine 2,3-dioxygenase. Immunol Lett *117*, 81-90.

Mole, D.J., Gungabissoon, U., Johnston, P., Cochrane, L., Hopkins, L., Wyper, G.M., Skouras, C., Dibben, C., Sullivan, F., Morris, A.*, et al.* (2016a). Identifying risk factors for progression to critical care admission and death among individuals with acute pancreatitis: a record linkage analysis of Scottish healthcare databases. BMJ Open *6*, e011474.

Mole, D.J., McFerran, N.V., Collett, G., O'Neill, C., Diamond, T., Garden, O.J., Kylanpaa, L., Repo, H., and Deitch, E.A. (2008). Tryptophan catabolites in mesenteric lymph may contribute to pancreatitis-associated organ failure. Br J Surg *95*, 855-867.

Mole, D.J., Olabi, B., Robinson, V., Garden, O.J., and Parks, R.W. (2009). Incidence of individual organ dysfunction in fatal acute pancreatitis: analysis of 1024 death records. HPB : the official journal of the International Hepato Pancreato Biliary Association *11*, 166-170.

Mole, D.J., Webster, S.P., Uings, I., Zheng, X., Binnie, M., Wilson, K., Hutchinson, J.P., Mirguet, O., Walker, A., Beaufils, B.*, et al.* (2016b). Kynurenine-3-monooxygenase inhibition prevents multiple organ failure in rodent models of acute pancreatitis. Nat Med *22*, 202-209.

Moroni, F., Cozzi, A., Sili, M., and Mannaioni, G. (2012). Kynurenic acid: a metabolite with multiple actions and multiple targets in brain and periphery. J Neural Transm (Vienna) *119*, 133-139.

Mounzer, R., Langmead, C.J., Wu, B.U., Evans, A.C., Bishehsari, F., Muddana, V., Singh, V.K., Slivka, A., Whitcomb, D.C., Yadav, D.*, et al.* (2012). Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. Gastroenterology *142*, 1476-1482; quiz e1415-1476.

Muddana, V., Whitcomb, D.C., Khalid, A., Slivka, A., and Papachristou, G.I. (2009). Elevated serum creatinine as a marker of pancreatic necrosis in acute pancreatitis. Am J Gastroenterol *104*, 164-170.

Munn, D.H., Shafizadeh, E., Attwood, J.T., Bondarev, I., Pashine, A., and Mellor, A.L. (1999). Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med *189*, 1363-1372.

Munn, D.H., Sharma, M.D., Lee, J.R., Jhaver, K.G., Johnson, T.S., Keskin, D.B., Marshall, B., Chandler, P., Antonia, S.J., Burgess, R.*, et al.* (2002). Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science *297*, 1867-1870.

Murakami, Y., and Saito, K. (2013). Species and cell types difference in tryptophan metabolism. Int J Tryptophan Res *6*, 47-54.

Neoptolemos, J.P., Raraty, M., Finch, M., and Sutton, R. (1998). Acute pancreatitis: the substantial human and financial costs. Gut *42*, 886-891.

Nguyen, N.T., Kimura, A., Nakahama, T., Chinen, I., Masuda, K., Nohara, K., Fujii-Kuriyama, Y., and Kishimoto, T. (2010). Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. Proc Natl Acad Sci U S A *107*, 19961-19966.

Niskanen, M., Kari, A., and Halonen, P. (1996). Five-year survival after intensive care--comparison of 12,180 patients with the general population. Finnish ICU Study Group. Crit Care Med *24*, 1962-1967.

Nøjgaard, C. (2010). Prognosis of acute and chronic pancreatitis - a 30-year follow-up of a Danish cohort. Dan Med Bull *57*, B4228.

Nojgaard, C., Becker, U., Matzen, P., Andersen, J.R., Holst, C., and Bendtsen, F. (2011). Progression from acute to chronic pancreatitis: prognostic factors, mortality, and natural course. Pancreas *40*, 1195-1200.

Nøjgaard, C., Matzen, P., Bendtsen, F., Andersen, J.R., Christensen, E., and Becker, U. (2011). Factors associated with long-term mortality in acute pancreatitis. Scand J Gastroenterol *46*, 495-502.

Ogawa, T., Matson, W.R., Beal, M.F., Myers, R.H., Bird, E.D., Milbury, P., and Saso, S. (1992). Kynurenine pathway abnormalities in Parkinson's disease. Neurology *42*, 1702-1706.

Okuda, S., Nishiyama, N., Saito, H., and Katsuki, H. (1998). 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity. J Neurochem *70*, 299-307.

Oxenkrug, G.F. (2007). Genetic and hormonal regulation of tryptophan kynurenine metabolism: implications for vascular cognitive impairment, major depressive disorder, and aging. Ann N Y Acad Sci *1122*, 35-49.

Pandharipande, P.P., Morandi, A., Adams, J.R., Girard, T.D., Thompson, J.L., Shintani, A.K., and Ely, E.W. (2009a). Plasma tryptophan and tyrosine levels are independent risk factors for delirium in critically ill patients. Intensive Care Med *35*, 1886-1892.

Pandharipande, P.P., Shintani, A.K., Hagerman, H.E., St Jacques, P.J., Rice, T.W., Sanders, N.W., Ware, L.B., Bernard, G.R., and Ely, E.W. (2009b). Derivation and validation of Spo2/Fio2 ratio to impute for Pao2/Fio2 ratio in the respiratory component of the Sequential Organ Failure Assessment score. Crit Care Med *37*, 1317-1321.

Papachristou, G.I., Muddana, V., Yadav, D., O'Connell, M., Sanders, M.K., Slivka, A., and Whitcomb, D.C. (2010). Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. Am J Gastroenterol *105*, 435-441; quiz 442.

Park, W.G. (2015). Pharmacologic therapy. In Prediction and Management of Severe Acute Pancreatitis, C.E. Forsmark, and T.B. Gardner, eds. (New York: Springer Science+Business Media), pp. 133-147.

Pastor, C.M., Matthay, M.A., and Frossard, J.L. (2003). Pancreatitis-associated acute lung injury: new insights. Chest *124*, 2341-2351.

Pearson, S.J., and Reynolds, G.P. (1992). Increased brain concentrations of a neurotoxin, 3-hydroxykynurenine, in Huntington's disease. Neurosci Lett *144*, 199-201.

Pedersen, E.R., Svingen, G.F., Schartum-Hansen, H., Ueland, P.M., Ebbing, M., Nordrehaug, J.E., Igland, J., Seifert, R., Nilsen, R.M., and Nygard, O. (2013). Urinary excretion of kynurenine and tryptophan, cardiovascular events, and mortality after elective coronary angiography. Eur Heart J *34*, 2689-2696.

Pellegrin, K., Neurauter, G., Wirleitner, B., Fleming, A.W., Peterson, V.M., and Fuchs, D. (2005). Enhanced enzymatic degradation of tryptophan by

indoleamine 2,3-dioxygenase contributes to the tryptophan-deficient state seen after major trauma. Shock *23*, 209-215.

Pezzilli, R., and Miglioli, M. (2001). Multicentre comparative study of two schedules of gabexate mesilate in the treatment of acute pancreatitis. Italian Acute Pancreatitis Study Group. Dig Liver Dis *33*, 49-57.

Piascik, M., Rydzewska, G., Milewski, J., Olszewski, S., Furmanek, M., Walecki, J., and Gabryelewicz, A. (2010). The results of severe acute pancreatitis treatment with continuous regional arterial infusion of protease inhibitor and antibiotic: a randomized controlled study. Pancreas *39*, 863-867.

Ploder, M., Spittler, A., Kurz, K., Neurauter, G., Pelinka, L.E., Roth, E., and Fuchs, D. (2010). Accelerated tryptophan degradation predicts poor survival in trauma and sepsis patients. Int J Tryptophan Res *3*, 61-67.

Poesen, R., Mutsaers, H.A., Windey, K., van den Broek, P.H., Verweij, V., Augustijns, P., Kuypers, D., Jansen, J., Evenepoel, P., Verbeke, K.*, et al.* (2015). The Influence of Dietary Protein Intake on Mammalian Tryptophan and Phenolic Metabolites. PLoS One *10*, e0140820.

Polyzogopoulou, E., Bikas, C., Danikas, D., Koutras, A., Kalfarentzos, F., and Gogos, C.A. (2004). Baseline hypoxemia as a prognostic marker for pulmonary complications and outcome in patients with acute pancreatitis. Dig Dis Sci *49*, 150-154.

Puolakkainen, P., Valtonen, V., Paananen, A., and Schroder, T. (1987). C-reactive protein (CRP) and serum phospholipase A2 in the assessment of the severity of acute pancreatitis. Gut *28*, 764-771.

Quasim, T., and McPeake, J. (2016). Intensive care Syndrome: Promoting Independence and Return to Employment (InS:PIRE). ICU Management & Practice *16*, 178-180.

Raghu, M.G., Wig, J.D., Kochhar, R., Gupta, D., Gupta, R., Yadav, T.D., Agarwal, R., Kudari, A.K., Doley, R.P., and Javed, A. (2007). Lung complications in acute pancreatitis. JOP *8*, 177-185.

Ranson, J.H., Rifkind, K.M., Roses, D.F., Fink, S.D., Eng, K., and Spencer, F.C. (1974a). Prognostic signs and the role of operative management in acute pancreatitis. Surg Gynecol Obstet *139*, 69-81.

Ranson, J.H., Roses, D.F., and Fink, S.D. (1973). Early respiratory insufficiency in acute pancreatitis. Ann Surg *178*, 75-79.

Ranson, J.H., Turner, J.W., Roses, D.F., Rifkind, K.M., and Spencer, F.C. (1974b). Respiratory complications in acute pancreatitis. Ann Surg *179*, 557-566.

Ranzani, O.T., Zampieri, F.G., Besen, B.A., Azevedo, L.C., and Park, M. (2015). One-year survival and resource use after critical illness: impact of organ failure and residual organ dysfunction in a cohort study in Brazil. Crit Care *19*, 269.

Renner, I.G., Savage, W.T., Pantoja, J.L., and Renner, V.J. (1985). Death due to acute pancreatitis. A retrospective analysis of 405 autopsy cases. Dig Dis Sci *30*, 1005-1018.

Richard, D.M., Dawes, M.A., Mathias, C.W., Acheson, A., Hill-Kapturczak, N., and Dougherty, D.M. (2009). L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications. Int J Tryptophan Res *2*, 45-60.

Ridley, S., Jackson, R., Findlay, J., and Wallace, P. (1990). Long term survival after intensive care. BMJ *301*, 1127-1130.

Ristagno, G., Latini, R., Vaahersalo, J., Masson, S., Kurola, J., Varpula, T., Lucchetti, J., Fracasso, C., Guiso, G., Montanelli, A.*, et al.* (2014). Early activation of the kynurenine pathway predicts early death and long-term outcome in patients resuscitated from out-of-hospital cardiac arrest. J Am Heart Assoc *3*.

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.-C., and Müller, M. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC bioinformatics *12*, 77.

Sainio, E.-L., Pulkki, K., and Young, S. (1996). L-Tryptophan: Biochemical, nutritional and pharmacological aspects. Amino Acids *10*, 21-47.

Salter, M., and Pogson, C.I. (1985). The role of tryptophan 2,3-dioxygenase in the hormonal control of tryptophan metabolism in isolated rat liver cells. Effects of glucocorticoids and experimental diabetes. Biochem J *229*, 499-504.

Samelson-Jones, B.J., and Yeh, S.R. (2006). Interactions between nitric oxide and indoleamine 2,3-dioxygenase. Biochemistry *45*, 8527-8538.

Santamaria, A., Jimenez-Capdeville, M.E., Camacho, A., Rodriguez-Martinez, E., Flores, A., and Galvan-Arzate, S. (2001). In vivo hydroxyl radical formation

after quinolinic acid infusion into rat corpus striatum. Neuroreport *12*, 2693-2696.

Schoenfeld, D. (1982). Partial residuals for the proportional hazards regression model. Biometrika *69*, 239-241.

Schwarcz, R. (2004). The kynurenine pathway of tryptophan degradation as a drug target. Curr Opin Pharmacol *4*, 12-17.

Schwarcz, R., Bruno, J.P., Muchowski, P.J., and Wu, H.Q. (2012). Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci *13*, 465-477.

Scotland (2000). Adults with Incapacity (Scotland) Act 2000--excerpt. Bull Med Ethics, 8-11.

Sekkai, D., Guittet, O., Lemaire, G., Tenu, J.P., and Lepoivre, M. (1997). Inhibition of nitric oxide synthase expression and activity in macrophages by 3-hydroxyanthranilic acid, a tryptophan metabolite. Arch Biochem Biophys *340*, 117-123.

Serpa Neto, A., Cardoso, S.O., Ong, D.S., Esposito, D.C., Pereira, V.G., Manetta, J.A., Slooter, A.J., and Cremer, O.L. (2013). The use of the pulse oximetric saturation/fraction of inspired oxygen ratio for risk stratification of patients with severe sepsis and septic shock. J Crit Care *28*, 681-686.

Severinghaus, J.W. (1979). Simple, accurate equations for human blood O2 dissociation computations. J Appl Physiol Respir Environ Exerc Physiol *46*, 599-602.

Shields, C.J., Winter, D.C., and Redmond, H.P. (2002). Lung injury in acute pancreatitis: mechanisms, prevention, and therapy. Curr Opin Crit Care *8*, 158-163.

Singh, V.K., Wu, B.U., Bollen, T.L., Repas, K., Maurer, R., Johannes, R.S., Mortele, K.J., Conwell, D.L., and Banks, P.A. (2009). A prospective evaluation of the bedside index for severity in acute pancreatitis score in assessing mortality and intermediate markers of severity in acute pancreatitis. Am J Gastroenterol *104*, 966-971.

Skouras, C., Davis, Z.A., Sharkey, J., Parks, R.W., Garden, O.J., Murchison, J.T., and Mole, D.J. (2016a). Lung ultrasonography as a direct measure of evolving respiratory dysfunction and disease severity in patients with acute pancreatitis. HPB (Oxford) *18*, 159-169.

Skouras, C., Hayes, A.J., Williams, L., Garden, O.J., Parks, R.W., and Mole, D.J. (2014). Early organ dysfunction affects long-term survival in acute pancreatitis patients. HPB (Oxford) *16*, 789-796.

Skouras, C., Zheng, X., Binnie, M., Homer, N.Z., Murray, T.B., Robertson, D., Briody, L., Paterson, F., Spence, H., Derr, L.*, et al.* (2016b). Increased levels of 3-hydroxykynurenine parallel disease severity in human acute pancreatitis. Sci Rep *6*, 33951.

Soldati, G., Copetti, R., and Sher, S. (2009). Sonographic interstitial syndrome: the sound of lung water. J Ultrasound Med *28*, 163-174.

Spanier, B.W., Dijkgraaf, M.G., and Bruno, M.J. (2008). Epidemiology, aetiology and outcome of acute and chronic pancreatitis: An update. Best Pract Res Clin Gastroenterol *22*, 45-63.

Stefanidis, K., Dimopoulos, S., Kolofousi, C., Cokkinos, D.D., Chatzimichail, K., Eisen, L.A., Wachtel, M., Karakitsos, D., and Nanas, S. (2012). Sonographic lobe localization of alveolar-interstitial syndrome in the critically ill. Critical care research and practice *2012*, 179719.

Steinberg, W., and Tenner, S. (1994). Acute pancreatitis. N Engl J Med *330*, 1198-1210.

Stone, T.W., and Darlington, L.G. (2002). Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov *1*, 609-620.

Stone, T.W., Stoy, N., and Darlington, L.G. (2013). An expanding range of targets for kynurenine metabolites of tryptophan. Trends Pharmacol Sci *34*, 136-143.

Takikawa, O., Yoshida, R., Kido, R., and Hayaishi, O. (1986). Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. J Biol Chem *261*, 3648-3653.

Talamini, G., Uomo, G., Pezzilli, R., Rabitti, P.G., Billi, P., Bassi, C., Cavallini, G., and Pederzoli, P. (1999). Serum creatinine and chest radiographs in the early assessment of acute pancreatitis. Am J Surg *177*, 7-14.

Tattevin, P., Monnier, D., Tribut, O., Dulong, J., Bescher, N., Mourcin, F., Uhel, F., Le Tulzo, Y., and Tarte, K. (2010). Enhanced indoleamine 2,3-dioxygenase activity in patients with severe sepsis and septic shock. J Infect Dis *201*, 956-966.

Taylor, M.W., and Feng, G.S. (1991). Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J *5*, 2516-2522.

Terness, P., Bauer, T.M., Rose, L., Dufter, C., Watzlik, A., Simon, H., and Opelz, G. (2002). Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med *196*, 447-457.

Thevandavakkam, M.A. (2011). Deciphering the Kynurenine-3-monooxygenase Interactome. PhD thesis (University of Leicester).

Thoner, J. (1987). Outcome and costs of intensive care. A follow-up study on patients requiring prolonged mechanical ventilation. Acta Anaesthesiol Scand *31*, 693-698.

Timmers, T.K., Verhofstad, M.H., Moons, K.G., and Leenen, L.P. (2011). Long-term survival after surgical intensive care unit admission: fifty percent die within 10 years. Ann Surg *253*, 151-157.

Toh, S.K., Phillips, S., and Johnson, C.D. (2000). A prospective audit against national standards of the presentation and management of acute pancreatitis in the South of England. Gut *46*, 239-243.

Tsubo, T., Yatsu, Y., Tanabe, T., Okawa, H., Ishihara, H., and Matsuki, A. (2004). Evaluation of density area in dorsal lung region during prone position using transesophageal echocardiography. Crit Care Med *32*, 83-87.

Uemura, T., and Hirai, K. (1998). L-kynurenine 3-monooxygenase from mitochondrial outer membrane of pig liver: purification, some properties, and monoclonal antibodies directed to the enzyme. J Biochem *123*, 253-262.

Uhl, W., Buchler, M.W., Malfertheiner, P., Beger, H.G., Adler, G., and Gaus, W. (1999). A randomised, double blind, multicentre trial of octreotide in moderate to severe acute pancreatitis. Gut *45*, 97-104.

Uhl, W., Warshaw, A., Imrie, C., Bassi, C., McKay, C.J., Lankisch, P.G., Carter, R., Di Magno, E., Banks, P.A., Whitcomb, D.C*., et al.* (2002). IAP Guidelines for the Surgical Management of Acute Pancreatitis. Pancreatology *2*, 565-573.

UK Working Party on Acute Pancreatitis (2005). UK guidelines for the management of acute pancreatitis. Gut *54 Suppl 3*, iii1-9.

Ulvik, A., Kvale, R., Wentzel-Larsen, T., and Flaatten, H. (2007). Multiple organ failure after trauma affects even long-term survival and functional status. Crit Care *11*, R95.

Valderrama, R., Perez-Mateo, M., Navarro, S., Vazquez, N., Sanjose, L., Adrian, M.J., and Estruch, J. (1992). Multicenter double-blind trial of gabexate mesylate (FOY) in unselected patients with acute pancreatitis. Digestion *51*, 65-70.

Van Berendoncks, A.M., Elseviers, M.M., Lins, R.L., and Group, S.S. (2010). Outcome of acute kidney injury with different treatment options: long-term follow-up. Clin J Am Soc Nephrol *5*, 1755-1762.

Via, G., Storti, E., Gulati, G., Neri, L., Mojoli, F., and Braschi, A. (2012). Lung ultrasound in the ICU: from diagnostic instrument to respiratory monitoring tool. Minerva Anestesiol *78*, 1282-1296.

Volpicelli, G., Mussa, A., Garofalo, G., Cardinale, L., Casoli, G., Perotto, F., Fava, C., and Frascisco, M. (2006). Bedside lung ultrasound in the assessment of alveolar-interstitial syndrome. Am J Emerg Med *24*, 689-696.

von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gotzsche, P.C., Vandenbroucke, J.P., and Initiative, S. (2007). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet *370*, 1453-1457.

Wang, G., Wen, J., Wilbur, R.R., Wen, P., Zhou, S.F., and Xiao, X. (2013). The effect of somatostatin, ulinastatin and Salvia miltiorrhiza on severe acute pancreatitis treatment. Am J Med Sci *346*, 371-376.

Wang, J., Simonavicius, N., Wu, X., Swaminath, G., Reagan, J., Tian, H., and Ling, L. (2006). Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem *281*, 22021-22028.

Wang, Q., Liu, D., Song, P., and Zou, M.H. (2015). Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation. Front Biosci (Landmark Ed) *20*, 1116-1143.

Wang, Q., Zhang, M., Ding, Y., Wang, Q., Zhang, W., Song, P., and Zou, M.H. (2014). Activation of NAD(P)H oxidase by tryptophan-derived 3-hydroxykynurenine accelerates endothelial apoptosis and dysfunction in vivo. Circ Res *114*, 480-492.

Wang, Y., Liu, H., McKenzie, G., Witting, P.K., Stasch, J.P., Hahn, M., Changsirivathanathamrong, D., Wu, B.J., Ball, H.J., Thomas, S.R.*, et al.* (2010). Kynurenine is an endothelium-derived relaxing factor produced during inflammation. Nat Med *16*, 279-285.

Wernecke, K., Galanski, M., Peters, P.E., and Hansen, J. (1987). Pneumothorax: evaluation by ultrasound--preliminary results. J Thorac Imaging *2*, 76-78.

Werner, E.R., Bitterlich, G., Fuchs, D., Hausen, A., Reibnegger, G., Szabo, G., Dierich, M.P., and Wachter, H. (1987). Human macrophages degrade tryptophan upon induction by interferon-gamma. Life Sci *41*, 273-280.

Werner-Felmayer, G., Werner, E.R., Fuchs, D., Hausen, A., Reibnegger, G., and Wachter, H. (1989). Characteristics of interferon induced tryptophan metabolism in human cells in vitro. Biochim Biophys Acta *1012*, 140-147.

Williams, T.A., Dobb, G.J., Finn, J.C., Knuiman, M.W., Geelhoed, E., Lee, K.Y., and Webb, S.A. (2008). Determinants of long-term survival after intensive care. Crit Care Med *36*, 1523-1530.

Williams, T.A., Dobb, G.J., Finn, J.C., and Webb, S.A. (2005). Long-term survival from intensive care: a review. Intensive Care Med *31*, 1306-1315.

Williams, T.A., and Leslie, G.D. (2011). Challenges and possible solutions for long-term follow-up of patients surviving critical illness. Aust Crit Care *24*, 175-185.

Wilson, C., Heads, A., Shenkin, A., and Imrie, C.W. (1989). C-reactive protein, antiproteases and complement factors as objective markers of severity in acute pancreatitis. Br J Surg *76*, 177-181.

Wilson, C., Heath, D.I., and Imrie, C.W. (1990). Prediction of outcome in acute pancreatitis: a comparative study of APACHE II, clinical assessment and multiple factor scoring systems. Br J Surg *77*, 1260-1264.

Wilson, P.G., Manji, M., and Neoptolemos, J.P. (1998). Acute pancreatitis as a model of sepsis. J Antimicrob Chemother *41 Suppl A*, 51-63.

Winslet, M., Hall, C., London, N.J., and Neoptolemos, J.P. (1992). Relation of diagnostic serum amylase levels to aetiology and severity of acute pancreatitis. Gut *33*, 982-986.

Wirleitner, B., Rudzite, V., Neurauter, G., Murr, C., Kalnins, U., Erglis, A., Trusinskis, K., and Fuchs, D. (2003). Immune activation and degradation of tryptophan in coronary heart disease. Eur J Clin Invest *33*, 550-554.

Wood, L. (2015). Rehabilitating patients after critical care. Nurs Times *111*, 12-14.

World Medical Association (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA *310*, 2191-2194.

Wright, J.C., Plenderleith, L., and Ridley, S.A. (2003). Long-term survival following intensive care: subgroup analysis and comparison with the general population. Anaesthesia *58*, 637-642.

Wu, B.U., Bakker, O.J., Papachristou, G.I., Besselink, M.G., Repas, K., van Santvoort, H.C., Muddana, V., Singh, V.K., Whitcomb, D.C., Gooszen, H.G., *et al.* (2011). Blood urea nitrogen in the early assessment of acute pancreatitis: an international validation study. Arch Intern Med *171*, 669-676.

Wu, B.U., and Banks, P.A. (2013). Clinical management of patients with acute pancreatitis. Gastroenterology *144*, 1272-1281.

Wu, B.U., Johannes, R.S., Sun, X., Tabak, Y., Conwell, D.L., and Banks, P.A. (2008). The early prediction of mortality in acute pancreatitis: a large population-based study. Gut *57*, 1698-1703.

Wunsch, H., Angus, D.C., Harrison, D.A., Linde-Zwirble, W.T., and Rowan, K.M. (2011). Comparison of medical admissions to intensive care units in the United States and United Kingdom. Am J Respir Crit Care Med *183*, 1666-1673.

Xu, J., Zhang, X., Pelayo, R., Monestier, M., Ammollo, C.T., Semeraro, F., Taylor, F.B., Esmon, N.L., Lupu, F., and Esmon, C.T. (2009). Extracellular histones are major mediators of death in sepsis. Nat Med *15*, 1318-1321.

Yadav, D. (2014). Acute pancreatitis: too many classifications-what is a clinician or researcher to do? Clin Gastroenterol Hepatol *12*, 317-319.

