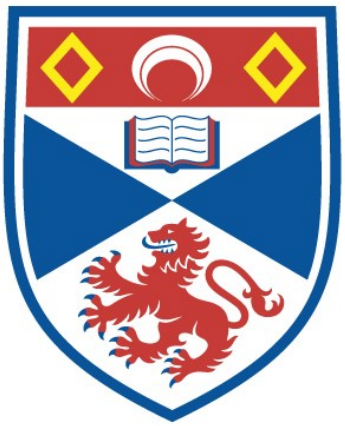
Characterisation of prognostic and cardiovascular markers in coronavirus disease 19

Alexander Qichen Liu

A thesis submitted for the degree of MD
at the
University of St Andrews



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Abbreviations

ACS	Acute coronary syndrome
AF	Atrial fibrillation
AUC	Area under the curve
ARDS	Acute respiratory distress syndrome
BAME	Black, Asian and minority ethnic
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 19
CRB-65	Risk score based on Confusion, Respiratory rate, Blood pressure, Age >65
CRP	C-reactive protein
CXR	Chest X-ray
HR	Hazard ratio
Hs-cTnT	High sensitivity cardiac troponin T
FLR	Ferritin-lymphocyte ratio
ICU	Intensive care unit
IHD	Ischaemic heart disease
LCR	Lymphocyte-CRP ratio
NIV	Non-invasive ventilation
PPE	Personal protective equipment
ROC	Receiver operating characteristics
Rt-PCR	Reverse transcriptase polymerase chain reaction
TIA	Transient ischaemic attack
WCC	White cell count

Abstract

Coronavirus disease 19 (COVID-19) is responsible for one of the worst pandemics of our time. Clinical risk stratification plays a pivotal role in guiding patient care decisions, such as admission vs. discharge from the hospital and the allocation of therapeutic resources. The development of novel biomarkers for assessing the prognostic impact of COVID-19 on patients is a clinical priority. This retrospective observational project identified cardiac, inflammatory, and risk-score-based biomarkers, and tested their prognostic value in a UK population of COVID-19 patients encountered during the first wave of the pandemic. The biomarkers included high-sensitivity cardiac troponin T (hs-cTnT), lymphocyte-CRP ratio (LCR), ferritin-lymphocyte ratio (FLR), and the non-invasive pneumonia severity score CRB-65. The results showed that hs-cTnT achieved a high negative predictive value for ruling out inpatient mortality. LCR and FLR were not superior to CRP for predicting adverse outcomes in COVID-19. Low CRB-65 scores showed high negative predictive values for ruling out both fatal and non-fatal outcomes, independent of chest X-ray findings. Five markers were shown to be independent predictors of inpatient mortality (hs-cTnT, oxygen requirement, CRB-65, FLR, and history of ischaemic heart disease). These markers were combined into a new risk score which performed well for predicting mortality in COVID-19 patients. Oxygen requirement was the only independent predictor of escalation to non-invasive ventilation, intubation/ventilation and intensive care unit admissions. Cardiac troponins, CRB-65 and the combined risk score with oxygen requirement deserve further validation for translating into clinically viable risk stratification tools in COVID-19.

Chapter 1: Introduction

Coronavirus disease 19 (COVID-19) is responsible for one of the worst pandemics in modern history,[1] with over 750 million people infected and over 7 million deaths recorded.[2] During the pandemic, healthcare services across the world endured immense pressures and medical resources were stretched to near breaking point.[3] The vast number of hospital admissions, the lack of effective treatments at the beginning of the pandemic and the heavy demands placed on acute medical services and intensive care units have overwhelmed healthcare systems across the world.[3]

While combating COVID-19, the world has suffered enormous financial costs.[4] The loss of an active workforce, repeated and costly lockdowns, and heavy restrictions on businesses have culminated in crippling effects on the global economy.[4] On an individual level, working on the "frontline" of the pandemic has pushed healthcare workers to the limits of their physical and psychological endurance.[3] Medical staff braced the risks of falling ill themselves and "battled on", often to exhaustion.[3]

1.1 Pathophysiological manifestations of COVID-19

COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which belongs to the *Coronaviridae* family and the *Sarbecovirus* genus.[5-7] Coronaviruses are large, single-stranded, positive-sense RNA viruses, which are responsible for previous outbreaks in Asia (SARS-CoV in 2003) and the Middle East (MERS-CoV in 2012).[8] SARS-CoV-2 is around 80% identical in genome to SARS-CoV but demonstrated more prolific lethality than its predecessors.[8] The transmission of

SARS-CoV-2 can take place through respiratory droplets or via the faecal-oral route.[8] The virus passes from person to person and even vertically from mother to foetus.[9]

1.1.1 Host cell invasion by SARS-CoV-2

The SARS-CoV-2 genome encodes a series of structural proteins including the membrane protein, the nucleocapsid protein, the envelope protein and the spike glycoprotein.[10] Viral invasion of the host cell begins with the S1 subunit of the viral spike protein binding to the angiotensin-converting enzyme II (ACE II) receptor on the host cell.[10] The spike protein is then cleaved by the transmembrane serine protease 2 (TMPRSS2).[11] The S2 subunit fuses the viral lipid bilayer with that of the host cell, facilitating the subsequent release of the SARS-CoV-2 ribonucleoprotein complex into the host cell.[10]

After gaining entry into the host cell cytoplasm, the viral RNA genome undergoes replication and translation into SARS-CoV-2 structural and accessory proteins.[12] The replication unit of the virus has membrane vesicles, which exert a shield-like function during the replication of viral RNA, preventing the detection of the transcription intermediates by the host cell pattern recognition receptors.[10] However, certain cytoplasmic pattern recognition receptors remain capable of detecting long double-stranded RNA, leading to interferon expression.[13]

The newly produced viral particles are transported in vesicles to the host cell surface membrane and released to infect further host cells.[12] ACE II receptors are present not only in cells within the respiratory tract but also in other organs such as the gastrointestinal tract, the heart, and the kidneys.[14] This indicates that SARS-CoV-2 has the potential to affect multiple organs.

Figure 1.1 summarises SARS-CoV-2 viral invasion of the host cell.

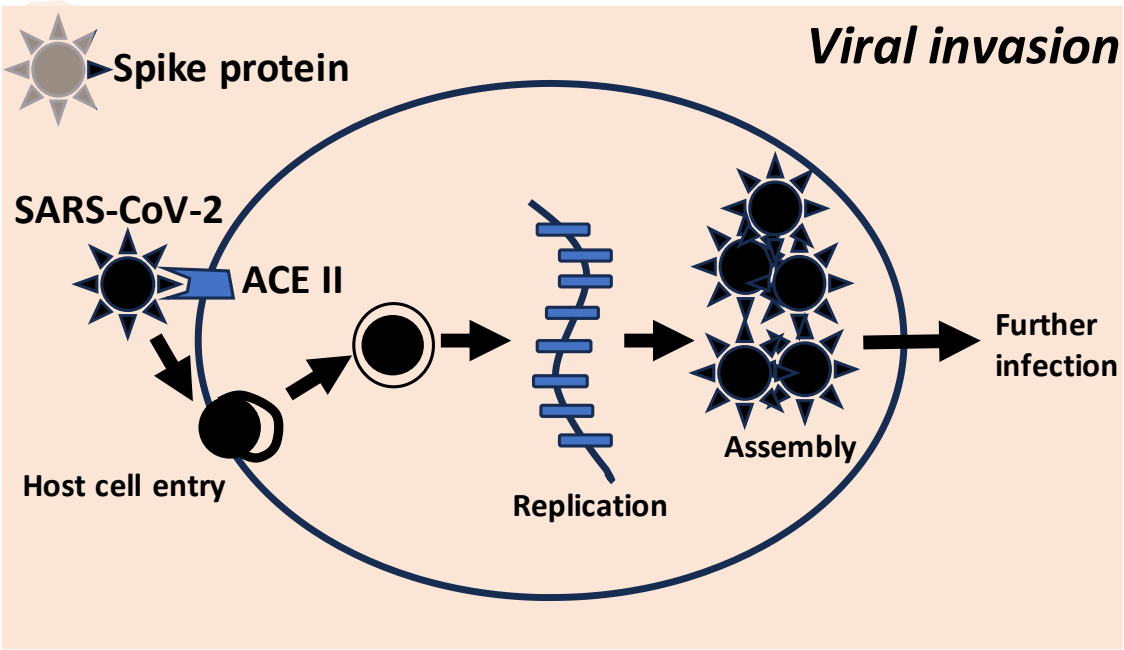


Figure 1.1: Invasion of host cell by SARS-CoV-2. ACE II: Angiotensin Converting Enzyme II receptor. After SARS-CoV-2 spike protein binds to the ACE II receptor, it is cleaved by the transmembrane serine protease 2.[11] The viral and host lipid membrane bilayers then fuse, allowing the release of the SARS-CoV-2 ribonucleoprotein complex into the host cell.[10]

1.1.2 Initial host response

The initial contact between SARS-CoV-2 and the host leads to activation of the innate immunity, as the body's frontline defence against invading micro-organisms.[12] Activation of the innate immunity curtails viral entry into host cells, limits viral replication, and signals infected cells for removal.[15] The innate immunity also acts as a broader mediator of downstream pro-inflammatory processes that lead to the priming of the adaptive immunity, which is designed for more specific viral removal.[12]

Like most pathogenic respiratory viruses, SARS-CoV-2 induces host cell death by direct cellular invasion of the respiratory tract epithelium (for instance the multi-ciliated cells),[16] leading to the release of signal molecules termed Pathogen Associated Molecular Patterns (PAMPs) and Damage Associated Molecular Patterns (DAMPs).[17] These signal molecules are then recognised by certain receptors which activate further inflammatory cascades.[17] PAMPs are recognised by pattern recognition receptors of the innate immunity, including the Toll-Like Receptors (TLR; in particular viral specific TLR 3 and 7)[18] and the cytosolic-related receptors (such as RIG-I-like receptors) expressed on macrophages and endothelial cells in the respiratory tract.[17 19] This process leads to the activation of pro-inflammatory cytokine pathways and interferon-dependent responses.[17 19] DAMPs are recognised by the nucleotide-binding domain leucine-rich repeat (NLR) proteins which lead to the activation of interleukin-1β (IL-1β) as a trigger for wider inflammatory pathways.[20]

Natural killer (NK) cells form an important part of the innate immunity, where they contribute to the first line of defence against SARS-CoV-2.[21] Major histocompatibility complex (MHC) class I molecules expressed in healthy cells activate inhibitory receptors on NK cells.[21] This process acts like a "host password" to prevent NK cell activation and leads to self-tolerance.[21] NK cells are activated to kill infected host cells by cytotoxic degranulation and release of proinflammatory cytokines.[21]

1.1.3 Proinflammatory response and cytokine storm

The initial contact between SARS-CoV-2 and the innate immunity leads to the activation of a variety of immune responses.[8] Proinflammatory cytokines are released, including monocyte chemoattractant proteins, interferon γ (IFN-γ) and IL-6.[8] There is increased

recruitment of macrophages and dendritic cells to the foci of infection, with immune activation and antigen presentation functions.[10] SARS-CoV-2 can also infect these incoming immune cells, leading to further and aggressive proinflammatory cytokine and chemokine activation.[10] The multi-faceted immune activation leads to elevated levels of circulating interleukins (including IL-2, IL-7, IL-10), granulocyte colony stimulating factors (G-CSF), macrophage inflammatory proteins (MIP) and tumour necrosis factor (TNF).[8 10] The communication between the innate immunity and the adaptive immunity takes place through cytokine release and antigen presentation, leading to the recruitment of antigen-specific T lymphocytes, which respond to and kill infected host cells, limiting the propagation of viral replication.[22]

Figure 1.2 summarises the activation of the innate immunity in response to cellular injury induced by SARS-CoV-2.

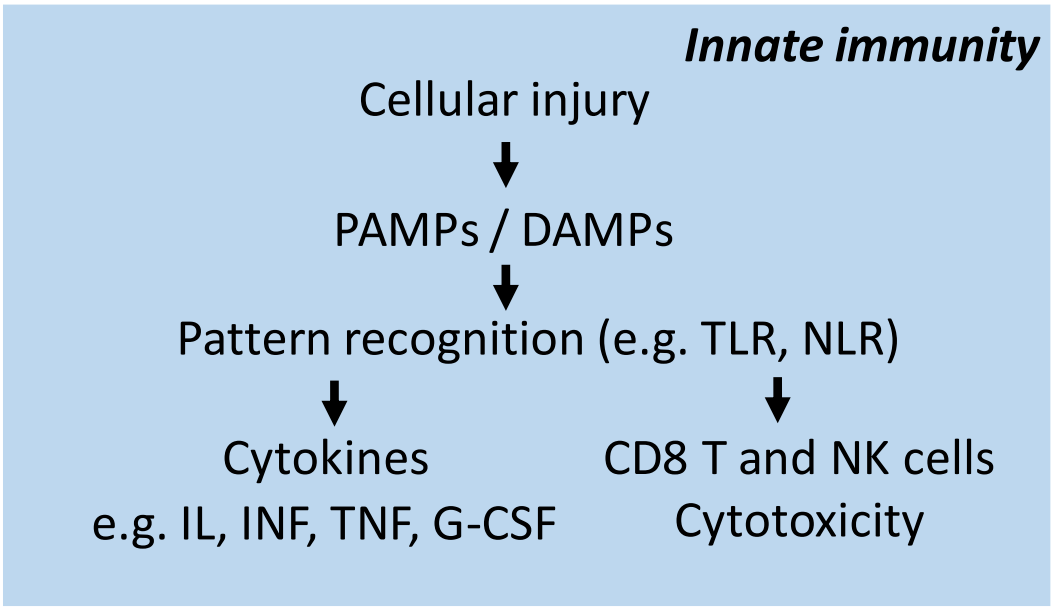


Figure 1.2: Innate immune responses to cellular injury induced by SARS-CoV-2. DAMPs: Damage Associated Molecular Patterns; G-CSF: Granulocyte Colony Stimulating Factors; IFN: interferons; IL: interleukins; NK: Natural Killer; NLR:

nucleotide-binding domain leucine-rich repeat proteins; PAMPs: Pathogen Associated Molecular Patterns; TLR: Toll-Like Receptor; and TNF: Tumour Necrosis Factor.

1.1.4 Innate to adaptive immunity

Although discussed in separate sections, the innate immunity and the adaptive immunity are not mutually exclusive; they are closely linked by complex cascades of activation.[8] Beyond the initial interaction with the innate immunity, SARS-CoV-2 faces further MHC-based host defences.[23] Class I MHCs are expressed on all nucleated cells and platelets, which present antigens to the T-cell receptors (TCR) of naïve $CD8^+$ T cells.[24] Upon antigen recognition, the naïve $CD8^+$ T cells undergo activation, clonal expansion and differentiation into effector cells, which can kill the target host cell or release proinflammatory cytokines.[24]

Class II MHCs are expressed on the membranes of antigen-presenting cells, such as macrophages, monocytes, dendritic cells, and B cells, which facilitate the activation, proliferation and differentiation of B cells and $CD4^+$ T cells.[23] The expression of class II MHCs is induced and modulated by IFN-γ, interleukins (e.g. IL-4, IL-10) and TNF-α.[23] The pro-inflammatory response leads to further activation, proliferation and differentiation of B and T lymphocytes, which facilitate the production of antibodies against SARS-CoV-2.[18 23]

T-helper cells ($CD4^+$) are highly important in the regulation of the adaptive immunity against SARS-CoV-2.[18] Viral antigens activate naïve $CD4^+$ T cells, which migrate to germinal centres to become follicular T-helper cells.[18] These interact with follicular B cells, which differentiate into antibody-producing plasma cells and memory B cells.[18] Immunoglobulins (Ig) M against the SARS-CoV-2 spike protein are produced first,

followed by their IgG counterparts,[25] which appear effective *in vitro* and may offer protection against re-infections.[26] Specific antibody production from the adaptive immunity is evident from around 7 days after infection.[27] Uncoordinated activation of the B/T lymphocytes has been linked to more severe forms of COVID-19.[18] Figure 1.3 summarises the proinflammatory processes in relation to the adaptive $CD4^+$ T cells against SARS-CoV-2.

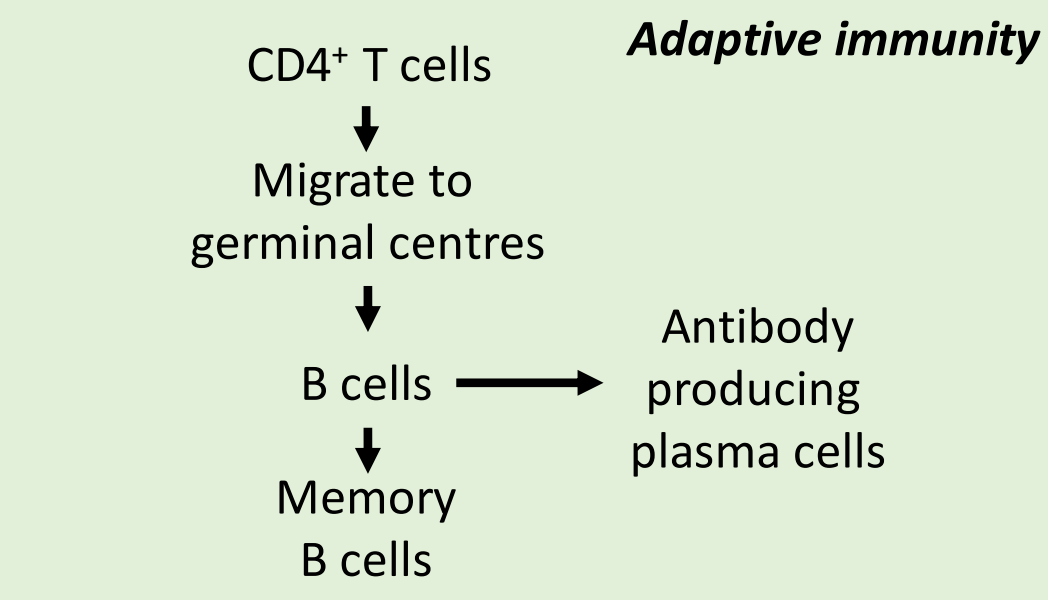


Figure 1.3: Summary of the $CD4^+$ T cell related adaptive immunity activation in COVID-19.

1.2 Organ-specific manifestations in COVID-19

An important characteristic of COVID-19 is that it is capable of affecting not only the respiratory system but also other areas such as the heart, the kidneys, the gastro-hepatic system, the haematological system, and the neurological system.[28-32] There are three possible mechanisms of organ injury in the context of SARS-CoV-2 infections: (1) direct host cell invasion by viruses, leading to cellular injury that affects organ function;[33-35] (2) cellular and organ injury secondary to the proinflammatory activation of the host

immunity by the SARS-CoV-2 infection, the ensuing cytokine storm and the septic response;[12 31 36] and (3) organ injury as a result of the thromboembolic phenomenon and the hypercoagulable state seen in COVID-19.[37-40]

1.2.1 Myocardial injury in COVID-19: possible mechanisms

Myocardial injury in COVID-19 patients has been reported since the early periods of the pandemic.[34 36 41-50] Markers of myocardial injury, e.g. cardiac troponins,[34 36 41-50] and markers of heart failure and cardiac strain, e.g. the B-type natriuretic peptide (BNP),[51-57] are known to be elevated in COVID-19 patients.

1.2.1.1 Direct viral-related injury of cardiomyocytes

The abundant expression of ACE II receptors on cardiomyocytes provides the means for direct cellular invasion by SARS-CoV-2.[14] *In vitro* studies have suggested a propensity for SARS-CoV-2 to target cardiomyocytes directly.[58] This is supported histologically by the presence of SARS-CoV-2 genome in endomyocardial biopsy samples.[59] In patients without overt cardiac symptoms during life, evidence of SARS-CoV-2 has been found in cardiomyocytes on autopsy, suggesting that viral invasion of the heart can be clinically silent.[60] Viral invasion can lead to down-regulation of genes encoding the cardiomyocyte contractile machinery, such as the sarcomeric proteins, which may manifest as cardiac dysfunction.[61] The direct invasion of cardiomyocytes can also disrupt the renin-angiotensin-aldosterone pathway by causing a reduction in ACE II, leading to excessive accumulation of angiotensin II and cardiomyocyte autophagy and apoptosis.[33] Whilst there is a body of evidence supporting direct cardiomyocyte invasion by SARS-CoV-2, it is not believed to be the only mechanism underlying myocardial injury in COVID-19.[34 36 62 63]

1.2.1.2 Myocardial injury in the context of cytokine storm

Systemic proinflammatory responses and cytokine storms in sepsis can lead to myocardial injury,[36] through processes such as endothelial dysfunction, excessive recruitment of immune cells and hypoxia related to ARDS.[36 64] Reports also suggest that a similar mechanism can potentially underpin myocardial injury in COVID-19 patients.[36 65] Elevation in myocardial injury markers, such as cardiac troponins, can be associated with a rise in markers of inflammatory response, including IL-6, which can drive the production of C-reactive protein (CRP) and ferritin.[63] This suggests that systemic inflammation and organ-specific myocardial injury may occur simultaneously.[63]

The release of IL-2, IL-6, IL-8, IL-10, and TNF-α can lead to severe systemic inflammation, promoting accelerated atherogenesis and the destabilisation of pre-existing coronary artery plaques.[37 66] The systemic proinflammatory response can also lead to catecholamine surges, microvascular damage, and stress-induced cardiomyopathy.[67] Both Takotsubo cardiomyopathy[68 69] and acute myocarditis[70-72] have been reported in the clinical context of severe systemic inflammation in COVID-19, though their prevalence is rare in imaging and autopsy studies.[73-75] It remains unclear whether it is the pro-inflammatory response or the myocardial injury (or both) that ultimately dictates the prognosis of patients.

1.2.1.3 Thromboembolic phenomenon and myocardial injury

Around a third of COVID-19 patients presenting with acute coronary syndromes (ACS) had unobstructed epicardial coronary arteries on invasive angiography.[76 77] Myocardial infarction with non-obstructive coronary arteries (MINOCA) is known to affect both adults and children with COVID-19.[78-81] Thrombotic occlusion of normal epicardial

coronary arteries has been detected during coronary angiography in patients with COVID-19,[78-80] supporting the aetiology being a hypercoagulable state instead of acute plaque rupture of atherosclerotic coronary disease.[78-80] Thrombotic occlusions (in the absence of significant atherosclerotic disease) can either be limited to only the coronary arteries or be involved in a wider systemic thromboembolic phenomenon also affecting other parts of the cardiovascular system (e.g. with concurrent left ventricular cavity thrombus or embolic ischaemic stroke).[78-81]

A postulated mechanism for the thrombotic phenomenon in COVID-19 involves the formation of microcirculatory thrombi, which may result from endothelial dysfunction and the proinflammatory hypercoagulable state generated by SARS-CoV-2 infections.[38 40] In autopsy studies, microthrombi within the coronary circulation have been co-localised to myocardial necrosis in COVID-19 patients.[40] Microthrombi aspirated from COVID-19 patients with ST-elevation myocardial infarction contain a greater degree of fibrin and complement molecules, which suggests a role played by the proinflammatory response in their pathogenesis.[40]

Spontaneous coronary artery dissection (SCAD) has also been reported in COVID-19 patients.[82-85] Although the underlying mechanism remains unclear. One possible pathophysiological process may involve the infiltration of the coronary artery adventitia and peri-adventitial tissue by T cells, leading to the activation of proteases and cytokines, and facilitating coronary vascular erosion and dissection.[86]

1.2.2 Heart failure and cardiogenic shock

Development of heart failure and/or cardiogenic shock in COVID-19 patients is multi-factorial, including potential mechanisms such as direct viral-mediated cardiomyocyte

injury; proinflammatory state of infection; supply and demand mismatch in sepsis; volume overload, and stress from critical illness.[87] Around a quarter of patients with COVID-19 can develop new-onset heart failure in the context of severe systemic inflammation.[28] Left ventricular systolic and diastolic dysfunction can occur in the presence of acute myocardial injury but without a prior history of heart failure.[88 89] COVID-19 patients who develop cardiogenic shock tend to have pre-existing co-morbidities such as diabetes mellitus and ischaemic heart disease.[90] Patients presenting with acute myocardial infarction and concurrent COVID-19 are more likely to develop cardiogenic shock,[91] possibly owing to delays in presentation, prolonged ischaemic injury, and the systemic inflammatory state.[92]

Patients with pre-existing heart failure can decompensate with COVID-19 infections.[93] Patients with heart failure have higher levels of ACE II expression and are therefore more susceptible to contracting COVID-19 and developing more severe infections.[88 93 94] Pulmonary infection, hypoxaemia, and ARDS in COVID-19 can lead to elevated right-sided cardiac pressures and right heart failure during acute infection.[95 96] Patients can also develop right ventricular strain and failure due to a combination of COVID-19 and mechanical ventilation.[95] Acute cor pulmonale is associated with a particularly poor prognosis in COVID-19 patients.[96-98]

1.2.3 Renal involvement in COVID-19

The expression of ACE II in the kidneys renders COVID-19 patients at risk of acute kidney injury, which is frequently reported.[99] Renal involvement in COVID-19 can range from mild, e.g. with small derangements in serum creatinine levels, to acute kidney injury (AKI) and/or failure.[99] AKI can affect up to 40% of critically ill COVID-19 patients in

ICU, in whom renal replacement therapy may be required.[100] Renal involvement is also linked to a worse prognosis in patients with COVID-19.[99]

Postmortem examinations of COVID-19 patients found potential evidence of direct viral invasion of the tubular epithelium and podocytes.[101] The cytokine storm in COVID-19 can lead to a systemic septic response which results in renal hypoperfusion and reduced glomerular filtration rates.[35] Cardiac dysfunction in severe COVID-19 can also be associated with renal hypoperfusion in the context of left ventricular failure or peripheral circulatory overload in right heart failure.[35] AKI occurring in the context of ARDS related to COVID-19 often confers a poor prognosis.[35]

1.2.4 Gastro-hepatic involvement in COVID-19

Gastrointestinal symptoms such as nausea, vomiting, and diarrhoea are commonly reported in COVID-19 patients.[30] Direct invasion of the gastrointestinal tract is a potential mechanism for these clinical presentations,[30] since the viral genome has been isolated from the gut epithelial cells.[102] It is unclear whether gastrointestinal symptoms have a significant overall bearing on the prognosis of the patient.

Liver injury can occur in COVID-19,[103] where deranged liver function tests are prevalent and are associated with the development of severe pneumonia.[104] Although SARS-CoV-2 viral particles have been found in the liver on post-mortem examinations,[105] the paucity of actual ACE II expression in hepatocytes makes direct invasion an unlikely aetiology of liver injury in COVID-19.[104] Further, despite ACE II being highly expressed in the cells of the bile duct, significant elevations of bile duct related injury markers, such as gamma glutamyl-transferase and alkaline phosphatase are not commonly reported.[106] Other possible mechanisms of liver injury include systemic cytokine storm, drug-induced

hepatotoxicity, or endothelial inflammation.[104] Elevated ferritin levels have been reported in COVID-19 patients who developed liver failure, however, a causative relationship has not been established.[107]

1.2.5 Haematological involvement in COVID-19

There is a high incidence of thrombotic complications in COVID-19 patients.[38] Coagulation abnormalities such as prolonged activated partial thromboplastin time (aPTT) and prothrombin time, elevated D-dimer, and thrombocytopenia have been reported.[38 108-110] The development of disseminated intravascular coagulation in COVID-19 is a sign of adverse prognosis.[31] Elevated levels of lupus anticoagulant have also been described, emphasising the presence of a hyper-coagulable state in patients with COVID-19.[111]

The inflammatory pathways, such as those involved in complement activation, are closely intertwined with the body's coagulation pathways.[38] Therefore, systemic inflammation secondary to SARS-Co-2 can be expected to lead to activation and dysregulation of the coagulation cascades.[38] The ensuing hypercoagulability is both a cause of thrombotic complications and a sign of impaired prognosis.[38] Both lung parenchymal injury and microvascular damage have been reported in patients with severe COVID-19.[112] As discussed previously in myocardial injury, a procoagulant status can also facilitate thrombosis within the cardiovascular system, such as the coronary or cerebral arteries.[78 80 81] Inflammatory cytokines can lead to dysregulation of the vascular endothelium, increased vascular permeability, and immune cell infiltration, which contribute further to the hypercoagulable state in patients with COVID-19.[38]

1.2.6 Neurological involvement in COVID-19

Neurological symptoms can occur in more than a third of COVID-19 patients, rising to close to half of patients with severe SARS-CoV-2 infections.[32] Commonly reported symptoms include headache, dizziness, unsteadiness, altered sense of smell, and loss of taste.[113 114] Guillain-Barre syndrome has also been described with SARS-CoV-2 infections though this is thought to be relatively rare.[115-117] Around 15-30% of patients with COVID-19 can develop impaired consciousness or altered mental state,[32 118] over half of whom can be older than 60 years.[118] Indeed, acute confusion can be the first presentation in the disease manifestation.[32 119] The occurrence of strokes in COVID-19 patients is thought to be mediated by endothelial activation and propagation of a hypercoagulable state.[120]

Most studies examining the cerebrospinal fluid (CSF) of COVID-19 patients did not detect a significant presence of SARS-CoV-2 viral RNA.[121] Evidence of direct injury by SARS-CoV-2 was also not found on brain autopsy examinations in COVID-19 patients.[122] It is thought that neurological injury is likely caused by inflammation rather than direct viral-mediated damage.[121] The CSF of COVID-19 patients did demonstrate increased expression of proinflammatory cytokines (such as IL-1 and IL-12) and the presence of activated NK cells and cytotoxic T cells.[123] Infiltration of the brain tissue by $CD8^+$ T cells can take place without evidence of cellular penetration by SARS-CoV-2.[121] The trigger for the immune activation directed at the central nervous system remains unclear.

1.3 Clinical factors in disease heterogeneity in COVID-19

Another important characteristic of COVID-19 is that patients can present with a range of disease severities.[124] Whilst most patients recover quickly without significant complications, others can go on to develop fulminant respiratory failure or multi-organ involvement.[124] The underlying aetiologies of this heterogeneous disease presentation are multi-factorial and highlight the importance of clinical risk stratification, to enable better delivery of healthcare resources to patients in need and potential early discharge of low-risk patients.[124] Numerous factors have been studied which may affect disease severity in COVID-19 patients.

1.3.1 Patient characteristics

There is established evidence suggesting that advanced patient age is associated with a greater risk of developing adverse clinical outcomes in COVID-19.[100 125-130] These outcomes include re-hospitalisation,[100] requirement for mechanical ventilation[131] and increased risk of mortality.[125 132] There have also been reports of gender-related effects on the prognosis of patients with COVID-19.[130 131 133] However, this topic has remained controversial.[130] Any trend suggestive of a protective effect of the female gender on COVID-19 prognosis disappeared when considering only patients with severe COVID-19.[133]

In observational studies, smoking has been linked to an elevated risk of developing adverse outcomes in patients with COVID-19.[134-136] This is supported by recent expert opinions affirming the damaging effects of cigarette smoking on hospitalisation and mortality risks in COVID-19 patients.[137] Such findings are in congruence with the well-known harmful effects of smoking on other lung diseases.[138]

1.3.2 Co-morbidities

Since the early pandemic, it has been known that clinical co-morbidities have a significant bearing on the likelihood of adverse outcome development in patients with COVID-19.[100 125 127 132 139-142] As the commonest co-morbidity, arterial hypertension affects up to half of patients with COVID-19.[143] Both hypertension and a prior history of cardiovascular diseases are associated with an elevated risk of developing severe COVID-19 and mortality.[142 144-147] Interest had developed in the potential for ACE inhibitors and/or angiotensin receptor blockers (ARB) to exert protective effects on hypertensive patients against SARS-CoV-2.[148] However, the discontinuation of ACE inhibitors or ARBs did not significantly affect prognosis in randomised controlled trials.[149] Complicated diabetes mellitus has been associated with an increased risk of mortality in patients with COVID-19, whilst the prognostic effect of uncomplicated diabetes was much weaker.[143] Obesity has also been associated with adverse clinical outcomes in COVID-19 patients, both alone and in combination with other risk factors.[143 150] Although lipid disorders are prevalent in COVID-19 patients, their prognostic value appears to be weak.[143]

Atrial fibrillation (AF) and atrial flutter, with a combined prevalence of around 10-15%, are the most common arrhythmias in patients with COVID-19.[151 152] COVID-19 patients with AF are known to exhibit greater levels of inflammatory markers and myocardial injury markers.[151] There is also evidence indicating that the presence of AF is associated with severe COVID-19 and an elevated mortality risk.[151-153]

Chronic obstructive pulmonary disease (COPD) is the commonest respiratory co-morbidity in COVID-19 patients.[154] COVID-19 patients with COPD have a higher risk of developing complications such as the requirement of mechanical ventilation and

admission to the intensive care unit (ICU).[154] [155] Further, COPD renders COVID-19 patients at a greater risk of inpatient mortality.[155] COVID-19 patients with asthma are also at a higher risk of requiring invasive ventilation, ICU admissions, and suffering inpatient mortality.[154] Finally, patients with chronic respiratory conditions can develop severe exacerbations when infected with SARS-CoV-2.[156] [157]

Patients with malignancies have greater risks of developing COVID-19.[158] Cancer sufferers who contract COVID-19 are more likely to develop adverse complications such as ICU admissions and inpatient mortality.[159] [160] The pathophysiological processes underlying these observed risks in cancer patients remain unclear,[161] which may involve complex interactions between cancer cells, the host immunity, and the acute SARS-CoV-2 invasion.[161] Pre-existing chronic kidney disease and dementia are also risk factors for inpatient mortality in COVID-19.[162]

It should be emphasised that the risk of developing adverse outcomes in patients with COVID-19 increases with multiple co-morbidities.[150] [163] It is likely that these patients have less physiological reserve to fight infections, and are therefore vulnerable to developing more severe disease manifestations.[164]

1.3.3 Oxygenation

Peripheral and arterial oxygenation readings can help to guide the decision for intubation in patients with severe COVID-19.[165] The target oxygen saturation range (92-96%) was extrapolated from patients with ARDS, where both low (<92%)[166] and high (>96%) saturation readings were found to be prognostically deleterious.[167] Peripherally detected hypoxia can help to guide the timing of intubation and mechanical ventilation.[168-172] In intubated COVID-19 patients, further reductions in oxygenation indicate greater

mortality risks[173] and prone ventilation therapy has been associated with improvements in oxygenation.[174]

Similar to peripherally measured oxygen saturations, both reduced and elevated arterial oxygen partial pressures (PaO_2) have been linked to the development of adverse outcomes in COVID-19 patients.[175] In ARDS, the ratio of PaO_2 and inhaled oxygen fraction (PaO_2/FiO_2) is an indicator of severity.[175] Reduced PaO_2/FiO_2 is also associated with adverse outcomes in COVID-19 patients, such as prolonged hospital admission[176] and the requirement for intubation.[169 177 178]

1.3.4 Limitations of oxygenation

Although oxygenation is a useful guide to a patient's risk of clinical deterioration, it has several limitations as a prognostic biomarker.[179-181] Hypoxia can develop rapidly in COVID-19 patients without the manifestation of symptoms or apparent increases in respiratory effort.[179-181] This means that patients can deteriorate "silently", potentially eluding clinical detection until severe deterioration has taken place.[179-181] Hypoxia is more difficult to detect using pulse oximeter alone in Afro-Caribbean patients, which could lead to significant deteriorations being missed and potentially introduce inequalities in access to healthcare treatments in the acute setting.[182]

In terms of arterial blood gas derived oxygenation parameters, heavy dependence on PaO_2 and PaO_2/FiO_2 in guiding intubation and ventilation remains controversial.[183] Intubation decisions are not determined by hypoxaemia alone.[184] Several clinical parameters, such as increased respiratory effort, hypercapnia, and reduced consciousness are also important in the overall decision-making for intubation in COVID-19 patients.[184]

1.3.5 Risk scores

Vital signs such as tachycardia, hypotension, and increased respiratory rate are considered adverse features in patients with COVID-19.[134 179 185 186] The Early Warning Score (EWS), based on vital signs alone, enables early detection of clinical deterioration before the COVID-19 era,[187] with extrapolatory use in the clinical assessment of patients during the pandemic.[180 188] However, the clinical reliability of the EWS in COVID-19 has been questioned. EWS relies on parameters such as heart rate to detect sequelae related to sepsis, which may not be sensitive in patients with milder forms of COVID-19.[189] However, these patients remain to be at significant risk of developing respiratory failure and other complications.[189]

During the pandemic, with high volumes of hospital admissions, the development of a biomarker that enables on-the-spot assessment of a patient's prognostic risk is highly attractive.[190 191] To this end, risk models were developed with simplicity in mind and the lack of a need to wait for investigations before a clinical decision could be made.[190 191]

As discussed earlier, advanced age has strong prognostic implications in COVID-19 patients.[100 125-130] After being admitted to the hospital, elderly patients are more likely to suffer complications of severe COVID-19 such as the requirement of mechanical ventilatory support.[131] After discharge, patients of advanced age are more likely to be re-admitted to the hospital.[100] The inpatient mortality rate is also significantly higher for elderly patients than for their younger counterparts.[125 132] For reasons still unclear, patients with advanced age are less likely to display characteristic COVID-19-related symptoms,[192] which may lead to diagnostic delays and impairments in prognosis.[192]

Hypotension and elevated respiratory rates are important bedside prognostic markers in COVID-19 patients.[134 179 185 186] The combination of these signs may have a greater association with adverse outcomes than a single abnormality alone.[193] From a pathological perspective, abnormal vital signs have been extensively tested in the context of ARDS,[166 194 195] which shares many similarities with the severe systemic manifestations of COVID-19.[196]

As discussed previously, acute confusion and altered mental state are relatively common and can manifest as the first presentation of COVID-19.[119] Delirium is also more common in the elderly population with COVID-19, which is linked to frailty and an adverse clinical course.[197] Cases of reported encephalitis in COVID-19 patients suggest that systemic inflammation may also localise to the brain, leading to seizures and an altered mental state.[198] Therefore, the assessment of confusion is an important parameter in building a risk model that incorporates brain involvement in COVID-19.