Yadav, D., and Lowenfels, A.B. (2006). Trends in the epidemiology of the first attack of acute pancreatitis: a systematic review. Pancreas *33*, 323-330.

Yadav, D., and Lowenfels, A.B. (2013). The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology *144*, 1252-1261.

Yang, C.Y., Chang-Chien, C.S., and Liaw, Y.F. (1987). Controlled trial of protease inhibitor gabexelate mesilate (FOY) in the treatment of acute pancreatitis. Pancreas *2*, 698-700.

Yasui, H., Takai, K., Yoshida, R., and Hayaishi, O. (1986). Interferon enhances tryptophan metabolism by inducing pulmonary indoleamine 2,3-dioxygenase: its possible occurrence in cancer patients. Proc Natl Acad Sci U S A *83*, 6622-6626.

Young, V.R., Hussein, M.A., Murray, E., and Scrimshaw, N.S. (1969). Tryptophan intake, spacing of meals, and diurnal fluctuations of plasma tryptophan in men. Am J Clin Nutr *22*, 1563-1567.

Zeden, J.P., Fusch, G., Holtfreter, B., Schefold, J.C., Reinke, P., Domanska, G., Haas, J.P., Gruendling, M., Westerholt, A., and Schuett, C. (2010). Excessive tryptophan catabolism along the kynurenine pathway precedes ongoing sepsis in critically ill patients. Anaesth Intensive Care *38*, 307-316.

Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K., and Hauser, C.J. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature *464*, 104-107.

Zhou, M.T., Chen, C.S., Chen, B.C., Zhang, Q.Y., and Andersson, R. (2010). Acute lung injury and ARDS in acute pancreatitis: mechanisms and potential intervention. World J Gastroenterol *16*, 2094-2099.

Zompatori, M., Ciccarese, F., and Fasano, L. (2014). Overview of current lung imaging in acute respiratory distress syndrome. Eur Respir Rev *23*, 519-530.

Zwilling, D., Huang, S.Y., Sathyasaikumar, K.V., Notarangelo, F.M., Guidetti, P., Wu, H.Q., Lee, J., Truong, J., Andrews-Zwilling, Y., Hsieh, E.W.*, et al.* (2011). Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. Cell *145*, 863-874.

Publications arising from this thesis

1. Early organ dysfunction affects long-term survival in acute pancreatitis patients. **Skouras C**, Hayes AJ, Williams L, Garden OJ, Parks RW, Mole DJ. *HPB (Oxford)*. **2014** Sep;16(9):789-96. doi: 10.1111/hpb.12259. Epub 2014 Apr 9.

2. Lung ultrasonography as a direct measure of evolving respiratory dysfunction and disease severity in patients with acute pancreatitis. **Skouras C**, Davis ZA, Sharkey J, Parks RW, Garden OJ, Murchison JT, Mole DJ. *HPB (Oxford)*. **2016** Feb;18(2):159-69. doi: 10.1016/j.hpb.2015.10.002. Epub 2015 Nov 18.

3. Prostaglandin E_2 constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. Duffin R, O'Connor RA, Crittenden S, Forster T, Yu C, Zheng X, Smyth D, Robb CT, Rossi F, **Skouras C**, Tang S, Richards J, Pellicoro A, Weller RB, Breyer RM, Mole DJ, Iredale JP, Anderton SM, Narumiya S, Maizels RM, Ghazal P, Howie SE, Rossi AG, Yao C. *Science*. **2016** Mar 18;351(6279):1333-8. doi: 10.1126/science.aad9903.

4. Increased levels of 3-hydroxykynurenine parallel disease severity in human acute pancreatitis. **Skouras C**, Zheng X, Binnie M, Homer NZM, Murray TBJ, Robertson D, Briody L, Paterson F, Spence H, Derr L, Hayes AJ, Tsoumanis A, Lyster D, Parks RW, Garden OJ, Iredale JP, Uings IJ, Liddle J, Wright WL, Dukes G, Webster SP, Mole DJ. *Sci Rep*. **2016 Sep** 27;6:33951. doi: 10.1038/srep33951**.**

EARLY ORGAN DYSFUNCTION AFFECTS LONG-TERM SURVIVAL IN ACUTE PANCREATITIS PATIENTS

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ORIGINAL ARTICLE

Early organ dysfunction affects long-term survival in acute pancreatitis patients

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Abstract

Background: The effect of early organ dysfunction on long-term survival in acute pancreatitis (AP) patients is unknown.

Objective: The aim of this study was to ascertain whether early organ dysfunction impacts on long-term survival after an episode of AP.

Methods: A retrospective analysis was performed using survival data sourced from a prospectively maintained database of patients with AP admitted to the Royal Infirmary of Edinburgh during a 5-year period commencing January 2000. A multiple organ dysfunction syndrome (MODS) score of ≥ 2 during the first week of admission was used to define early organ dysfunction. After accounting for in-hospital deaths, long-term survival probabilities were estimated using the Kaplan–Meier test. The prognostic significance of patient characteristics was assessed by univariate and multivariate analyses using Cox's proportional hazards methods.

Results: A total of 694 patients were studied (median follow-up: 8.8 years). Patients with early organ dysfunction (MODS group) were found to have died prematurely [mean survival: 10.0 years, 95% confidence interval (CI) 9.4–10.6 years] in comparison with the non-MODS group (mean survival: 11.6 years, 95% CI 11.2–11.9 years) (log-rank test, $P = 0.001$) after the exclusion of in-hospital deaths. Multivariate analysis confirmed MODS as an independent predictor of long-term survival [hazard ratio (HR): 1.528, 95% CI 1.72–2.176; $P = 0.019$] along with age (HR: 1.062; $P < 0.001$), alcohol-related aetiology (HR: 2.027; $P = 0.001$) and idiopathic aetiology (HR: 1.548; $P = 0.048$).

Conclusions: Early organ dysfunction in AP is an independent predictor of long-term survival even when in-hospital deaths are accounted for. Negative predictors also include age, and idiopathic and alcohol-related aetiologies.

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Introduction

Acute pancreatitis (AP) is a common disease characterized by acute inflammation of the pancreas, usually triggered by the passage of gallstones or excessive alcohol consumption.[1,2] Usually an episode of AP will resolve with supportive measures, but the disease may also be fatal and results in reported mortality of 6–9% in the UK.[3–6]

Many systems have been devised to stratify the subgroup of patients who present with, or are predicted to develop, the severe form of AP, but these systems focus on the severity of the index episode. According to the widely accepted and updated definition of severe acute pancreatitis (SAP),[7] which incorporates evidence of persistent organ failure, approximately one in four patients with AP will experience a severe episode.[5,8,9] Unsurprisingly, mortality rates in SAP are considerably higher than in mild disease and range from 14% to 30%.[5,10,11] It is well established that the majority of deaths occur early in the disease course; up to 60% of all AP deaths occur during the first 7 days of hospital admission and are mostly attributable to deteriorating organ function.[3,4,6,12–14] It is

understandable, therefore, that clinical research in AP to date has largely focused on improving early outcomes; relatively little is known about late deaths amongst hospital survivors.

Few contemporary studies have investigated long-term survival and the relevant prognostic factors in AP.[15,16] Long-term studies have for the most part examined morbidity outcomes, such as progression to chronic pancreatitis, or have focused upon a severe pancreatitis subgroup. Those that do report survival suggest that SAP hospital survivors may carry a reduced life expectancy.[17] After the exclusion of inpatient mortality, death rates amongst AP patients are reported to be in the order of one death in every four to eight cases, over 5–8 years.[17,18] Late deaths seem to be most commonly attributed to cardiovascular disease or malignancy,[15,16] but the factors that reduce long-term survival are likely to be complex.

The aim of this study was to determine whether early acute organ dysfunction is associated with altered long-term survival in hospital survivors of AP.

Materials and methods

Study approval

Caldicott Guardian approval was obtained to allow the use of confidential patient data. The study was assessed by the University of Edinburgh/National Health Service (NHS) Lothian ACCORD Research and Development Office and the South East Scotland Research Ethics Service and was declared exempt from requirements for formal research ethics committee review as a clinical audit.

Data collection and inclusion criteria

In 2013, a retrospective analysis was performed using long-term survival data sourced from a prospectively maintained database of patients with AP admitted to the Royal Infirmary of Edinburgh during the 5-year period between January 2000 and December 2004. This historical cohort was originally identified from the Lothian Surgical Audit database,[19] and its clinical details and immediate outcomes have been previously reported.[20] For this cohort, existing data on age, gender, organ dysfunction, aetiology of pancreatitis, necrosectomy and disease severity were supplemented with survival data. Patients for whom data on these characteristics were unavailable were excluded from subsequent analysis. For those patients with recurrent attacks of AP, the earliest episode during the study period was taken as the index episode for the purposes of survival analysis.

Patient inclusion in the original patient cohort was based on the presence of clinical features compatible with AP, supported by the finding of elevated serum amylase (three times higher than the upper limit of the laboratory reference range). In instances in which a strong clinical suspicion of AP in the context of a non-diagnostic amylase result existed, radiological evidence of AP by means of computed tomography or the finding of pancreatitis at laparotomy was required for inclusion. Patients admitted with chronic pancreatitis were excluded.

Definitions: severity, aetiology and organ dysfunction

Severity stratification was performed in accordance with the original version of the Atlanta consensus definition,[21] which was the classification system in use at the time when patients in the study cohort presented with AP. Patients were not retrospectively reclassified according to the revised 2012 Atlanta criteria[7] in order to avoid introducing error. Patients with an admission APACHE II (Acute Physiology and Chronic Health Evaluation) score of ≥ 8 were classified as predicted severe.

Gallstones were considered to be the precipitating cause of pancreatitis when gallbladder or bile duct calculi were detected by any imaging procedure. In the absence of gallstones and when excessive consumption of alcohol was reported by the patient or the patient's family, the pancreatitis was classified as alcohol-induced. When no definite cause was identified, the disease was characterized as idiopathic. Rarer established causes were collated under the category 'other'.

Organ dysfunction scores were calculated for all patients at 24 h, 48 h and 7 days, based on the most extreme laboratory values or clinical measurements during each 24-h period, for five (respiratory, cardiovascular, renal, haematological, central nervous system) of six organ systems that constitute the multiple organ dysfunction score (MODS).[22] Hepatic dysfunction (serum bilirubin) was excluded in order to avoid the confounding effects of biliary obstruction. A MODS score of ≥ 2 (based on dysfunction of a single or more than one organ system) at one or more time-points was considered to define organ dysfunction. The duration and persistence of organ dysfunction could not be retrospectively ascertained from the existing database; attempts to retrieve archived primary records to gather these data were not successful.

Follow-up and mortality

In-hospital mortality, overall mortality and long-term survival were the main outcome measures of the present study. NHS Lothian electronic patient records were individually reviewed; for surviving patients, the date of the most recent general practitioner and/or hospital visit, outpatient clinic attendance or hospital discharge were defined as the point of last known contact. Dates of death were retrieved and recorded for non-survivors.

Statistical analysis

Data for continuous variables are presented as the mean ± standard deviation (SD) or as the median and interquartile range (IQR). Categorical variables are presented as absolute and relative frequencies. Comparisons between groups were performed using Student's *t*-test, the Mann–Whitney *U*-test, the Kolmogorov–Smirnov test and the chi-squared test, as appropriate.

The Kaplan–Meier method was used to estimate survival probabilities. The generalized Wilcoxon test was used to detect early

death rate differences between groups and the log-rank test was applied to detect differences manifesting throughout the entire follow-up period. Survival times were calculated from the date of admission to the date of death from any cause or date of last contact in survivors. Patients lost from follow-up were censored at the date of confirmed last contact. The prognostic impact of patient characteristics on survival was assessed by univariate and multivariate Cox's proportional hazards regression. For univariate analyses, the proportional hazards assumption was examined graphically with the use of pairwise Cox log(-log) plots, revealing approximately parallel curves between groups for each of the categorical covariates MODS, gender, severity and aetiology. Linear regression was implemented to test for goodness-of-fit of Schoenfeld partial residuals[23] against natural logarithmic survival times for each of the aforementioned covariates; no violation of the proportional hazards assumption was revealed. The linearity assumption for age was confirmed graphically by examining the respective Cox log(-log) plots, after transformation to a categorical variable with six strata. Similar procedures were used for multivariate analyses.

Selection of variables for the multivariate Cox regression model was performed in accordance with the 'purposeful selection' algorithm,[24,25] originally described by Hosmer and Lemeshow.[26] Variables with a univariate *P*-value of > 0.25 were originally selected as candidates for multivariate analysis. Covariates were subsequently removed from the model if they were found to be non-significant at the $\alpha = 0.1$ level and not to be confounders. Confounding was defined as a change of > 15% in any of the remaining covariate estimates. Variables that were not selected for the original model were returned one at a time, and any that were significant at the $\alpha = 0.15$ level were retained.[24] This procedure resulted in a multivariate model containing age, MODS, gender, severity and aetiology. The necrosectomy variable did not satisfy the proportional hazards assumption and was examined with the use of stratified Cox regression.

Results of the Cox models are presented as hazard ratios (HRs) with 95% confidence intervals (95% CIs) together with the *P*-values from Wald's tests. All statistical tests were based on a two-sided α-value of 0.05. Statistical analysis was performed using IBM SPSS Statistics Version 19.0 (IBM Corp., Armonk, NY, USA). Figures were designed using GraphPad Prism Version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). This double-arm cohort study followed the principles of the STROBE (*st*rengthening the *r*eporting of *ob*servational studies in *e*pidemiology) statement.[27]

Results

Demographics

Unique patient identifiers with corresponding survival data and variables of interest were available for 694 of 759 (91.4%) AP patients in the database and were included in the present study. With regard to classification of disease severity according to APACHE II scores, 256 (36.9%) patients were predicted to have a severe attack, although ultimately 268 (38.6%) suffered from some degree of organ dysfunction during the first week of admission. A total of 235 (33.9%) patients had a MODS score of ≥ 2 at one or more time-points during the first week of their hospital stay and were classified as having organ dysfunction (denoted as the 'MODS group'). Sixty-nine (9.9%) patients underwent one or more necrosectomy procedures during the study interval. These were performed predominantly as open procedures because minimally invasive retroperitoneal pancreatic (MIRP) necrosectomy was not routinely performed in the department during the study period. Patients' demographic characteristics are summarized in Table 1.

Study follow-up

The median follow-up in the whole patient series was 8.8 years. After excluding in-hospital deaths, median follow-up was 9.0 years. This period was marginally comparable between the MODS group (8.7 years, IQR: 4.7–10.5 years) and non-MODS group (9.1 years, IQR: 6.6–10.6 years) after the exclusion of in-hospital deaths (Mann–Whitney *U*-test, $P = 0.046$; Kolmogorov–Smirnov test, $P = 0.105$).

Overall survival

There were 36 (5.2%) in-hospital deaths in total, 35 of which occurred during the first 2.2 months. During the follow-up period, 179 patients died, giving an overall mortality rate of 31.0% (215 of 694 patients). Median survival in the overall patient sample remained undefined because more than 50% of patients were still alive at the end of follow-up. The overall mean survival was 10.6 years (95% CI 10.2–10.9 years); after the exclusion of in-hospital deaths this increased to 11.1 years (95% CI 10.8–11.5 years).

Organ failure and survival

Analysis of the overall patient sample demonstrated a clear difference in survival between the MODS and non-MODS groups (Fig. 1). Mean survival in the MODS group (8.6 years, 95% CI 7.9–9.3 years) was significantly lower than that in the non-MODS group (11.5 years, 95% CI 11.1–11.9 years) (Wilcoxon test, $P < 0.001$; log-rank test, $P < 0.001$). The highest frequency of death occurred during the in-hospital period; the majority of these patients had a MODS score of ≥ 2. Four (11.1%) of the 36 patients who died as inpatients had a MODS score of < 2.

To investigate long-term mortality, inpatient deaths were removed from the analysis. Interestingly and importantly, with this exclusion, mean survival in MODS group hospital survivors (10.0 years, 95% CI 9.4–10.6 years) remained significantly lower than that in non-MODS group hospital survivors (11.6 years, 95% CI 11.2–11.9 years) (Wilcoxon test, $P = 0.002$; log-rank test, $P = 0.001$). Furthermore, the survival curves continued to diverge several years after the index episode of AP (Fig. 2). Again, median

Table 1 Demographic characteristics of patients with acute pancreatitis

Characteristics	Overall sample	Non-MODS group[a]	MODS group[a]	In-hospital deaths	Survivors[a]	Non-survivors[a]
Sample size, *n* (%)	694 (100%)	455 (69.1%)	203 (30.9%)	36 (5.2%)	479 (72.8%)	179 (27.2%)
Age, years, median (IQR)	57.1 (43.2–71.2)	54.3 (40.3–69.3)	59.9 (47.7–74.0)	70.9 (79.9–57.4)	52.1 (39.0–65.0)	66.7 (79.5–54.2)
Gender, *n* (%)						
Male	355 (51.2%)	228 (50.1%)	106 (52.2%)	21 (58.3%)	236 (49.3%)	98 (54.7%)
Female	339 (48.8%)	227 (49.9%)	97 (47.8%)	15 (41.7%)	243 (50.7%)	81 (45.3%)
Aetiology of pancreatitis, *n* (%)						
Gallstones	337 (48.6%)	223 (49.0%)	104 (51.2%)	10 (27.8%)	237 (49.5%)	90 (50.3%)
Alcohol	223 (32.1%)	159 (34.9%)	54 (26.6%)	10 (27.8%)	158 (33.0%)	55 (30.7%)
Idiopathic	92 (13.3%)	54 (11.9%)	27 (13.3%)	11 (30.6%)	54(11.3%)	27 (15.1%)
Other	42 (6.1%)	19 (4.2%)	18 (8.9%)	5 (13.9%)	30 (6.3%)	7 (3.9%)
Severity, *n* (%)						
Mild	438 (63.1%)	380 (83.5%)	56 (27.6%)	2 (5.6%)	338 (70.6%)	98 (54.7%)
Severe	256 (36.9%)	75 (16.5%)	147 (72.4%)	34 (94.4%)	141 (29.4%)	81 (45.3%)
Necrosectomy, *n* (%)	69 (9.9%)	17 (3.7%)	42 (20.7%)	10 (27.8%)	43 (9.0%)	16 (8.9%)
In-hospital deaths, *n* (%)	36 (5.2%)	N/A	N/A	N/A	N/A	N/A
Post-discharge deaths, *n* (%)	179 (25.8%)	107 (59.8%)	72 (40.2%)	N/A	N/A	179 (100%)
MODS, *n* (%)	235 (33.9%)	N/A	N/A	32 (88.9%)	131 (27.3%)	72 (40.2%)

[a]After exclusion of in-hospital deaths.
MODS, multiple organ dysfunction syndrome; IQR, interquartile range; N/A, not available.

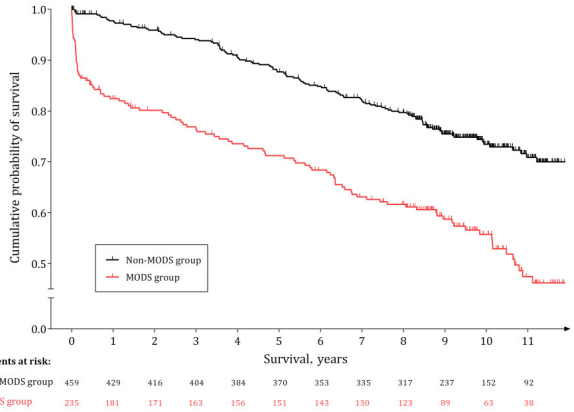

Figure 1 Kaplan–Meier survival plot by group of patients (non-MODS group, $n = 459$; MODS group, $n = 235$), representing cumulative percentages of surviving patients by time in years. A distinct, steep decrease is evident on the MODS curve during the first months of the study, corresponding to in-hospital deaths. A clear difference in survival is observed between the two groups. Vertical tick-marks represent right-censored patients. MODS, multiple organ dysfunction syndrome

survival was not defined; however the 75th percentiles of survival were 6.5 years and 9.9 years in the MODS and non-MODS groups, respectively. Survival rates in each group at 1, 3, 5 and 10 years are shown in Table 2.

To account for the difference in mean ± SD age between the MODS (59.9 ± 17.1 years) and non-MODS (54.3 ± 17.9 years) groups ($P < 0.001$), and because other AP characteristics may impact on long-term survival, a multivariate analysis was per-

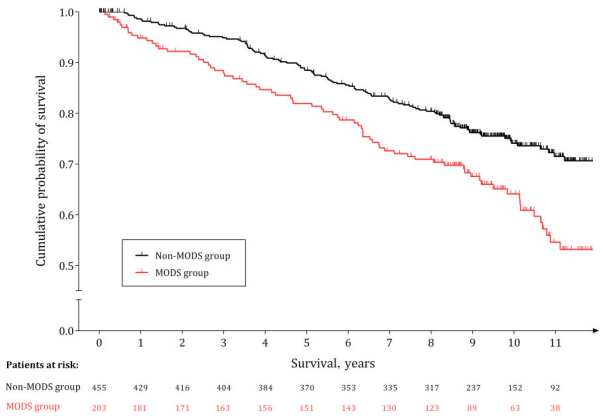

Figure 2 Kaplan–Meier survival plot by group of patients, after the exclusion of in-hospital deaths (non-MODS group, $n = 455$; MODS group, $n = 203$), representing the percentages of surviving patients by time in years. A clear, moderately increasing difference in survival is observed between the two groups. Vertical tick-marks represent right-censored patients. MODS, multiple organ dysfunction syndrome

Table 2 Number of patients at risk and survival rates for each group at 1, 3, 5 and 10 years of follow-up, after exclusion of in-hospital deaths

Follow-up	MODS group			Non-MODS group		
	Patients at risk	Cumulative proportion of patients surviving	Standard error	Patients at risk	Cumulative proportion of patients surviving	Standard error
1 **year**	181	0.92	0.02	429	0.97	0.01
3 **years**	163	0.85	0.03	404	0.91	0.01
5 **years**	151	0.79	0.03	370	0.86	0.02
10 **years**	63	0.55	0.04	152	0.72	0.02

MODS, multiple organ dysfunction syndrome.

formed. The MODS score was shown to be an independent predictor of patient survival, with the MODS group carrying a higher level of risk for post-discharge death than the non-MODS group in both univariate and multivariate analyses. Age and cause of pancreatitis were also established as individual predictors, whereas severity and gender were identified as confounding factors (Table 3). When necrosectomy was added by stratification on the multivariate Cox regression model, a negligible effect was observed. Specifically, the resulting HR for the MODS group was 1.573 (95% CI 1.102–2.246; $P = 0.013$).

A supplementary univariate Cox regression analysis was performed in the MODS group alone (after the exclusion of in-hospital deaths) in order to examine whether the magnitude of the MODS score (worst MODS score value of days 1, 2 and 7 of admission) or APACHE II score on the day of admission had any effect on long-term survival. No significant effects were revealed for either the MODS score (HR: 0.876, 95% CI 0.748–1.026; $P = 0.102$) or APACHE II score (HR: 1.019, 95% CI 0.954–1.088; $P = 0.573$).

Discussion

This study demonstrates that organ dysfunction has a lasting negative impact on long-term survival after an attack of AP. Importantly, this effect is seen after the exclusion of in-hospital deaths and is independent of age, aetiology and gender. In addition, age and an alcohol-related or idiopathic aetiology were also found to negatively influence long-term survival.

The extent to which the consequences of critical illness contribute to post-discharge mortality is poorly understood,[28–31] especially in AP. No previous study has specifically investigated the impact of early organ dysfunction as a predictive marker for long-term mortality. Findings of the available long-term AP studies have been equivocal in associating multiple organ failure or various surrogate markers of organ dysfunction with long-term survival. Lund *et al.*[32] found that an increased Ranson's score and length of stay in the intensive care department were associated with an increased long-term incidence of mortality in univariate

Table 3 Association of patient characteristics with overall survival, after exclusion of in-hospital deaths

Factors	Overall survival					
	Univariate analyses			Multivariate analysis		
	Crude HR	95% CI	*P*-value	Adjusted HR	95% CI	*P*-value
MODS						
Yes	1.661	1.232–2.240	**0.001**	1.528	1.072–2.176	**0.019**
No	1			1		
Gender						
Male	1.181	0.879–1.585	0.269	1.302	0.946–1.793	0.106
Female	1			1		
Age	1.050	1.040–1.061	< **0.001**	1.062	1.049–1.075	< **0.001**
Severity						
Severe	1.862	1.387–2.499	< **0.001**	0.850	0.587–1.231	0.390
Mild	1			1		
Cause						
Gallstones	1			1		
Alcohol	0.927	0.662–1.296	0.656	2.027	1.359–3.024	**0.001**
Idiopathic	1.350	0.878–2.076	0.171	1.548	1.004–2.387	**0.048**
Other	0.788	0.365–1.701	0.544	1.488	0.679–3.263	0.321

HR, hazard ratio; 95% CI, 95% confidence interval; MODS, multiple organ dysfunction syndrome.

but not multivariate analyses. Conversely, Halonen *et al.*[17] reported that a smaller proportion of late-death patients had suffered multiple organ failure than had long-term survivors. There was no statistical analysis of this frequency as the study had other objectives pertaining to quality of life outcomes, but this is a surprising result and is difficult to rationalize. However, median follow-up in this study was limited to 61 months, the rate of capture of events (deaths) was subsequently low and the number of participants relatively small.[17] Furthermore, acute organ dysfunction has been shown to be associated with reduced life expectancy amongst survivors of critical illness compared with matched controls in a number of other conditions.[33,34]

In addition to early organ dysfunction, increasing age was observed to be an independent predictor of reduced survival. It is important not to dismiss this finding as obvious, albeit expected, because the observation refers to more than just the fact that older people die sooner from all causes. Although age has been established as an independent predictor in the present and previous studies,[35–37] it has been suggested that poorer outcomes in elderly patients may reflect residual functional disability and dysfunction of organ systems caused by critical illness.[38] This may be supported by the findings of Lankisch *et al.*, who reported that the most common causes of death were cardiovascular and cerebrovascular diseases.[16] This finding was echoed by Nøjgaard *et al.* with reference to cardiovascular disease.[39] The pathophysiological mechanisms and exact long-term confounding effects of critical illness on individual patient subgroups, particularly elderly patients, deserve further investigation.