CRB-65 (based on confusion, respiratory rate, blood pressure and advanced age) is a simple, history and observation-based risk score that has been validated for the assessment of the severity of community-acquired pneumonia.[199] CRB-65 can be used to indicate the mortality risk of patients with pneumonia.[199] It can also act as a quick and practical gatekeeper to aid clinicians in deciding whether patients require hospital admission or management in the community.[199] CRB-65 has been investigated both in the inpatient and outpatient settings for clinical risk stratification of patients with pneumonia.[200]

CRB-65 can provide an estimation of clinical risk without the need for blood tests or other investigations.[199 200] This means that the score can be calculated rapidly, often within

minutes of meeting the patient or be derived from common variables already recorded in the admission clinical notes.[199 200] This is particularly advantageous for the management of COVID-19 since clinical deterioration during the acute or progressive phases of the illness can take place rapidly.[179-181]

1.4 Serum biomarkers

Serum biomarkers provide rapid and quantitative clinical data that allow clinicians to assess the severity of COVID-19 and, in many cases, the prognosis of the patient.[201] Numerical thresholds can be set as benchmarks for disease outcomes, which are of value in deciding the clinical risk of patients and guiding clinical decisions.[191] Many serum biomarkers have shown prognostic value in COVID-19 based on retrospective analyses.[191]

1.4.1 Inflammatory markers

Serum inflammatory markers can assess the degree of host immune response activation in COVID-19 and are therefore potentially informative regarding disease severity.[22 140 202-205] Common examples of inflammatory markers include white cell counts (WCC) and C-reactive protein (CRP), which were already important for assessing the severity of common infections and their responses to treatment before the onset of the pandemic.[206 207] In COVID-19 patients, WCC and CRP remain useful tests owing to their widespread availability, low cost, and familiarity amongst clinical staff.[203 208 209]

The use of conventional inflammatory markers to prognosticate individual patients with COVID-19 remains unclear. The existing literature does not suggest that inflammatory

markers can accurately predict adverse outcomes in individual COVID-19 patients.[209 210] This is an important weakness.[191]

1.4.1.1 C-reactive protein (CRP)

CRP is an acute-phase protein made in the hepatocytes.[207] CRP can also be produced by macrophages, lymphocytes, endothelial cells, smooth muscle cells, and adipocytes.[207] Production of CRP is mainly stimulated by IL-6, and also by IL-1 and TNF-α.[207] Patients with raised serum CRP levels have a greater risk of developing severe COVID-19[203] and suffering inpatient mortality.[209] Patients with elevated CRP and low lymphocyte counts are at a higher risk of developing respiratory failure, as compared to patients without this particular pattern.[211] Similarly, patients with severe COVID-19 tend to have higher CRP levels than their counterparts with milder forms of disease manifestation.[212] Non-survivors of COVID-19 also have higher CRP levels than survivors.[212]

The association between COVID-19 severity and CRP varies between different patient races and genders.[213-216] In Caucasian and Asian patients with COVID-19, CRP levels were shown to be higher in non-survivors, as compared to survivors.[213] This trend could not be replicated in Afro-Caribbean patients.[213] Male patients tend to exhibit higher CRP levels than female patients,[214 217 218] which may reflect a more aggressive activation of innate immunity.[214-216]

Despite clinical familiarity amongst healthcare professionals, one of the major limitations of CRP as a standalone risk assessor in COVID-19 lies in its non-specific nature.[191] Further, the heterogeneities in CRP values across different patient characteristics render the derivation of a uniform prognostic cut-off highly challenging in COVID-19.[213-216]

1.4.1.2 Ferritin

Ferritin is an iron-storage protein synthesised in the liver and can be found in the bloodstream, cellular mitochondria, and the cytosol.[219] Ferritin synthesis increases with elevated cellular iron levels.[220] As a non-specific acute phase protein, ferritin production also rises in response to infection, where the secretion of cytokines such as IL-6 and TNF-α can lead to the release of ferritin into the circulation.[220 221]

The level of ferritin within the body is dynamic and dependent on the organ site assessed.[220] Serum ferritin levels are significantly higher in patients with COVID-19, as compared to controls,[222] whilst hepatic accumulation of ferritin has been shown in patients with severe COVID-19 and liver failure.[223] Elevated serum ferritin can occur with low serum iron levels, suggesting the presence of inflammation rather than iron overload in this context.[219] Ferritin levels rise acutely in response to SARS-CoV-2 infections and return to near baseline levels within a few months post-recovery.[107]

During acute infections, ferritin may serve to limit the access of pathogens to vital iron stores required for metabolism and multiplication.[221] Active production of ferritin may also have certain immunomodulatory roles.[221 224 225] Several reports have suggested an association between raised serum ferritin levels and an adverse clinical course in COVID-19 patients.[107 226] However, these reports are also countered by evidence that failed to report such prognostic link.[227] Ferritin is not a specific marker of viral infection and like other acute phase reactants, such as CRP, its role as a standalone marker for prognosticating COVID-19 patients remains unclear.[228]

1.4.2 Platelet counts

Thrombocytopenia is a common observation in COVID-19 patients, which is linked to an elevated mortality risk.[1 39 229-234] Reduced platelet counts can be related to systemic inflammatory responses[235] and represent manifestations of immune-mediated coagulopathy.[38] Thrombotic thrombocytopenic purpura and haemolytic uraemia syndrome are rarer causes of thrombocytopenia in severe COVID-19, which carry an adverse prognosis.[108]

1.4.3 Combination of inflammatory biomarkers

The concept that a combination can achieve a greater desired effect than its constituents is ubiquitous in healthcare.[236] In therapeutic medicine, this concept often takes the form of combination drug regimens, e.g. multiple antibiotic therapy for infective endocarditis or for tuberculosis, which are more effective than single-drug treatments.[237 238]

In COVID-19 patients, combinations of inflammatory biomarkers have been assembled recently and tested for predicting adverse outcomes.[239-242] Two such combinations include the lymphocyte-CRP ratio (LCR) and the ferritin-lymphocyte ratio (FLR), which have shown some early promise.[243 244]

LCR was designed as a novel biomarker for assessing the prognosis of patients suffering from gastrointestinal cancers.[245] LCR may reflect immunological host-tumour interactions and indicate cancer severity.[245] Recent studies have also suggested that LCR is linked to severe disease manifestations and the risk of death in COVID-19 patients.[239 240 243] This cross-specialty versatility makes LCR a combination biomarker worthy of further characterisation.

FLR is another combination biomarker with potential prognostic value in COVID-19 patients.[244] It has recently been shown to predict adverse clinical outcomes with high diagnostic performance (with an area under the receiver operating characteristics curve of around 0.9).[244] However, the results of this study have not yet been replicated in another patient population, which if confirmed, could have important implications in the clinical risk stratification of COVID-19 patients.

Research on combination biomarkers is in its early days and most of the studies testing their use were relatively small.[239 240] There was no published study comparing novel combination biomarkers with conventional inflammatory markers. Therefore, the incremental value of combination biomarkers over the existing standard practice remains unclear.

1.4.4 Cardiac troponins

In COVID-19 patients, the presence of myocardial injury is associated with an increased risk of mortality.[41 50 246-251] Cardiac troponins are proteins that form part of the myocardial contractile apparatus.[252] In the event of myocardial injury, troponins are released into the circulation which can be detected using serum bioassays.[253 254] For more than ten years, high-sensitivity cardiac troponin (hs-cTn) assays have undergone extensive validation for the assessment of patients with suspected ACS.[255] Hs-cTn is now a routinely-measured frontline biomarker for the acute risk-stratification of patients with suspected myocardial injury.[256] Despite its widespread use and familiarity to medical practitioners, the application of hs-cTn for the risk-stratification of COVID-19 patients remains unclear, despite significant volumes of evidence supporting the occurrence of myocardial injury with SARS-CoV-2 infections.[34 250]

Several studies have indicated that COVID-19 patients with elevated hs-cTn have worse inpatient survival than those with normal hs-cTn.[41 48 50 257 258] However, despite this pattern of results, hs-cTn has not transitioned into routine practice for guiding clinical decision-making. The reasons behind this failed transition are unclear. Further work is required to improve our understanding of the diagnostic properties of hs-cTn in COVID-19 for predicting adverse clinical outcomes. Moreover, the optimal way of using hs-cTn for prognosticating COVID-19 patients (either as a rule-in or rule-out test for adverse events) remains under-explored.[191]

1.4.5 COVID-19 progression and serum biomarker evolution

The clinical evolution of COVID-19 from initial infection to severe disease manifestation can traverse through 3 broad stages.[191] In the initial stage of SARS-CoV-2 infection, the patient may display little or no symptoms.[259] During this incubation period, the infection can remain undetected.[191] Global disease screening, testing, and education of the public have enabled early detection of COVID-19 in the disease progression.[260] During the progressive stage, pulmonary involvement and/or non-pulmonary involvement can occur.[261-263] Later in the infection, systemic complications may ensue, such as ARDS and/or multiorgan disease involvement.[259 264]

Changes in serum biomarkers are associated with the progressive evolution of COVID-19.[191] The initial stage of viral incubation can last up to 2 weeks, during which the patient may remain asymptomatic or demonstrate only lymphopenia.[1 201 265]

After the initial stage, COVID-19 can become progressive,[201] characterised by elevated inflammatory markers including C-reactive protein (CRP), ferritin, IL-6, and procalcitonin.[209 266 267] Cytokines, such as IL-2, IL-6, IL-8, IL-10, interferons, and tumour

necrosis factor alpha (TNF-α), are released into the bloodstream and can be detected during this stage, when the innate immunity has been activated.[209] Serum biomarkers tend to fall or recover within 10 days of hospital admission in COVID-19 survivors but remain elevated in critically unwell patients.[268]

Persistent elevations of D-dimer, liver function tests, creatinine, cardiac troponins, and b-type natriuretic peptides indicate the transition to a systemic stage where organ-specific and/or multi-organ involvement is evident.[49 264 269] Markers of critical illness, such as the rise in creatine kinase and the development of anaemia, are further adverse signs of systemic illness.[267] Figure 1.4 summarises the potential changes in serum markers during the stages of COVID-19 infection.

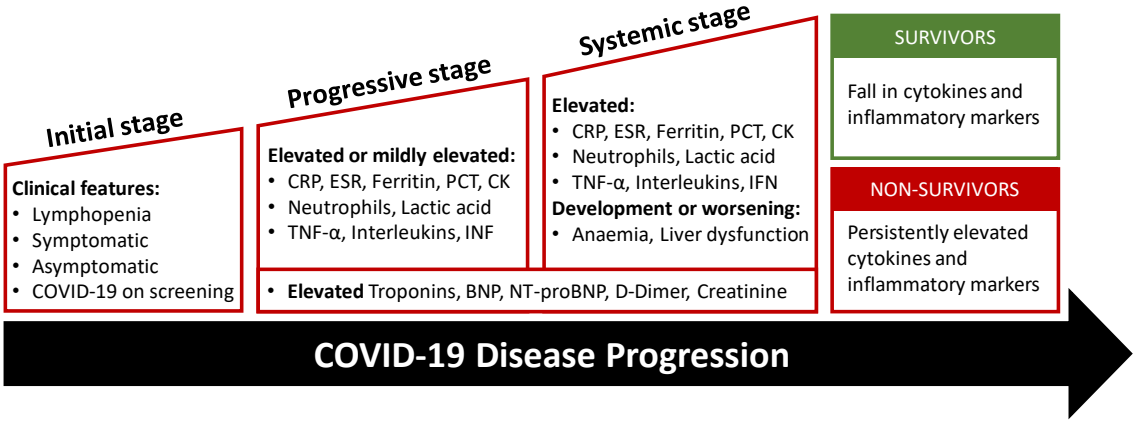


Figure 1.4: Dynamic changes in inflammatory biomarkers during the evolution of COVID-19 infections.[191] BNP: B-type natriuretic peptide; CK: creatine kinase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; INF: interferons; NT-proBNP: N-terminal pro-B-type natriuretic peptides; PCT: procalcitonin and TNF-α: tumour necrosis factor α.

1.5 Chest Radiograph (CXR)

In COVID-19 patients, the so-called "ground-glass" opacifications and/or shadowing suggestive of consolidation can occur on CXR,[270] which may be unilateral or bilateral in lung field distribution.[270-274] Despite CXR abnormalities being perceived as a marker of severity in COVID-19,[270-274] the practice of relying on the presence of CXR abnormalities to diagnose COVID-19 is inferior to laboratory testing using nasopharyngeal swabs and reverse transcription polymerase chain reaction (rt-PCR) based methods.[274 275] When referenced to rt-PCR tests, the presence of CXR abnormalities achieved a suboptimal diagnostic accuracy for detecting COVID-19.[274] This is likely because a significant proportion of patients with COVID-19 can have normal appearances on CXR, i.e. without the conventional changes expected from COVID-19 pneumonia.[270-274 276] The widespread availability and familiarity with CXR render it an important clinical test in patients with COVID-19.[270-274 276]

Recent reports also suggested a role for CRB-65 in the early risk stratification of patients with COVID-19 pneumonia, by highlighting its ability to predict adverse clinical outcomes in this patient group.[277-280] However, in COVID-19 patients who presented with normal CXR findings,[270-274 276] the clinical value of CRB-65 remains unclear, which significantly limits the widespread clinical applications of this biomarker. Further work is required to elucidate the effect of CXR abnormalities on the prognostic value of CRB-65 in COVID-19.

1.6 Project Conception and Clinical Motivation

Since early 2020, I have worked on the "frontline" of the COVID-19 pandemic. Like many colleagues, I regularly spent several hours in personal protective equipment (PPE)

looking after COVID-19 patients, many of whom were critically unwell or passed away during their hospital stays. I also noticed that a significant proportion of COVID-19 patients were only mildly symptomatic or asymptomatic, who needed minimal medical treatment, and went home to recover uneventfully.

I often wondered "*How can we identify which patients are at the highest risk of deteriorating or dying and which patients could be discharged from hospital?*" If we knew the answer to this question, we might be able to target more intensive therapies to patients who need them and protect low-risk patients from the potential harm of unnecessary hospitalisation.[281-283] Indeed, elucidating methods for clinical risk stratification of COVID-19 patients was a global priority.[163 251 284 285] However, not many biomarkers have been brought into clinical practice to guide clinical decision-making. As a result, patients continued to suffer. I decided to devote my efforts to improving this area.

1.6.1 Idea development

Armed with the aim to improve clinical risk stratification during the pandemic, I wanted to gain a better understanding of the link between clinical biomarkers and prognosis in COVID-19 patients. The first question I asked was: "*<u>What</u> type of biomarkers would be most useful on the frontline?"* I thought of two possible strategies: either to re-develop existing biomarkers to make them fit to prognosticate COVID-19 patients or to develop completely novel biomarkers to assess disease risk. Due to the urgent need for biomarkers on the frontline, I decided that it was most time-efficient to invest efforts into biomarker re-purposing.

The second question I asked was: "*<u>Why</u> was there no effective prognostic biomarkers in wide use for guiding the clinical management of COVID-19 patients*". The answer to this

question lies in the lack of clinical translation of existing biomarkers. For conventional acute illnesses such as myocardial infarction or pneumonia, the prognostic data of routine investigations such as blood tests and CXR were already known, which support their clinical use.[286 287] Clinicians could use these tests with confidence to risk-stratify patients and guide clinical management. However, since COVID-19 was a new disease entity, the prognostic data of routine tests were unclear. As a result, these tests cannot be used to guide clinical decisions based on prognosis (Figure 1.5).

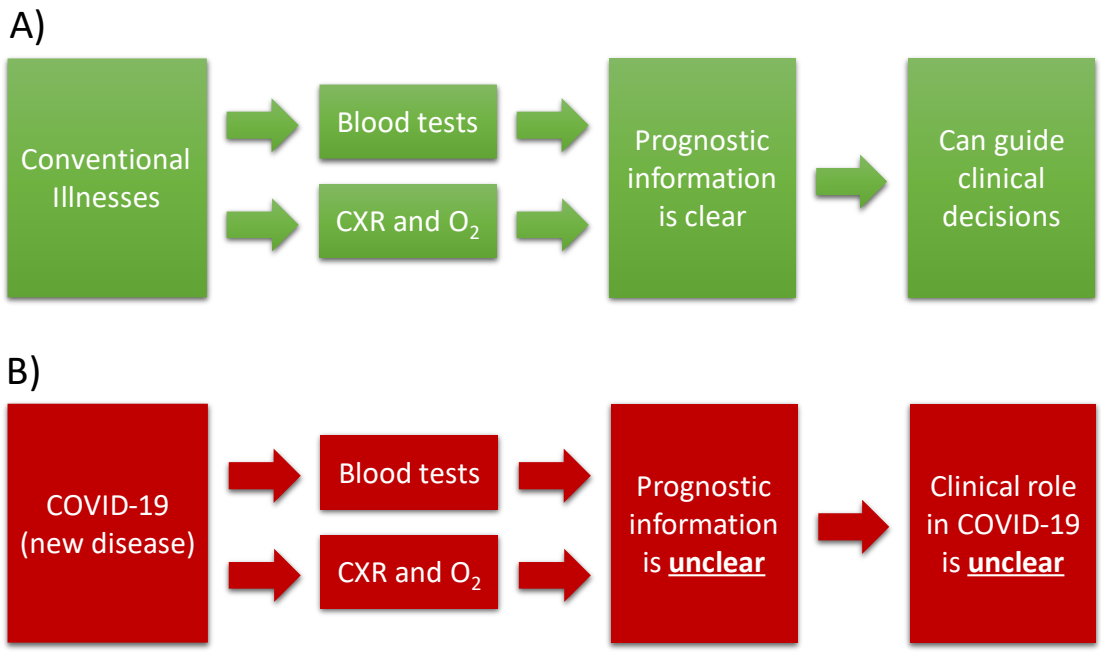


Figure 1.5: Conceptual reasons why no biomarker could give evidence-based guidance on clinical decisions in coronavirus disease 19 (COVID-19). The fact that COVID-19 was a new disease entity meant that routine clinical tests such as blood tests, oxygenation, and imaging had limited prognostic or clinical evidence in the management of COVID-19. This was a major knowledge gap that prevented the development or translation of biomarkers to the pandemic frontline. CXR: chest X-ray; O_2: oxygen saturation.

As the pandemic continued, increasing numbers of studies were published that showed population-based prognostic values of routine clinical tests in COVID-19.[288] However, whilst tests such as cardiac troponins were linked to a worse prognosis in COVID-19,[42] these were not translated into clinical practice or guidelines to positively influence decision-making. Therefore, improving the clinical application of the routine frontline biomarkers in COVID-19 was a clinical priority. This could take place in two stages. Firstly, to elucidate the reasons behind the apparent failure of clinical translation of biomarkers with prognostic value in COVID-19. Secondly, to improve the clinical suitability of these biomarkers in order to move them closer to the frontline (Figure 1.6).

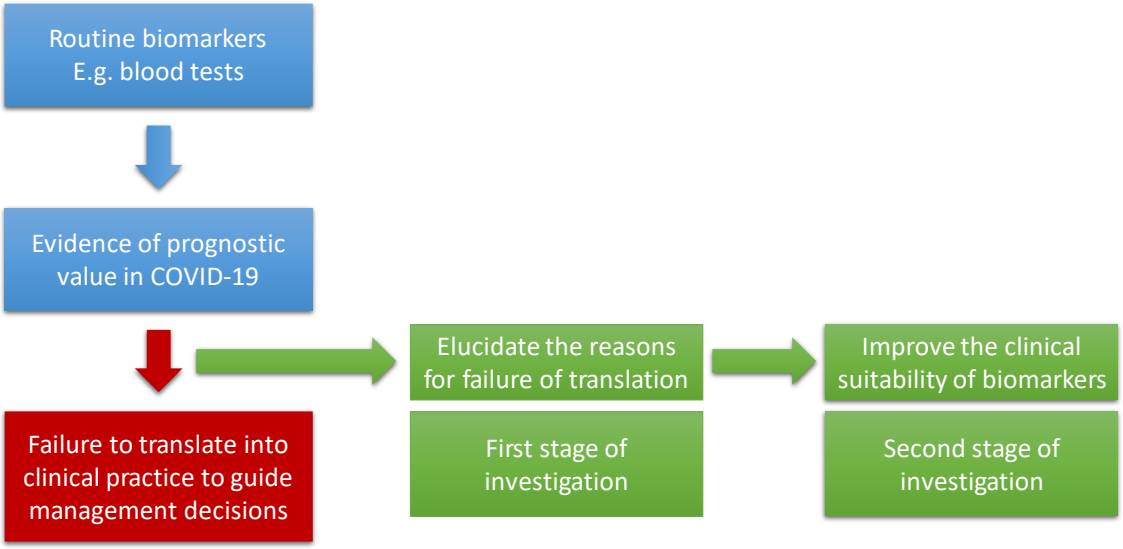


Figure 1.6: The two-stage strategy for improving the clinical applicability of routine biomarkers for assessing patients with COVID-19. The study was aimed at gathering data that could propel prospective studies to bring biomarkers into eventual clinical practice.