It is known that the aetiology of AP can influence morbidity amongst hospital survivors, but the influence of aetiology on long-term mortality has been less well researched. Compared with biliary pancreatitis, alcohol seems to have greater deleterious effects in AP with regard to exocrine insufficiency and morphological changes to the pancreas[16] and thus it may be unsurprising that patients with alcohol-related pancreatitis have a life expectancy lower than that reported for gallstone pancreatitis. Nøjgaard *et al.*[39] reported significantly higher long-term mortality in patients with AP related to high alcohol consumption, in keeping with the results of the present study.

The longitudinal design of the present study, its long follow-up with a median of approximately 9 years, and the fact that it was conducted in a single centre and included consecutive AP patients regardless of disease severity or treatment are amongst its strengths. Potential weaknesses include the study's retrospective design, although a prospective long-term survival study of this duration would be a major undertaking. Secondly, there is little information regarding the mode of death or any subsequent comorbidity of survivors. The latter detail would certainly merit further investigation. Furthermore, it was not feasible to retrieve and include in the analysis data on the comorbid status of the present cohort. It is therefore possible, and indeed one might argue reasonable, that patients with comorbidities may have been at higher risk for organ dysfunction during AP, which as a consequence resulted in shorter survival. Future studies would benefit from including details on important confounders such as detailed patient comorbid status, the presence of pancreatic necrosis, occurrence of pancreatic insufficiency and other long-term sequelae, as well as specific organ system dysfunction duration and severity. Notwithstanding these weaknesses, the present study identifies an interesting and important long-term negative impact

of organ dysfunction in AP, which extends beyond the injury seen in the critical care unit during the index episode. This work reinforces the importance of the early identification, stratification and correct management of extrapancreatic organ dysfunction in AP.

Early organ dysfunction in AP is associated with a shortened lifespan. It is hypothesized that patients who sustain organ dysfunction during their index episode, and who survive, accrue systemic changes which impact negatively on life expectancy in comparison with hospital survivors without multiple organ dysfunction. In AP, the effects of critical illness do not cease after the initial insult has subsided. This study reinforces the cardinal need to identify and pre-empt organ dysfunction, and introduces the need to consider interventions that impact on long-term outcomes in high-risk individuals.

Acknowledgement

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Conflicts of interest

None declared.

References

1. Goldacre MJ, Roberts SE. (2004) Hospital admission for acute pancreatitis in an English population, 1963–98: database study of incidence and mortality. *BMJ* 328:1466–1469.
2. Steinberg W, Tenner S. (1994) Acute pancreatitis. *N Engl J Med* 330:1198–1210.
3. McKay CJ, Evans S, Sinclair M, Carter CR, Imrie CW. (1999) High early mortality rate from acute pancreatitis in Scotland, 1984–1995. *Br J Surg* 86:1302–1305.
4. Mofidi R, Duff MD, Wigmore SJ, Madhavan KK, Garden OJ, Parks RW. (2006) Association between early systemic inflammatory response, severity of multiorgan dysfunction and death in acute pancreatitis. *Br J Surg* 93:738–744.
5. Toh SK, Phillips S, Johnson CD. (2000) A prospective audit against national standards of the presentation and management of acute pancreatitis in the south of England. *Gut* 46:239–243.
6. Johnson CD, Abu-Hilal M. (2004) Persistent organ failure during the first week as a marker of fatal outcome in acute pancreatitis. *Gut* 53:1340–1344.
7. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG *et al*. (2013) Classification of acute pancreatitis – 2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 62:102–111.
8. Winslet M, Hall C, London NJ, Neoptolemos JP. (1992) Relation of diagnostic serum amylase levels to aetiology and severity of acute pancreatitis. *Gut* 33:982–986.
9. Neoptolemos JP, Kemppainen EA, Mayer JM, Fitzpatrick JM, Raraty MG, Slavin J *et al*. (2000) Early prediction of severity in acute pancreatitis by urinary trypsinogen activation peptide: a multicentre study. *Lancet* 355:1955–1960.
10. Ashley SW, Perez A, Pierce EA, Brooks DC, Moore FD Jr, Whang EE *et al*. (2001) Necrotizing pancreatitis: contemporary analysis of 99 consecutive cases. *Ann Surg* 234:572–579; discussion 579–580.
11. Isenmann R, Rau B, Beger HG. (1999) Bacterial infection and extent of necrosis are determinants of organ failure in patients with acute necrotizing pancreatitis. *Br J Surg* 86:1020–1024.
12. Buter A, Imrie CW, Carter CR, Evans S, McKay CJ. (2002) Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. *Br J Surg* 89:298–302.
13. Mole DJ, Olabi B, Robinson V, Garden OJ, Parks RW. (2009) Incidence of individual organ dysfunction in fatal acute pancreatitis: analysis of 1024 death records. *HPB* 11:166–170.
14. Renner IG, Savage WT 3rd, Pantoja JL, Renner VJ. (1985) Death due to acute pancreatitis. A retrospective analysis of 405 autopsy cases. *Dig Dis Sci* 30:1005–1018.
15. Nøjgaard C. (2010) Prognosis of acute and chronic pancreatitis – a 30-year follow-up of a Danish cohort. *Dan Med Bull* 57:B4228.
16. Lankisch PG, Breuer N, Bruns A, Weber-Dan B, Lowenfels AB, Maisonneuve P. (2009) Natural history of acute pancreatitis: a long-term population-based study. *Am J Gastroenterol* 104:2797–2805; quiz 806.
17. Halonen KI, Pettilä V, Leppäniemi AK, Kemppainen EA, Puolakkainen PA, Haapiainen RK. (2003) Long-term health-related quality of life in survivors of severe acute pancreatitis. *Intensive Care Med* 29:782–786.
18. Lankisch PG, Burchard-Reckert S, Petersen M, Lehnick D, Schirren CA, Stöckmann F *et al*. (1996) Aetiology and age have only a limited influence on the course of acute pancreatitis. *Pancreas* 13:344–349.
19. Aitken RJ, Nixon SJ, Ruckley CV. (1997) Lothian surgical audit: a 15-year experience of improvement in surgical practice through regional computerized audit. *Lancet* 350:800–804.
20. Mofidi R, Madhavan KK, Garden OJ, Parks RW. (2007) An audit of the management of patients with acute pancreatitis against national standards of practice. *Br J Surg* 94:844–848.
21. Bradley EL 3rd. (1993) A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, GA, September 11 through 13, 1992. *Arch Surg* 128:586–590.
22. Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. (1995) Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit Care Med* 23:1638–1652.
23. Schoenfeld D. (1982) Partial residuals for the proportional hazards regression model. *Biometrika* 69:239–241.
24. Bursac Z, Gauss CH, Williams DK, Hosmer DW. (2008) Purposeful selection of variables in logistic regression. *Source Code Biol Med* 3:17.
25. Heinze G. Medical Biostatistics 2. Available at http://www.academia.edu/2658157/Medical_Biostatistics_2 (last accessed 4 November 2013).
26. Hosmer JDW, Lemeshow S, May S. (2008) *Applied Survival Analysis: Regression Modeling of Time to Event Data*. New York, NY: Wiley.
27. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. (2007) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 370:1453–1457.
28. Keenan SP, Dodek P, Chan K, Hogg RS, Craib KJ, Anis AH *et al*. (2002) Intensive care unit admission has minimal impact on long-term mortality. *Crit Care Med* 30:501–507.
29. Kaplan V, Angus DC. (2002) Surviving intensive care. *Crit Care Med* 30:703–705.

30. Angus DC, Carlet J, Brussels Roundtable Participants. (2003) Surviving intensive care: a report from the 2002 Brussels Roundtable. *Intensive Care Med* 29:368–377.
31. Williams TA, Dobb GJ, Finn JC, Webb SA. (2005) Long-term survival from intensive care: a review. *Intensive Care Med* 31:1306–1315.
32. Lund H, Tønnesen H, Tønnesen MH, Olsen O. (2006) Long-term recurrence and death rates after acute pancreatitis. *Scand J Gastroenterol* 41:234–238.
33. Ulvik A, Kvåle R, Wentzel-Larsen T, Flaatten H. (2007) Multiple organ failure after trauma affects even long-term survival and functional status. *Crit Care* 11:R95.
34. Wright JC, Plenderleith L, Ridley SA. (2003) Long-term survival following intensive care: subgroup analysis and comparison with the general population. *Anaesthesia* 58:637–642.
35. Niskanen M, Kari A, Halonen P. (1996) Five-year survival after intensive care – comparison of 12,180 patients with the general population. Finnish ICU Study Group. *Crit Care Med* 24:1962–1967.
36. Thoner J. (1987) Outcome and costs of intensive care. A follow-up study on patients requiring prolonged mechanical ventilation. *Acta Anaesthesiol Scand* 31:693–698.
37. Dragsted L. (1991) Outcome from intensive care. A five-year study of 1308 patients. *Dan Med Bull* 38:365–374.
38. Ridley S, Jackson R, Findlay J, Wallace P. (1990) Long-term survival after intensive care. *BMJ* 301:1127–1130.
39. Nøjgaard C, Matzen P, Bendtsen F, Andersen JR, Christensen E, Becker U. (2011) Factors associated with long-term mortality in acute pancreatitis. *Scand J Gastroenterol* 46:495–502.

LUNG ULTRASONOGRAPHY AS A DIRECT MEASURE OF EVOLVING RESPIRATORY DYSFUNCTION AND DISEASE SEVERITY IN PATIENTS WITH ACUTE PANCREATITIS

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ORIGINAL ARTICLE

Lung ultrasonography as a direct measure of evolving respiratory dysfunction and disease severity in patients with acute pancreatitis

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Abstract

Background: The value of lung ultrasonography in the diagnosis of respiratory dysfunction and severity stratification in patients with acute pancreatitis (AP) was investigated.

Methods: Over a 3-month period, 41 patients (median age: 59.1 years; 21 males) presenting with a diagnosis of potential AP were prospectively recruited. Each participant underwent lung ultrasonography and the number of comet tails was linked with contemporaneous clinical data. Group comparisons, areas under the curve (AUC) and respective measures of diagnostic accuracy were investigated.

Results: A greater number of comet tails were evident in patients with respiratory dysfunction ($P = 0.021$), those with severe disease ($P < 0.001$) and when contemporaneous and maximum CRP exceeded 100 mg/L ($P = 0.048$ and $P = 0.003$ respectively). Receiver-operator characteristic plot area under the curve (AUC) was greater when examining upper lung quadrants, using respiratory dysfunction and AP severity as variables of interest (AUC = 0.783, 95% C.I.: 0.544–0.962, and AUC = 0.996, 95% C.I.: 0.982–1.000, respectively). Examining all lung quadrants except for the lower lateral resulted in greater AUCs for contemporaneous and maximum CRP (AUC = 0.708, 95% C.I.: 0.510–0.883, and AUC = 0.800, 95% C.I.: 0.640–0.929).

Discussion: Ultrasonography of non-dependent lung parenchyma can reliably detect evolving respiratory dysfunction in AP. This simple bedside technique shows promise as an adjunct to severity stratification.

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Introduction

Acute pancreatitis (AP) complicated by organ dysfunction has a high mortality, which ranges from 14% to 30%.[1–4] Up to 53% of all deaths occur during the first week of admission, as a result of progressive organ failure.[5,6] Respiratory dysfunction is the most prevalent and one of the most deleterious systemic manifestations of severe acute pancreatitis (SAP). Lung injury may be subclinical, manifest as mild hypoxaemia or, in extreme cases, severe acute respiratory distress syndrome (ARDS).[7–9] Therefore, it is important to identify patients with developing respiratory dysfunction in order to implement appropriate monitoring and supportive measures early in the course of the disease.[10]

Pulmonary conditions with diffuse involvement of the interstitium and impairment of the alveolo-capillary exchange capacity can be classified under the term Alveolar-Interstitial Syndrome (AIS) and are common in the critically ill.[11] This syndrome includes acute pulmonary oedema, ARDS, interstitial pneumonia, exacerbation of chronic interstitial lung disease, and other miscellaneous conditions.[9,12–14] The use of transthoracic lung ultrasonography (LUSS) has been proposed as a useful non-

*: Denotes joint senior authors.

invasive method for diagnosing AIS in critically ill patients, based on the detection and quantification of comet-tail artefacts, generated by the ultrasound beam reverberation.[11,12,14] Although the accuracy of LUSS has been evaluated in generic critical care populations,[15] the specific diagnostic features of lung sonography in patients with AP have not been examined to date.

The aim of the present study was to investigate the value of LUSS in the diagnosis of evolving respiratory dysfunction and severity stratification in patients with AP.

Methods

Study approval

The present study was performed with Research Ethics Committee approval (REC reference number: 13/SS/0136) and institutional regulatory approval (ACCORD Project No: 2013/0098). Written consent was obtained from all participants or in cases of Adults with Incapacity, the named individual responsible for their welfare.

Recruitment

Over a three-month period from September 2013 to December 2013, adult patients presenting with potential AP were identified. The initial screening criterion was a presentation serum amylase >100 U/L, following which a definite diagnosis of AP, the definition of organ failure and severity stratification were performed in accordance with the revised Atlanta guidelines for AP, published by the Acute Pancreatitis Classification Working Group in 2013.[16] Patient recruitment to this study was by convenience, with no conscious or deliberate selection bias. Data collection was planned in advance and was performed prospectively.

Transthoracic lung ultrasound scans

All scans were performed by two consultant radiologists (JS, JTM) and a specialist registrar in radiology (ZAD). Bilateral intercostal LUSS were performed with participants in the supine position, after applying acoustic gel on the skin to provide an airless interface. In order to optimize imaging, the intercostal spaces were widened by raising each patient's ipsilateral arm up to or above the head level during the procedure, and the transducer was held perpendicular to the skin surface. For the purposes of the study, either a Micromaxx® portable ultrasound system fitted with a C60/5-2 MHz transducer (SonoSite, Inc., WA, USA), an Acuson S2000™ system with a 4C1 transducer (Siemens Medical Solutions USA, Inc., CA, USA) or an Acuson Antares™ Premium Edition ultrasound system with a CH4-1 transducer (Siemens Medical Solutions USA, Inc., CA, USA) was used.

Each hemithorax was divided into anterior and lateral, upper and lower areas (Fig. 1). For each hemithorax, the anterior area was delineated between the clavicle and the diaphragm and from the parasternal to the anterior axillary line. The lateral area was delineated between the axilla and the diaphragm and from the anterior to the posterior axillary line. The upper quadrants were demarcated from the 1st to the 3rd intercostal space and the lower quadrants from the 4th to the 6th intercostal space. Each of the 8 chest areas where visualised during normal respiration. The pattern analysed was the comet-tail artefact arising from the lung–wall interface (the hyperechogenic interface between the

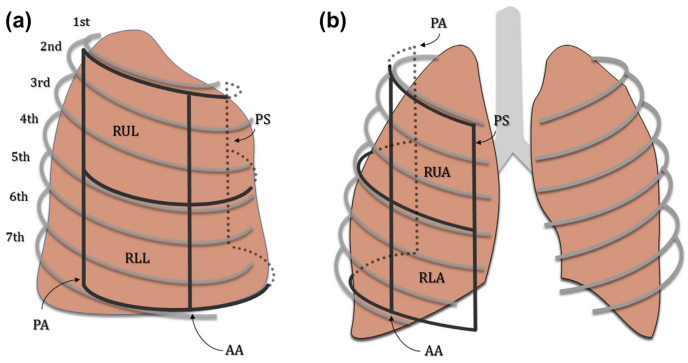

Figure 1 **a**: Lateral view of the right lung. Schematic representation of the areas scanned in relation to ribs (numbered) and intercostal spaces. **b**: Anterior view of the lungs. Schematic representation of the areas scanned (right hemithorax) in relation to ribs and intercostal spaces. (**PA**: posterior axillary line; **AA**: anterior axillary line; **PS**: parasternal line; **RUL**: right upper lateral area; **RLL**: right lower lateral area; **RUA**: right upper anterior area; **RLA**: right lower anterior area)

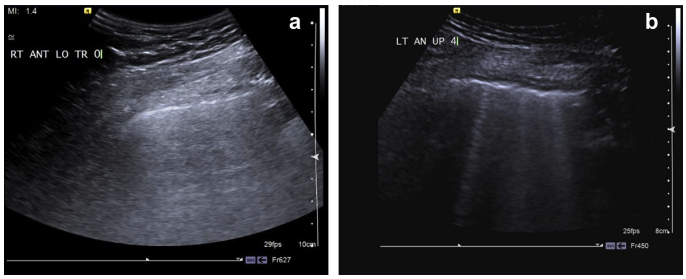

Figure 2 **a**: Sonographic image of normal lung. **b**: Anterior view of the lungs from a patient with AIS: Sonographic pattern of comet-tail artefacts vertical fanning out from the lung–wall interface and spreading up to the edge of the screen (B-lines)

chest wall and the lung surface), which was defined as a hyperechogenic narrow-based reverberation artefact, spreading like a laser-ray up to the edge of the screen[14] (Fig. 2). For each of the 8 quadrants, the number of comet-tail artefacts present was recorded and was linked with contemporaneous, prospectively collected clinical data.

Based on findings from previous studies,[14,15,17] comet-tail artefacts may be present in dependent regions of normally aerated lungs, and can be observed in healthy patients. Therefore, in order to optimize the protocol for sensitive detection of the comet-tail artefacts, three scan zones for each participant were compared, namely: (i). All lung quadrants, (ii). All lung quadrants except for the lower lateral, and (iii). Upper lung quadrants only, and the results were analysed.

Definitions

C-reactive protein

Plasma levels of C-reactive protein (CRP) have been shown to correlate well with the presence of pancreatic necrosis and severity of AP. However due to the late peak (36–72 h after admission) admission levels of CRP may not be useful in assessing severity.[18] Therefore, both CRP contemporaneous with the LUSS (highest value within the same 24-h period) and maximum CRP value during the first week of admission were used as surrogate markers of AP severity, by using 100 mg/L as the cut-off for the binary classification of the cohort.[19,20]

Alveolar-interstitial syndrome

Pulmonary diseases with involvement of the alveolar space and the interstitium are grouped under the term alveolar-interstitial syndrome (AIS) and include ARDS, pneumonia, acute cardiogenic pulmonary oedema, exacerbation of chronic interstitial lung disease and miscellaneous other pulmonary conditions.[11,14] The radiological diagnosis of AIS was based on the presence of alveolar opacities (ill-defined shadowing, confluent opacities with air bronchograms) and/or interstitial opacities (septal lines, linear, reticular, or nodular opacities) on chest X-ray (CXR), as previously proposed by Lichtenstein *et al.*[14]

Respiratory dysfunction

The worst value of PaO_2/FiO_2 for the 24-h period when each LUSS was performed was used as a metric of respiratory dysfunction. In accordance with the modified Multiple Organ Dysfunction Syndrome (MODS) score recommended in the revised Atlanta guidelines for AP,[16] a threshold value of 300 was used to divide the cohort into two groups, since those with $PaO_2/FiO_2 < 300$ have a modified MODS score equal or greater than 2. When an arterial blood gas (ABG) measurement was not available, the partial arterial pressure of oxygen (PaO_2) was extrapolated from the pulse oximetric oxygen saturation ($SatO_2$) by applying the method described by Severinghaus,[21] previously used in different settings.[22] For patients receiving supplemental oxygen, the fraction of inspired oxygen (FiO_2) was estimated as described Banks *et al.*,[16] modified as shown in Table 1.

Statistical analysis

Data for continuous variables are presented as mean ± standard deviation (SD) or as median and interquartile range (IQR).

Table 1 Fraction of inspired oxygen for non-ventilated patients (modification of the values provided in the revised Atlanta guidelines for acute pancreatitis)

Supplemental oxygen (L/min)	FiO_2 (ratio)
Room air	0.21
1–2	0.25
3–4	0.30
5–8	0.40
9–10	0.50
11–12	0.60
15	0.90

Categorical variables are presented as absolute and relative frequencies. Comparisons between independent groups were performed using the Mann–Whitney U-test. Spearman's rho was used to examine correlations between pairs of non-parametric variables. All statistical tests were based on a two-sided α-value of 0.05.

For early and late scans, independent samples analysis was performed with the cohort stratified by, (i) respiratory dysfunction ($PaO_2/FiO_2 < 300$ versus $PaO_2/FiO_2 \geq 300$), (ii) disease severity (severe versus non-severe), based on the revised Atlanta criteria for AP (modified MODS score <2 vs. score ≥2), (iii) CRP value contemporaneous with LUSS (contemporaneous CRP < 100 mg/L vs. CRP ≥ 100 mg/L), and, (iv) maximum CRP value within the first week of admission (maximum CRP < 100 mg/L vs. CRP ≥ 100 mg/L).

Receiver Operator Characteristic (ROC) curves were plotted for each comparison. Areas under the curve (AUC) and measures of diagnostic accuracy with respective 95% confidence intervals (95% C.I.) were calculated, with the use of 'pROC' package[23] of RStudio version 0.98.1091 (RStudio, Inc.), after identifying ROC curve thresholds by the use of Youden's index. AUC for paired ROC curves were compared with the DeLong method.[24] Additional statistical analyses were performed with IBM SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA). Figures were designed using GraphPad Prism Version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Formal blinding of the LUSS readers was not feasible due to the nature of the investigations. The principles of the STARD (*STAndards for the Reporting of Diagnostic accuracy studies*) statement have been adhered to.[25]

Results

Demographics

Forty-one patients were enrolled in the study. Based on the revised Atlanta guidelines, 32 of 41 (78%) participants had a clear diagnosis of AP, whereas the remaining 9 patients had hyperamylasaemia that did not satisfy the revised Atlanta definition of AP. All patients were included in the analysis. Of the patients with definite AP, 12 participants (37.5%) had mild AP, 15 (46.9%) had moderately severe and 5 (15.6%) had severe disease. The demographic characteristics are summarized in Table 2.

Fifty-one LUSS were performed, of which 33 (64.7%) were performed early in the disease course, and 18 (35.3%) were performed at a late stage. With the exception of one patient who underwent an early scan on day 5 and a late scan on day 11 after recruitment, scans performed within 4 days from patient recruitment were defined as early scans and those performed later than 4 days were defined as late. Early scans were performed after a median of 3 days from the initial finding of elevated amylase (IQR: 1.5–4 days), and late scans after a median of 6 days (IQR: 5–9.3 days). With regard to onset of symptoms, early scans were performed after a median of 5 days (IQR: 3–7 days), and late scans after a median of 8.5 days (IQR: 6–12.3 days). Twenty-three participants (53.7%) underwent an early scan only, 8 (22%) underwent a late scan and 10 (24.4%) underwent both. One late scan performed on day 4 after recruitment (on a participant who underwent an early scan on day 1) was excluded from further analysis.

Early scans

In patients with respiratory dysfunction (n = 7), a greater number of comet tails were evident on LUSS when compared to patients without respiratory dysfunction (n = 26), both when all lung quadrants except for the lower lateral were examined, and when upper quadrants alone were considered (P = 0.030 and P = 0.021 respectively). When all lung quadrants were taken into consideration the difference did not reach statistical significance (P = 0.067). Conversely, when contemporaneous CRP was examined, borderline significant difference was shown with the methods using all lung quadrants and all quadrants except for the lower lateral (P = 0.048 for both methods), but only a tendency for the method using only upper quadrants (P = 0.074). When severity status was used as the defining parameter for the cohort, patients with severe AP (n = 5) had a greater number of comet tails than others (n = 28), by all three methods. Lastly, the number of comet tails was significantly different by all three methods when maximum CRP value of the first week of admission was examined. The number of comet tails identified by each method is summarized in Table 3 and group comparisons are depicted in Fig. 3.

With respect to respiratory dysfunction and disease severity, the AUC was greater for the method examining upper lung quadrants alone (AUC = 0.783, 95% C.I.: 0.544–0.962, P = 0.023 and AUC = 0.996, 95% C.I.: 0.986–1.000, P < 0.001, respectively). Pairwise AUC comparisons between methods examining all quadrants, all quadrants except the lower lateral and upper quadrants alone, did not reveal superiority for either variable of interest (Fig. 4).