Once I decided on these steps, I proceeded to hypothesis generation. Much of this process depended on the extent to which the existing literature investigated the prognostic value

of certain biomarkers. To elucidate the reasons behind the failure of clinical translation of the biomarkers, I needed to first have a firm grasp of the evidence in the published literature and critically appraise it to identify the gaps in knowledge.

1.6.2 Clinical audit

At the time, I also had plans with my education supervisor in the hospital (Royal Berkshire Hospital, Reading) to perform a clinical audit. This audit was to assess the measurement of cardiac biomarkers such as troponins and B-type natriuretic peptides in COVID-19 patients, to see how well our practices adhered to clinical guidelines and how that affected the clinical outcomes of patients. Under guidance from my educational supervisor, I recruited and led a task force of junior doctor colleagues working with me on the frontline. In our spare time on evenings and weekends, we collected the audit data on a wide range of clinical parameters on COVID-19 patients.

1.6.3 Turning audit data into research

With the clinical audit data, I realised that there was a clear opportunity to examine the research hypotheses I had. After a discussion with my educational supervisor, we applied for ethical approval from the Health Research Authority via a fast-track COVID-19 program to use the data for research purposes. This application was successful in August 2020 when we were granted ethical approval to conduct formal research using the dataset (Appendix I). The work that followed has led to the scientific content of this thesis.

1.7 Publication of literature search

The literature search was published as a first-author Review article in the Journal of Internal Medicine (See Appendix II).[191]

1.8 Biomarker selection

After thoroughly reviewing the clinical literature on biomarkers in COVID-19, there were three important aspects I wanted to pursue in biomarker development.

Firstly, I wanted to further explore the clinical potential of cardiac troponin. Despite the existing evidence linking it to adverse prognosis in COVID-19, it was still not used in clinical practice for prognosticating COVID-19 patients.[191] Troponin is a commonly performed biomarker in practice, it was of clinical benefit to further develop its utility.

Secondly, I wanted to better characterise combination inflammatory biomarkers. LCR and FLR were two biomarkers that showed potential.[243 244] LCR had potential cross-specialty versatility in being also prognostic in gastric cancer patients and its potential value in COVID-19 was attractive.[243 245] FLR has been shown to demonstrate high diagnostic performance in predicting adverse outcomes in COVID-19.[244] I wanted to test its value in a COVID-19 UK population and better understand how FLR compared against other inflammatory markers.

Thirdly, I wanted to develop a rapid access biomarker that may enable risk stratification without the need to wait for investigations such as blood tests or radiology results. CRB-65 was a promising biomarker that had a proven record in pneumonia.[200] Its wider applicability in COVID-19 required it to function in patients with normal CXR since this represented a significant proportion of patients.[273]

The common characteristic of these biomarkers was that they were practical and could be performed using tests already available on the pandemic frontline, which may help to facilitate clinical translation. Hs-cTnT is a cardiac biomarker that is already in widespread

clinical use. LCR is a ratio based on lymphocyte counts and CRP, both of which are inflammatory markers already familiar to clinical staff. FLR is a ratio between ferritin and lymphocyte counts which are also markers of inflammation and acute phase reactants. CRB-65 is a biomarker based on observations and clinical history that combines confusion, respiratory rate, blood pressure, and advanced age into the risk prediction model. CRB-65 can be easily performed both in the hospital and primary care settings, without the need to wait for blood tests or other investigations.

These biomarkers also provided a good coverage of different aspects of acute COVID-19. For instance, combination biomarkers LCR and FLR focused on the inflammatory components of the acute infection, hs-cTnT could offer an indication of myocardial involvement, and CRB-65 could potentially assess the systemic manifestations of COVID-19 pneumonia and clinical risk.

The selection of biomarkers in this project is illustrated in Figure 1.7 below.

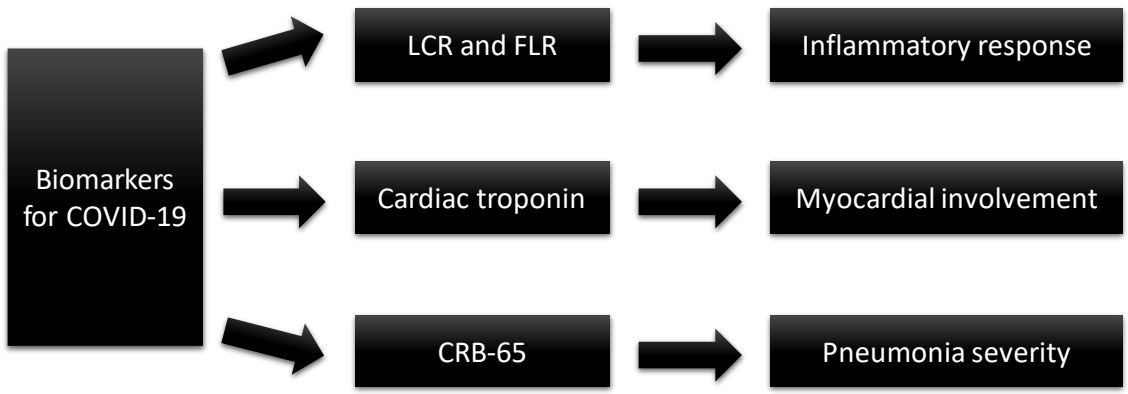


Figure 1.7: the selection of biomarkers and their potential pathological and/or clinical coverage in the assessment of COVID-19 patients.

1.9 Project Objectives and hypotheses

The major objective of the project was to characterise a range of biomarkers with prognostic value in COVID-19 patients. The overarching aim was to identify biomarkers that demonstrate potential for clinical translation, that could be brought forward for future validation in guiding the management of COVID-19 patients.

1.9.1 Specific study objectives

There were three main objectives:

1. To characterise the potential clinical usefulness of high-sensitivity cardiac troponin T (hs-cTnT) for assessing prognosis in COVID-19 patients.
2. To determine the efficacy of combination inflammatory biomarkers (LCR and FLR) for prognosticating COVID-19 patients.
3. To investigate the prognostic value of the clinical risk score CRB-65 in COVID-19 patients with normal and abnormal CXR.

1.9.2 Study hypotheses

In this study, I hypothesised that in COVID-19 patients:

1. High-sensitivity cardiac troponin (hs-cTn) can predict inpatient mortality.
2. Lymphocyte-CRP ratio (LCR) can predict adverse clinical outcomes.
3. Ferritin-Lymphocyte ratio (FLR) can predict adverse clinical outcomes.
4. CRB-65 score can predict adverse clinical outcomes independent of CXR.

Chapter 2: Methods

Establishing an accurate clinical database was the most important initial target of the project. This was performed as a Trust-wide clinical audit at the time. Once the database was established, the data was felt to be suitable to answer the research questions in this study. The next task was working with the local Research and Development (R&D) department in applying for ethical approval for using the database for research purposes. Once the ethical approval was granted, research-orientated analysis of the database could begin. Once the initial analysis took place, the results were independently validated by a medical statistician before manuscripts were submitted.

Figure 2.1 illustrates the processes that took place in the project, which will be explained in greater detail in the following sections.

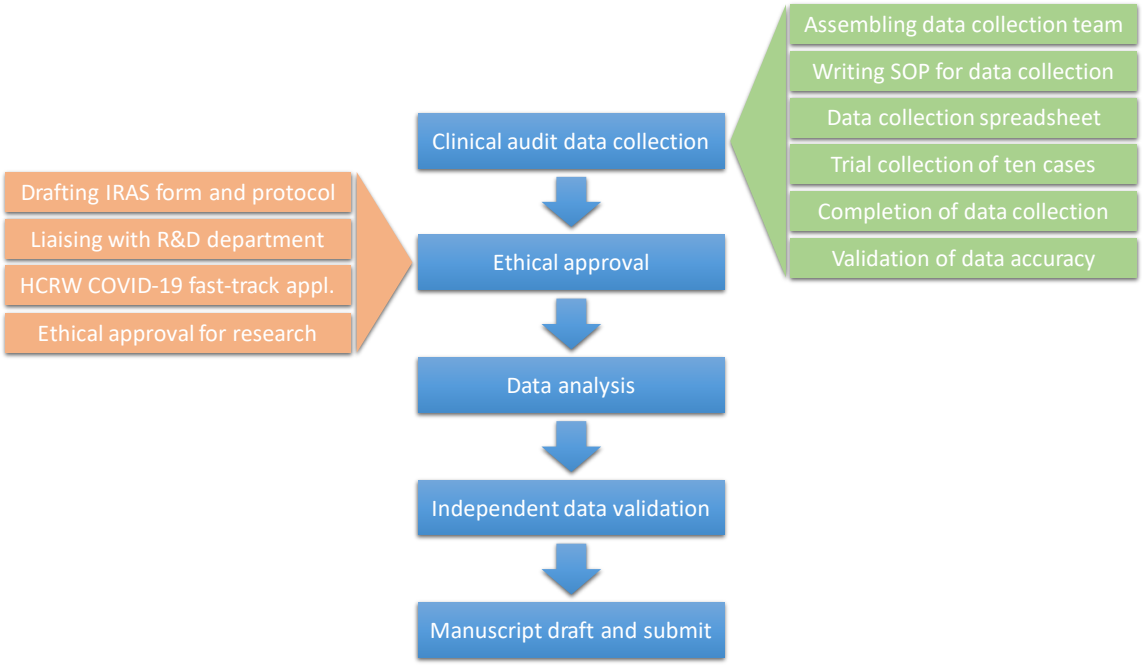


Figure 2.1: Overview of project processes. IRAS: Integrated Research Application System; SOP: Standard Operating Procedure; R&D: Research and Development.

2.1 Data collection

Ensuring that all the data collected were accurate according to the patient medical records was the key factor in ensuring the validity of the scientific interpretation of the data and the conclusions that could be drawn from the subsequent analysis.

Substantial attention was given to the planning, coordination, and execution of the data collection process. These included (i) the assembly of a competent and dependable data collection team; (ii) the careful design of the initial data collection spreadsheet with a comprehensive selection of data fields; (iii) the writing of a standardised operating procedure (SOP) document for data collection; (iv) a trial period of initial data collection in ensuring familiarity and accuracy; and (v) the further validation of the collected data against the original patient records. The process of data collection was performed with high integrity, with several measures in place to ensure accuracy.

2.1.1 Assembling the data collection team

The data collection team was carefully selected and assembled to consist of junior doctors working on the frontline of the COVID-19 pandemic at the time in the Royal Berkshire Hospital. They were doctors who had worked with me within the hospital. Since I had not performed any audit or research work with the potential team members in the past, I selected suitable members according to the presence of transferrable qualities I observed whilst working with them in clinical practice. These included:

- *Demonstrating the quality of paying attention to detail when reviewing patient records in clinical work.* The team members demonstrate the ability to perform detailed reviews of clinical notes on ward rounds and when seeing clinical referrals

as cardiology registrars, detailed and accurate review and presentation of clinical details of patients on ward rounds and during practice as senior house officers and house officers. It was important that I could trust the team members to pay attention to detail when reviewing patient records and potentially translate this skill to handling data collection in the project.

- *Dependability in performing tasks in their clinical duties during the time I have worked with them.* This quality included following clinical guidelines and senior advice when managing clinical patients and "going the extra mile" to ensure that clinical tasks were performed comprehensively. I believed that possessing this quality meant that the team members were more likely to take responsibility and show initiative in ensuring data accuracy when advised to do so.
- *Clear and accurate clinical documentation in practice.* This transferrable skill could help to ensure that data entry is performed in a tidy and clear manner, and potentially minimise any errors in the data transcription process.
- *Good team players in the clinical environment,* which was an important transferrable characteristic that would ensure the efficient conduct of the project.

I held a meeting with each potential team member I identified as suitable to participate in the project. In this informal "interview" process as the team leader, the objectives of the project were explained to the team members. Participation was voluntary and the potential team members were free not to take part. The final data collection team included one cardiology registrar, a senior house officer, and several house officers, offering a range of experiences and expertise. Most of the team had some experience in clinical audits and research either at the undergraduate or post-graduate levels.

2.1.2 Inclusion and exclusion criteria

The inclusion criteria for patients were:

- Age 18 years old or over.
- Admitted to the Royal Berkshire Hospital between January 2020 to May 2020.
- Tested positive for COVID-19 by a rt-PCR of nasopharyngeal swab in the hospital.
- Underwent clinical assessments, serum blood tests and CXR.

The exclusion criteria for patients were:

- Equivocal or indeterminant rt-PCR tests for COVID-19.
- Tested positive for COVID-19 more than 48 hours before or after the biomarker of interest was performed, when the potential relationship between the COVID-19 diagnosis and the biomarkers tested may be weakened.
- Transferred to another hospital during admission but without repatriation such that clinical outcome could not be reliably determined and/or potential bias may be introduced.
- Patients tested for COVID-19 in the community without hospital admission or clinical or biomarker assessments.

2.1.3 Data collection fields

I created a data collection spreadsheet consisting of many data fields. These are broken down into the following categories:

- Demographics data on admission

    - Age, gender and ethnic origins
    - Height and weight on admission
    - Date of admission
    - Date of COVID-19 diagnosis
- Symptomology on admission (YES/NO)
    - Chest pain, dyspnoea, palpitations, cough, fever, diarrhoea, anosmia/ageusia, other
    - Duration of longest symptoms
- Co-morbidities
    - Ischaemic heart disease, heart failure, hypertension, diabetes, atrial fibrillation
    - Hypercholesterolaemia, smoking status (current / ex-smoker / never-smoker)
    - Chronic kidney disease, chronic obstructive pulmonary disease, asthma
    - Stroke / transient ischaemic attack, dementia, cancer (type)
- Regular medications
    - Angiotensin-converting enzyme inhibitor (ACEi)
    - Angiotensin receptor blocker (ARB)
    - Beta-blocker, calcium channel blockers, spironolactone or eplerenone, statins
    - Aspirin, digoxin, warfarin, direct oral anticoagulant, nitrates
    - We did not specifically assess whether patients were taking corticosteroid therapy since at the time of data collection, clinical trials showing the benefit of such therapy had not yet been published.
- Electrocardiogram (ECG)
    - Heart rate, sinus rhythm, AF
    - Axis, PR-interval, QRS-duration, QTc, bundle branch block

    - Screenshot of anonymised 12-lead ECG
- Chest X-ray (CXR)
    - Clear (YES/NO), comparison to previous CXR
    - Abnormalities (Side [Left/right/bilateral]; Region [Upper/Middle/Lower])
        - Consolidation
        - Ground glass opacification
        - Atelectasis
    - Pleural effusion (Side [Left/right/bilateral])
    - Lymphadenopathy
    - CXR report pasted text
- Blood test results
    - Haemoglobin, haematocrit, white cell count, platelet count, lymphocyte count
    - Sodium, potassium, creatinine, glomerular filtration rate, d-dimer, creatine kinase
    - Ferritin, vitamin D, procalcitonin, high sensitivity cardiac troponin
    - C-reactive protein, B-type natriuretic peptide
- Observations on admission
    - Temperature, systolic blood pressure, diastolic blood pressure, respiratory rate
    - Glasgow coma scale, oxygen requirement
- Clinical outcomes data
    - Requirement for non-invasive ventilation (NIV)
    - Requirement for intubation and mechanical ventilation
    - Requirement for intensive care unit (ICU) admission
    - Inpatient mortality, date of mortality

- Date of discharge alive from hospital

A summary of the data fields used in this project is illustrated in Figure 2.2.

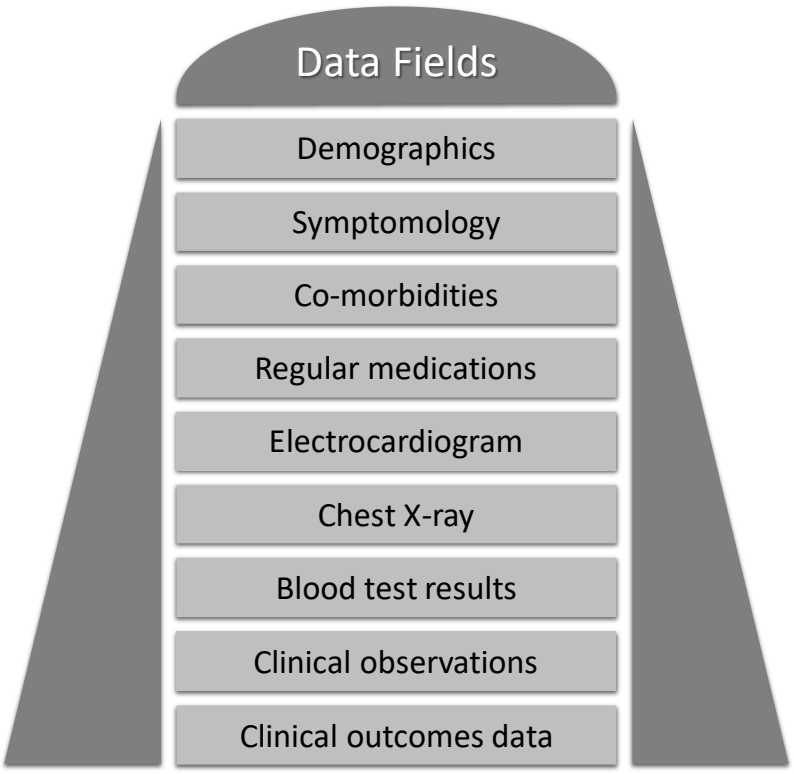


Figure 2.2: A summary of the data fields used for data collection in this project.

2.1.4 Standard Operating Procedure (SOP) for data collection

I wrote a data collection SOP (see Appendix III) which was reviewed and approved in consensus by all the team members. Data collection then took place using the pre-created data field spreadsheet in accordance with the SOP to standardise the process. The data were collected for each patient in the study from the electronic patient records (EPR) in the Royal Berkshire Hospital.

2.1.5 Trial data collection

I obtained a list of all patients who were tested for SARS-CoV-2 in the Royal Berkshire Hospital laboratory during the first wave of the pandemic in 2020 from the clinical database registry. I then drafted an Excel spreadsheet containing the data fields to be collected. This was again discussed amongst the data collection team to reach a consensus agreement. Where certain additional fields that were felt necessary, these were added to the data collection Excel sheet.

Each member of the team was allocated approximately 100 patients to collect data for. All members of the data collection team were then instructed by me to collect data according to the SOP for the first ten cases they were given. This formed the trial collection process whereby the initial ten cases were checked for accuracy against the EPR and validated for data format against the SOP by me, independent of the data collector.

I offered feedback to each team member on their trial 10-case data collection and where necessary discussed in detail how the collection should continue according to the SOP. All members of the data collection team successfully completed the trial data collection; no member was disqualified from the study. Most of the issues identified were related to the format of data entry rather than numerical accuracy. Once it was felt that the team members were suitably able to continue with data collection, they were instructed to finish collecting for the remaining patients in their allocated list.

2.1.6 Further data validation and anonymisation

Once the data collection was completed, the data from each team member were joined together into one complete database. To further ensure data accuracy, samples of data

(around 10%) were selected at random and further validated against the electronic patient records jointly by myself and another observer, independent of other data collectors.

Once the data checking process was complete, the database was fully anonymised whereby all patient identifiable information, such as name, date of birth, and hospital/NHS numbers, were removed.

2.2 Ethical approval application

The ethical approval application process began with a discussion with the R&D department of the Royal Berkshire Hospital, which took place during a meeting I arranged with the R&D research officer. An agreement was soon given by the local R&D team to support the ethical approvals application to use the clinical audit data for research purposes.

After initial meetings, I drafted an IRAS form and a study protocol for the project. I searched on the Website and found the HRCW COVID-19 fast-track approvals service and informed the R&D of my intentions to apply through this system, which was approved.