The greatest AUC for contemporaneous and maximum CRP resulted form examining all lung quadrants except for the lower lateral (AUC = 0.708, 95% C.I.: 0.510–0.883, P = 0.047, and AUC = 0.800, 95% C.I.: 0.640–0.929, P = 0.004 respectively), as depicted on Fig. 4. For contemporaneous CRP, pairwise comparison did not reveal superiority of either of the three methods whereas for maximum CRP, examining all quadrants except for the lower lateral was superior to upper quadrants alone (P = 0.011).

For each method, the derived threshold for the number of comet tails, the corresponding sensitivity (SE) and specificity (SP), positive (PPV) and negative predictive values (NPV), along with respective 95% C.I., as well as the correlation coefficients of the number of comet tails with PaO_2/FiO_2, modified MODS score, contemporaneous and maximum CRP are presented in Table 4.

Table 2 Demographic characteristics of study participants

	Overall Patient sample	Hyperamylasaemia[a]	Mild AP	Moderate AP	Severe AP
n	41	9	12	15	5
Age (years)					
Median	59.1	59.1	69.7	57	49.4
IQR	49.2–67.5	50.7–64.6	50–81.6	49.2–67.5	45.8–63.9
Gender – ratio, (%)					
Males	21/41 (51.2)	5/9	6/12	8/15	2/5
Females	20/41 (48.8)	4/9	6/12	7/15	3/5
BMI					
Median	28	26	27	29	32
IQR	23–32	23–28	23–30	24–35	30–33
AP Aetiology – ratio, (%)					
Gallstones	19/32 (59.4)	N/A	7/12	11/15	1/5
Alcohol	6/32 (18.8)	N/A	2/12	1/15	3/5
Idiopathic	5/32 (15.6)	N/A	2/12	2/15	1/5
Other	2/32 (6.25)	N/A	1/12	1/15	0/5
N/A	9/41 (22.0)	9/9	N/A	N/A	N/A
Severity – ratio, (%)					
Mild	12/41 (29.3)	N/A	16/16	N/A	N/A
Moderately Severe	15/41 (35.6)	N/A	N/A	11/11	N/A
Severe	5/41 (12.2)	N/A	N/A	N/A	5/5
N/A	9/41 (22.0)	9/9	N/A	N/A	N/A
Amylase (IU/L)[b]					
Median	507	145	1045	642	669
IQR	230–1143	138–197	434–1435	324–2314	654–1143
CRP (mg/L)[c]					
Median	66	13	29	154	133
IQR	24–146	6–26	12–71	46–241	119–146
APACHE II score[d]					
Median	10	12	9	10	17
IQR	8.5–16.5	9–17	6–12	9–15	10–31
SIRS[e]					
Ratio (%)	23/41 (56.1%)	6/9	6/12	6/15	5/5
Modified MODS score					
Median	1	2	1	1	3
IQR	1–2	1–3	1–2	1–2	2–8
PaO_2/FiO_2 ratio[f]					
Median	328	360	360	319	223
IQR	282–361	268–390	333–390	291–337	95–223

BMI, body mass Index; **LOS**, length of hospital stay; **CRP**, C-reactive protein; **MODS**, multiple organ dysfunction syndrome; **APACHE**, Acute Physiology and Chronic Health Evaluation score; **PaO_2**, partial arterial pressure of oxygen; **FiO_2**, fraction of inspired oxygen; **IQR**, interquartile range; **N/A**, not applicable.
[a] Participants who did not meet the criteria for acute pancreatitis, according to the 2012 revised Atlanta guidelines.
[b] Worst value of serum amylase on the first 24 h of admission.
[c] Worst value of CRP on the first 24 h of admission upon recruitment.
[d] Based on most extreme values of the first 24 h of admission.
[e] Based on most extreme values of the first 24 h of admission.
[f] As defined in methods section.

Table 3 Descriptive summary of the number of comet tails identified by each method (early scans)

	Respiratory dysfunction							
	PaO_2/FiO_2 < 300			PaO_2/FiO_2 ≥ 300			Sub-group comparison[a] P-value	Correlation[b] with PaO_2/FiO_2 (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	7	9	7–10	26	2	1–8	0.067	–0.456 (0.008)
All lung quadrants except for the lower lateral	7	7	5–9	26	2	0–6	0.030	–0.470 (0.006)
Upper lung quadrants	7	5	3–9	26	1	0–3	0.021	–0.409 (0.018)
	Disease severity							
	Severe			Non-severe			Sub-group comparison[a] P-value	Correlation[b] with modified MODS score (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	5	11	10–20	28	2	1–8	0.001	0.388 (0.026)
All lung quadrants except for the lower lateral	5	10	9–13	28	2	0–6	0.001	0.426 (0.014)
Upper lung quadrants	5	8	7–9	28	1	0–3	<0.001	0.396 (0.023)
	Contemporaneous CRP							
	≥100			<100			Sub-group comparison[a] P-value	Correlation[b] with CRP on the day of LUSS (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	13	9	6–10	20	2	1–8	0.048	0.406 (0.019)
All lung quadrants except for the lower lateral	13	6	2–9	20	2	0–5	0.048	0.354 (0.043)
Upper lung quadrants	13	4	1–6	20	1	0–3	0.074	0.306 (0.083)
	Maximum CRP							
	≥100			<100			Sub-group comparison[a] P-value	Correlation[b] with maximum CRP (P-value)
	n	Median	IQR	n	Median	IQR		
All lung quadrants	20	8	4–10	13	2	0–2	0.005	0.395 (0.023)
All lung quadrants except for the lower lateral	20	6	2–9	13	0	0–2	0.003	0.391 (0.024)
Upper lung quadrants	20	3	1–6	13	0	0–2	0.040	0.323 (0.066)

[a] Independent samples Mann–Whitney U test.
[b] Spearman correlation coefficient.

Late scans

None of the 19 patients who underwent LUSS late in the course of the disease had concurrent respiratory dysfunction, therefore it was not feasible to examine the diagnostic accuracy of late scans in this context. No significant correlation between the number of late LUSS comet tails and either corresponding disease severity (contemporaneous modified MODS score), respiratory dysfunction (PaO_2/FiO_2), disease severity on admission (as defined by the revised Atlanta criteria), or contemporaneous and maximum CRP was discovered, regardless of the method used (data not presented).

X-rays

Twenty-eight of the 33 participants (84.8%) who underwent an early LUSS, also had at least one prior CXR as part of their routine clinical management, and 15 (53.6%) of these showed AIS. Although LUSS were performed a median of 2 days (IQR: 1–3 days) after the CXR, a satisfactory diagnostic association was demonstrated between CXR diagnosed AIS and LUSS findings (Table 4).

Discussion

Respiratory dysfunction is the most frequent and clinically relevant manifestation of extra-pancreatic organ injury in AP.[6,7,26] In patients with SAP, it constitutes a major determinant of mortality and often precedes renal, cardiovascular and hepatic dysfunction.[7,27,28] Several hypotheses have been explored and several associated inflammatory mediators and signalling pathways have been identified regarding the underlying pathophysiology,[8,29,30] however the exact mechanisms remain incompletely understood. The spectrum of sequelae of AP-associated respiratory dysfunction ranges from subclinical hypoxaemia to severe ARDS,[31,32] and radiologically evident complications such as

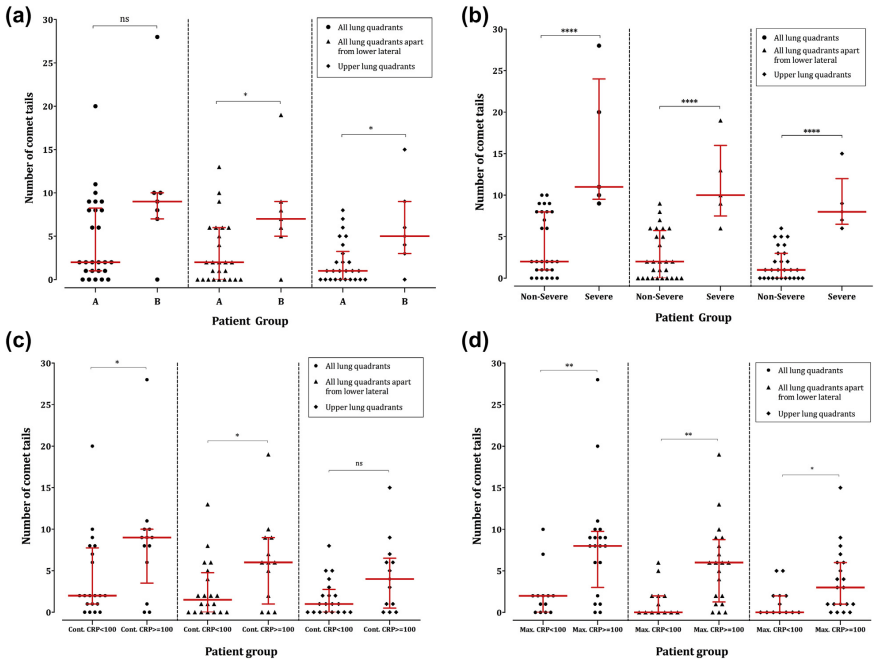

Figure 3 Scatterplot of the total number of comet tails per patient group as determined by: **a**. The respiratory dysfunction status ($PaO_2/FiO_2 < 300$ vs. $PaO_2/FiO_2 \geq 300$). Red lines represent median and IQR for each patient group. **A**: $PaO_2/FiO_2 \geq 300$; **B**: $PaO_2/FiO_2 < 300$; **ns**: $P \geq 0.05$; *: $P < 0.05$ – **b**. The severity status (Severe vs. Non-severe). Red lines represent median and interquartile range for each patient group. ****: $P < 0.001$ – **c**. Contemporaneous CRP – **d**. Maximum value of CRP. **: $P < 0.01$

atelectasis, pleural effusion, and pulmonary infiltrates and/or oedema may frequently be present, amongst others.[33,34] Nevertheless, hypoxaemia may precede radiological findings and has been described as an early indicator of pathological pulmonary changes.[10] Consequently, a timely and accurate detection of evolving respiratory dysfunction may aid in identifying high risk patients and may expedite the implementation of appropriate management.

Although the lung parenchyma has historically been considered not amenable to assessment by ultrasound, lung sonography has undergone rapid development and its diagnostic potential has improved in recent years.[13] Several studies exploring the value of LUSS in the diagnosis of AIS in acutely ill patients have emerged,[11,12,14,15,35] with reports of high sensitivity and specificity.[11,14,36] The sonographic diagnosis of AIS relies on the detection of multiple and diffuse comet tail B lines,[12] described as vertical artefacts fanning out from the lung–wall interface and spreading up to the edge of the screen, resulting from thickened interlobular septa and extravascular lung water.[14] In addition, LUSS has been proposed as advantageous to other imaging techniques; bedside X-rays pose diagnostic challenges[14,15] whereas CT can be time-consuming and hazardous for critically ill patients requiring transfer,[11,37] and is associated with significant radiation exposure.

Stratifying severity in AP is challenging. Early stratification into moderately severe (transient organ dysfunction) and severe AP (persistent organ dysfunction) disease groups for clinical and academic purposes is bound by limitations inherent in the definitions, such as the requirement for a period of at least 48 h before a definite conclusion about severity has been reached. Similar to several previous classification systems,[31,38–40] the revised Atlanta guidelines use the long established PaO_2/FiO_2 ratio as a measure of respiratory dysfunction, which although undoubtedly invaluable, constitutes a relatively non-specific prognostic marker, a fact

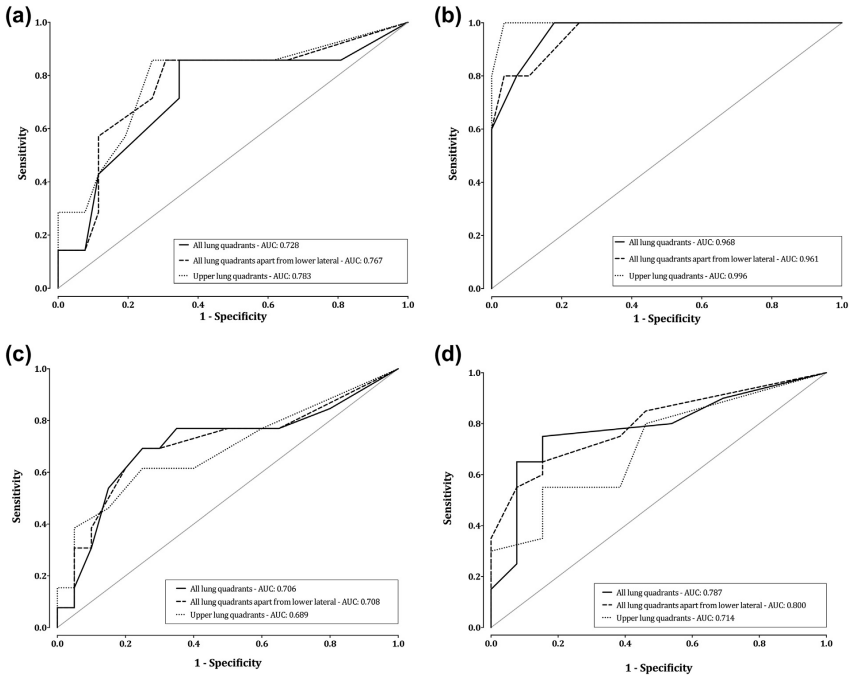

Figure 4 Receiver operator characteristic curves of the number of comet tails by: **a**. Respiratory dysfunction status – **b**. Disease severity status – **c**. Contemporaneous CRP – **d**. Maximum value of CRP

already noted by Ranson *et al.* four decades ago.[41] The introduction of transthoracic lung ultrasound as a method supplementary to PaO_2/FiO_2 would aim to increase prognostic efficiency in AP. The hope is that it may potentially provide a valid and accurate measure of respiratory dysfunction and severity early in the disease course, by differentiating between established pathological pulmonary changes and transient physiological impairment. If validated, this non-invasive, easily repeatable bedside method would allow for a more timely patient stratification in comparison to and/or complementary to existing scores.

The findings of the present study demonstrate a useful diagnostic association of early LUSS with disease severity and respiratory dysfunction, for all three methods applied, particularly when all lung quadrants excluding the lower lateral and when upper quadrants only were examined. More comet tail artefacts are present in patients with respiratory dysfunction, severe disease and/or elevated CRP, and thresholds for the number of comet-tails have been proposed. The routine use of LUSS in the early stage of AP appears to be attractive as an initial proposition for further research, as a non-invasive, easily repeatable bedside adjunct that provides an accurate evaluation of disease severity and respiratory dysfunction.

The present study has several potential limitations. The cohort size is relatively small, however as a pilot evaluation of this diagnostic approach it is considered adequate. The number of late scans performed is insufficient to comment on its utility in the late phase of the disease and the small number of patients who underwent both early and late scans (n = 10) has not allowed for a valid comparison between these two groups. However, given that organ dysfunction and mortality are early events in AP, this fact does not obviate the utility of this approach, and a possibly slower resolution of the pathological pulmonary changes in comparison to the anticipated clinical recovery of patients with non-severe AP may raise questions on the potential application of late scans in monitoring disease progress.

Table 4 Areas under the curve, thresholds for the number of comet tails and parameters of diagnostic accuracy for each method, with regard to respiratory dysfunction, disease severity status and CRP. In order to examine the diagnostic association between the scan findings and each dichotomous variable, a value equal to or greater than the respective threshold of comet tails has been applied

		AUC (95% C.I.)	Threshold	Sensitivity (ratio; 95% C.I.)	Specificity (ratio; 95% C.I.)	PPV (ratio)	NPV (ratio)	Likelihood ratio
Respiratory dysfunction	All lung quadrants	0.728 (0.481–0.926)	7	0.857 (6/7; 0.571–1.000)	0.654 (17/26; 0.462–0.809)	0.400 (6/15)	0.944 (17/18)	2.5
	All lung quadrants except for the lower lateral	0.767 (0.544–0.948)	5	0.857 (6/7; 0.571–1.000)	0.692 (18/26; 0.500–0.846)	0.429 (6/14)	0.947 (18/19)	2.8
	Upper lung quadrants	0.783 (0.544–0.962)	3	0.857 (6/7; 0.571–1.000)	0.731 (19/26; 0.539–0.885)	0.462 (6/13)	0.950 (19/20)	3.2
Severity	All lung quadrants	0.968 (0.893–1.000)	9	1.000 (5/5; 1.000–1.000)	0.821 (23/28; 0.679–0.964)	0.714 (5/7)	1.000 (26/26)	5.6
	All lung quadrants except for the lower lateral	0.961 (0.868–1.000)	9	0.800 (4/5; 0.400–1.000)	0.964 (27/28; 0.893–1.000)	0.800 (4/5)	0.964 (27/28)	22.2
	Upper lung quadrants	0.996 (0.986–1.000)	6	1.000 (5/5; 1.000–1.000)	0.964 (27/28; 0.893–1.000)	0.833 (5/6)	1.000 (27/27)	27.8
CRP contemporaneous with early USS	All lung quadrants	0.706 (0.494–0.892)	8	0.692 (9/13; 0.462–0.923)	0.750 (15/20; 0.550–0.950)	0.643 (9/14)	0.789 (15/19)	2.8
	All lung quadrants except for the lower lateral	0.708 (0.510–0.883)	5	0.692 (9/13; 0.462–0.923)	0.750 (15/20; 0.550–0.900)	0.643 (9/14)	0.789 (15/19)	2.8
	Upper lung quadrants	0.689 (0.513–0.889)	3	0.615 (8/13; 0.308–0.846)	0.750 (15/20; 0.550–0.900)	0.615 (8/13)	0.750 (15/20)	2.5
Maximum CRP of the first 7 days of admission	All lung quadrants	0.787 (0.610–0.937)	4	0.750 (15/20; 0.550–0.900)	0.846 (11/13; 0.615–1.000)	0.882 (15/17)	0.689 (11/16)	4.9
	All lung quadrants except for the lower lateral	0.800 (0.640–0.929)	3	0.650 (13/20; 0.450–0.850)	0.846 (11/13; 0.615–1.000)	0.867 (13/15)	0.611 (11/18)	4.2
	Upper lung quadrants	0.714 (0.529–0.864)	3	0.550 (11/20; 0.350–0.750)	0.846 (11/13; 0.615–1.000)	0.846 (11/13)	0.550 (11/20)	3.6
CXR diagnosed AIS	All lung quadrants	0.859 (0.705–0.980)	8	0.733 (11/15; 0.467–0.933)	0.923 (12/13; 0.769–1.000)	0.917 (11/12)	0.750 (12/16)	9.5
	All lung quadrants except for the lower lateral	0.818 (0.639–0.949)	3	0.733 (11/15; 0.467–0.933)	0.846 (11/13; 0.615–1.000)	0.846 (11/13)	0.733 (11/15)	4.8
	Upper lung quadrants	0.810 (0.631–0.946)	3	0.667 (10/15; 0.400–0.867)	0.923 (12/13; 0.769–1.000)	0.909 (10/11)	0.706 (12/17)	8.7

AUC, area under the curve; **95% C.I.**, 95% confidence interval, ×2000 bootstrap; **PPV**, positive predictive value; **NPV**, negative predictive value; **N/A**, not applicable.

Moreover, the inter-observer variability of scan findings has not been examined, and despite the fact that satisfactory results are already available from previous studies,[35] such data would have provided useful information on a method generally characterized by operator dependency. With regard to methodology, the convention used to extrapolate PaO_2/FiO_2 from $SatO_2$ may have introduced unknown error in the analysis, as the Severinghaus equation has not been validated for calculation

of the modified MODS score in AP patient cohorts. Nevertheless, $SatO_2$ has previously been used for similar approximations, with reasonable accuracy.[42,43] Additionally, the timing of CXRs for study participants was not altered from ordinary clinical care. LUSS were in general performed after CXRs, therefore the accuracy of the association between the findings of the two imaging methods may be confounded by this discrepancy.

The fact that early scans were performed a median of 3 days after the first finding of an elevated serum amylase, may have concealed an even greater prognostic association with early respiratory dysfunction. The exact prognostic features of early LUSS deserve further investigation and future studies are likely to benefit from standardizing the timing of scans within the first 48 h. Nevertheless, the fact that scans performed very early may carry a high false negative ratio has to be taken into consideration, as at that stage developing pulmonary involvement may not yet be identifiable with this method.

In conclusion, ultrasonography of non-dependent lung parenchyma can reliably detect evolving respiratory dysfunction in AP, and shows promise as an adjunct to severity stratification. This simple, easily repeatable bedside test warrants further validation in a larger cohort.

Meetings

Results from this study have been presented in the following meetings:

1. Annual Scientific Meeting of the Pancreatic Society of Great Britain and Ireland (26th–28th of November 2014) – Poster and short oral presentation
2. Alpine Liver and Pancreatic Surgery Meeting 2015 (ALPS 2015 – 4th–8th of February 2015) – Oral presentation
3. 27th European Congress of Radiology (4th–8th of March 2015) – e-poster
4. Digestive Disorders Federation 2015 meeting (DDF 2015–22nd–25th of June 2015) – Oral presentation

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Sources of funding

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Conflicts of interest

None declared.

References

1. Goldacre MJ, Roberts SE. (2004) Hospital admission for acute pancreatitis in an English population, 1963–98: database study of incidence and mortality. *BMJ* 328:1466–1469.
2. Toh SK, Phillips S, Johnson CD. (2000) A prospective audit against national standards of the presentation and management of acute pancreatitis in the South of England. *Gut* 46:239–243.
3. Ashley SW, Perez A, Pierce EA, Brooks DC, Moore, FD, Jr., Whang EE *et al*. (2001) Necrotizing pancreatitis: contemporary analysis of 99 consecutive cases. *Ann Surg* 234:572–579. discussion 9–80.
4. Isenmann R, Rau B, Beger HG. (1999) Bacterial infection and extent of necrosis are determinants of organ failure in patients with acute necrotizing pancreatitis. *Br J Surg* 86:1020–1024.
5. McKay CJ, Evans S, Sinclair M, Carter CR, Imrie CW. (1999) High early mortality rate from acute pancreatitis in Scotland, 1984–1995. *Br J Surg* 86:1302–1305.
6. Mole DJ, Olabi B, Robinson V, Garden OJ, Parks RW. (2009) Incidence of individual organ dysfunction in fatal acute pancreatitis: analysis of 1024 death records. *HPB* 11:166–170.
7. Johnson CD, Abu-Hilal M. (2004) Persistent organ failure during the first week as a marker of fatal outcome in acute pancreatitis. *Gut* 53: 1340–1344.
8. Zhou MT, Chen CS, Chen BC, Zhang QY, Andersson R. (2010) Acute lung injury and ARDS in acute pancreatitis: mechanisms and potential intervention. *World J Gastroenterol* 16:2094–2099.
9. Pastor CM, Matthay MA, Frossard JL. (2003) Pancreatitis-associated acute lung injury: new insights. *Chest* 124:2341–2351.
10. Polyzogopoulou E, Bikas C, Danikas D, Koutras A, Kalfarentzos F, Gogos CA. (2004) Baseline hypoxemia as a prognostic marker for pulmonary complications and outcome in patients with acute pancreatitis. *Dig Dis Sci* 49:150–154.
11. Stefanidis K, Dimopoulos S, Kolofousi C, Cokkinos DD, Chatzimichail K, Eisen LA *et al*. (2012) Sonographic lobe localization of alveolar-interstitial syndrome in the critically ill. *Crit Care Res Pract* 2012:179719.
12. Volpicelli G, Mussa A, Garofalo G, Cardinale L, Casoli G, Perotto F *et al*. (2006) Bedside lung ultrasound in the assessment of alveolar-interstitial syndrome. *Am J Emerg Med* 24:689–696.
13. Soldati G, Copetti R, Sher S. (2009) Sonographic interstitial syndrome: the sound of lung water. *J Ultrasound Med* 28:163–174.
14. Lichtenstein D, Meziere G, Biderman P, Gepner A, Barre O. (1997) The comet-tail artifact. An ultrasound sign of alveolar-interstitial syndrome. *Am J Respir Crit Care Med* 156:1640–1646.
15. Bouhemad B, Zhang M, Lu Q, Rouby JJ. (2007) Clinical review: bedside lung ultrasound in critical care practice. *Crit Care* 11:205.
16. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG *et al*., Acute Pancreatitis Classification Working Group. (2013) Classification of acute pancreatitis – 2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 62:102–111.
17. Wernecke K, Galanski M, Peters PE, Hansen J. (1987) Pneumothorax: evaluation by ultrasound–preliminary results. *J Thorac Imaging* 2:76–78.
18. Banks PA, Freeman ML, & Practice Parameters Committee of the American College of G. (2006) Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 101:2379–2400.
19. Puolakkainen P, Valtonen V, Paananen A, Schroder T. (1987) C-reactive protein (CRP) and serum phospholipase A2 in the assessment of the severity of acute pancreatitis. *Gut* 28:764–771.
20. Mayer AD, McMahon MJ, Bowen M, Cooper EH. (1984) C reactive protein: an aid to assessment and monitoring of acute pancreatitis. *J Clin Pathol* 37:207–211.
21. Severinghaus JW. (1979) Simple, accurate equations for human blood O_2 dissociation computations. *J Appl Physiol* 46:599–602.

22. Dean NC, Jones JP, Aronsky D, Brown S, Vines CG, Jones BE *et al*. (2012) Hospital admission decision for patients with community-acquired pneumonia: variability among physicians in an emergency department. *Ann Emerg Med* 59:35–41.

23. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC *et al*. (2011) pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinform* 12:77.

24. DeLong ER, DeLong DM, Clarke-Pearson DL. (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*, 837–845.

25. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM *et al*., Standards for Reporting of Diagnostic Accuracy. (2003) Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD Initiative. *Radiology* 226:24–28.

26. Renner IG, Savage, WT, III, Pantoja JL, Renner VJ. (1985) Death due to acute pancreatitis. *Dig Dig Sci* 30:1005–1018.

27. Buter A, Imrie CW, Carter CR, Evans S, McKay CJ. (2002) Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. *Br J Surg* 89:298–302.

28. Bhatia M, Brady M, Shokuhi S, Christmas S, Neoptolemos JP, Slavin J. (2000) Inflammatory mediators in acute pancreatitis. *J Pathol* 190: 117–125.

29. Shields CJ, Winter DC, Redmond HP. (2002) Lung injury in acute pancreatitis: mechanisms, prevention, and therapy. *Curr Opin Crit Care* 8:158–163.

30. Chooklin S. (2009) Pathogenic aspects of pulmonary complications in acute pancreatitis patients. *Hepatobiliary Pancreat Dis Int* 8:186–192.

31. Imrie CW, Benjamin IS, Ferguson JC, McKay AJ, Mackenzie I, O'Neill J *et al*. (1978) A single-centre double-blind trial of Trasylol therapy in primary acute pancreatitis. *Br J Surg* 65:337–341.

32. Ranson JH, Turner JW, Roses DF, Rifkind KM, Spencer FC. (1974) Respiratory complications in acute pancreatitis. *Ann Surg* 179:557–566.

33. Basran GS, Ramasubramanian R, Verma R. (1987) Intrathoracic complications of acute pancreatitis. *Br J Dis Chest* 81:326–331.

34. Raghu MG, Wig JD, Kochhar R, Gupta D, Gupta R, Yadav TD *et al*. (2007) Lung complications in acute pancreatitis. *JOP* 8:177–185.

35. Via G, Storti E, Gulati G, Neri L, Mojoli F, Braschi A. (2012) Lung ultrasound in the ICU: from diagnostic instrument to respiratory monitoring tool. *Minerva Anestesiol* 78:1282–1296.

36. Lichtenstein D, Goldstein I, Mourgeon E, Cluzel P, Grenier P, Rouby JJ. (2004) Comparative diagnostic performances of auscultation, chest radiography, and lung ultrasonography in acute respiratory distress syndrome. *Anesthesiology* 100:9–15.

37. Beckmann U, Gillies DM, Berenholtz SM, Wu AW, Pronovost P. (2004) Incidents relating to the intra-hospital transfer of critically ill patients. An analysis of the reports submitted to the Australian Incident Monitoring Study in Intensive Care. *Intensive Care Med* 30:1579–1585.

38. Ranson JH, Rifkind KM, Roses DF, Fink SD, Eng K, Spencer FC. (1974) Prognostic signs and the role of operative management in acute pancreatitis. *Surg Gynecol Obstet* 139:69–81.

39. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. (1985) APACHE II: a severity of disease classification system. *Crit Care Med* 13: 818–829.

40. Harrison DA, D'Amico G, Singer M. (2007) The Pancreatitis Outcome Prediction (POP) Score: a new prognostic index for patients with severe acute pancreatitis. *Crit Care Med* 35:1703–1708.

41. Ranson JH, Roses DF, Fink SD. (1973) Early respiratory insufficiency in acute pancreatitis. *Ann Surg* 178:75–79.

42. Neto AS, Cardoso SO, Ong DS, Espósito DC, Pereira VG, Manetta JA *et al*. (2013) The use of the pulse oximetric saturation/fraction of inspired oxygen ratio for risk stratification of patients with severe sepsis and septic shock. *J Crit Care* 28:681–686.

43. Pandharipande PP, Shintani AK, Hagerman HE, St Jacques PJ, Rice TW, Sanders NW *et al*. (2009) Derivation and validation of SpO_2/FiO_2 ratio to impute for PaO_2/FiO_2 ratio in the respiratory component of the Sequential Organ Failure Assessment score. *Crit Care Med* 37: 1317–1321.

INCREASED LEVELS OF 3-HYDROXYKYNURENINE PARALLEL DISEASE SEVERITY IN HUMAN ACUTE PANCREATITIS

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Increased levels of 3-hydroxykynurenine parallel disease severity in human acute pancreatitis

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Inhibition of kynurenine 3-monooxygenase (KMO) protects against multiple organ dysfunction (MODS) in experimental acute pancreatitis (AP). We aimed to precisely define the kynurenine pathway activation in relation to AP and AP-MODS in humans, by carrying out a prospective observational study of all persons presenting with a potential diagnosis of AP for 90 days. We sampled peripheral venous blood at 0, 3, 6, 12, 24, 48, 72 and 168 hours post-recruitment. We measured tryptophan metabolite concentrations and analysed these in the context of clinical data and disease severity indices, cytokine profiles and C-reactive protein (CRP) concentrations. 79 individuals were recruited (median age: 59.6 years; 47 males, 59.5%). 57 met the revised Atlanta definition of AP: 25 had mild, 23 moderate, and 9 severe AP. Plasma 3-hydroxykynurenine concentrations correlated with contemporaneous APACHE II scores ($R^2 = 0.273$; Spearman rho = 0.581; $P < 0.001$) and CRP ($R^2 = 0.132$; Spearman rho = 0.455, $P < 0.001$). Temporal profiling showed early tryptophan depletion and contemporaneous 3-hydroxykynurenine elevation. Furthermore, plasma concentrations of 3-hydroxykynurenine paralleled systemic inflammation and AP severity. These findings support the rationale for investigating early intervention with a KMO inhibitor, with the aim of reducing the incidence and severity of AP-associated organ dysfunction.

Acute pancreatitis (AP) is a sterile, localized inflammation of the pancreas. In approximately 25% of affected patients, AP results in a systemic inflammatory response leading to multiple organ dysfunction syndrome (MODS)[1,2]. MODS commonly develops early after the onset of AP[3]. Persons who develop AP–MODS require invasive monitoring and organ support in a critical care environment, with an in-hospital mortality rate that may reach 36–50%[4,5]. Moreover, survivors of AP-MODS require a prolonged hospital stay and have a reduced overall life expectancy compared to those with mild AP[6]. Therefore, early recognition and stratification of AP severity is essential.

The exact pathological mechanisms that drive AP–MODS remain unclear, but the kynurenine pathway of tryptophan metabolism (Fig. 1) is emerging as a potentially important contributory mechanism[7,8]. In an

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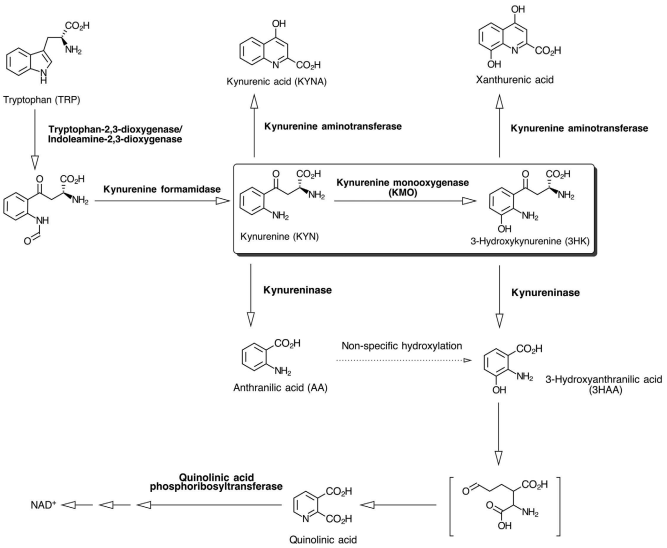

Figure 1. The kynurenine pathway of tryptophan metabolism. The key step of kynurenine conversion to 3-hydroxykynurenine via the action of the gate-keeper enzyme kynurenine 3-monooxygenase (KMO), is highlighted.

inflammatory context, metabolism of tryptophan through the kynurenine pathway is dramatically increased through induction of indoleamine 2,3-dioxygenase (IDO) expression by pro-inflammatory cytokines, in particular, interferon gamma (IFN-γ)[9]. IDO upregulation increases substrate flux through downstream enzymes, in particular kynurenine 3-monooxygenase (KMO), thereby generating biologically–active metabolites that participate in multiple physiological and pathological processes[10–15]. In rodent models of AP, 3-hydroxykynurenine, the product of the gate-keeper enzyme kynurenine 3-monooxygenase (KMO), appears to be critical to the pathogenesis of AP-MODS[7]. 3-hydroxykynurenine causes tissue injury via oxidative stress and pathological cross-linking of proteins[16]. Therapeutic blockade of KMO by genetic deletion in mice or pharmacological inhibition in rats reduces 3-hydroxykynurenine formation and protects against lung and kidney injury in experimental models of AP[7]. Other metabolites in the kynurenine pathway have been postulated to mediate immunological tolerance dependent on signalling via the aryl hydrocarbon receptor, whilst kynurenine specifically may contribute to T-helper Th17 and T-regulatory lymphocyte differentiation[17,18].

In critically ill individuals, rapid and profound tryptophan degradation can occur[19]. Indeed, tryptophan depletion and/or elevated concentrations of kynurenine metabolites have been detected in the plasma of major trauma patients, and in patients following cardiac bypass surgery, while elevated serum kynurenine concentrations are associated with the need for renal replacement therapy and invasive ventilation in persons with AP-MODS[8,11,20,21]. However, the detailed profile of the kynurenine pathway and its role in the pathophysiology of AP has not been elucidated. In considering the therapeutic potential of KMO inhibition for AP, it is important to establish the precise timing and magnitude of activation of the kynurenine pathway in relation to the onset of AP and the development of AP-MODS, the inflammatory burden and overall AP severity. The aim of the present study, therefore, was to define the precise temporal and quantitative relationship between kynurenine pathway metabolism and the onset and severity of AP-associated organ dysfunction.

Results

Recruitment Performance. A total of 79 patients were recruited, 57 patients (72.2%) of whom were diagnosed with AP in accordance with the revised Atlanta guidelines ("true AP" – tAP). Of the 57 tAP patients, 10 had an amylase level below 300 upon presentation, and either a subsequent amylase rise or a confirmation of AP by imaging. The demographic characteristics of the study participants are summarized in Supplementary Table S1, and a CONSORT diagram is shown in Fig. 2. There was no effect of day of the week ($P = 0.317$) or time of day ($P = 0.397$) on recruitment. Moreover, time of day for recruitment (T0) followed a random distribution. The cumulative frequency of participant enrolment by calendar day of the recruitment phase and the relative frequency of enrolment by weekday are shown in Fig. 3a and Supplementary Table S2, respectively. No systematic bias was present for the twenty-nine potentially eligible patients that were not recruited. Thirty patients declined

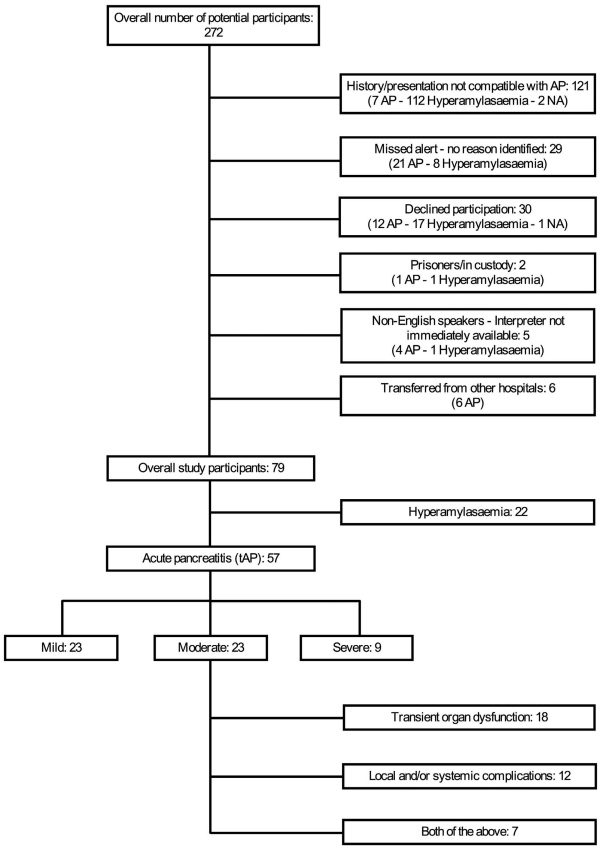

Figure 2. CONSORT diagram of the IMOFAP study. Reasons for patient exclusion are summarised, with post-hoc diagnoses for each exclusion category included in brackets (NA = not available). Patients of the moderate AP severity group were divided to three further sub-groups according to the revised Atlanta guidelines for explanatory purposes.

to participate, of whom 12 had AP and 3 required admission to critical care. One male participant formally withdrew from the study within 12 hours from recruitment.

Sampling performance was efficient, and a total of 508 samples were obtained and analysed. Due to the high temporal resolution design of the study, some sample time-points were missed, but no systematic bias was identified for the non-obtained samples. The median time from acute presentation to hospital (pre-recruitment sample) to recruitment was 5 hours 34 minutes (IQR: 182–611 minutes) for tAP participants (Fig. 3b). This interval included examination and consultation by the direct clinical care team(s), routinely collected acute presentation sample (pre-recruitment) receipt, processing and analysis, verification of the result by the hospital laboratory staff and contact with the study team for verification of the clinical history, and informed consent.

Inflammatory Indices, Composite Scores & Cytokines. A clear rise in CRP level was evident for tAP participants (standardized AUC for CRP concentration, median (IQR): hyperamylasaemia participants: 18.9 (5.75–45.4) mg/L; tAP participants: 126.0 (49.9–201.1); $P < 0.001$), the magnitude of which was proportionate to AP severity (Fig. 4a) and in keeping with a substantial systemic inflammatory insult (standardized AUC for CRP concentration, median (IQR): mild: 58.9 (26.3–131.2) mg/L; moderate: 148.1 (74.5–243.9) mg/L; severe: 225.6

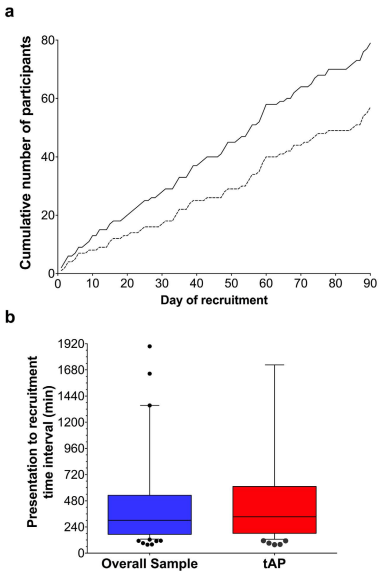

Figure 3. (**a**) Cumulative number of enrolled participants per calendar day of the recruitment phase of the study. Solid line: overall study participants; dashed line; tAP participants. (**b**) Box & whiskers plot of the time interval from patient presentation to hospital to study recruitment, in minutes. Blue box: overall study participants; red box tAP participants. Boxes: 1st and 3rd quartile; horizontal lines: median values; error bars: 1st and 9th deciles; dots: outlying values beyond 1st and 9th decile.

(174.9–260.2) mg/L; P = 0.001). The acute presentation (pre-recruitment) mean CRP concentrations were higher in severe AP, but this difference was not statistically significant (CRP concentration, median (IQR): mild: 11 (5–34) mg/L; moderate: 19 (9–39) mg/L; severe: 59 (30–65) mg/L; P = 0.166) (Fig. 4a and Supplementary Table S3). Resolution of inflammation, indicated by a fall in CRP, occurred between 48 and 72 hours for the majority of patients. Conversely, serum amylase was more than three times higher than the upper limit of the normal range upon recruitment, and normalised within 24 hours for the majority of participants. There was no association between serum amylase level upon presentation and disease severity (Fig. 4b). Furthermore, mean and minimum concentrations of albumin, as well as the corresponding mean standardized AUC for albumin were significantly lower in the severe AP group (Supplementary Table S3).

Severe AP patients had a significantly greater modified MODS score at presentation, when compared to the other two participant groups (mean MODS score, (SD; range): mild: 0.8 (0.7; 0–3); moderate: 0.8 (0.9, 0–4); severe: 2.8 (2.5; 0–8); P = 0.003), followed by a further rise during the initial 12-hour period after recruitment and apparent resolution (a reduction in MODS score to $\leq$2) beyond the 48-hour mark (Fig. 4c). No association was discovered between the time interval from the onset of symptoms to recruitment and the modified MODS score upon presentation of severe AP patients (Spearman's rho = −0.037, P = 0.926). Both day 1 APACHE II and T_{minus} APACHE II scores were higher in the severe AP group (day 1 APACHE II score, mean (SD): mild: 8.2 (4.4); moderate: 11.4 (3.8); severe 19.3 (11.0); P = 0.001, and T_{minus} APACHE II score, mean (SD): mild: 6.0 (3.6); moderate: 7.6 (3.8); severe: 12.0 (5.6); P = 0.002). APACHE II scores also declined after 48 hours, with the decrease being more prominent from T72 onwards (Fig. 4d). Importantly, the decline of both modified MODS and APACHE II scores (calculated for each time-point) coincided with and is due to the deaths of two patients in the severe AP group, who died within 55 and 101 hours after recruitment. The respective scores for the surviving severe AP patients remained relatively unchanged for the duration of their participation in the study.

The inflammatory burden of the study cohort was further quantified by supplementary serial measurements of pro- and anti-inflammatory cytokines over the same time-points. The severe AP patient group was found to have significantly higher levels of peak and mean concentrations, as well as standardized AUC levels of IL-1B, IL-6, IL-10, TFF3, and CD163 (Supplementary Table S3).

Tryptophan Metabolites. Summary measures for the concentration of tryptophan and all studied tryptophan metabolites are presented in Table 1. Tryptophan depletion was marked and proportionate to disease

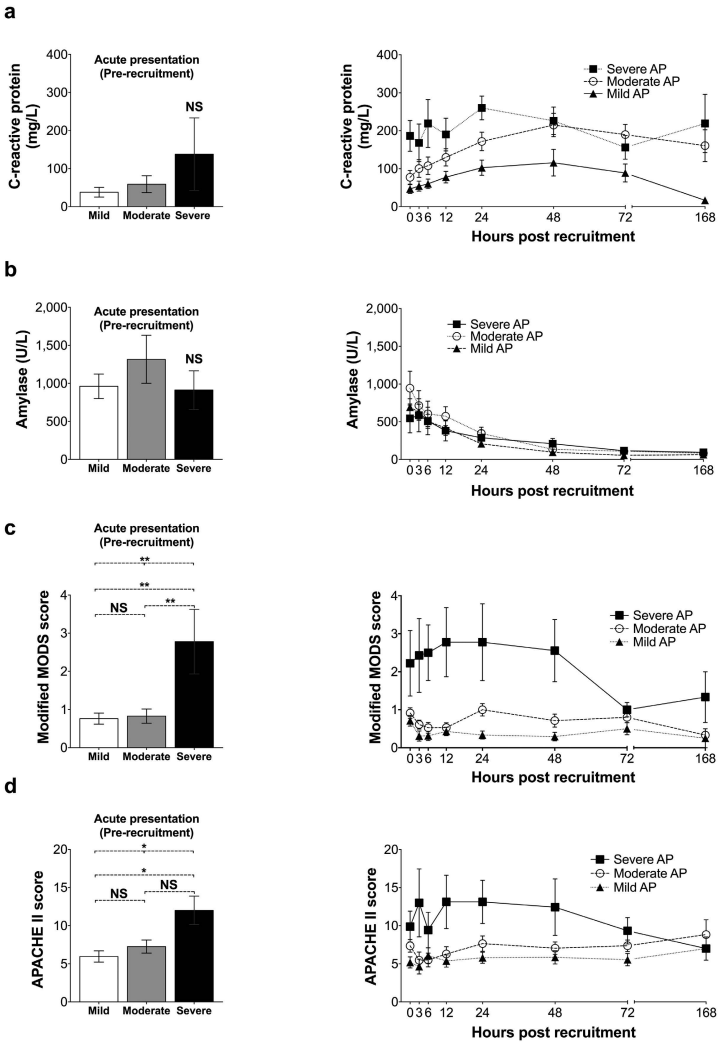

Figure 4. Panel of comparisons between mild (n = 25), moderate (n = 23) and severe AP (n = 9) participant groups for (**a**) C-reactive protein; (**b**) Amylase; (**c**) Modified MODS score (as described in the revised Atlanta guidelines); (**d**) APACHE II score. Bar charts on the left hand side of the panel represent Tminus sample means (acute presentation – pre-recruitment), while right-hand side plots depict the time-course for each variable mean per participant group. NS: not statistically significant difference; *0.05 > P > 0.01; **P < 0.01; error bars represent standard errors of the mean.

severity and especially profound in severe AP (standardised AUC for tryptophan concentration, mean (SD): mild: 24423.9 (8803.9) ng/mL; moderate: 19721.4 (8140.5) ng/mL; severe: 13782.4 (3789.8) ng/mL; P = 0.004).

	Summary Measure	Mild	Moderate	Severe	P-value	HV
Tryptophan (ng/mL)	Minimum values, median (IQR)	16407.2 (13334.9–20463.6)	13993.6 (10714–20941.9)	8820.4 (7295.3–11355.6)	0.071	39763.4
	Mean values, mean (SD)	23840.4 (8970.0)	20346.2 (8144.3)	14421.2 (4317.7)	**0.014**	25215.8 (21675.9–33362.4)
	Time-to-Minimum, median hours (95% C.I.)	12 (3.5–20.5)	12 (0.0–27.7)	12 (3.2–20.8)	0.542	—
	Standardized AUC, mean (SD)	24423.9 (8803.9)	19721.4 (8140.5)	13782.4 (3789.8)	**0.004**	—
Kynurenine (ng/mL)	Peak values, median (IQR)	694.9 (429.4–857.9)	677.8 (408.2–900.8)	742.0 (626.6–1182.8)	0.680	622.3
	Mean values, median (IQR)	520.5 (338.8–708.0)	470.6 (302.1–874.4)	599.4 (547.1–912.4)	0.533	429.2 (353.0–463.1)
	Time-to-Peak, median hours (95% C.I.)	12 (0.0–29.6)	24 (6.3–41.7)	12 (0.0–25.2)	0.288	—
	Standardized AUC, median (IQR)	554.8 (360.3–734.0)	522.7 (370.8–782.5)	634.3 (570.4–840.7)	0.584	—
3-Hydroxykynurenine (ng/mL)	Peak values, median (IQR)	13.5 (8.6–17.8)	20.0 (12.1–46.6)	25.2 (14.3–35.6)	**0.013**	10.5
	Mean values, median (IQR)	10.7 (8.1–13.5)	15.6 (8.6–38.3)	22.3 (11.8–27.0)	**0.039**	8.1 (7.6–9.0)
	Time-to-Peak, median hours (95% C.I.)	12 (6.7–17.3)	46.0* (32.9–59.0)	24 (0.0–49.0)	**0.019**	—
	Standardized AUC, median (IQR)	10.8 (7.4–13.9)	18.2 (11.4–38.1)	21.6 (11.7–28.0)	**0.015**	—
Kynurenic Acid (ng/mL)	Peak values, median (IQR)	211.5 (165.0–404.3)	184.8 (48.2–364.4)	198.4 (182.4–201.6)	0.303	372.1
	Mean values, median (IQR)	194.9 (146.0–364.4)	141.8 (41.8–184.8)	186.4 (172.1–197.5)	0.104	301.6 (267.7–327.5)
	Time-to-Peak value, median hours (95% C.I.)	6 (3.2–8.9)	6 (2.7–9.3)	3 (0.0–7.2)	0.102	—
	Standardized AUC, median (IQR)	296.7 (155.4–406.6)	74.1 (34.8–203.9)	208.3 (173.8–374.3)	0.110	—
3-Hydroxyantranilic Acid (ng/mL)	Peak values, median (IQR)	48.8 (37.3–118.6)	48.8 (33.4–64.7)	65.0 (46.2–394.2)	0.236	54.3
	Mean values, median (IQR)	38.4 (25.9–85.3)	34.8 (26.0–62.8)	53.6 (41.9–215.1)	0.186	37.5 (28.7–48.8)
	Time-to-Peak value, median hours (95% C.I.)	6 (0.0–12.1)	6 (0.0–15.1)	6 (0.0–14.8)	0.686	—
	Standardized AUC, median (IQR)	45.7 (28.0–98.4)	34.9 (26.8–56.0)	51.2 (44.1–107.5)	0.165	—
3-Hydroxykynurenine/ Tryptophan ratio x1000	Peak values, median (IQR)	0.673 (0.417–1.523)	0.878 (0.532–4.515)	1.876 (1.505–3.600)	0.052	0.46
	Minimum values, median (IQR)	0.341 (0.201–0.804)	0.428 (0.283–1.513)	0.872 (0.637–1.420)	**0.005**	0.18
	Mean value, median (IQR)	0.429 (0.321–1.255)	0.645 (0.409–2.902)	1.550 (0.831–1.769)	**0.022**	0.326 (0.248–0.384)
	Time-to-Peak value, median hours (95% C.I.)	12.0 (0.0–26.6)	48 (21.6–74.4)	12 (0.0–24.5)	0.110	—
	Standardized AUC, median (IQR)	0.447 (0.262–0.892)	0.734 (0.506–2.635)	1.667 (0.930–1.841)	**0.003**	—

Table 1. Summary analysis measures[1] of serial tryptophan and tryptophan metabolite concentrations for the three patient groups of AP severity (T0 to T168). Standardised AUC were calculated as detailed in the methods section. T168 values were excluded from Time-to-Peak/Minimum analyses. P-values correspond to results from independent samples Kruskal-Wallis H tests or one-way ANOVA comparisons, as appropriate. **HV**: Healthy Volunteers.