With support from my educational supervisor at the time Dr Jim Stirrup as local principal investigator and support from the R&D department, I submitted the application. The ethical approval to conduct research work on the audit data was granted on 14 August 2020. Please see the Appendix section for the approval letter.

2.3 Data analysis

The data analysis was divided into three main domains (as summaries in Figure 2.3). The first domain was the definition of study endpoints, which were based on the clinical outcomes data in the project. The second domain was made up of the statistical data analysis performed and checked by me. The third domain of data analysis was the independent validation of the data by the medical statistician.

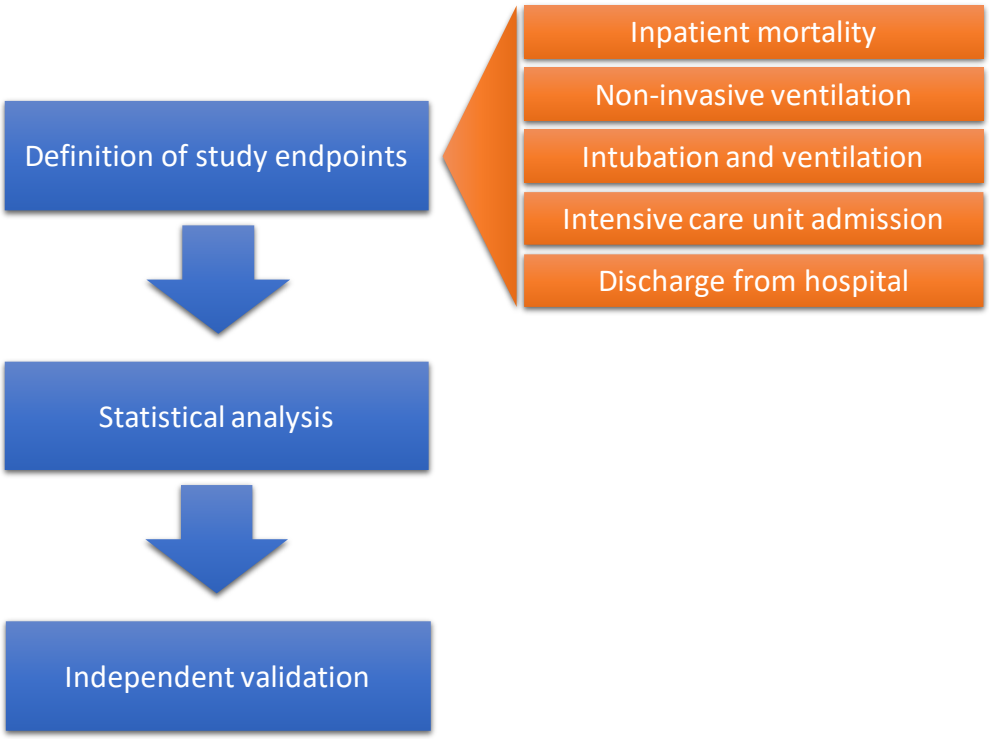


Figure 2.3: A figure to summarise the three data analysis domains in the project.

2.3.1 Study endpoints

The primary study endpoint was inpatient mortality related to COVID-19. This was considered the hardest clinical outcome endpoint.

The secondary study endpoints included:

- Requirement for non-invasive ventilation (NIV)
- Requirement for intubation and mechanical ventilation
- Requirement for admission to intensive care unit (ICU)
- Discharge alive from hospital

Where appropriate, a composite endpoint was used consisting of any combination of the above study endpoints.

A STROBE statement checklist is provided in Appendix IV.

2.3.2 Statistical analysis

Continuous data were assessed for normality using the Kolmogorov-Smirnov test.[289] Parametric data were displayed as mean (SD).[48 246 290] Non-parametric data were expressed as median with inter-quartile range (IQR).[48 246 290] Two groups of parametric data were compared using the Student's t-test, paired where necessary.[290] Two groups of non-parametric data were compared using the Mann-Whitney test.[290] Two groups of categorical data were compared using the Chi-square test or the Fisher Exact test.[290 291]

Inpatient survival patterns attributable to biomarker thresholds were evaluated using Kaplan-Meier curves.[292] The Kaplan-Meier curves were compared using the log-rank test. The diagnostic performance of biomarkers for predicting adverse clinical outcomes was assessed using the Receiver-Operating Characteristics (ROC) curves. The area under the ROC curves was expressed with either 95% confidence intervals or as one standard deviation.[293]

Multiple regression analysis was performed to assess the partial correlation ($R^{partial}$) between different biomarkers. Where appropriate, the multi-variate Cox proportional-

hazard regression analysis was used to assess inpatient mortality risk, with the hazard ratios (HR) displayed with 95% confidence intervals.[294]

Statistical significance was denoted by $p<0.05$. All initial statistical analyses were performed using the commercially available MedCalc software (Version 12.7.8.0).

2.3.3 Independent validation of data analysis and results

Once the initial data analysis was performed and checked by me, the data and results were handed over to an experienced medical statistician. The medical statistician then validated the results by performing an independent analysis. The data validation was performed using the Stata software (Basic Edition version 17.0, Statacorp LLC, Texas USA).

Where the results agreed between the initial analysis and the validation analysis, the results were accepted as final. Where results disagreed between the initial analysis and the validation analysis, these were reviewed by the statistician and me before a consensus set of results was agreed and accepted as final.

2.3.4 The dataset selection

A list of 1043 patients was initially generated from the IT system. These patients had nasopharyngeal swabs tested in the hospital laboratory for SARS-CoV-2. However, many patients could not be included in data collection due to either (i) having “low level” or “inhibited/indeterminant” nasopharyngeal SARS-CoV-2 swabs (i.e. a COVID-19 diagnosis was not made); or (ii) having swabs taken in the community which were tested positive in the hospital laboratory, but these patients did not attend hospital for clinical assessment (i.e. had no biomarkers measured). These were part of the documented study

exclusion criteria. After the exclusion of these patients, a total of 650 patients were eligible and underwent data collection.

Chapter 3: Overall description of total dataset and validation of high sensitivity cardiac troponin in prognosticating COVID-19 patients

In the first part of this data Chapter, I will present the characteristics of the entire study cohort to provide an overview. In the second part of this data Chapter, I will then present the data on cardiac troponin.

3.1 Characteristics and data on the whole patient cohort

Of the 1043 patients in the original patient list, 650 patients were eligible to undergo data collection. The characteristics of the total patient cohort are displayed below in Table 3.1.

Figure 3.1 Patient characteristics of the entire study cohort in the project.

	All Patients (n = 650)
Age (years)	71 (56 – 83)
Male (%)	368 (57)
BMI (kg/m^2)	25.7 (22.1 – 30.1)
Symptoms	
Chest pain	73/648 (11)
Dyspnoea	343/649 (53)
Palpitations	10/647 (2)
Fatigue	152/648 (23)
Cough	352/648 (54)
Fever	312/648 (48)
Diarrhoea	91/648 (14)
Comorbidities	
IHD	97/648 (15)
Heart failure	71/648 (11)
Hypertension	297/650 (46)
Diabetes	176/645 (27)
Dyslipidaemia	74/649 (11)
Current Smoker	44/650 (7)
Ex-Smoker	188/650 (29)
AF	106/647 (16)
CKD	149/649 (23)

COPD	78/634 (12)
Asthma	78/650 (12)
CVD	65/644 (10)
Dementia	92/647 (14)
Cancer	19/594 (3)
Medications	
ACEi / ARB	161/649 (25)
Beta-Blockers	154/650 (24)
CCB	111/650 (17)
Aspirin/Clopidogrel	83/638 (13)
Digoxin	13/650 (2)
Warfarin	31/649 (5)
DOAC	86/650 (13)
MRA	26/649 (4)
Nitrates	17/650 (3)
Statins	212/649 (33)

Hs-cTnT: high sensitivity cardiac troponin T; BMI: body mass index; IHD: ischaemic heart disease; AF: atrial fibrillation; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVD: cerebral vascular disease; ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker; DOAC: direct oral anticoagulant; and MRA: mineralocorticoid receptor agonist.

The observations, test results and clinical outcome data are shown in Table 3.2 below.

Table 3.2: Observations, test results and clinical outcome data of the whole study cohort

	All Patients (n = 650)
Admission Observation	
Temperature (^{o}C)	37.0 (36.6 – 37.8)
SBP (mmHg)	127 (113 – 145)
DBP (mmHg)	74 (66 – 82)
Respiratory Rate (/min)	20 (18 – 24)
Significant Hypoxia	98/625 (16)
Chest radiograph	
Consolidation	113/605 (19)
Opacification	200/600 (33)
Atelectasis	51/606 (8)
Pleural Effusion	44/606 (7)
Laboratory Results	
Haemoglobin (g/L)	126 (109 – 142)
Haematocrit	0.38 (0.34 – 0.42)
WCC (10^{9}/L)	7.6 (5.6 – 10.8)
Platelet Count (10^{9}/L)	224 (175 – 293)

Lymphocyte Count ($\times 10^9$/L)	0.9 (0.6 – 1.3)
Sodium (mmol/L)	138 (135 – 140)
Potassium (mmol/L)	4.2 (3.8 – 4.6)
Creatinine (μmol/L)	86 (66 – 128)
Ferritin (μg/L)	709 (265 – 1272)
CRP (mg/L)	101 (39 – 191)
Complications	
NIV requirement	84/649 (13)
ICU admission	66/647 (10)
Intubation	36/648 (6)
Inpatient Mortality	164/649 (25)

Hs-cTnT: high sensitivity cardiac troponin T; SBP: systolic blood pressure; DBP: diastolic blood pressure; WCC: white cell count; CRP: C-reactive protein; NIV: non-invasive ventilation; ICU: intensive care unit. Significant hypoxia was defined as an oxygen requirement of greater than 50%.[295]

3.2 Validation of cardiac troponins in COVID-19 patients

A paper based on the work in this chapter has been published in a peer-reviewed journal (PLOS ONE). I am the first author of the paper (see Appendix II).[296] This paper evaluated the diagnostic value of high-sensitivity cardiac troponin T (hs-cTnT) for predicting the occurrence of inpatient mortality in COVID-19 patients admitted to the hospital. Of the 650 patients in the study, 191 patients had hs-cTnT performed which were selected for this Chapter.

3.3 Introduction

For the clinical management of patients with acute COVID-19, reducing unnecessary hospitalisations is an important clinical priority.[285 297-299] The ability to select patients with a low mortality risk can help to reduce hospital admissions and may facilitate safe and early discharge of patients.[285 297-299]

Myocardial injury is a reported risk factor for inpatient mortality in COVID-19 patients.[45 48 246 248 250 251 257 258] The ability to detect myocardial injury is potentially important for identifying patients who are at high risk for developing adverse clinical events.[48] Over the last ten years, high sensitivity cardiac troponins (hs-cTn) have become important biomarkers of acute myocardial injury,[300] as well as the underlying cardiovascular comorbidities of patients.[64 286 301-303] Elevated hs-cTn has been linked to a heightened risk of inpatient mortality in COVID-19 patients, with the assumption that this biomarker is detecting underlying myocardial injury as the cause of the apparent adverse prognosis.[64]

Despite the reported prognostic signal observed with this widely available biomarker in COVID-19, most of the data in the literature are based on population differences.[34 41-43] In other words, the apparent heightened prognostic risk is predominantly observed when groups of patients with elevated hs-cTn are compared to groups of patients with normal hs-cTn.[34 41-43] The diagnostic value of hs-cTn in the individual patient remains unclear. I.e. the probability of a single patient developing inpatient mortality if he or she presents with a certain hs-cTn remains unclear.[34 41-43] This is a major drawback of hs-cTn since it is difficult to extrapolate population-based data to the individual patient who presents to the emergency department. As a result, the translation of this important biomarker into a clinical risk stratification tool in COVID-19 patients has yet to take place. The precise reasons behind the lack of clinical translation of hs-cTn deserve further investigation, which forms one of the important objectives of this study.

The work in this chapter sought to elucidate the diagnostic value of hs-cTn for predicting the likelihood of inpatient mortality in COVID-19. The study hypothesis was that hs-cTn can accurately predict inpatient mortality in acute COVID-19 patients.

3.4 Methods

This chapter details the retrospective analysis of consecutive patients with laboratory-confirmed acute COVID-19 who had high-sensitivity cardiac troponin T (hs-cTnT) measurement. Patients were admitted to the Royal Berkshire National Health Services (NHS) Foundation Trust (Reading, UK) between March and May 2020.

3.4.1 Diagnosis of COVID-19

COVID-19 was diagnosed only using nasopharyngeal swabs of patients.[44-46] The swabs were analysed in the laboratory using real-time reverse transcriptase polymerase chain reaction (rt-PCR) tests for SARS-CoV-2, as previously described.[44-46] I opted for this strict laboratory-based COVID-19 diagnostic criteria which is consistent with other similar studies.[44-46]

Although a proportion of patients may have displayed clinical symptoms suspicious of COVID-19 or radiological abnormalities suggestive of COVID-19, the inclusion of clinical symptoms as a mandate for study inclusion may risk the exclusion of asymptomatic patients who tested positive for COVID-19 in nasopharyngeal swabs. This would be disadvantageous to the potential scientific value of the results since asymptomatic COVID-19 patients are an important disease group to study. Further, evidence suggests that the use of radiological features to diagnose COVID-19 may also be inferior to rt-PCR testing.[274] Therefore, a considered decision was made to restrict the inclusion of patients in this study based only on a positive nasopharyngeal rt-PCR test.

3.4.2 Assessment of hs-cTnT

The hs-cTnT assays were analysed using a Roche Cobas e801 analyser (Roche Diagnostics, Mannheim, Germany). This information was provided with kind support from the consultant biochemist of the hospital (Royal Berkshire Hospital) where the test was performed. The analytical range of this hs-cTnT assay was between 3ng/L to 10,000 ng/L, whereby a value less than 3ng/L falls out of the detection range of the assay analysis to accurately quantify.

The hs-cTnT tests were ordered as part of the clinical care of the patients. The COVID-19 patients were categorised as having either a normal hs-cTnT value or an elevated hs-cTnT value. The cut-off between normal and elevated hs-cTnT was based on an established cut-off value that was produced from a healthy population, which is as previously described.[304 305] Accordingly, a normal hs-cTnT test was defined as one that returned a value ≤14 ng/L (i.e. below the 99th percentile of healthy normal population).[304 305] An elevated hs-cTnT test was defined as one that returned a value >14ng/L (i.e. above the 99th percentile of a healthy normal population), as previously described.[304 305]

3.4.3 Data Acquisition

The data acquisition process was based on the study criteria and was performed with strict adherence to a pre-defined study protocol as outlined previously in the Methods section of the thesis. Of all the processes in this Chapter, data acquisition was one of the most important steps since the data and their accuracy form the foundation of all subsequent analysis.

3.4.4 Data parameters

Data were collected from the electronic clinical records on:

- Demographic information of patients, which included parameters such as age, gender, and body mass index.
- Clinical presentation of patients, which included information such as presenting symptoms, pre-existing co-morbidities, smoking history, and the medications they were taking.
- Laboratory blood tests, which included hs-cTnT, full blood count, renal function, inflammatory markers, and creatine kinase. D-dimer and ferritin.
- Chest x-rays and electrocardiogram findings.
- Clinical outcomes data, which included inpatient mortality, date of discharge from hospital if discharged alive, the requirement for non-invasive ventilation during admission, the requirement for intubation and mechanical ventilation during admission, and intensive care unit admissions.

3.4.5 Data collection process

A standardised data collection protocol was constructed, and a spreadsheet template was designed and circulated to all data collectors. Each data collector was asked to collect ten initial trial cases. These trial cases were validated by me against the medical records. I offered feedback where necessary. Each data collector needed to pass the trial collection and have shown that they have taken on board the feedback in correcting further data collection before being allowed to continue further in the study. All data collectors passed the trial period and were allowed to continue and complete collecting the remaining data. To ensure further data accuracy, once the data were all collected, these were validated

again as referenced to electronic patient records, by myself and another data collector (independent of the other data collectors); any errors detected on validation were corrected and logged (date the error was found and reason) in the master study database.

3.4.6 Ethical approvals

This work was granted COVID-19 Fast-Track Approval by the Health Research Authority (HRA) and Health and Care Research Wales (HCRW), UK. IRAS project ID: 287103. This study only involved a retrospective analysis of already collected anonymised data, no informed consent was required.

3.4.7 Statistical Analysis

Continuous variables were assessed for normality using the Kolmogorov-Smirnov test.[289] Parametric data were displayed as mean (SD).[48 246 290] Non-parametric data were displayed as median with inter-quartile range (IQR).[48 246 290] Comparisons between two groups of parametric data were performed using the unpaired Student's t-test.[290] Comparisons between two groups of non-parametric data were performed using the Mann-Whitney test.[290] Two groups of categorical data were compared using the Chi-square test or the Fisher Exact test as appropriate.[290 291] The Kaplan-Meier curves were used to assess inpatient survival in COVID-19 patients, as categorised by normal vs elevated hs-cTnT,[292] which were compared using the Log-rank test. Multi-variate Cox proportional-hazard regression analysis was used to assess the independent predictors of inpatient mortality risk for hs-cTnT and other possible risk factors, with hazard ratios (HR) presented with 95% confidence intervals (CI).[294] Receiver-Operating Characteristics (ROC) analysis was performed to assess the diagnostic performance of hs-cTnT for predicting inpatient mortality in COVID-19 patients. The area under the

ROC curve (AUC) was presented with 95% CI.[293] Statistical significance was defined as $p<0.05$. The statistical analysis was performed by me (using MedCalc; Version 12.7.8.0), which was then independently validated by a medical statistician (using Stata; Basic Edition version 17.0, Statacorp LLC, Texas USA).

3.5 Results

3.5.1 Patient characteristics

A total of 191 COVID-19 patients (mean age 65.8 ± 16.3 years; 62.3% male) had hs-cTnT measured on admission. Patients with elevated troponins had lower BMI and a lower proportion of patients from Black Asian and Minority Ethnics (BAME) than patients with normal troponins. Patients with elevated troponin had a lower prevalence of symptoms such as chest pain, dyspnoea, cough, and fever (Table 3.3). The patient characteristics are summarised in Table 3.3.

Table 3.3 Baseline patient characteristics.