Importantly, upon presentation to hospital, no statistically significant difference in tryptophan concentration levels was evident among the three groups (tryptophan concentration, mean (SD): mild 29796.6 (10939.7) ng/mL; moderate: 26133.0 (9973.8) ng/mL; severe: 24273.7 (9926.4) ng/mL; $P = 0.281$). Moreover, the observed effect on tryptophan levels was not a result of dilution due to resuscitation, as no statistically significant difference was observed in the standardised AUC of haematocrit[22] between the three groups (standardized AUC for haematocrit, mean (SD): mild 0.361 (0.04); moderate: 0.350 (0.045); severe 0.330 (0.034); $P = 0.161$).

No significant differences in peak level, mean level or standardized AUC of kynurenine, kynurenic acid, and 3-hydroxyanthranilic acid were detected among the three patient groups (Table 1). In specific, the observed difference of 3-hydroxyanthranilic acid concentration plots on Fig. 5e, although visually substantial, did not reflect a statistically significant difference, and was driven by the extreme values on two individual participants in the severe AP group.

The severe AP patient group had a significantly higher peak 3-hydroxykynurenine concentration across all study time-points (peak 3-hydroxykynurenine concentration, median (IQR): mild: 13.5 (8.6–17.8) ng/mL; moderate: 20.0 (12.1–46.6) ng/mL; severe: 25.2 (14.3–35.6) ng/mL; $P = 0.013$). The standardized AUC for 3-hydroxykynurenine was also significantly different among the three groups, although only a marginal difference was observed between the moderate and the severe group, as shown on Table 1 (standardized AUC for 3-hydroxykynurenine concentration, median (IQR): mild: 10.81 (7.4–13.9) ng/mL; moderate: 18.2 (11.4–38.1) ng/mL; severe: 21.6 (11.7–28.0) ng/mL; $P = 0.015$). The median time interval required to reach peak 3-hydroxykynurenine concentration was 24 hours (95% C.I.: 0.0–49.0 hours) in the severe AP group, compared to 12.0 hours (95% C.I.: 6.7–17.3 hours) in the mild AP group and to a mean time interval of 46.0 hours (95% C.I.: 32.9–59.0 hours) in the moderate group ($P = 0.019$).

To provide an indication of flux through KMO we calculated the 3-hydroxykynurenine/tryptophan ratio, multiplied by 1000 for practical purposes. We observed a significant increase in KMO flux in proportion to disease severity (standardized AUC for 3-hydroxykynurenine/tryptophan ratio x 1000, median (IQR): mild: 0.45 (0.26–0.89); moderate: 0.73 (0.51–2.64); severe: 1.67 (0.93–1.84); $P = 0.003$). Time-plots for the concentration of each analysed metabolite are depicted on Fig. 5.

When considering all samples obtained from T0 up to and including T48, logarithmic 3-hydroxykynurenine levels correlated well with contemporaneous CRP ($R^2 = 0.132$; $rho = 0.455$, $P < 0.001$) (Fig. 6a) and APACHE II score ($R^2 = 0.250$; $rho = 0.583$; $P < 0.001$) (Fig. 6b). Furthermore, moderate correlation of logarithmic 3-hydroxykynurenine was discovered with contemporaneous levels of TFF3 ($rho = 0.604$, $P < 0.001$), albumin ($rho = -0.568$, $P < 0.001$), creatinine ($rho = 0.531$, $P < 0.001$), TNF-a ($rho = 0.462$, $P < 0.001$), and RAGE ($rho = 0.455$, $P < 0.001$), and weaker correlation with IL-10 ($rho = 0.357$, $P < 0.001$), IL-6 ($rho = 0.352$, $P < 0.001$), IL-17a ($rho = 0.349$, $P < 0.001$), CD163 ($rho = 0.298$, $P < 0.001$), IL-8 ($rho = 0.293$, $P < 0.001$), TNFS10 ($rho = -0.259$, $P < 0.001$), chemerin ($rho = 0.247$, $P < 0.001$), IL-1 ($rho = 0.220$, $P < 0.001$), insulin C-peptide ($rho = 0.230$, $P < 0.001$), cardiac troponin I ($rho = 0.217$, $P < 0.001$), CXCL12 ($rho = 0.195$, $P = 0.001$), and CD40 ligand ($rho = 0.181$, $P = 0.003$). Conversely, no correlation was found with insulin ($rho = 0.057$, $P = 0.352$), CA 15-3 ($rho = -0.079$, $P = 0.199$), B7-H1 ($rho = -0.062$, $P = 0.312$), and IFN-γ ($rho = -0.019$, $P = 0.758$). Differences between participant groups for all studied cytokines are summarized in Supplementary Table S3, and post-hoc pairwise comparisons of significantly different standardized AUC are depicted on Supplementary Table 4.

Discussion

The kynurenine pathway of tryptophan metabolism and especially KMO, the enzyme that determines the metabolic fate of kynurenine, is increasingly recognized as a key contributor to the pathogenesis of AP-MODS. The present study precisely defines the temporal profile of kynurenine pathway metabolite concentrations in peripheral blood in relation to the onset and severity of AP in humans. Our data show a noteworthy association between plasma concentrations of 3-hydroxykynurenine and AP severity as defined by standard classification systems. Furthermore, an important correlation was discovered between 3-hydroxykynurenine concentrations and the systemic inflammatory burden measured by CRP levels. Additionally, we observed the classical paradigm pro-inflammatory cytokine profile in AP, namely TNF-α and IL-6, as well as RAGE and TFF3, that correlated strongly with 3-hydroxykynurenine concentrations in this cohort. Our novel findings add depth to the previously reported association between serum kynurenine concentrations and the requirement for invasive renal and respiratory support during AP-MODS[8]. Moreover, these findings build on our recent discovery that genetic deletion of KMO in mice and administration of a highly-specific KMO inhibitor protects against lung and kidney injury in experimental rodent models of AP[7]. Together, these data strongly support the translational potential of KMO inhibition as a therapeutic strategy to protect against MODS in human AP.

Strengths of this study include the efficiency of recruitment, sampling process, sampling frequency and high coverage, thus minimal sampling bias. Based on the knowledge that AP and the consequent systemic inflammatory response evolve rapidly, we hypothesise that KMO inhibition is likely to be most effective when delivered as early as possible in the course of the disease and therefore sought to define the evolving trajectory of the inflammatory response with high definition during the early phase.

The burden of systemic inflammation in the cohort as measured by the magnitude of CRP rise was clear. The fact that APACHE II score correlated well with disease severity as classified by the revised Atlanta criteria, and that the modified MODS score followed the same trajectory as CRP, provide additional reassurance that interpreting plasma concentrations of 3-hydroxykynurenine and other kynurenine metabolites in this context is valid and appropriate.

In the present study, the magnitude of tryptophan depletion was proportional to AP severity and was more prominent over time, suggesting increased tryptophan metabolism. Although tryptophan levels can fluctuate diurnally[23,24], diurnal variation does not account for the difference in tryptophan metabolism between AP severity strata, because the time of day at which recruitment occurred was distributed randomly. Furthermore, although it is possible that fasting due to hospital admission and/or critical illness may in theory account for part of the observed tryptophan depletion, Poesen *et al.*[25] previously demonstrated that plasma levels of tryptophan, kynurenine, and kynurenic acid were not significantly different in healthy humans with low or high protein intake, and one would not expect to observe a correlation with AP severity if fasting was the cause. The extent of tryptophan depletion is too great to be due to haemodilution after intravenous fluid resuscitation, and there was no observed change in the haematocrit or dilution effect seen in other plasma analytes. It therefore seems reasonable to conclude that the observed decrease in tryptophan levels is likely to be the result of increased metabolism.

The increase in steady state 3-hydroxykynurenine concentrations in plasma is consistent with increased flux through the kynurenine pathway. This change is proportionate to disease severity and augments with time.

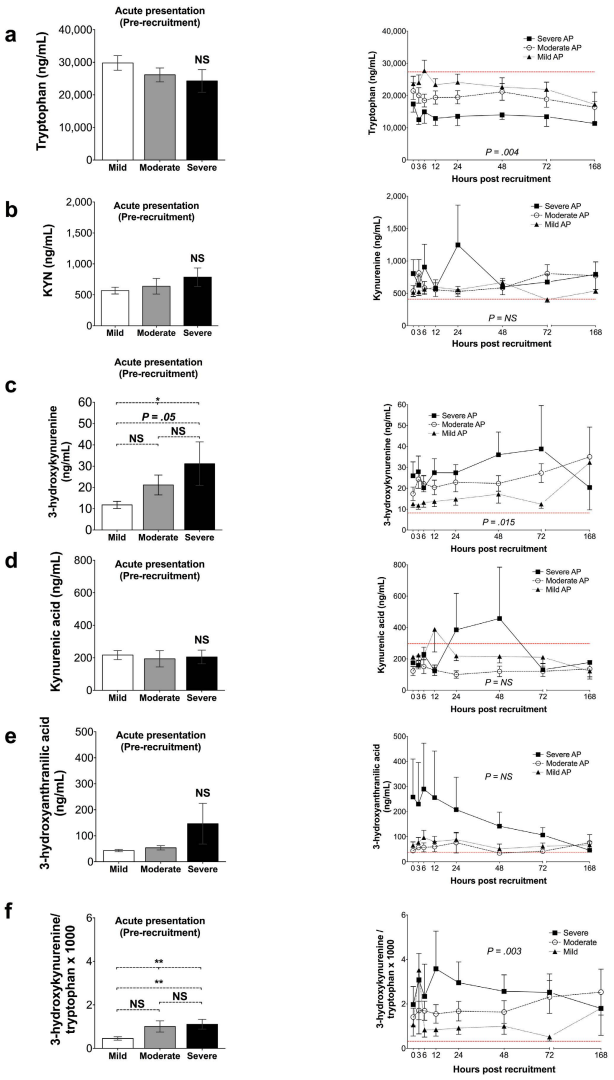

Figure 5. Plasma concentrations of tryptophan and the kynurenine pathway metabolites in tAP participants over time, grouped by AP severity according to the revised Atlanta criteria (group sizes at recruitment: mild n = 25; moderate n = 23; severe n = 9 individuals). (**a**) Tryptophan; (**b**) Kynurenine; (**c**) 3-hydroxykynurenine; (**d**) Kynurenic acid; (**e**) 3-hydroxyanthranilic acid; (**f**) 3-hydroxykynurenine to tryptophan ratio multiplied by 1000. For all panels, data points represent means and error bars represent standard errors of the mean. P-values for between-group comparisons of standardised AUC are appended. NS: not statistically significant. Dashed red lines represent healthy volunteer (n = 8) mean concentration of each metabolite.

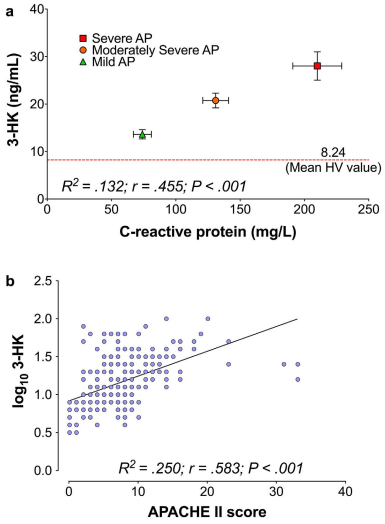

Figure 6. Dot-plots of correlations between (**a**) Plasma concentrations of C-reactive protein and 3-hydroxykynurenine, for the tAP cohort grouped by AP severity (mild AP n = 25; moderate AP n = 23; severe AP n = 9). Data points represent means; error bars show standard deviation on both axes. The dashed red line represents mean 3-hydroxykynurenine plasma concentration in healthy volunteers (n = 8). (**b**) APACHE II score and contemporaneous $\log_{10}$ 3-hydroxykynurenine plasma concentrations. Due to the non-normal distribution of plasma concentrations of of 3-hydroxykynurenine, a logarithmic transformation was used for the correlation to provide a better fit. For both correlations, results from samples obtained from T0 up to and including T48 were analysed. Results of respective Spearman correlations have been appended on each figure of the panel.

Importantly, elevated 3-hydroxykynurenine is associated with increased inflammatory burden, as indicated by the correlation of plasma 3-hydroxykynurenine levels with contemporaneous CRP concentrations. While it is curious that no significant changes were observed in other tryptophan metabolites, the indications from studies in rodents are that KMO represents the predominant route for this pathway. For this reason, the ratio between 3-hydroxykynurenine to tryptophan (multiplied by 1000 for practical purposes) was used to try and express the pathway flux through KMO. This ratio was found to be time-dependent and proportionate to AP severity, with a lead-time of 12 hours when compared to the timing of the peak serum CRP that occurred at 24 hours for the severe AP group. Lastly, the elevation in 3-hydroxykynurenine/tryptophan ratio was greater in those few patients with specific respiratory, renal and cardiac dysfunction as defined by those specific components of the MODS score.

In conclusion, this study demonstrates that metabolic flux through KMO is elevated proportionately to disease severity in human AP. Plasma concentrations of 3-hydroxykynurenine correlate with the burden of inflammation, incidence of organ dysfunction and AP severity. These findings reinforce the rationale for investigating early phase KMO inhibition as a therapeutic strategy to protect against AP-MODS in human AP.

Methods

Ethics & Regulatory Approvals. This study was approved by the Scotland A Research Ethics Committee (REC) (REC reference number: 13/SS/0136, amended REC REF AM01), and NHS Lothian Research & Development committee (Project Number: 2013/0098 and SA1). The study sponsor was the Academic and Clinical Central Office for Research and Development (ACCORD), a collaboration between the University of Edinburgh and NHS Lothian. Permission to access confidential medical records was granted by the NHS Lothian Caldicott Guardian. Written informed consent was obtained from all participants or their legal representative. Prior to commencing the study, a summary of the study protocol was registered in the public domain on the UK Clinical Trials Gateway (former UK Clinical Research Network) (registration number: 16116)[26]. The study was conducted in accordance with the University of Edinburgh and NHS Lothian guidance. Adults without the capacity to give informed consent were recruited in accordance with the Adults with Incapacity (Scotland) Act 2000, Part 5[27]. Informed consent was sought when capacity was regained.

Research Sites. Recruitment of participants was undertaken in the Emergency Department (ED), and General Surgery wards at the Royal Infirmary of Edinburgh (RIE). The Wellcome Trust Clinical Research Facility (WTCRF) provided research nurse support for recruitment and consent, data collection, sample handling and storage.

Inclusion and Exclusion Criteria. Any patient over the age of 16 years with a potential or confirmed new diagnosis of AP was identified as a potential participant. For the diagnosis of potential AP, two of the following three features were required:

(i) A clinical history of symptoms compatible with AP (i.e. abdominal pain, nausea, and/or vomiting);
(ii) Serum amylase concentration greater than the upper limit of the reference range (>100 IU/L). This threshold was decided upon in order to capture potential participants with an amylase level below the threshold of the revised Atlanta definition for AP, due to a late or atypical presentation. A serum amylase concentration in excess of 300 IU/L was required as a feature for the diagnosis of true AP (tAP).
(iii) Evidence of AP on computerised tomography (CT) and/or abdominal ultrasound scan (AUSS).

Persons under the age of 16 years, prisoners and persons in police custody were excluded from the study.

Recruitment. In order to capture potential participants as early as possible in the course of the disease, an automatic alert was initiated from the biochemistry laboratory for any serum sample with a measured elevated amylase concentration and transmitted to the WTCRF research nurses on a dedicated study mobile telephone. The dedicated study telephone number was also distributed to all ED and surgical team members. Upon each alert, the investigator or WTCRF nurse checked the clinical history of each potential study participant via his or her electronic records on InterSystems TrakCare®. If the clinical presentation was compatible with AP, the patient was visited and suitability for recruitment was confirmed. A member of the direct clinical care team made the initial approach to each potential participant, prior to informed consent and recruitment by the WTCRF nurse team.

The recruitment phase lasted for 90 calendar days – from 17th September 2013 to 16th December 2013 – and was carried out 24 hours per day, 7 days per week with support from 30 WTCRF research nurses on a 12-hour shift rota supported by 2 clinical support workers and a clinical technician.

Healthy Volunteers. Healthy volunteers over the age of 18 years were recruited with ethical approval (REC reference number: 08/S1103/38, United Kingdom). Volunteers were excluded from participation on the basis of the presence of any of the following: renal dysfunction (eGFR < 30 mL/min); hepatic dysfunction (Child-Pugh score B or C); pregnancy or breast feeding; blood dyscrasia or anaemia (haemoglobin <12 g/dL); active malignancy; chronic inflammatory condition; any intercurrent illness; and any recent surgical procedure.

Data Collection and Data Management. Data were collected from individual patient charts, clinical case notes and the following electronic sources:

(i) WardWatcher software (Scottish Intensive Care Society Audit Group - SICSAG).
(ii) TrakCare Patient Management System (InterSystems, Massachusetts, USA).
(iii) SCI-Store data repository (SCI NHS National Services Scotland).
(iv) Picture Archiving and Communication System (PACS).
(v) Emergency Care Summary (ECS) database (National Information Systems Group).
(vi) iLaboratory information system (iLab or APEX).

Data were entered into a dedicated, password-protected and software-encrypted study laptop with regular back-up. Participant data were link-anonymized and securely stored separately from the identifiers in accordance with the secure data protection principles of the sponsor and the WTCRF. A bespoke data collection template was created in Java™ with set limits to avoid the potential for mistyping data entry and mandatory completion to ensure comprehensive recording. Input from the data collection software was made automatically to a custom Microsoft® Access® study database. A diarized alert/reminder system was activated on initiation of a new patient at the time of recruitment, that sent email and screen notifications to the study nurses to ensure time points were not missed. This was especially important in cases of simultaneously enrolled and overlapping participants to ensure all time points were met (Supplementary Figure S5). In addition, WTCRF study nurses kept a hand-written log to enter free text, notes and other information pertinent to the study for which there was no appropriate data field in the electronic study database. Cross-referencing with the study database was performed at intervals.

Sampling and Sample Handling. Peripheral blood was sampled at recruitment (T0) and 3, 6, 12, 24, 48, 72 and 168 hours (7 days) afterwards. In addition, the serum sample obtained at presentation to hospital (pre-recruitment, T_{minus}) was retained by the Biochemistry Department and subsequently retrieved by WTCRF staff. Blood was sampled into gel clot-activator tubes for serum and into EDTA tubes for plasma and subsequently centrifuged at 3200 rpm for 8–10 minutes. All centrifuges were serviced and calibrated prior to the start of the study. Serum and plasma were aliquoted and immediately frozen at −80 °C until transfer on dry ice to long-term storage in a monitored, dedicated −80 °C freezer. A sample history and tracking log was kept for all events. There were no breaches of the sample handling/storage protocol. Additionally, routine haematological and biochemical analyses were performed in accordance with standard hospital protocols used in clinical care. Serum C-reactive protein (CRP) was used to define the inflammatory burden of the cohort.

Composite Clinical Scores. For each participant, the APACHE II score was calculated on the day of admission, by using the most extreme values of the variables of interest (day 1 APACHE II) and on per time-point basis[28]. Similarly, the Marshall multiple organ dysfunction syndrome (MODS) score, modified as per the revised Atlanta Classification of AP, was calculated for each patient at each time-point and on a daily basis[4,29].

Tryptophan Metabolite Measurement. Kynurenine pathway metabolites were measured in plasma by two separate liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods. For the detection of tryptophan, kynurenine, kynurenic acid and 3-hydroxyantranilic acid, plasma (5 μl) was initially diluted in phosphate-buffered saline (45 μl). The diluted plasma was added to 5 mM ammonium formate containing 0.1% trifluoroacetic acid (130 μl). Protein was precipitated by the addition of ice-cold 100% trichloroacetic acid (20 μl), samples were vortexed briefly, incubated for 30 minutes at 4 °C and centrifuged to obtain the supernatant. Separate standard curves were used to quantify levels of the individual metabolites. For tryptophan, the standard curve consisted of serial dilutions of d5-tryptophan (as surrogate analyte of tryptophan) in 10% pooled human plasma. For kynurenine, 1% BSA in phosphate-buffered saline was used. For kynurenic acid and 3-hydroxyanthranilic acid, 0.2% BSA in phosphate-buffered saline was used. Samples (10 μl) were injected onto a Waters Select HSS XP column (3 mm × 100 mm, 2.5 μm, Waters, Elstree, Herts) using a Waters Acquity UHPLC system, coupled to an ABSciex QTRAP 5500 mass analyser. The flow rate was 0.35 mL/min at 25 °C. Separation was carried out using a water:methanol gradient (both containing 0.1% formic acid). Chromatographic conditions were 50% methanol rising to 60% over 60 seconds, then to 65% over 180 seconds; held for 110 seconds, returned to 50% over 10 seconds and re-equilibrated for a further 200 seconds, giving a total run time of 10.2 minutes. The mass spectrometer was operated in positive electrospray ionization mode. The multiple reaction monitoring (MRM) transitions for the protonated analytes were tryptophan (*m/z* 205-188), kynurenine (*m/z* 209-146), kynurenic acid (*m/z* 190-144) and 3-hydroxyanthranilic acid (*m/z* 154-136). The transitions for d5-tryptophan standard were (*m/z* 210-122). Collision energies were 11, 29, 31, 33 and 37 eV respectively.

For the analysis of 3-hydroxykynurenine, plasma (100 μl) was added to a 40:60 mixture of acetonitrile in water containing 0.1% formic acid (130 μl) and 50 ng/ml of internal standard. Protein was precipitated by the addition of trichloroacetic acid (33 μl), samples were vortexed briefly, incubated for 30 minutes at 4 °C and centrifuged to obtain the supernatant. A standard curve containing serial dilutions of 3-hydroxykynurenine in 10% BSA diluted with phosphate-buffered saline enriched with 50 ng/ml internal standard was used. 10 μL volumes of each sample were injected onto a Waters Select HSS XP column (30 mm × 100 mm, 2.5 μm, Waters Corp, Elstree, Herts) using a Waters Acquity UHPLC system, coupled to an ABSciex QTRAP 5500 mass analyser. The flow rate was 0.8 mL/min at 30 °C. Separation was carried out using a water:methanol gradient (both containing 0.1% formic acid). Conditions were 5% methanol rising to 95% over 150 seconds, returning to 5% over 30 seconds, giving a total run time of 4 minutes. The mass spectrometer was operated in positive ion electrospray mode. The transitions for the protonated analytes were 3-hydroxykynurenine (*m/z* 225-208) and internal standard (*m/z* 228-211). Collision energies were 20 and 12 eV respectively.

Data were acquired and processed using Analyst quantitation software 1.3 (ABI Sciex). Results from retrieved acute presentation (pre-recruitment) samples were excluded from the time-course analysis since serum-gel activator tube containers were routinely used by the clinical care teams, a fact that may have led to discrepancy upon comparison with results from the dedicated K3-Ethylenediaminetetraacetic acid containers that were used for the study.

Four samples obtained from two patients in the severe AP group returned values of kynurenine concentration that were greater than 20 standard deviations away from the remaining concentration values of the severe AP cohort, including temporally adjacent samples from the same patient. These samples were deemed to have resulted from contamination during sample analysis, were designated as extreme outliers and were removed from further analysis.

Cytokine Analysis. Plasma cytokines were analysed using a custom-designed Human Magnetic Luminex Screening Assay according to the manufacturer's instructions (R&D Systems, MN, USA). Levels of insulin, interleukin-1 beta (IL-1B), insulin C-peptide and IFN-γ were measured using kit LXSAHM-4, cancer antigen 15-3 (CA 15-3) using kit LXSAHM-1 and tumour necrosis factor alpha (TNFα), B7 homolog 1 (B7-H1), chemokine (C-X-C motif) ligand 12 (CXCL 12), cluster of differentiation 163 (CD163), tumour necrosis factor superfamily member 10 (TNFS10), receptor for advanced glycation endproducts (RAGE), trefoil factor 3 (TFF3), cluster of differentiation 40 ligand (CD40 ligand), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 17A (IL-17A), cardiac troponin I and chemerin using kit LXSAHM-15, respectively. The concentrations of each cytokine were determined using Bio-Rad Bio-Plex 200 system (BioRad, CA, USA).

Statistical Analysis. A data analysis plan was formulated prospectively and was adhered to. Quantitative data are presented as median and interquartile range (IQR) or mean and standard deviation (SD), as appropriate. Qualitative data are presented by utilising absolute and/or relative frequencies. Data conformity to the normal distribution was analysed by one-sample Kolmogorov-Smirnov testing, and comparisons between patient groups were performed by using one-way ANOVA or the independent samples Kruskal-Wallis H test, as appropriate. For statistically significant differences, post-hoc pairwise comparisons were performed by using Tukey's honestly significant difference test or Dunn's test, respectively. Spearman's rho was used to examine bivariate correlations of quantitative variables. Logarithmic transformations of non-normally distributed variables were used to establish the best linear regression fit during correlation. For the analysis of serial metabolite measurements, the method of summary measures was used[30]. The area under the curve (AUC) for each metabolite was calculated for each participant by using the trapezium rule, as described by Matthews *et al.*[30]. These were standardized by duration

of participation in the study, defined by the ultimate time-point of blood sampling for each participant. For the computation of each AUC, any uttermost missing values were omitted, whereas for any middle missing values AUCs were calculated between the immediately adjacent available values. The Kaplan-Meier method was used to compare time-to peak and time-to-minimum metabolite values between groups, and the log-rank test was applied to detect differences; only time-points T0 up to and including T72 were included in the latter analyses.

All statistical tests were based on a two-sided α-value of 0.05. Data were analysed using SPSS® Statistics version 22.0 (IBM Corp., Armonk, NY, USA) and graphs drawn using GraphPad Prism® version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Achieved statistical power was calculated post-hoc using the G-Power algorithm: F tests - ANOVA: Fixed effects, omnibus, one-way[31]. The effect size was computed from measured 3-hydroxykynurenine concentrations in tAP as $f = 5.78$, with α error probability $= 0.05$. The noncentrality parameter, $\lambda = 1902.25$; Critical $F = 3.17$; numerator $df = 2$; denominator $df = 54$; computed power $(1-\beta$ error probability$) = 1.00$.

References

1. Norman, J. The role of cytokines in the pathogenesis of acute pancreatitis. *Am. J. Surg.* **175,** 76–83 (1998).
2. Granger, J. & Remick, D. Acute pancreatitis: models, markers, and mediators. *Shock* **24,** Suppl 1, 45–51 (2005).
3. Buter, A., Imrie, C. W., Carter, C. R., Evans, S. & McKay, C. J. Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. *Br. J. Surg.* **89,** 298–302 (2002).
4. Banks, P. A. *et al.* Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. *Gut* **62,** 102–111 (2013).
5. Harrison, D. A., D'Amico, G. & Singer, M. Case mix, outcome, and activity for admissions to UK critical care units with severe acute pancreatitis: a secondary analysis of the ICNARC Case Mix Programme Database. *Crit. Care* **11,** Suppl 1: S1, doi: 10.1186/cc5682 (2007).
6. Neoptolemos, J. P., Raraty, M., Finch, M. & Sutton, R. Acute pancreatitis: the substantial human and financial costs. *Gut* **42,** 886–891 (1998).
7. Mole, D. J. *et al.* Kynurenine-3-monooxygenase inhibition prevents multiple organ failure in rodent models of acute pancreatitis. *Nat. Med.* **22,** 202–209 (2016).
8. Mole, D. J. *et al.* Tryptophan catabolites in mesenteric lymph may contribute to pancreatitis-associated organ failure. *Br. J. Surg.* **95,** 855–867 (2008).
9. Murakami, Y. & Saito, K. Species and cell types difference in tryptophan metabolism. *Int. J. Tryptophan Res.* **6,** 47–54 (2013).
10. Schwarcz, R. The kynurenine pathway of tryptophan degradation as a drug target. *Curr. Opin. Pharmacol.* **4,** 12–17 (2004).
11. Pellegrin, K. *et al.* Enhanced enzymatic degradation of tryptophan by indoleamine 2,3-dioxygenase contributes to the tryptophan-deficient state seen after major trauma. *Shock* **23,** 209–215 (2005).
12. Wang, Y. *et al.* Kynurenine is an endothelium-derived relaxing factor produced during inflammation. *Nat. Med.* **16,** 279–285 (2010).
13. Bellac, C. L., Coimbra, R. S., Christen, S. & Leib, S. L. Inhibition of the kynurenine-NAD+ pathway leads to energy failure and exacerbates apoptosis in pneumococcal meningitis. *J. Neuropathol. Exp. Neurol.* **69,** 1096–1104 (2010).
14. Grohmann, U. *et al.* CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol.* **3,** 1097–1101 (2002).
15. Moffett, J. R. & Namboodiri, M. A. Tryptophan and the immune response. *Immunol. Cell Biol.* **81,** 247–265 (2003).
16. Mizdrak, J., Hains, P. G., Truscott, R. J., Jamie, J. F. & Davies, M. J. Tryptophan-derived ultraviolet filter compounds covalently bound to lens proteins are photosensitizers of oxidative damage. *Free Radic. Biol. Med.* **44,** 1108–1119 (2008).
17. Nguyen, N. T. *et al.* Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc. Natl. Acad. Sci. USA* **107,** 19961–19966 (2010).
18. Mezrich, J. D. *et al.* An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.* **185,** 3190–3198 (2010).
19. Zeden, J. P. *et al.* Excessive tryptophan catabolism along the kynurenine pathway precedes ongoing sepsis in critically ill patients. *Anaesth. Intensive Care* **38,** 307–316 (2010).
20. Logters, T. T. *et al.* Increased plasma kynurenine values and kynurenine-tryptophan ratios after major trauma are early indicators for the development of sepsis. *Shock* **32,** 29–34 (2009).
21. Forrest, C. M. *et al.* Kynurenine metabolism predicts cognitive function in patients following cardiac bypass and thoracic surgery. *J. Neurochem.* **119,** 136–152 (2011).
22. Aggarwal, A., Manrai, M. & Kochhar, R. Fluid resuscitation in acute pancreatitis. *World J. Gastroenterol.* **20,** 18092–18103 (2014).
23. Fernstrom, J. D. *et al.* Diurnal variations in plasma concentrations of tryptophan, tryosine, and other neutral amino acids: effect of dietary protein intake. *Am. J. Clin Nutr.* **32,** 1912–1922 (1979).
24. Young, V. R., Hussein, M. A., Murray, E. & Scrimshaw, N. S. Tryptophan intake, spacing of meals, and diurnal fluctuations of plasma tryptophan in men. *Am. J. Clin Nutr.* **22,** 1563–1567 (1969).
25. Poesen, R. *et al.* The Influence of Dietary Protein Intake on Mammalian Tryptophan and Phenolic Metabolites. *PLoS One* **10,** e0140820, doi: 10.1371/journal.pone.0140820 (2015).
26. *UK Clinical Trials Gateway.* https://www.ukctg.nihr.ac.uk/trials/trial-details/trial-details?trialId=30762&query=%257B%2522query%2522%253A%252216116%2522%252C%2522facetDef%2522%253A%257B%257D%252C%2522rows%2522%253A%252225%2522%252C%2522offset%2522%253A50%252C%2522openurl%2522%253A%2522yes%2522%257D (2016).
27. Scotland. Adults with Incapacity (Scotland) Act 2000–excerpt. *Bull. Med. Ethics* **157,** 8–11 (2000).
28. Knaus, W. A., Draper, E. A., Wagner, D. P. & Zimmerman, J. E. APACHE II: a severity of disease classification system. *Crit. Care Med.* **13,** 818–829 (1985).
29. Marshall, J. C. *et al.* Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit. Care Med.* **23,** 1638–1652 (1995).
30. Matthews, J. N., Altman, D. G., Campbell, M. J. & Royston, P. Analysis of serial measurements in medical research. *BMJ* **300,** 230–235 (1990).
31. Faul, F., Erdfelder, E., Lang, A. G. & Buchner, A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39,** 175–191 (2007).

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Author Contributions

C.S.: study design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis; obtained funding; critical revision of the manuscript for important intellectual content; administrative, technical, or material support; study supervision. X.Z.: acquisition of data; critical revision of the manuscript for important intellectual content; administrative, technical and material support. M.B.: acquisition of data; critical revision of the manuscript for important intellectual content; administrative, technical and material support. N.Z.M.H.: acquisition of data; critical revision of the manuscript for important intellectual content; administrative, technical and material support. T.B.J.M.: acquisition of data; critical revision of the manuscript for important intellectual content; administrative support. D.R.: obtained funding; critical revision of the manuscript for important intellectual content. L.B.: acquisition of data; administrative, technical, and material support. F.P.: acquisition of data; administrative, technical, and material support. H.S.: acquisition of data; administrative, technical, and material support. L.D.: acquisition of data; administrative, technical, and material support. A.J.H.: critical revision of the manuscript for important intellectual content, administrative support. A.T.: technical and administrative support. D.L.: administrative and material support. R.W.P.: critical revision of the manuscript for important intellectual content; study supervision. O.J.G.: critical revision of the manuscript for important intellectual content; study supervision. J.P.I.: critical revision of the manuscript for important intellectual content; study supervision. I.J.U.: analysis and interpretation of data; critical revision of the manuscript for important intellectual content. J.L.: analysis and interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; study supervision. W.L.W.: analysis and interpretation of data; material and technical support; critical revision of the manuscript for important intellectual content. G.D.: analysis and interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; study supervision. S.P.W.: acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, and material support. D.J.M.: study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; administrative support; study supervision.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: D.R. was an employee of GSK during the course of the study. I.J.U., J.L., W.L.W. and G.D. are employed by GSK. S.P.W. is engaged in a separately funded Discovery Partnership with Academia collaboration with GlaxoSmithKline. D.J.M. is engaged in a separately funded Discovery Partnership with Academia collaboration with GlaxoSmithKline. The University of Edinburgh receives milestone and royalty payments according to the phase of the project, as governed by the University of Edinburgh revenue sharing policy.

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Presentations arising from this thesis

1. **International Hepato-Pancreato Biliary Association (IHPBA) 2014**
   "Early Organ Dysfunction Affects Long-Term Survival of Acute Pancreatitis Patients". **Skouras C**, Hayes AJ, Williams L, Garden OJ, Parks RW, Mole DJ.

2. **Alpine Liver and Pancreatic Surgery (ALPS) meeting 2014**
   "Early Organ Dysfunction Affects Long-Term Survival of Acute Pancreatitis Patients". **Skouras C**, Hayes AJ, Williams L, Garden OJ, Parks RW, Mole DJ.

3. **Pancreatic Society of Great Britain and Ireland 2014**
   "Lung ultrasonography as a direct measure of evolving respiratory dysfunction in patients with acute pancreatitis". **Skouras C**, Davis ZA, Sharkey J, Garden OJ, Parks RW, Murchison JT, Mole DJ.

4. **Association of Surgeons of Great Britain and Ireland (ASGBI) 2014**
   "Independent Predictors of Long-term Survival in Acute Pancreatitis". **Skouras C**, Williams L, Garden OJ, Parks RW, Mole DJ. Abstract published in the British Journal of Surgery.

5. **Scottish Intensive Care Society Audit Group (SICSAG) annual conference 2014.** "Acute Pancreatitis and Critical Care in Scotland". Mole DJ, Skouras C.

6. **Alpine Liver and Pancreatic Surgery (ALPS) meeting 2015**
   "Lung ultrasonography as a direct measure of evolving respiratory dysfunction in patients with acute pancreatitis". **Skouras C**, Davis ZA, Sharkey J, Garden OJ, Parks RW, Murchison JT, Mole DJ.

7. **Digestive Disorders Federation (DDF) 2015**

   "Lung ultrasonography as a direct measure of evolving respiratory dysfunction in patients with acute pancreatitis". **Skouras C**, Davis ZA, Sharkey J, Garden OJ, Parks RW, Murchison JT, Mole DJ. Abstract published in Gut.

8. **Association of Surgeons of Great Britain and Ireland (ASGBI) 2016**

   "The Kynurenine Pathway of Tryptophan Metabolism in Patients with Acute Pancreatitis". **Skouras C**, Mole DJ, on behalf of the UoE-GSK DPAc collaboration. Abstract published in the British Journal of Surgery. **Moynihan prize finalist.**

Appendix

PARTICIPANT CONSENT FORM

PARTICIPANT CONSENT FORM – V 2.0 – 14.08.2013

Study Title: Inflammation, Metabolism and Organ Failure in Acute Pancreatitis

Name of Researcher	
Site	
Patient study identification number	

Please **initial** each box

1. I confirm that I have read and understand the information leaflet dated 14th August 2013 (version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Edinburgh, from regulatory authorities or from the NHS Board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree that the samples I have given and the information gathered about me can be stored by Mr. Damian Mole at the University of Edinburgh and that the surplus samples and information may be used in the future for other ethically approved research projects. ☐
5. I understand that some research may be carried out, using my anonymised samples and information, by clinical, academic or commercial researchers in the UK and overseas. ☐
6. I understand that I will not benefit financially from any research that may lead to the development of a new treatment or medical tests in the future. ☐
7. I understand that as part of my standard care I may undergo tests that will expose me to ionizing radiation. These will be decided only by the doctors looking after me, but the researchers will have access to the results of these tests. ☐
8. I agree to take part in the above study. ☐
9. I agree to my GP being informed of my participation in the study. ☐

	YES	NO
Genetic Consent (optional) – *please **initial** either "Yes" or "No" box:* I agree to provide research blood, saliva, urine samples for the genetic analysis.	☐	☐
Use of data and already collected samples after withdrawal (optional) – *please **initial** either "Yes" or "No" box:* In case I decide to withdraw, I give my permission for the ongoing use of already collected data and samples and for the future recording and use of routinely collected clinical data.	☐	☐

Name of Participant…… Signature…… Date……

Name of Investigator…… Signature…… Date……

Original – to Investigator Site File *First Copy – to participant* *Second Copy – To Hospital Notes*

IMOFAP
Participant Consent Form
Version 2.0 – 14th of August 2013

Page **1** of **1**

RELATIVE/WELFARE GUARDIAN CONSENT FORM

RELATIVE/WELFARE GUARDIAN CONSENT FORM – V 2.0 – 14.08.2013

Study Title: Inflammation, Metabolism and Organ Failure in Acute Pancreatitis

Name of Researcher	
Site	
Patient study identification number	

Please **initial** each box

1.	I confirm that I have read and understand the information leaflet dated 14th August 2013 (version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my relative's/ward's participation is voluntary and that I am free to withdraw at any time without giving any reason, without my relative's/ward's medical care or legal rights being affected.	☐
3.	I understand that relevant sections of my relative's/ward's medical notes and data collected during the study may be looked at by individuals from the University of Edinburgh, from regulatory authorities or from the NHS Board, where it is relevant to his/her taking part in this research. I give permission for these individuals to have access to my relative's/ward's records.	☐
4.	I agree that the samples collected and the information gathered about relative/ward can be stored by Mr. Damian Mole at the University of Edinburgh and that the surplus samples and information may be used in the future for other ethically approved research projects.	☐
5.	I understand that some research may be carried out, using my relative's/ward's anonymised samples and information, by clinical, academic or commercial researchers in the UK and overseas.	☐
6.	I understand that I or my relative/ward will not benefit financially from any research that may lead to the development of a new treatment or medical tests in the future.	☐
7.	I understand that as part of my relative's/ward's standard care he/she may undergo tests that will expose him/her to ionizing radiation. These will be decided only by the doctors looking after my relative/ward, but the researchers will have access to the results of these tests.	☐
8.	I give consent for my relative/ward to take part in the above study.	☐
9.	I agree to my relative's/ward's GP being informed of my participation in the study.	☐

	YES	NO
Genetic Consent (optional) – *please **initial** either "Yes" or "No" box:* I agree to provide research blood, saliva, urine samples for the genetic analysis.	☐	☐
Use of data and already collected samples after withdrawal (optional) – *please **initial** either "Yes" or "No" box:* In case I decide to withdraw, I give my permission for the ongoing use of already collected data and samples and for the future recording and use of routinely collected clinical data.	☐	☐

I confirm that I am the nearest relative for ..... and that no other nearest relative or welfare attorney or guardian exists.

Relationship to patient: .....

I confirm that I am the Welfare Attorney or Guardian for: .....

Name of Relative/Welfare Guardian..... Signature..... Date.....

Name of Investigator..... Signature..... Date.....

Original – to Investigator Site File *First Copy – to participant* *Second Copy – To Hospital Notes*

PARTICIPANT WITH RECOVERED CAPACITY CONSENT FORM

PARTICIPANT WITH RECOVERED CAPACITY CONSENT FORM – V 2.0 – 14.08.2013

Study Title: Inflammation, Metabolism and Organ Failure in Acute Pancreatitis

Name of Researcher	
Site	
Patient study identification number	

Please **initial** each box

1.	I confirm that I have read and understand the information leaflet dated 14th August 2013 (version 2.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
3.	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Edinburgh, from regulatory authorities or from the NHS Board, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4.	I agree that the samples I have given and the information gathered about me can be stored by Mr. Damian Mole at the University of Edinburgh and that the surplus samples and information may be used in the future for other ethically approved research projects.	☐
5.	I understand that some research may be carried out, using my anonymised samples and information, by clinical, academic or commercial researchers in the UK and overseas.	☐
6.	I understand that I will not benefit financially from any research that may lead to the development of a new treatment or medical tests in the future.	☐
7.	I understand that as part of my standard care I may undergo tests that will expose me to ionizing radiation. These will be decided only by the doctors looking after me, but the researchers will have access to the results of these tests.	☐
8.	I agree to continue participating in the above study.	☐
9.	I agree to my GP being informed of my participation in the study.	☐

	YES	NO
Genetic Consent (optional) – *please **initial** either "Yes" or "No" box:* I agree to provide research blood, saliva, urine samples for the genetic analysis.	☐	☐
Use of data and already collected samples after withdrawal (optional) – *please **initial** either "Yes" or "No" box:* In case I decide to withdraw, I give my permission for the ongoing use of already collected data and samples and for the future recording and use of routinely collected clinical data.	☐	☐

Name of Participant…… Signature…… Date……

Name of Investigator…… Signature…… Date……

Original – to Investigator Site File *First Copy – to participant* *Second Copy – To Hospital Notes*

PARTICIPANT INFORMATION SHEET

Study Title: Inflammation, Metabolism and Organ Failure in Acute Pancreatitis
Participant information sheet – V 2.0 – 14/08/2013

We are inviting you to take part in research. Before you decide, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully and discuss with a member of the research team if you would like any further explanation.

Purpose of our study

You have been invited to participate in this research study because your doctors believe that you may be having acute pancreatitis. The aim of this study is to investigate how this disease causes problems with other organs, for example lung damage or kidney failure. This study is being conducted by researchers at the University of Edinburgh and NHS Lothian University Hospitals. It has been reviewed and has been given a favourable opinion by an independent group of health professionals, the *Scotland A Research Ethics Committee,* in order to protect your interests.

What is involved if I decide to take part?

We would like to take some samples of blood, urine and saliva from you, in order to perform some specialized tests. All research samples will be in addition to the ones taken by the doctors looking after you, and will be collected at specific stages of your admission. We will also perform two ultrasound scans of your lungs, to check if they have been affected. These scans will not expose you to any radiation and do not cause any pain, but you may have some discomfort when the blood samples are being taken. Furthermore, we will process the first samples taken by your doctors on this admission and we will record information about you (for example your date of birth, any medical conditions, possible causes of your pancreatitis, information about how you are recovering).

You don't have to do anything special and your participation will end after 7 days, or on your discharge, whichever comes first. The doctors looking after you may decide to organise some X-Rays, CT scans and/or a special camera test called ERCP for you, if this is necessary for your care. These tests will expose you to low doses of radiation, but the risk is very small. The researchers will have access to the results of these tests, but you can still take part in the study even if none of these tests are required in your case. If your acute pancreatitis was discovered during one of these tests, the research team would like to have access to those results.

Do I have to take part in this research?

It is up to you to decide whether you wish to join this research study or not and your care will be the same whether you agree to participate or not. If you agree to take part, we will ask you to sign a consent form, but if at any time you decide that you no longer wish to participate, you are free to withdraw, without giving a reason and without any effect on your care. In case you decide to withdraw, with your permission we may continue using any samples and information we have already collected and in the future we may look up information about how you have recovered on the hospital computer or in your notes.

Will my details be kept confidential?

All the information you give to the research staff and all the information collected from your records will be kept confidential and will be stored in a secure electronic database of the University of Edinburgh. You will be allocated a unique study participation number and the samples will be coded. Therefore no names or identifying facts about you will be used on any research documentation or samples. You will not be able to be identified by the researchers who will be using the tissue samples in their laboratories. It is however important that the

research team hold the link relating your participant number and sample codes to your personal details because it is possible that some participants may take part more than once in our studies over a period of time and this would enable this important information to be confirmed.

With your permission, we will inform your GP that you have accepted to participate in this study. It is also a requirement that your records in this research are made available for scrutiny by monitors from NHS Lothian, whose role is to check that research is properly conducted and the interests of participants are protected.

How might my samples be used?

Your samples will be used to measure the levels of substances called "kynurenines" and to perform other common tests. These results will be analysed at different stages of your disease, together with your scan results and all collected information. With your permission, we will also use your samples for genetic research to see if the genes responsible for these substances can tell us how quickly you might recover and if you are more likely to get any complications. If you do not wish your samples to undergo genetic analysis, you may still take part in the main study.

The information, results and samples will be stored for up to 5 years at the University of Edinburgh by the Chief Investigator (Mr. Damian Mole) and we ask for your permission to use these in future research, after further approval from the Research Ethics Committee. This will provide an invaluable resource which may be used by researchers many times in several different studies. Future studies cannot be specified in detail at present, but will be in the field of acute pancreatitis and/or organ failure. Some studies may involve collaboration(s) with laboratories in other universities or commercial companies in the U.K. or overseas. At the end of the 5 year period and if your samples cannot be used for further research, they will be destroyed according to the recommendations of the University of Edinburgh.

Can I benefit from taking part in this research?

This is study is non-therapeutic and you cannot expect any benefit. If researchers for example, develop a new drug, treatment or test it will not be possible for you to claim any money because you participated. However, any new drug, treatment or test would hopefully benefit many people in the future. We do not expect that any of the study findings will be of importance to you and your own medical health, however with your permission we will inform your GP if any significant information is found unexpectedly.

Individual participants will not be personally made aware of the research findings. However, if the overall research programme progresses to potential therapies, this will be made public through the media through the University of Edinburgh and NHS Lothian Press Offices.

What if there is a problem?

If you have any concerns about this study, the researchers will be glad to discuss with you in person or by telephone, on 0131 242 3616. If you would like to discuss with a doctor who is not involved in the study, you can contact Dr. Jonathan Fallowfield on 0131 242 6655. If you remain unhappy and wish to complain formally, you can contact the NHS Lothian Complaints Team at Waverley Gate, 2-4 Waterloo Place, Edinburgh, EH13EG (Tel: 01315363370; Email: complaints.team@nhslothian.scot.nhs.uk).

Who is organising and funding the research?

The University of Edinburgh and NHS Lothian will be co-sponsoring this study, through the established joint research and development office (ACCORD). Support staff and running costs will be funded by an industry collaborator, GlaxoSmithKline.

Researcher contact details

- **Mr. Damian Mole – Senior Lecturer in Surgery & Consultant Surgeon**
  Tel: 0131 242 3616, Email: damian.mole@ed.ac.uk
- **Mr. Christos Skouras – Clinical/Research Fellow, Clinical Surgery**
  Tel: 0131 242 3616, Email: christos.skouras@ed.ac.uk

THANK YOU FOR READING THIS INFORMATION SHEET

RELATIVE/WELFARE GUARDIAN INFORMATION SHEET

Study Title: Inflammation, Metabolism and Organ Failure in Acute Pancreatitis
Relative/welfare guardian information sheet – V 2.0 – 14/08/2013

As the relative or welfare guardian of **INSERT NAME** you are being invited to give your consent for **INSERT NAME** to take part in research, because **HE/SHE** currently lacks capacity. Before you decide, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully and discuss with a member of the research team if you would like any further explanation.

Purpose of our study

INSERT NAME has been invited to participate in this research study because **HIS/HER** doctors believe that **HE/SHE** may be having acute pancreatitis. The aim of this study is to investigate how this disease causes problems with other organs, for example lung damage or kidney failure. This study is being conducted by researchers at the University of Edinburgh and NHS Lothian University Hospitals. It has been reviewed and has been given a favourable opinion by an independent group of health professionals, the *Scotland A Research Ethics Committee,* in order to protect each research participant's interests.

What is involved if I decide to permit participation of my relative/ward?

We would like to take some samples of blood, urine and saliva from **INSERT NAME**, in order to perform some specialized tests. All research samples will be in addition to the ones taken by the doctors looking after **HIM/HER**, and will be collected at specific stages of **HIS/HER** admission. We will also perform two ultrasound scans of **HIS/HER** lungs, to check if they have been affected. These scans will not expose **HIM/HER** to any radiation and do not cause any pain, but **HE/SHE** may have some discomfort when the blood samples are being taken. Furthermore, we will process the first samples taken by **HIS/HER** doctors on this admission and we will record information about **HIM/HER** (for example **INSERT NAME's** date of birth, any medical conditions, possible causes of **HIS/HER** pancreatitis, information about how **HE/SHE** is recovering).

You don't have to do anything special and **INSERT NAME's** participation will end after 7 days, or on **HIS/HER** discharge, whichever comes first. The doctors looking after **HIM/HER** may decide to organise some X-Rays, CT scans and/or a special camera test called ERCP for **HIM/HER**, if this is necessary for **HIS/HER** care. These tests will expose **HIM/HER** to low doses of radiation, but the risk is very small. The researchers will have access to the results of these tests, but **INSERT NAME** can still take part in the study even if none of these tests are required in **HIS/HER** case. If **HIS/HER** acute pancreatitis was discovered during one of these tests, the research team would like to have access to those results.