	All Patients (n = 191)	**Normal hs-cTnT (n = 67)**	**Elevated hs-cTnT (n = 124)**	**P value**
Age (years)	65.8 ± 16.3	53.6 ± 13.6	72.4 ± 13.7	<0.0001
Male (%)	119 (62.3)	35 (52.2)	84 (67.7)	0.035
BMI (kg/m^2)	27.5 (23.8–31.9)	29.8 (25.1–33.2)	26.4 (22.4–30.3)	0.012
BAME	48/174 (27.6)	24/60 (40.0)	24/114 (21.1)	0.008
Symptoms				
Chest pain	40/190 (21.1)	21/66 (31.8)	19 (15.3)	0.015
Dyspnoea	128/190 (67.4)	54/66 (81.8)	74 (59.7)	0.002
Palpitations	5/190 (2.6)	2/66 (3.0)	3 (2.4)	1.000
Fatigue	41/190 (21.6)	13/66 (19.7)	28 (22.6)	0.65
Cough	116/190 (61.1)	56/66 (84.9)	60 (48.4)	<0.0001
Fever	101/190 (53.2)	50/66 (75.8)	51 (41.1)	<0.0001
Diarrhoea	29/190 (15.3)	12/66 (18.2)	17 (13.7)	0.41
Anosmia/Ageusia+	8/119 (6.7)	4/38 (10.5)	4/81 (4.9)	0.265
Comorbidities				
IHD	34/188 (18.1)	3/65 (4.6)	31/123 (25.2)	0.0003
Heart failure	22/188 (11.7)	0 (0)	22/123 (17.8)	<0.0001
Hypertension	84/189 (44.4)	13/65 (20.0)	71 (57.3)	<0.0001
Diabetes	60/188 (31.9)	9/64 (14.1)	51 (41.1)	<0.0001
Dyslipidaemia	19/188 (10.1)	4/64 (6.3)	15 (12.1)	0.213

Current Smoker	10/179 (5.6)	3/60 (5.0)	7/119 (5.9)	1.000
Ex-Smoker	45/179 (25.1)	10/60 (16.7)	35/119 (29.4)	0.06
AF	25/189 (13.2)	2/65 (3.1)	23 (18.6)	0.003
CKD	44/189 (23.3)	1/65 (1.5)	43 (34.7)	<0.0001
COPD	17/189 (9.0)	1/65 (1.5)	16 (12.9)	0.009
Asthma	25/189 (13.2)	12/65 (18.5)	13 (10.5)	0.12
CVD	18/189 (9.5)	1/65 (1.5)	17 (13.7)	0.007
Dementia	17/189 (9)	2/65 (3.1)	15 (12.1)	0.04
Cancer	12/189 (6.4)	3/65 (4.6)	9 (7.3)	0.55
Medications				
ACEi / ARB	48/186 (25.8)	10/65 (15.4)	38/121 (31.4)	0.017
Beta-Blockers	50/186 (26.9)	6/65 (9.2)	44/121 (36.4)	<0.0001
CCB	46/186 (24.7)	6/65 (9.2)	40/121 (33.1)	<0.0001
Aspirin/Clopidogrel	28/186 (15.1)	5/65 (7.7)	23/121 (19.0)	0.04
Digoxin +	3/186 (1.6)	0 (0)	3/121 (2.5)	0.553
Warfarin +	8/186 (4.3)	0 (0)	8/121 (6.6)	0.052
DOAC	25/186 (13.4)	3/65 (4.6)	22/121 (18.2)	0.01
MRA +	6/186 (3.2)	0 (0)	6/121 (5.0)	0.093
Nitrates +	5/186 (2.7)	0 (0)	5/121 (4.1)	0.164
Statins	66/186 (35.5)	15/65 (23.1)	51/121 (42.2)	0.01

Hs-cTnT: high sensitivity cardiac troponin T; BMI: body mass index; BAME: Black, Asian and minority ethnic; IHD: ischaemic heart disease; AF: atrial fibrillation; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVD: cerebral vascular disease; ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker; DOAC: direct oral anticoagulant; MRA: mineralocorticoid receptor agonist. + categorical data was compared using Fisher's exact test; all other categorical data were compared using the Chi-squared test.

Patients with elevated troponins had a higher prevalence of co-morbidities, such as ischaemic heart disease (IHD), hypertension, heart failure, diabetes mellitus (DM), and atrial fibrillation (AF). Other co-morbidities such as chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), cerebrovascular disease, and dementia were also more frequently observed in patients with elevated troponins (Table 3.3). A greater proportion of patients with elevated troponins took regular medications (Table 3.3).

3.5.2 Patient clinical data

Patients with elevated troponins and patients with normal troponins had similar temperature, systolic blood pressures, respiratory rate, and prevalence of hypoxia, on admission (Table 3.4). The prevalence of chest X-ray abnormalities was also similar between the two patient cohorts. Patients with elevated troponins also had wider QRS complexes and longer QTc intervals on 12-lead electrocardiograms compared to patients with normal troponins. Haemoglobin, haematocrit, lymphocyte count, creatinine, and d-dimer were higher in patients with elevated troponins (Table 3.4).

Table 3.4 Patient observations, investigation results, and complications.

	All Patients (n = 191)	**Normal hs-cTnT (n = 67)**	**Elevated hs-cTnT (n = 124)**	**P value**
Admission Observation				
Temperature (ºC)	37.3 ± 1.2	37.3 ± 1.3	37.3 ± 1.3)	0.97
SBP (mmHg)	127 (114–143)	129 (118, 140)	125 (112–144)	0.244
DBP (mmHg)	75 ± 14	78 ± 12	74 ± 14	0.06
Respiratory Rate (/min)	22 (19–28)	22 (19–28)	23 (19–28)	0.289
Significant Hypoxia	45/185 (24.3)	11/66 (16.7)	34/119 (28.6)	0.07
Chest radiograph				
Consolidation	41/185 (22.2)	16/66 (24.2)	25/119 (21.0)	0.612
Opacification	73 (38)	28 (42)	45 (36)	0.533
Atelectasis	18/185 (9.7)	4/66 (6.1)	14/119 (11.8)	0.210
Pleural Effusion	10/185 (5.4)	0 (0)	10/119 (8.4)	0.016
ECG				
Heart rate (bpm)	88 (75–102)	88 (77–101)	90 (75–104)	0.590
PR interval (ms)	155 (140-174)	155 (138-174)	155 (141–176)	0.456
QRS duration (ms)	97 (87–108)	96 (86–102)	100 (88–114)	0.011
QTc duration (ms)	410.8 ± 38.5	396.8 ± 37.1	418.1 ± 37.4	0.0004
Laboratory Results				
Haemoglobin (g/L)	128 (113 – 144)	141 (124 – 150)	123 (107-139)	<0.0001
Haematocrit	0.39 (0.35-0.44)	0.41 (0.38-0.44)	0.37 (0.34-0.42)	0.0001
WCC (10^9/L)	7.6 (5.5–10.3)	7.6 (5.1-9.8)	7.7 (5.6–11.5)	0.238
Platelet Count (10^9/L)	228 (178–299)	225 (181-293)	231 9177-302)	0.97
Lymphocyte Count ($\times 10^9$/L)	0.90 (0.69–1.29)	1.08 (0.89–1.58)	0.80 (0.54–1.14)	<0.0001
Sodium (mmol/L)	138 (135–140)	138 (134–140)	138 (135–141)	0.31
Potassium (mmol/L)	4.2 (3.9–4.5)	4.1 (3.9–4.3)	4.2 (3.8–4.6)	0.374
Creatinine (μmol/L)	86 (67–137)	71 (62–85)	105 (77–185)	<0.0001
Ferritin (μg/L)	753 (297–1493)	775 (161–1409)	739 (394–1657)	0.295
CRP (mg/L)	115 (45–212)	92 (28–207)	131 (55–229)	0.090
D-Dimer (ng/ml)	1104 (663–3037)	885 (550–1377)	1605 (800–3676)	0.004
Creatine Kinase (U/L)	100 (64–241)	102 (56–240)	96 (71–271)	0.566
Complications				

NIV requirement	47 (24.6)	13 (19.4)	34 (27.4)	0.220
ICU admission	33 (17.3)	15 (22.4)	18 (14.5)	0.170
Intubation	15 (7.9)	6 (9.0)	9 (7.3)	0.677
Inpatient Mortality	51 (26.7)	4 (6.0)	47 (37.9)	<0.0001

Hs-cTnT: high sensitivity cardiac troponin T; SBP: systolic blood pressure; DBP: diastolic blood pressure; ECG: electrocardiogram; WCC: white cell count; CRP: C-reactive protein; NIV: non-invasive ventilation; ICU: intensive care unit. Significant hypoxia was defined as an oxygen requirement of greater than 50%.[295]

Of the 191 COVID-19 patients with troponin measured, 47 (24.6%) required non-invasive ventilation (NIV), 33 (17.3%) were admitted to the intensive care unit (ICU), 15 (7.9%) required intubation, and 51 (26.7%) suffered inpatient mortality. Inpatient mortality was significantly more prevalent in patients with elevated troponins than patients with normal troponins (37.9% vs 6.0%, $p<0.0001$). Patients with elevated troponins and patients with normal troponins had similar requirements for NIV (27.4% vs 19.4%, $p=0.220$), ICU admissions (14.5% vs 22.4%, $p=0.170$), and intubation (7.3% vs 9.0%, $p=0.677$; Table 3.4).

3.5.3 Survival analysis

On Kaplan Meier analyses, COVID-19 patients with elevated troponins had worse inpatient survival than patients with normal troponins ($p=0.0014$, by log-rank test comparison; Fig 3.1).

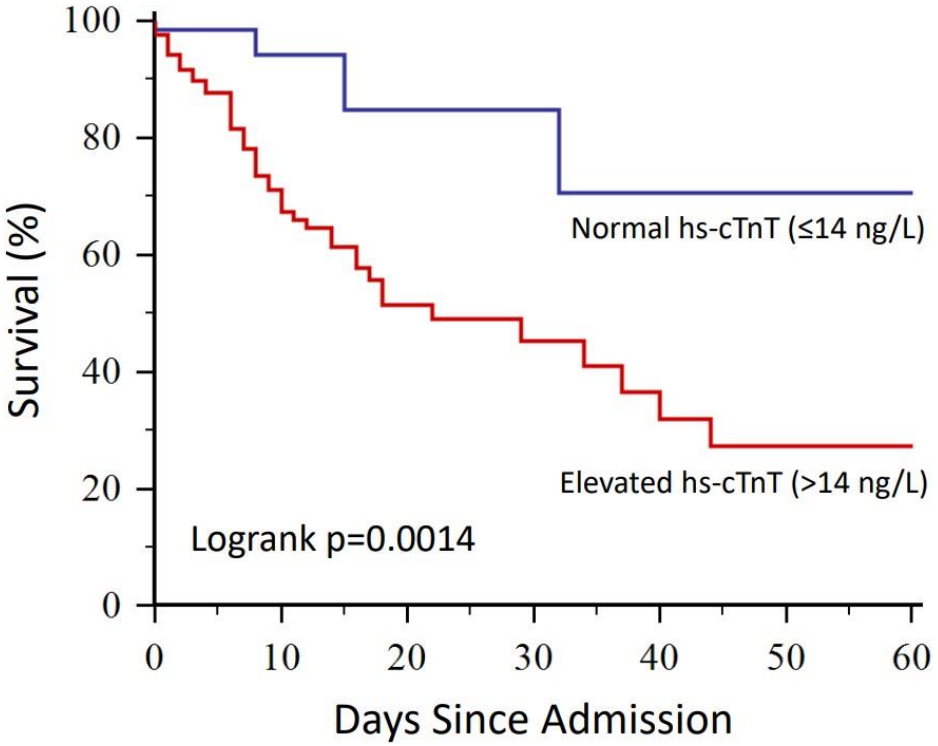


Figure 3.1 Inpatient survival in COVID-19 patients with normal vs elevated troponin. Hs-cTnT: high sensitivity cardiac troponin T.

A Cox multivariate regression analysis was carried out which included several clinically relevant variables such as patient age, BAME status, CKD, COPD, diabetes mellitus, stroke or TIA, smoking status, heart failure, hypertension, IHD, AF, dementia, and troponins. Patients with elevated troponins had a significantly higher risk of inpatient mortality than patients with normal troponins (with a hazard ratio of 5.84, 95% CI 1.29–26.5, p=0.023; Fig 3.2), which was independent of the other variables in the analysis. Other independent predictors of inpatient mortality in COVID-19 patients were IHD (HR 2.24, 95% CI 1.02–4.94, p=0.047) and COPD (HR 2.56, 95% CI 1.03–6.38], p=0.045; Fig 3.2).

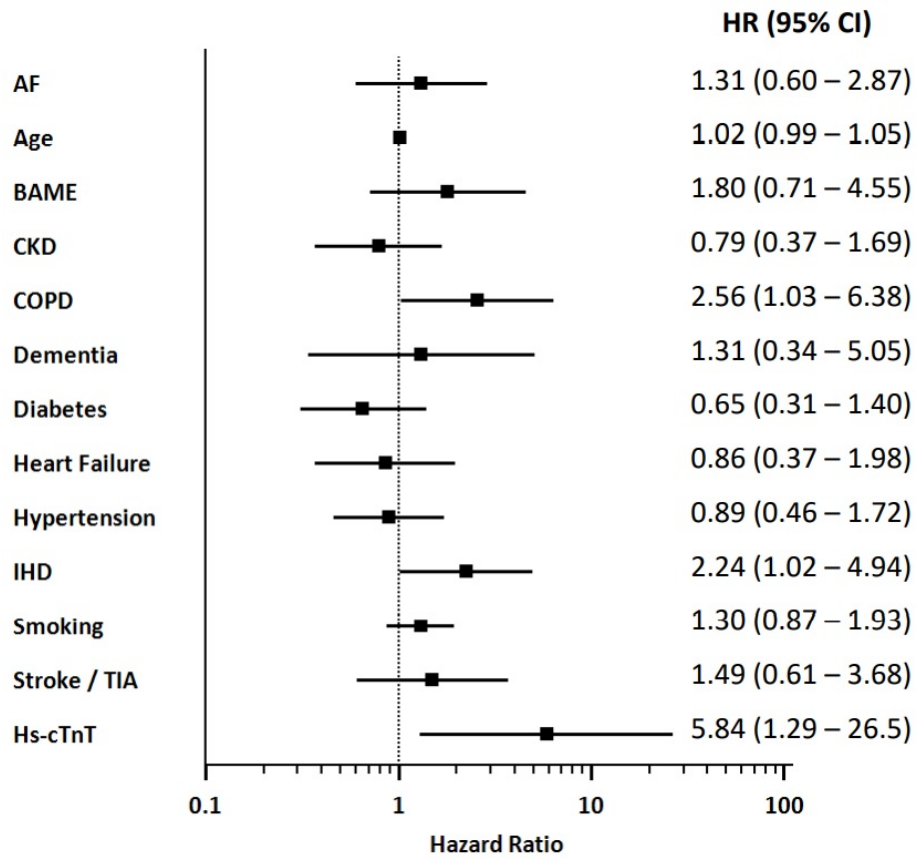


Figure 3.2 Hazard ratios (HR) of risk factors for inpatient mortality assessed using Cox proportional hazard regression multivariate model. AF: atrial fibrillation; BAME: black, Asian and minority ethnic; CI: confidence interval; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; IHD: ischaemic heart disease; TIA: transient ischaemic attack; Hs-cTnT: high sensitivity cardiac troponin T.

3.5.4 Predictive value of hs-cTnT for inpatient mortality

For the prediction of inpatient mortality in COVID-19 patients (AUC = 0.75 on ROC analysis, 95% CI: 0.68–0.81; Figure 3.3), a normal troponin (hs-cTnT <14 ng/L) achieved a sensitivity of 92% (95% CI: 81-98%), a specificity of 45% (95% CI: 37-54%), a positive predictive value (PPV) of 38% (95% CI: 29–47%) and a negative predictive value (NPV) of 94% (95% CI: 85–98%).

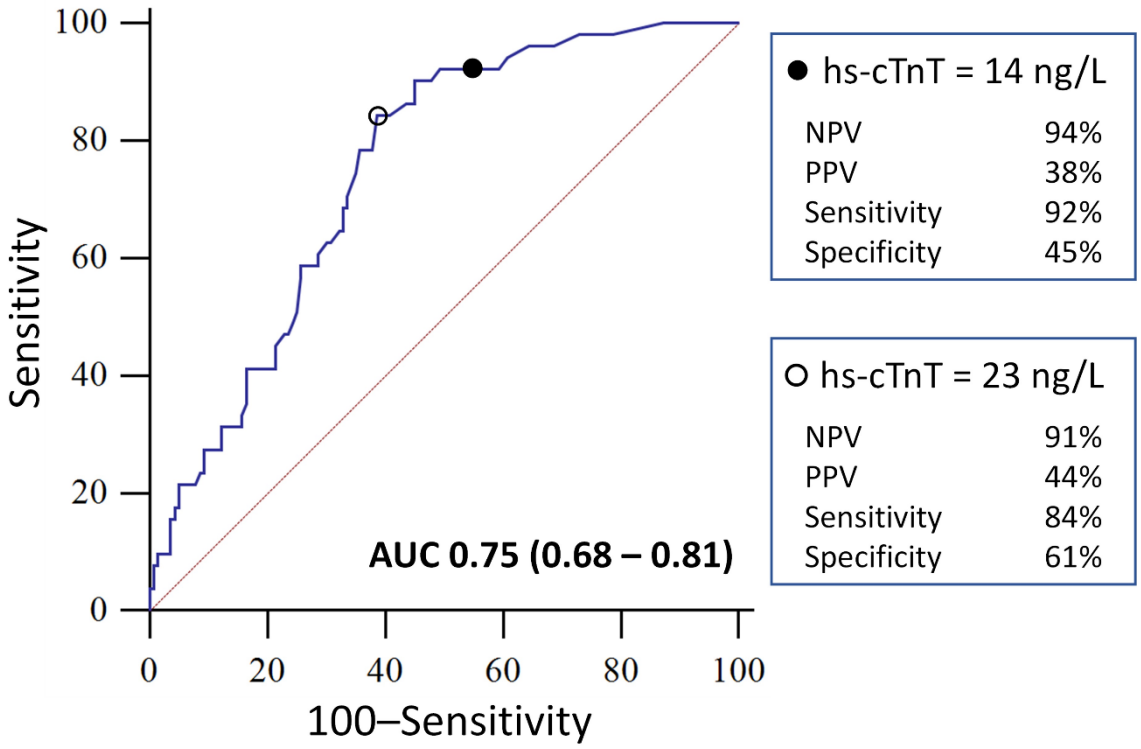


Figure 3.3 Diagnostic performance of high sensitivity cardiac troponin T (hs-cTnT) for predicting mortality in patients with COVID-19. AUC: area under the ROC curve. The 95% confidence interval of the AUC are shown in brackets.

The diagnostic values of a range of troponin levels for predicting inpatient mortality are shown in Table 3.5. As the troponin level threshold reduced, there was an increase in sensitivity and negative predictive value (Table 3.5). As the troponin threshold increased, there was an increase in specificity while the positive predictive value changed minimally (Table 3.5).

Table 3.5 Diagnostic values of different hs-cTnT thresholds for predicting inpatient mortality in COVID-19 patients.

Hs-cTnT (ng/L)	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI	+LR	95% CI	-LR	95% CI
3	100	93–100	5	2–10	28	21–35	100	59–100	1.05	1.0–1.1	0	-
5	100	93–100	13	8–20	30	23–37	100	81–100	1.15	1.1–1.2	0	-
10	94	84–99	39	31–48	36	28–45	95	86–99	1.55	1.3–1.8	0.15	0.05–0.5
14 (Normal)	92	81–98	45	37–54	38	29–47	94	85–98	1.68	1.4–2.0	0.17	0.07–0.5
20	86	74–94	55	46–63	41	32–51	92	84–97	1.92	1.5–2.4	0.25	0.1–0.5
23 (Youden)	84	71–93	61	53–69	44	34–55	91	84–96	2.19	1.7–2.8	0.26	0.1–0.5
25	78	65–89	64	56–72	44	34–55	89	81–94	2.20	1.7–2.9	0.34	0.2–0.6
30	69	54–81	67	59–75	43	32–55	86	78–92	2.09	1.5–2.8	0.47	0.3–0.7
35	65	50–78	68	59–76	42	31–54	84	76–90	2.01	1.5–2.8	0.52	0.4–0.8
40	59	44–72	72	64–79	44	32–56	83	75–89	2.11	1.5–3.0	0.57	0.4–0.8
45	57	42–71	74	66–81	45	32–58	83	75–89	2.21	1.5–3.2	0.58	0.4–0.8
50	45	31–60	79	71–85	43	30–58	80	72–86	2.10	1.4–3.3	0.70	0.5–0.9
55	41	28–56	79	71–85	41	28–56	79	71–85	1.92	1.2–3.0	0.75	0.6–1.0
60	41	28–56	81	74–88	45	30–60	79	72–86	2.22	1.4–3.6	0.72	0.6–0.9

CI: confidence interval; hs-cTnT: high sensitivity cardiac troponin T; +LR: positive likelihood ratio; -LR: negative likelihood ratio; NPV: negative predictive value; PPV: positive predictive value. Normal represents the hs-cTnT threshold from the 99th percentile of healthy individuals. Youden represents the optimal threshold based on the receiver operating characteristics curve.

3.6 Discussion

By the time this work was performed, there was a significant volume of data suggesting an association between elevated high-sensitivity cardiac troponins and a heightened risk of mortality on a patient group level.[41 45 48 50 257 306] I.e. if one were to select a group of COVID-19 patients with elevated troponins and compared their overall prognosis against patients with normal troponins, the patient group with elevated troponins had a worse overall prognosis.[41 45 48 50 257 306]

Despite this volume of evidence, the clinical translation of this important biomarker, cardiac troponin, for guiding clinical decision-making in acute COVID-19 has not taken place. The work in this Chapter showed that having an elevated troponin level did not necessarily mean that an individual patient would go on to suffer inpatient mortality. Cardiac troponin was an inaccurate predictor of mortality, in that an elevated troponin level had a low positive predictive value for any individual COVID-19 patient dying from the disease. This finding hinders the development of a clinical threshold for identifying high-risk patients and means that it is unlikely that we can use a positive troponin value in clinical practice to risk-stratify patients.

The second, perhaps more clinically useful, finding from this work was that a normal high-sensitivity cardiac troponin excluded inpatient mortality (at least in this retrospective cohort) with a good negative predictive value. This observation meant that cardiac troponins could be better developed as a rule-out test for inpatient mortality, to identify low-risk COVID-19 patients.

The aim of these findings naturally desires to shift our thinking and move us forward from the observed group-based link between troponins and mortality in the existing literature to a more

practical understanding of the diagnostic value of cardiac troponins in COVID-19 and how we could exploit it for clinical translation.

It is important to emphasise that the results in this Chapter do not advocate the direct use of cardiac troponins in clinical decision-making in COVID-19 patients at present. I do think that it is vital that any clinical diagnostic test (new or old) should undergo robust prospective testing before it can be used in practice to guide clinical management. This robust testing is lacking for cardiac troponins at present in COVID-19. The importance of robust clinical testing should be emphasised further in that the clinical stakes in patient care are high and I am therefore of the firm opinion that we should not introduce any clinical arbiters into practice, such as routinely performed blood tests that could affect millions upon introduction for a new indication, until we are categorically certain of its validated use.

To this end, it is important to say that the data in this study is a stepping stone toward clinical translation and is *not* the finished product of clinical translation. That said, this study does set the stage for an important prospective validation of a normal troponin as a rule-out test. Practically speaking, this future study would potentially facilitate admission avoidance and early safe discharge of COVID-19 patients (Figure 3.4).

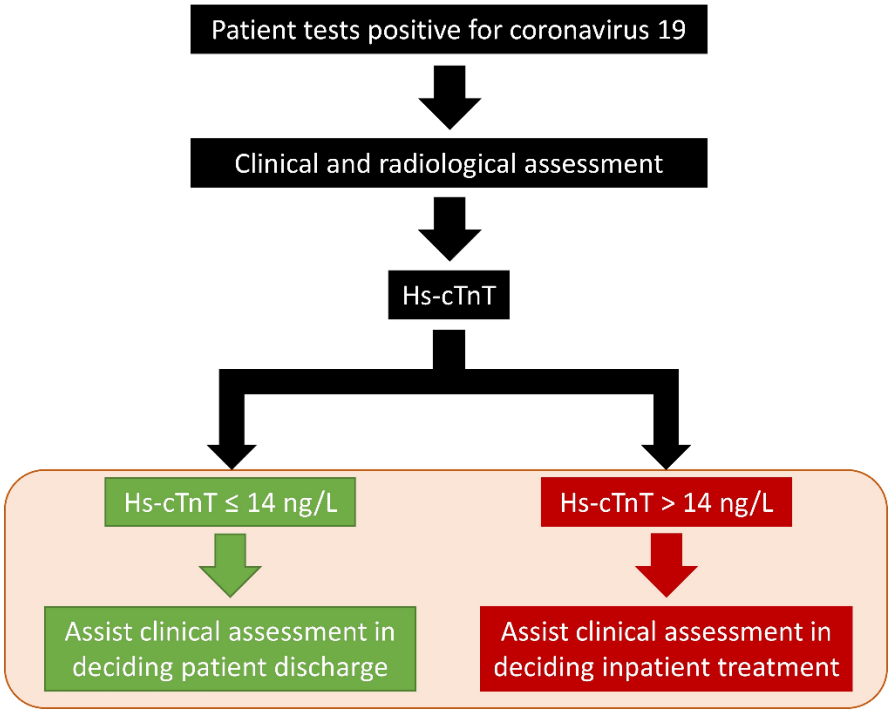


Figure 3.4 Model for prospective validation. Hs-cTnT: high sensitivity cardiac troponin T.

3.6.1 Cardiac troponin is an inaccurate predictor of mortality risk in COVID-19

Only a small proportion of patients with an elevated troponin suffered mortality (~39.7%), which translated to a poor positive predictive value on ROC analysis. Even at a higher troponin level of 60 ng/L, the positive predictive value for patient mortality only reached 45%. An elevated troponin is therefore an inaccurate predictor of inpatient mortality on an individual patient basis in acute COVID-19.

The reasons underlying the poor positive predictive value of cardiac troponins for predicting mortality in COVID-19 remain unclear. One explanation may be that an elevated troponin in an unselected patient population can be non-specific in aetiology[307] and can be caused by a wide range of pathological processes.[307] These can include cardiac causes, for instance, ischaemic heart disease, hypertension, atrial fibrillation, and heart failure.[307] Further, non-

cardiac causes, for instance, CKD, COPD, and cerebrovascular disease can also be associated with elevated cardiac troponin levels.[307] Each of these diseases could lead to a different prognosis in COVID-19.[29 308 309]

Indeed, recent studies have shown that non-cardiac sequelae such as septic shock and multi-organ failure are the commonest causes of death in COVID-19 patients.[140 310] Cardiac troponin elevations are commonly associated with these end-stage illnesses.[140 307 311].

The non-specific aetiology of an elevated troponin and the range of disease processes it represents will continue to serve as an obstacle to any meaningful clinical translation. Patients presenting to the hospital will continue to have co-morbidities that cannot be controlled. Therefore, an elevated troponin cannot be used as a laboratory test to directly risk stratify COVID-19 patients.

Patients with elevated troponin levels and patients with normal troponin levels had similar rates of non-fatal complications such as requirement for NIV, intubation, and ICU admissions, despite significantly different mortality rates. This finding is in line with other studies.[46 312] The reason behind this finding is unclear. The end-stage cause of death in COVID-19 patients is often multi-factorial.[310] COVID-19 patients who go on to develop respiratory failure requiring NIV, intubation, or ICU admissions do not necessarily suffer inpatient mortality.[310] Whilst COVID-19 patients can develop severe respiratory failure, patients are more likely to die from sepsis, myocardial injury, multi-organ failure, or secondary bacterial infections, which may not result in NIV/intubation or ICU requirement.[310] The complex interplay between respiratory complications in COVID-19, multi-organ failure and sepsis, and the eventual mortality events remain incompletely understood and deserve further investigation.

3.6.2 Cardiac troponin: a potentially useful rule-out test in COVID-19

Cardiac troponin tests have a rapid turnaround time of less than an hour,[313] which can enable timely clinical decision-making.[314] It is already established for the assessment of acute coronary syndromes which is a disease that requires rapid diagnosis to enable effective management.[255 315] The results in this Chapter strongly indicate that the clinical utility of troponin in COVID-19 may be to rule out mortality (Table 3.5). A negative troponin may also lessen the effect of any cardiac and non-cardiac co-morbidities on prognosis.

3.6.3 Limitations

The retrospective nature of the results means that the effect of a negative troponin for guiding clinical management is unclear and this, as aforementioned, requires a further prospective validation study. The single centre-basis of the results should also be tested in other centres and on a multi-centre basis. The troponin assays were requested at the discretion of the clinical treating team and mostly in the context of COVID-19 patients presenting with suspected ACS. Since cardiac troponin was not routinely measured in all patients, the results would invariably be vulnerable to sampling bias. The lack of repeat measurements of troponins during admission (since it was not routinely tested in COVID-19 patients) meant that patterns such as rise and fall in troponin could not be assessed, which could offer further insights into true myocardial injury[316] and chronically elevated troponin levels.[317] This patient sample was studied in the pre-vaccination periods and further studies are needed to investigate the diagnostic properties of cardiac troponins in vaccinated patients. Finally, treatment strategies for COVID-19 were not standardised at the time of collection, and the effects of troponins on the risk stratifications of patients who now benefit from current and emerging therapies could not be investigated, which is a substrate for further work.

3.6.4 Conclusion

Cardiac troponin is an inaccurate test for predicting inpatient mortality in acute COVID-19 patients. The potential clinical value of this diagnostic test appears to rely on ruling out mortality. This finding requires prospective validation for guiding clinical management.

Chapter 4: Characterisation of Lymphocyte-CRP ratio (LCR) in acute COVID-19 patients

A paper based on the work in this Chapter has been published in a peer-reviewed journal (Journal of Personalised Medicine). I am the first author (see Appendix II).[318] The work characterised the lymphocyte-CRP ratio (LCR) for predicting adverse outcomes in COVID-19. Of the 1043 patients in the original patient list, 650 patients were eligible to undergo data collection. Of these patients, 413 patients with LCR measured were included in the study.

4.1 Introduction

In COVID-19 patients, serum inflammatory biomarkers enable the assessment of the severity of infection.[319-321] C-reactive protein (CRP) is commonly used in clinical practice and elevated CRP levels have been associated with severe COVID-19 disease and elevated mortality risk.[319-322] However, CRP is a non-specific indicator of immune activation and does not specifically reflect the effect of viral infections on adaptive immunity.[319-322]

The Lymphocyte-to-CRP ratio, or LCR for short, is a new combination inflammatory marker with the potential to assess activation of the innate immune system and adaptive immunity.[245 323 324] LCR may provide a better assessment of the overall inflammatory changes that take place in viral infections.[245 323 324] Based on components from conventional laboratory test results, LCR has recently been shown to demonstrate prognostic value in acute COVID-19 patients.[239-243 325] Reports have indicated that a low LCR value is associated with an elevated risk of patients needing invasive ventilation, admission to intensive care units (ICU), and inpatient mortality due to COVID-19.[239-243 325]

Despite the early promise shown by LCR in the respective reports, this new biomarker has not been compared to CRP (the established inflammatory marker already in routine practice) for

assessing the prognosis of acute COVID-19 patients.[239 245] This limits the possible clinical use of LCR for patient risk stratification.[239-243 325] The work in this Chapter aimed to address this knowledge gap by providing a direct comparison of LCR against CRP for assessing the prognosis of COVID-19 patients.

4.2 Methods

4.2.1 Study Subjects

The work was conducted as detailed in the Methods Chapter. In brief, adult patients (≥ 18 years) with laboratory-confirmed COVID-19 who were admitted to the Royal Berkshire NHS Foundation Trust (UK) between 14th March 2020 to 9th May 2020 were studied. COVID-19 was diagnosed by real-time reverse transcriptase polymerase chain reaction (PCR) testing for SARS-CoV-2 in nasopharyngeal swabs, as described previously.[239] Patients were included if they had laboratory inflammatory markers assessed on admission. Patients were excluded if they had admission laboratory tests >48 hours from their positive SARS-CoV-2 PCR test (n=222); did not have lymphocyte count or serum CRP assessment (n=10); or had unmeasurable CRP levels at <1 mg/L (n=5) which precludes LCR calculation. A total of 413 patients were included. The study flowchart is shown in Figure 4.1.

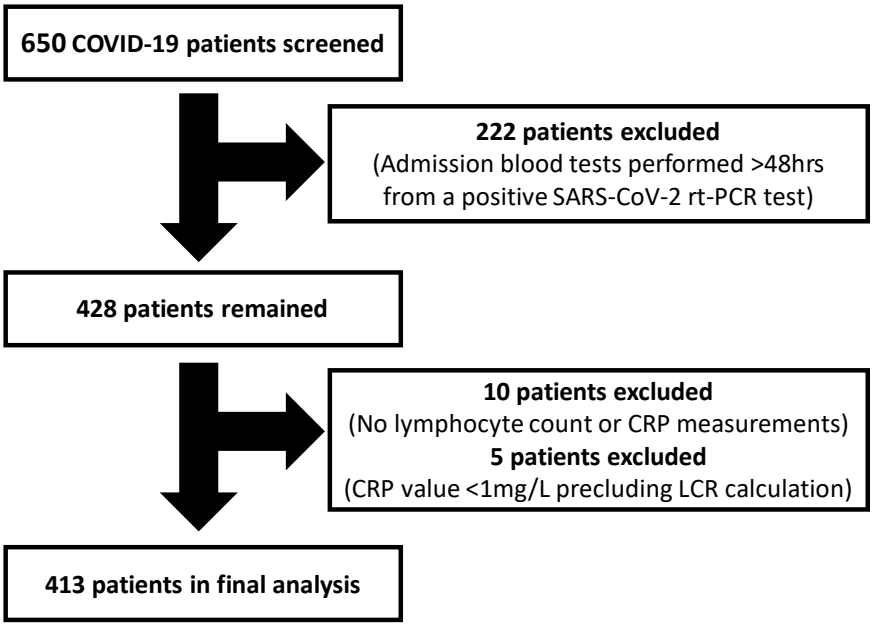


Figure 4.1. Study flowchart showing the patient selection process.

4.2.2 Data Collection

This study was granted COVID-19 Fast-Track Approval by the Health Research Authority (HRA) and Health and Care Research Wales (HCRW), UK. Demographic data, clinical symptoms, and laboratory test results were collected according to a standardised data collection protocol and spreadsheet template as outlined previously in the Methods Chapter.

4.2.3 Endpoints

The primary endpoint was inpatient mortality related to acute COVID-19. The secondary endpoints were a composite of inpatient mortality, requirement for non-invasive ventilation (NIV), intubation/mechanical ventilation, and intensive care unit (ICU) admission related to acute COVID-19. A composite endpoint was included to assess the diagnostic accuracy of LCR and CRP for ruling in and ruling out all major adverse outcomes associated with COVID-19. LCR values were derived using the existing formula: lymphocyte count (number/μL) divided by CRP (mg/dL), as previously described.[245]

4.2.4 Statistical Analysis

The statistical analysis was performed as previously described in the Methods Chapter.

4.3 Results

4.3.1 Baseline patient characteristics

In the 413 COVID-19 patients (median age 70 years old [IQR 56-82]; 58% males) used in the final analyses, there were a total of 313 (76%) survivors and 100 (24%) non-survivors (Table 4.1). Non-survivors were significantly older and had a lower frequency of symptoms such as chest pain and fever, in comparison to the survivors (Table 4.1). Non-survivors also had a lower prevalence of patients suffering from asthma but a significantly higher burden of sufferers of atrial fibrillation, ischaemic heart disease, chronic kidney disease, and chronic obstructive airway disease, in comparison to the survivors (Table 4.1). Other symptoms, co-morbidities, and medication history were similar between the two patient groups (Table 4.1).

Table 4.1. Patient demographics table.

	All Patients (n = 413)	Survivors (n = 313)	Non-survivors (n = 100)	P value
Age	70 (56-82)	66 (52-81)	79 (71-86)	<0.0001
Male	240 (58)	180 (58)	60 (60)	0.66
BMI	26 (22-30)	27 (22-30)	25 (21-30)	0.164
Symptoms				
Chest pain	45 (11)	40 (13)	5 (5)	0.030
Cough	257 (62)	199 (64)	58 (58)	0.317
Dyspnoea	250 (61)	184 (59)	66 (66)	0.199
Fatigue	106 (26)	78 (25)	28 (28)	0.539
Fever	219 (53)	177 (57)	42 (42)	0.011
Comorbidities				
Atrial fibrillation	61 (15)	37 (12)	24 (24)	0.003
Ischaemic heart disease	60 (15)	38 (12)	22 (22)	0.015
Heart failure	44 (11)	30 (10)	14 (14)	0.213
Hypertension	188 (46)	135 (43)	53 (53)	0.084
Diabetes	111 (27)	80 (26)	31 (31)	0.301
Dyslipidaemia	50 (12)	33 (11)	17 (17)	0.087
Smoker (current and ex)	119 (31)	81 (28)	38 (38)	0.077
CKD	99 (24)	66 (21)	33 (33)	0.016
COPD	47 (12)	26 (9)	21 (21)	0.001

Asthma	58 (14)	51 (16)	7 (7)	0.020
CVA/TIA	38 (9)	27 (9)	11 (11)	0.475
Dementia	56 (14)	39 (13)	17 (17)	0.248
Medications				
ACEi / ARB	105 (25)	76 (24)	29 (29)	0.345
Warfarin	19 (5)	11 (4)	8 (8)	0.095
DOAC	47 (11)	31 (10)	16 (16)	0.095
Aspirin	57 (14)	42 (14)	15 (15)	0.690
Statins	145 (35)	107 (34)	38 (38)	0.487

BMI: body mass index; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVA: cerebrovascular accident; ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; DOAC: direct oral anticoagulant.

4.3.2 Laboratory blood tests and clinical outcomes

LCR was significantly lower in non-survivors of COVID-19 than in survivors (median non-survivor LCR 42 [IQR 21-84] vs. median survivor LCR 119 [51-351], $p<0.001$, Figure 4.2). Non-survivors also had lower lymphocyte counts (0.67×10^9/L [0.45-1.00] vs. 0.94×10^9/L [0.65-1.36], $p<0.001$) and platelet counts (188×10^9/L [143-271] vs. 224×10^9/L [178-289], $p<0.001$) compared to the survivors of COVID-19.

Non-survivors had significantly higher median CRP levels (169mg/L [IQR 92-269] vs. 81mg/L [33-152], $p<0.001$, Figure 4.2) and serum creatinine (118µmol/L [80-173] vs 85µmol/L [66-112], $p<0.001$), compared to the survivors.