Do my relative/ward have to take part in this research?

It is up to you to decide whether you wish **INSERT NAME** to join this research study or not and **HIS/HER** care will be the same whether you agree for **HIM/HER** to participate or not. If you agree to take part, we will ask you to sign a consent form, but if at any time you decide that you no longer wish your relative/ward to participate, you are free to withdraw, without giving a reason and without any effect on **HIS/HER** care. In case you decide to withdraw, with your permission we may continue using any samples and information we have already collected and in the future we may look up information about how **HE/SHE** has recovered on the hospital computer or in your notes. If **INSERT NAME** regains capacity during his participation in the study, we will seek HIS/HER consent, in order to remain in the study.

Will HIS/HER details be kept confidential?

All the information collected from **HIS/HER** records will be kept confidential and will be stored in a secure electronic database of the University of Edinburgh. **INSERT NAME** will be allocated a unique study participation number and the samples will be coded. Therefore no names or identifying facts about **HIM/HER** will be used on any research documentation or samples. **HE/SHE** will not be able to be identified by the researchers who will be using the tissue samples in their laboratories. It is however important that the research team hold the link relating **HIS/HER** participant number and sample codes to **HIS/HER** personal details because it is possible that some participants may take part more than once in our studies over a period of time and this would enable this important information to be confirmed.

With your permission, we will inform **HIS/HER** GP that you have accepted our invitation for **INSERT NAME** to participate in this study. It is also a requirement that **HIS/HER** records in this research are made available for scrutiny by monitors from NHS Lothian, whose role is to check that research is properly conducted and the interests of participants are protected.

How might my relative's/ward's samples be used?

All samples will be used to measure the levels of substances called "kynurenines" and to perform other common tests. These results will be analysed at different stages of **INSERT NAME**'s disease, together with the scan results and all collected information. With your permission, we will also use the samples for genetic research to see if the genes responsible for these substances can tell us how quickly **HE/SHE** might recover and if **HE/SHE** is more likely to get any complications. If you do not wish **INSERT NAME's** samples to undergo genetic analysis, you may still agree for **HIM/HER** to take part in the main study.

The information, results and samples will be stored for up to 5 years at the University of Edinburgh by the Chief Investigator (Mr. Damian Mole) and we ask for your permission to use these in future research, after further approval from the Research Ethics Committee. This will provide an invaluable resource which may be used by researchers many times in several different studies. Future studies cannot be specified in detail at present, but will be in the field of acute pancreatitis and/or organ failure. Some studies may involve collaboration(s) with laboratories in other universities or commercial companies in the U.K. or overseas. At the end of the 5 year period and if the samples cannot be used for further research, they will be destroyed according to the recommendations of the University of Edinburgh.

Can my relative/ward benefit from taking part in this research?

This is study is non-therapeutic and you cannot expect any benefit. If researchers for example, develop a new drug, treatment or test it will not be possible for you or your relative/ward to claim any money because you participated. However, any new drug, treatment or test would hopefully benefit many people in the future. We do not expect that any of the study findings will be of importance to INSERT NAME and **HIS/HER** own medical health, however with your permission we will inform your GP if any significant information is found unexpectedly.

Individual participants will not be personally made aware of the research findings. However, if the overall research programme progresses to potential therapies, this will be made public through the media through the University of Edinburgh and NHS Lothian Press Offices.

What if there is a problem?

If you have any concerns about this study, the researchers will be glad to discuss with you in person or by telephone, on 0131 242 3616. If you would like to discuss with a doctor who is not involved in the study, you can contact Dr. Jonathan Fallowfield on 0131 242 6655. If you remain unhappy and wish to complain formally, you can contact the NHS Lothian Complaints Team at Waverley Gate, 2-4 Waterloo Place, Edinburgh, EH13EG (Tel: 01315363370; Email: complaints.team@nhslothian.scot.nhs.uk).

Who is organising and funding the research?

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Researcher contact details

- **Mr. Damian Mole – Senior Lecturer in Surgery & Consultant Surgeon**
  Tel: 0131 242 3616, Email: damian.mole@ed.ac.uk
- **Mr. Christos Skouras – Clinical/Research Fellow, Clinical Surgery**
  Tel: 0131 242 3616, Email: christos.skouras@ed.ac.uk

THANK YOU FOR READING THIS INFORMATION SHEET

PARTICIPANT WITH RECOVERED CAPACITY INFORMATION SHEET

Study Title: Inflammation, Metabolism and Organ Failure in Acute Pancreatitis
Participant information sheet – Recovered Capacity – V 2.0 – 14/08/2013

We are inviting you to consider continuing to take part in research. Before you decide, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully and discuss with a member of the research team if you would like any further explanation.

Why am I already in this study?

During your recent admission to hospital you were unable to give consent for participation in a study; we therefore asked your nearest relative or welfare attorney or guardian who gave consent on your behalf. This is permissible under the Adults with Incapacity Act (Scotland) 2000. You are now capable of making an informed decision about whether you wish to continue in the study or not.

Purpose of our study

You have been invited to participate because your doctors believe that you may be having acute pancreatitis. The aim of this study is to investigate how this disease causes problems with other organs, for example lung damage or kidney failure. This study is being conducted by researchers at the University of Edinburgh and NHS Lothian University Hospitals. It has been reviewed and has been given a favourable opinion by an independent group of health professionals, the *Scotland A Research Ethics Committee,* in order to protect your interests.

What is involved if I decide to continue taking part?

We would like to take some samples of blood, urine and saliva from you, in order to perform some specialized tests. All research samples will be in addition to the ones taken by the doctors looking after you, and will be collected at specific stages of your admission. We will also perform two ultrasound scans of your lungs, to check if they have been affected. These scans will not expose you to any radiation and do not cause any pain, but you may have some discomfort when the blood samples are being taken. **We have already taken X samples and performed X scans of your lungs.** Furthermore, we will process the first samples taken by your doctors on this admission and we will record information about you (for example your date of birth, any medical conditions, possible causes of your pancreatitis, information about how you are recovering).

You don't have to do anything special and your participation will end after 7 days, or on your discharge, whichever comes first. The doctors looking after you may decide to organise some X-Rays, CT scans and/or a special camera test called ERCP for you, if this is necessary for your care. These tests will expose you to low doses of radiation, but the risk is very small. The researchers will have access to the results of these tests, but you can still take part in the study even if none of these tests are required in your case. If your acute pancreatitis was discovered during one of these tests, the research team would like to have access to those results.

Do I have to take part in this research?

It is up to you to decide whether you wish to join this research study or not and your care will be the same whether you agree to participate or not. If you agree to take part, we will ask you to sign a consent form, but if at any time you decide that you no longer wish to participate, you are free to withdraw, without giving a reason and without any effect on your care. In case you decide to withdraw, with your permission we may continue using any samples and information we have already collected and in the future we may look up information about how you have recovered on the hospital computer or in your notes.

Will my details be kept confidential?

All the information you give to the research staff and all the information collected from your records will be kept confidential and will be stored in a secure electronic database of the University of Edinburgh. You will be allocated a unique study participation number and the samples will be coded. Therefore no names or identifying facts about you will be used on any research documentation or samples. You will not be able to be identified by the researchers who will be using the tissue samples in their laboratories. It is however important that the research team hold the link relating your participant number and sample codes to your personal details because it is possible that some participants may take part more than once in our studies over a period of time and this would enable this important information to be confirmed.

With your permission, we will inform your GP that you have accepted to participate in this study. It is also a requirement that your records in this research are made available for scrutiny by monitors from NHS Lothian, whose role is to check that research is properly conducted and the interests of participants are protected.

How might my samples be used?

Your samples will be used to measure the levels of substances called "kynurenines" and to perform other common tests. These results will be analysed at different stages of your disease, together with your scan results and all collected information. With your permission, we will also use your samples for genetic research to see if the genes responsible for these substances can tell us how quickly you might recover and if you are more likely to get any complications. If you do not wish your samples to undergo genetic analysis, you may still take part in the main study.

The information, results and samples will be stored for up to 5 years at the University of Edinburgh by the Chief Investigator (Mr. Damian Mole) and we ask for your permission to use these in future research, after further approval from the Research Ethics Committee. This will provide an invaluable resource which may be used by researchers many times in several different studies. Future studies cannot be specified in detail at present, but will be in the field of acute pancreatitis and/or organ failure. Some studies may involve collaboration(s) with laboratories in other universities or commercial companies in the U.K. or overseas. At the end of the 5 year period and if your samples cannot be used for further research, they will be destroyed according to the recommendations of the University of Edinburgh.

Can I benefit from taking part in this research?

This is study is non-therapeutic and you cannot expect any benefit. If researchers for example, develop a new drug, treatment or test it will not be possible for you to claim any money because you participated. However, any new drug, treatment or test would hopefully benefit many people in the future. We do not expect that any of the study findings will be of importance to you and your own medical health, however with your permission we will inform your GP if any significant information is found unexpectedly.

Individual participants will not be personally made aware of the research findings. However, if the overall research programme progresses to potential therapies, this will be made public through the media through the University of Edinburgh and NHS Lothian Press Offices.

What if there is a problem?

If you have any concerns about this study, the researchers will be glad to discuss with you in person or by telephone, on 0131 242 3616. If you would like to discuss with a doctor who is not involved in the study, you can contact Dr. Jonathan Fallowfield on 0131 242 6655. If you remain unhappy and wish to complain formally, you can contact the NHS Lothian Complaints Team at Waverley Gate, 2-4 Waterloo Place, Edinburgh, EH13EG (Tel: 01315363370; Email: complaints.team@nhslothian.scot.nhs.uk).

Who is organising and funding the research?

The University of Edinburgh and NHS Lothian will be co-sponsoring this study, through the established joint research and development office (ACCORD). Support staff and running costs will be funded by an industry collaborator, GlaxoSmithKline.

Researcher contact details

- **Mr. Damian Mole – Senior Lecturer in Surgery & Consultant Surgeon**
  Tel: 0131 242 3616, Email: damian.mole@ed.ac.uk
- **Mr. Christos Skouras – Clinical/Research Fellow, Clinical Surgery**
  Tel: 0131 242 3616, Email: christos.skouras@ed.ac.uk

THANK YOU FOR READING THIS INFORMATION SHEET

GENERAL PRACTITIONER LETTER

IMOFAP GP Information Letter – V 1.0 – 03/07/2013

[GP Surgery]

[Recipient's name & address]

MRC Centre for Inflammation Research,
W2.16, Queen's Medical Research Institute,
The University of Edinburgh,
47 Little France Crescent,
Edinburgh, EH16 4TJ,
United Kingdom

Date **[date]**
Enquiries to: Mr. Damian J. Mole
Direct Line +44 (0)131 242 3616
Fax +44 (0)131 242 3617

Dear Dr. **[Recipient]**

RE: [Patient's Details]

NOTIFICATION OF RECRUITMENT TO A CLINICAL RESEARCH STUDY – Please note that this letter is not intended to be part of direct patient care correspondence and that a clinical discharge summary will follow in due course.

I would like to inform you that your patient has been admitted in the Royal Infirmary of Edinburgh with a likely diagnosis of acute pancreatitis. [He/She] has been recruited as a participant in a Clinical Study of the Department of Clinical Surgery of the University of Edinburgh, entitled: "Inflammation, Metabolism and Organ Failure in Acute Pancreatitis". This study has been approved by the designated Research Ethics Committee. This study is observational only and there is no randomisation or intervention or any departure from the usual clinical care received by participants.

A formal discharge summary will follow, and no action is required on your behalf.

Please feel free to contact me should you require any further information.

Yours sincerely,

Mr. Damian J. Mole, BMedSc, MBChB, PhD, FRCS

Clinician Scientist Fellow and Honorary Consultant Surgeon
Royal Infirmary of Edinburgh
The University of Edinburgh

LAY-PERSON SUMMARY

Inflammation, Metabolism, and Organ Failure in Acute Pancreatitis

One Page Study Summary – V3.0 – 21/08/2013

What is acute pancreatitis? Acute pancreatitis (AP) is inflammation of the pancreas usually triggered by gallstones or drinking excessive alcohol. Most people who have an episode of AP get better without having any complications, but approximately 1 out of every 4 people with AP will have problems with the pancreas or with other organs, for example the lungs and kidneys, and require treatment in high dependency or intensive care.

Why are we doing this study? We are doing research to try and reduce the harm done during a severe attack of AP. We have discovered that a particular aspect of the immune system is involved in AP and we are working with a drug company (GSK), to develop better medicines to treat AP. We are **not** testing any new medicines or drugs in this study but we want to see how part of the immune system changes in AP.

Who is regulating this study? This study has been examined and approved by the Scotland A Research Ethics Committee, the University of Edinburgh, NHS Lothian ACCORD R&D Office and the Caldicott Guardian (patient confidentiality authority).

Where is the study taking place? At the Royal Infirmary of Edinburgh.

Is this a clinical trial? No, we are not testing or comparing any new medicines or treatments. We are gathering information and clinical samples.

Will participation affect my treatment? No, participation will not affect the way any person with AP is looked after.

How long does the study last? Each participant will be in the study for 1 week, or less if you are discharged from hospital before that. We will continue to check routinely collected information (e.g. about any subsequent illnesses) relevant to the study for up to 5 years.

Who can participate? Most adults (aged 16 years or over) with AP can be considered for participation. Certain individuals belonging to vulnerable groups e.g. prisoners will not be able to participate.

What is involved if I decide to participate?

- We will collect detailed clinical information about your health and your episode of AP, including collecting information from scans done as part of your normal care
- We will invite you to donate a series of blood, urine and saliva samples, at regular time points over one week making a total of 8 blood samples, 8 urine samples and 1 saliva sample.
- We will invite you to have 2 lung ultrasound scans during the week, in addition to scans organised by the team looking after you.
- If you participate, you will remain in the study for a total of seven days or until hospital discharge, whichever comes first, but we will continue to gather relevant information from your records for a up to 5 years.
- The samples will be analysed for biochemical markers of inflammation and to see how cells change the way they use their genes during AP. If you have a specific wish not to have any genetic analysis done on your samples, then you can opt out of that part of the study but still participate in the rest of the study.

Can I see my own results from the study? We are not able to disclose any results to individual participants, but we will be able to inform you of the outcome of the analysis of the whole study when it is complete.

Where can I get more information? We would be very happy to answer any questions about the study. Please get in touch with Mr Christos Skouras, Postgraduate research fellow and Investigator, or Mr Damian Mole, Chief Investigator on tel: 0131 242 3616, or by email (christos.skouras@ed.ac.uk or damian.mole@ed.ac.uk)

Thank you for reading this information sheet.

HEALTHCARE PROFESSIONAL SUMMARY

Inflammation, Metabolism, and Organ Failure in Acute Pancreatitis

Study Summary – V2.0 – 21/08/2013

PLEASE NOTE THAT THIS DOCUMENT IS NOT A PATIENT INFORMATION SHEET AND DOES NOT FORM PART OF THE INFORMED CONSENT PACK.

Introduction

Acute pancreatitis (AP) is inflammation of the pancreas usually triggered by gallstones or drinking excessive alcohol. Most people who have an episode of AP experience a short self-limiting illness, but approximately 1 out of every 4 will develop dysfunction of one or more organ systems and often require treatment in high dependency or intensive care. The overall mortality in AP is approximately 7%, but for those who get organ dysfunction, the mortality approaches 20%.

We are investigating ways in which we can reduce the proportion of patients who suffer a severe attack and reduce the harm done during a severe attack. We have identified that a particular metabolic pathway that also affects the immune system (called the kynurenine pathway of tryptophan metabolism) is important in experimental models of AP and in preliminary studies involving patients. We are working with GSK, a drug company, to develop new ways of altering that metabolic pathway to prevent or reduce organ dysfunction and this work is at an early stage. In order to understand this inflammatory process in more detail and build a better medicine, we are examining exactly how and when this metabolic pathway is altered in patients with AP.

Approvals

This study will have been reviewed and prior to commencement will have confirmed approval from the Scotland A Research Ethics Committee, University of Edinburgh/NHS Lothian ACCORD R&D Office and the Office of the Caldicott Guardian for NHS Lothian.

Study design and endpoints:

1. The present study is an observational, prospective, clinical study, which involves no intervention or randomisation.
2. The study will be performed in the Royal Infirmary of Edinburgh.
3. **No deviation from the usual process of care should occur as a result of participation in this study and direct clinical care teams should act as they normally would while caring for any patient with AP.**
4. The whole duration of the study is one year. The recruitment period will last three months. The estimated start date is 15th September 2013.
5. We will be recruiting adult (aged 16 years or over) patients with acute pancreatitis, regardless of aetiology and as early as possible after their presentation. Patients with and without capacity will be included. Prisoners will be excluded.
6. The recruitment will be performed on 24/7 basis.
7. We are aiming to recruit up to 70 patients.

Participant Identification:

In order to i) maximise the number of patients recruited, ii) to recruit as early as possible in the disease course and, iii) to capture as many possible potential participants in order to reduce bias, we have designed the following protocol:

1. An alert for any patient with raised serum amylase result will be given from the biochemistry laboratory to the research team member on call for the study (the "investigator" – usually a research nurse from the WT CRF).
2. The investigator will review the clinical notes (electronic and/or hand-written) to ascertain eligibility.
3. If patient is eligible for recruitment, the investigator will liaise with a member of the clinical care team and ask them to make the initial approach to the potential participant as follows:
    a. For patients with capacity, a member of the clinical care team will approach the potential participant and will ask for their permission for a discussion with the investigator.
    b. For patients with incapacity (e.g. due to critical illness or significant alcohol intoxication), the first point of contact to the relative/welfare guardian will be a member of the clinical care team.
4. After permission has been obtained, the investigator will undertake recruitment and informed consent, both for patients with and without capacity.

Participation in the study:

1. Detailed clinical information for each recruited patient will be collected.
2. Participants will be subjected to a series of blood, urine and saliva sampling, at pre-defined time-points (a total of eight blood and urine samples and one saliva sample for each participant).
3. Two lung ultrasound scans will also be performed on each participant at pre-defined time-points.
4. Apart from lung ultrasound scans, all other imaging examinations will be decided by the clinical care team based on clinical indications and are not a pre-requisite for participation in the study. The research team will however obtain access to the results of these tests.
5. Each participant will remain in the study for a total of seven days or until hospital discharge, whichever comes first.

Please do not hesitate to contact the project lead and Co-Investigator, Mr Christos Skouras (Postgraduate research fellow), or the Chief Investigator, Mr Damian Mole (Clinician Scientist Fellow and Hon Cons Surgeon) if you have any questions, concerns or wish to clarify any aspect of this study.

Mr Christos Skouras MBBS MRCS
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Mr Damian J. Mole BMedSc MBChB PhD FRCS
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MRC Centre for Inflammation Research W2.16
Queen's Medical Research Institute
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Mobile: 07775 801868
Tel: 0131 242 6685 or 242 3616
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SAMPLE HANDLING PROTOCOL – BLOOD SAMPLES

IMOFAP – Sample handling protocol

Special samples

V1.0 – 02/09/2013

Biological sample type:

Venous blood

Equipment:

- Venepuncture equipment (gloves, alcohol swabs, tourniquet, sharps bin, etc.)
- Sarstedt Monovette tubes:
    - **Plasma:** RED top (Haematology Potassium EDTA – Tube volume 7.5 mL – reference No: 01.1605.001)
      → **x1 tube – Blood volume: 6 mL**
    - **Serum:** BROWN top (Serum-Gel Clotting Activator – Tube volume 7.5 mL – reference No: 01.1602)
      → **x1 tube – Blood volume: 6 mL**
- NUNC Cryo-Tubes (ThermoScientific – Tube volume 1.8 mL – Reference No: 375418)
  ➔ At least 2-4 NUNC tubes – **Aliquot volume: 1 mL for each tube**.

Method:

1. Aseptic technique
2. Venepuncture with Sarstedt system
3. Fill tubes at aforementioned volumes at least and label
4. **CENTRIFUGE** S-Monovette tubes on 3200 rpm for 8-10 minutes
5. Label NUNC tubes with participant ID and time-point (e.g. #0005, T+12)
6. After spinning carefully remove cap from S-Monovette tubes and aliquot 1 mL of serum/plasma from each tube into labelled NUNC freezer tubes.
7. Mark (black dot on lid) the partially filled NUNC tube(s)
8. Discard the red call pellet
9. Transfer NUNC tubes containing serum/plasma **IMMEDIATELY to -80º freezer** (or to -20º freezer until the morning for overnight samples, then to -80º freezer)

- *For patients with a Central Venous Line in situ, blood sampling can be performed via the CVL, as per local guidelines.*

SAMPLE HANDLING PROTOCOL – SALIVA SAMPLES

IMOFAP – Sample handling protocol

Saliva samples

V1.0 – 02/09/2013

Biological sample type:

Saliva

Equipment:

- BLUE top Sarstedt® Salivette® tubes for cortisol determination (Ref No: 51.1534.500)
- Nunc™ CryoTubes™ (Thermo Scientific™ – Tube volume 1.8 mL – Ref No: 375418)
  ➔ At least 2 Nunc™ tubes

Method:

1. Perform only on T0 (for conscious patients only)
2. Patient or investigator removes synthetic swab from Salivette® tube
3. Swab is placed in patient's mouth and is chewed for about 45 seconds to stimulate salivation
4. Return swab with absorbed saliva to the Salivette® tube and replace stopper
5. Label Salivette® tubes with participant ID and T0 (e.g. #0005, T0)
6. **CENTRIFUGE** for 2 minutes at 1000 g
7. After spinning open Salivette® tube and discard swab
8. Aliquot collected precipitate from the tip of the Salivette® tube in Nunc™ CryoTubes™
9. Mark filled Nunc™ tubes (black dot on lid)
10. Label Nunc™ tubes with participant ID and time-point (e.g. #0005, T0)
11. **Freeze filled Nunc™ CryoTubes™ in -80°C freezer**
12. Discard Salivette® tube

SAMPLE HANDLING PROTOCOL – URINE SAMPLES

IMOFAP – Sample handling protocol

Urine samples

V1.0 – 02/09/2013

Biological sample type:

Urine

Equipment:

- WHITE top urine tubes (Universal container)
- Nunc™ CryoTubes™ (Thermo Scientific™ – Tube volume 1.8 mL – Ref No: 375418)
  ➔ At least 2 Nunc™ tubes

Method:

1. Aseptic technique
2. Collect urine sample from participant, either by directly asking for a sample, or by retrieving sample from catheter bag in catheterised participants
3. Label white top urine tube with patient ID and time-point (e.g. #0005, T24)
4. **NO CENTRIFUGE**
5. Dip urine and record results results to dedicated study laptop
6. Carefully aliquot 1 mL of urine into each of the 2 Nunc™ CryoTubes™
7. Mark filled Nunc™ tubes (black dot on lid)
8. Label Nunc™ tubes with participant ID and time-point (e.g. #0005, T24)
9. **Freeze filled Nunc™ CryoTubes™ in -80°C freezer**
10. Safely discard white top urine tube and remaining sample

SAMPLING SUMMARY

	Container	Time-point	T0	T3	T6	T12	T24	T48	T72	T168
VENOUS BLOOD – NHS LABORATORY	**RED top 2.7 mL** (Haematology Potassium EDTA)	No spinning No freezing Send to NHS Lab	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
	GREEN top 3.0 mL (Coagulation Sodium Citrate)		3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL
	BROWN top 4.9 mL (Serum-Gel Clotting Activator)		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL
	Glucose strip	Blood sugar machine	x1 drop	x1 drop	x1 drop	x1 drop	x1 drop	x1 drop	x1 drop	x1 drop
URINE	**Dipstick**	No spinning	x1	x1	x1	x1	x1	x1	x1	x1
	White top	**NO SPIN ALIQUOT FREEZE**	x2 aliquots	x2 aliquots	x2 aliquots	x2 aliquots	x2 aliquots	x2 aliquots	x2 aliquots	x2 aliquots
VENOUS BLOOD – SPECIAL TESTS	**RED top 7.5 mL** (Haematology Potassium EDTA)	**SPIN ALIQUOT FREEZE**	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL
	BROWN top 7.5 mL (Serum-Gel Clotting Activator)		6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL
	RNA (PAXgene **RNA** Tube)	**SEE MANUAL**	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL	2.5 mL
	DNA (PAXgene **DNA** Tube)	**SEE MANUAL**	8.5 mL							
SALIVA	**BLUE top** (Salivette)	**SPIN ALIQUOT FREEZE**	x1							
ABG	**ABG tube**	Routine processing	x1	x1	x1	x1	x1	x1	x1	x1

For urine samples 1 aliquot of at least 1 mL was obtained and was frozen to -80°C. For serum special tests 2 aliquots of at least 1 mL each were obtained and were spin at 3200 rpm for 8 minutes, then frozen to -80°C. For plasma special tests 2 aliquots of at least 1 mL each were obtained. RNA & DNA samples were only obtained from participants who had given consent for genetic testing. Arterial blood samples (ABG) were obtained only when an A-line was in situ by decision of the direct clinical care team.