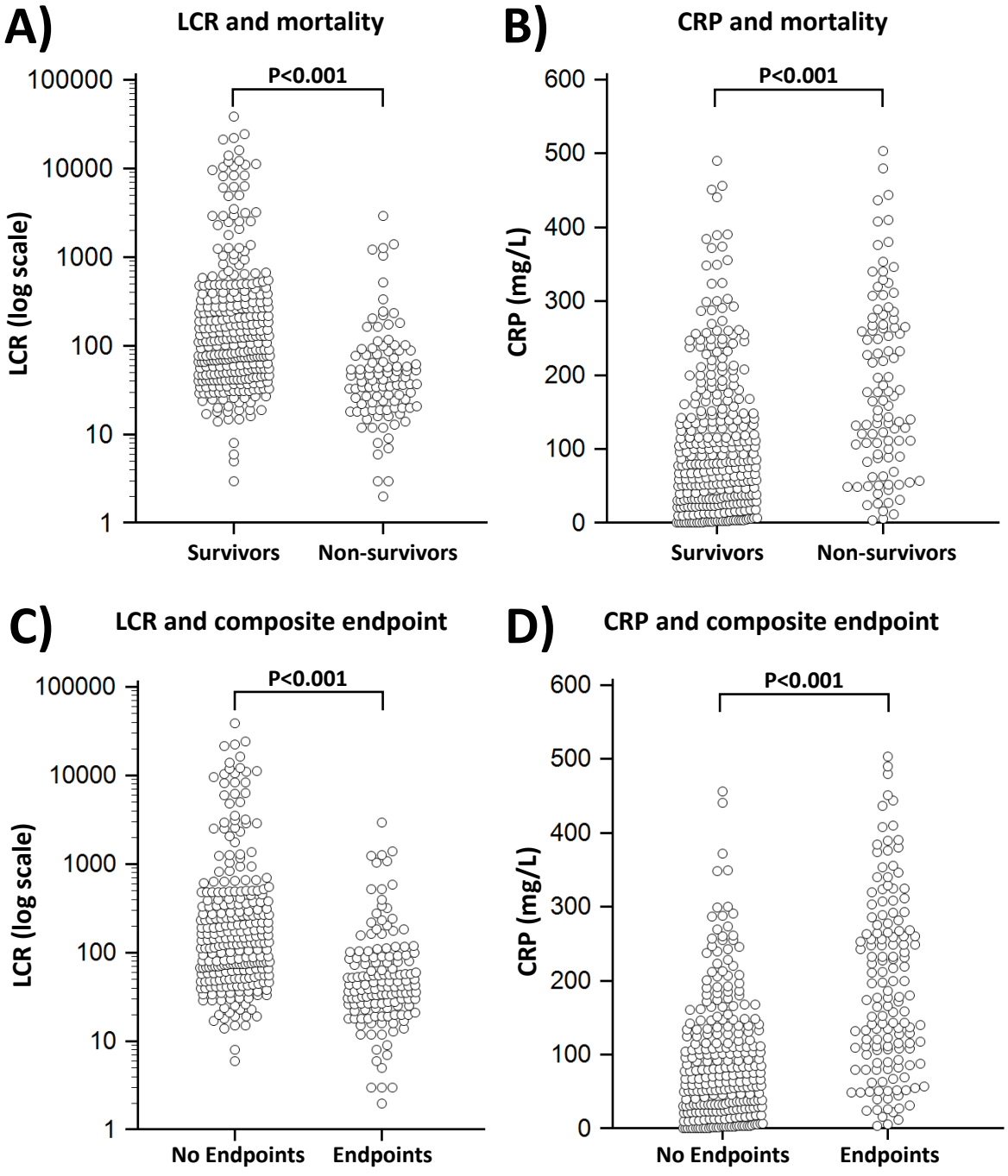


Figure 4.2. C-reactive protein (CRP) and lymphocyte CRP ratio (LCR) relations with inpatient mortality (Panels A and B) and composite endpoint (Panels C and D) in acute COVID-19 patients. Each point represents data from a single COVID-19 patient.

NIV requirement was significantly more prevalent in the non-survivors of COVID-19 compared to the survivors (27% vs 11%, respectively, p<0.001), whilst the frequency of intubation requirement and ICU admissions was similar between the two patient cohorts (Table 4.2).

Table 4.2. Patient clinical observations, laboratory blood results and adverse outcomes.

	All Patients (n = 413)	**Survivors (n = 313)**	**Non-survivors (n = 100)**	**P value**
Observations on admission				
Temperature	37.1 (36.6-37.9)	37.1 (36.7-37.9)	37.1 (36.5-37.9)	0.389
SBP	129 ± 24	130 ± 24	124 ± 24	0.0305
DBP	74 ± 14	75 ± 14	70 ± 15	0.0007
Respiratory Rate	22 (18-26)	20 (18-24)	24 (20-28)	<0.001
Laboratory Results				
LCR	82 (41-264)	119 (51-351)	42 (21-83)	<0.001
Lymphocyte Count	0.90 (0.60-1.31)	0.94 (0.65-1.36)	0.67 (0.45-1.00)	<0.001
CRP	102 (41-187)	81 (33-152)	169 (92-269)	<0.001
Haemoglobin	127 (111-143)	129 (114-145)	121 (108-134)	<0.001
WCC	7.2 (5.3-10.1)	7.0 (5.3-10.0)	8.0 (5.2-11.5)	0.240
Platelet Count	216 (171-286)	224 (178-289)	188 (143-271)	<0.001
Sodium	138 (134-140)	138 (134-140)	138 (135-140)	0.733
Potassium	4.2 (3.9-4.5)	4.2 (3.9-4.5)	4.2 (3.8-4.7)	0.094
Creatinine	89 (67-128)	85 (66-112)	118 (80-173)	<0.001
Complications				
NIV requirement	60 (15)	33 (11)	27 (27)	<0.001
ICU admission	42 (10)	29 (9)	13 (13)	0.282
Intubation	24 (6)	16 (5)	8 (8)	0.283

SBP: systolic blood pressure; DBP: diastolic blood pressure; WCC: white cell count; CRP: C-reactive protein; LCR: lymphocyte-CRP ratio; NIV: non-invasive ventilation; ICU: intensive care unit.

4.3.3 Prognostic data

On ROC analysis, LCR (AUC 0.74, 95% CI: 0.70-0.78) and CRP (AUC 0.71. 95% CI: 0.66-0.75) performed similarly for predicting inpatient mortality in COVID-19 patients, p=0.049 (Figure 4.3A).

For predicting the composite endpoint of inpatient mortality, NIV requirement, intubation/mechanical ventilation requirement, and/or ICU admissions, LCR (AUC 0.76, 95% CI: 0.71-0.80) also performed similarly to CRP (AUC 0.76, 95% CI: 0.71-0.80), p=0.812 (Figure 4.3B).

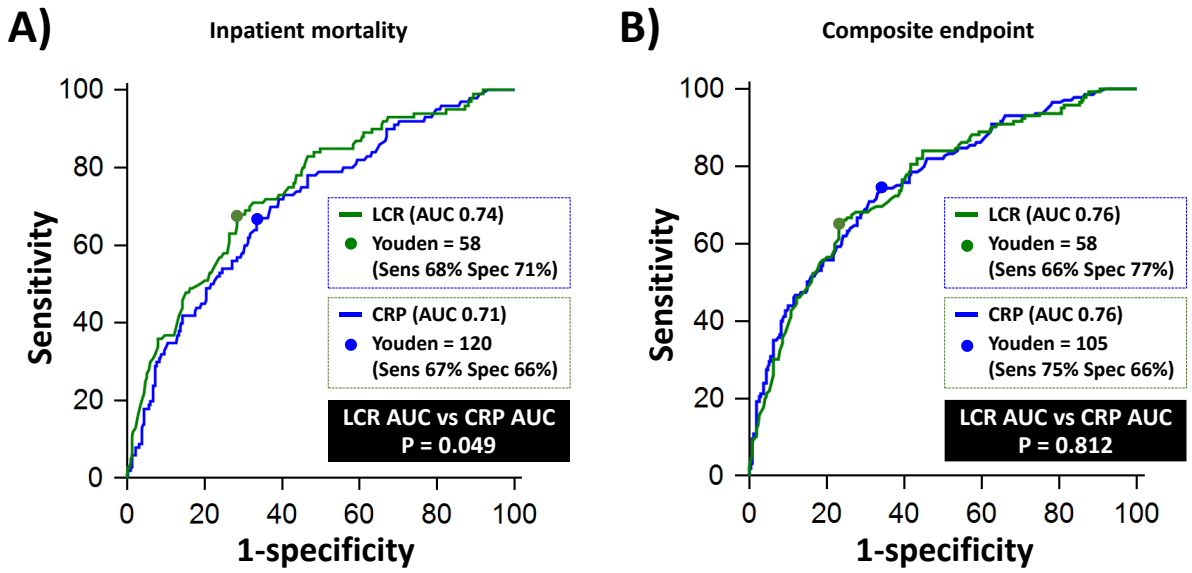


Figure 4.3. Receiver operating characteristics (ROC) curves demonstrating the diagnostic performance of C-reactive protein (CRP) and lymphocyte CRP ratio (LCR) for predicting inpatient mortality and composite endpoint. Panel A (left) shows the ROC curves of LCR and CRP for predicting inpatient mortality. Panel B (right) shows the ROC curves of LCR and CRP for predicting a composite of inpatient mortality, requirement for non-invasive ventilation (NIV), intubation/mechanical ventilation, and/or intensive care unit (ICU) admission. AUC: area under the ROC curve.

For predicting inpatient mortality, an LCR cut-off value of 58 produced a sensitivity of 68% (95% CI: 58-77%) and a specificity of 71% (95% CI: 66-76%); and a CRP cut-off value of 120mg/L yielded a sensitivity of 67% (95% CI: 57-76%) and a specificity of 67% (95% CI: 61-72%; Table 4.3).

For predicting the composite endpoint, an LCR cut-off of 58 yielded a sensitivity of 66% (95% CI: 57-73%) and a specificity of 77% (95% CI: 71-81%), whilst a CRP cut-off of 105mg/L yielded a sensitivity of 75% (95% CI: 67-81%) and a specificity of 66% (95% CI: 60-71%; Table 4.3).

Table 4.3. Diagnostic value of LCR and CRP for predicting clinical outcomes.

	For predicting mortality		**For predicting composite endpoint**	
	LCR	CRP (mg/L)	LCR	CRP (mg/L)
Optimal cut-off (Youden)	58	120	58	105
Sensitivity (95% CI)	68% (58-77)	67% (57-76)	66% (57-73)	75% (67-81)
Specificity (95% CI)	71% (66-76)	67% (61-72)	77% (71-81)	66% (60-71)
Positive LR (95% CI)	2.4 (1.9-3.0)	2.0 (1.6-2.5)	2.8 (2.2-3.6)	2.2 (1.8-2.6)
Negative LR (95% CI)	0.5 (0.3-0.6)	0.5 (0.4-0.7)	0.5 (0.4-0.6)	0.4 (0.3-0.5)
PPV (95% CI)	43% (35-51)	39% (32-47)	60% (52-68)	54% (47-61)
NPV (95% CI)	88% (83-91)	86% (81-90)	80% (75-85)	83% (77-88)

CI: confidence interval; CRP: C-reactive protein; LCR: lymphocyte-CRP ratio; NPV: negative predictive value; PPV: positive predictive values. Composite endpoints included inpatient mortality, requirement for non-invasive ventilation (NIV), intubation/mechanical ventilation and/or intensive care unit (ICU) admission.

In terms of non-fatal endpoints, CRP achieved higher performance than LCR for foretelling the need for NIV (AUC 0.74 vs. 0.68, p=0.022) and for predicting the later need for intubation, mechanical ventilation, or admission to ICU (AUC 0.75 vs. 0.67, p<0.001, Figure 4.4).

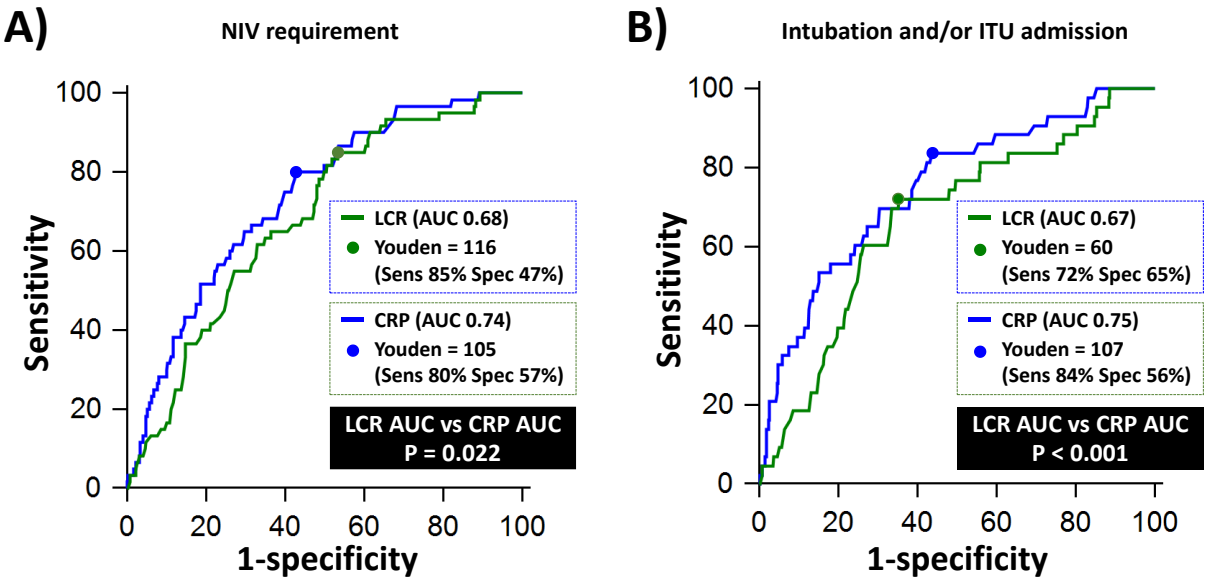


Figure 4.4. Diagnostic performance of CRP and LCR for predicting non-fatal clinical outcomes. Panel A shows the Receiver Operating Characteristics (ROC) curves of LCR and CRP for predicting NIV requirements. Panel B shows the ROC curves of LCR and CRP for predicting the requirement for intubation/mechanical ventilation and/or ICU admission. AUC: area under the ROC curve.

LCR performed significantly better than both lymphocyte counts (AUC 0.74 vs. 0.66, p=0.002), platelet counts (AUC 0.74 vs. 0.61, p=0.003), and the WCC (AUC 0.74 vs. 0.54, p<0.001) for predicting the occurrence of inpatient mortality (Figure 4.5). Although CRP outperformed platelet counts (p=0.043) and WCC (p<0.001) for predicting inpatient mortality, CRP did not outperform lymphocyte counts (AUC 0.71 vs 0.66, p=0.283) for predicting mortality (Figure 4.5). For predicting the composite endpoint in COVID-19 patients, LCR and CRP both performed significantly better than lymphocyte counts, platelet counts, and WCC on ROC analysis (all p<0.001; Figure 4.5).

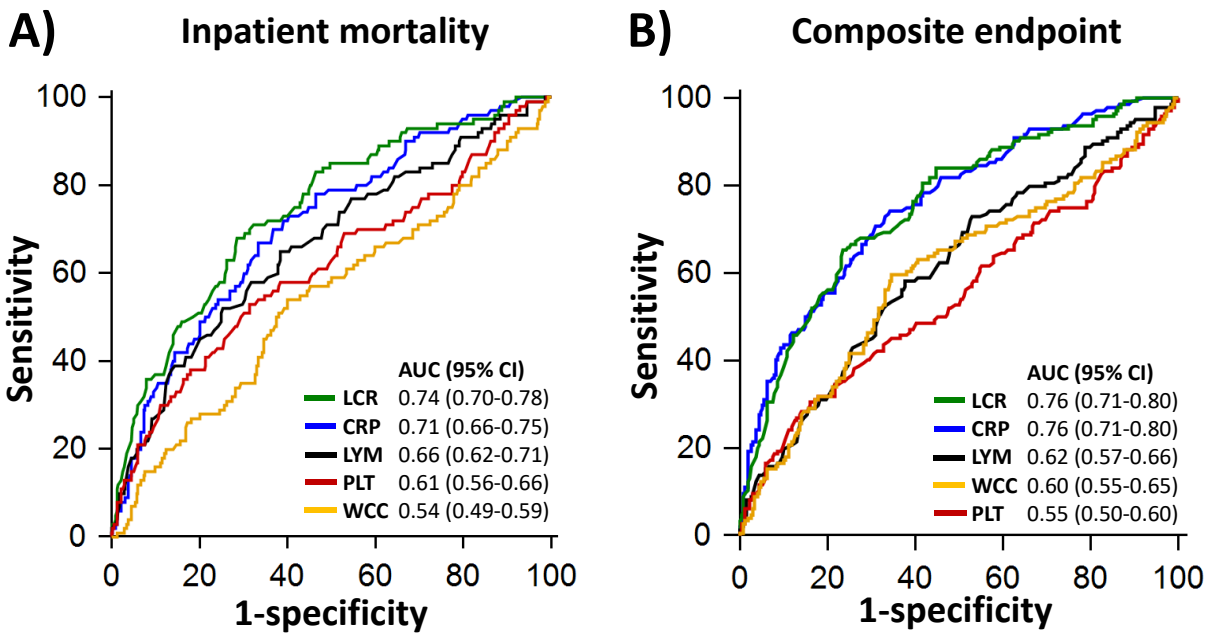


Figure 4.5. Diagnostic performance of inflammatory markers for inpatient mortality (Panel A) and composite endpoint (Panel B). AUC: area under the ROC curve; CRP: C-reactive protein; LCR: lymphocyte CRP ratio; LYM: lymphocyte counts; PLT: platelet count; WCC: white cell count.

4.3.4 Inpatient survival analysis of LCR and CRP

Using Kaplan Meier analysis, acute COVID-19 patients with LCR values below 58 (the Youden cut-off value as defined by the ROC analysis) had worse inpatient survival compared to patients with LCR values of 58 or above, $p<0.001$ (Figure 4.6).

Patients with CRP values above 120mg/L also had significantly worse inpatient survival compared to patients with CRP values of 120 or below, $p=0.012$ (Figure 4.6).

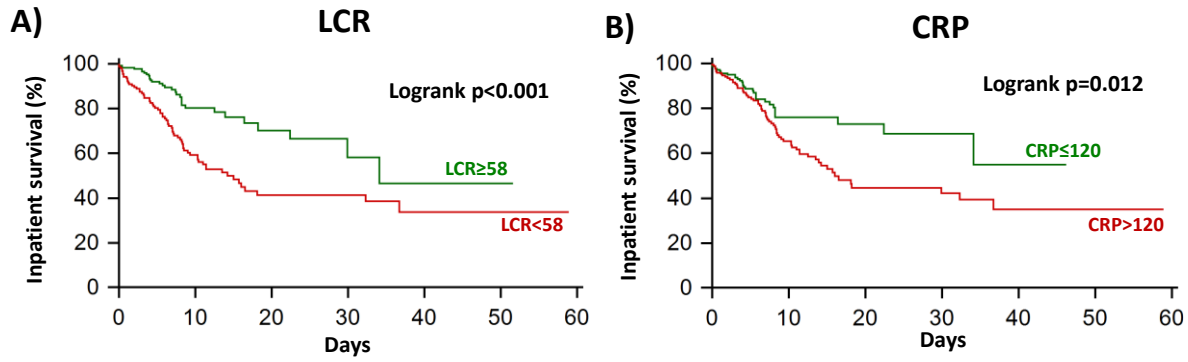


Figure 4.6. Kaplan Meier curves showing inpatient 60-day survival of LCR (Panel A) and CRP (Panel B). The thresholds for LCR and CRP were as derived from the Youden point cut-off values from the ROC analysis shown previously in the Chapter.

4.4 Discussion

The work in this Chapter showed a direct, head-to-head, comparison of the value of LCR against CRP for prognosticating a UK population of acute COVID-19 patients.

The main findings include:

1. As biomarkers, LCR and CRP performed similarly for predicting inpatient mortality and a composite of mortality and non-fatal outcomes.
2. CRP appears superior to LCR for predicting non-fatal clinical outcome endpoints.
3. LCR outperformed other inflammatory markers such as white cell count, lymphocyte counts and platelets for predicting both fatal and non-fatal clinical endpoints.
4. Patients with low LCR (<58 cut-off derived by ROC analysis) had worse inpatient survival compared to patients with higher LCR values.

LCR is a novel and potentially useful combination biomarker for prognosticating acute COVID-19 patients and should be prospectively validated in a larger and multi-centre study.

4.4.1 LCR: from cancer to COVID-19

There are two important reasons behind the development of LCR as a biomarker for risk stratification. The first reason rests on the weakness of CRP, which although being an established clinical inflammatory marker,[322 326] it is non-specific for assessing viral infections and the interaction between adaptive immunity and viral invasion of the human body.[326] From a mechanistic viewpoint, and because COVID-19 is a relatively novel version of coronavirus, a better marker that could more comprehensively assess adaptive immunity is highly desirable. LCR has already been used as a potential prognostic indicator in patients with gastrointestinal cancers, where it may act as a surrogate for host-tumour interactions.[245 323 324] Lymphocytes are important in combating both cancer and viral infections,[327] and therefore LCR may be useful in both cancer patients as well as patients suffering from viral infections such as COVID-19.[245]

4.4.2 LCR: a combination biomarker

As a combination biomarker, LCR may exploit the prognostic value of both CRP and lymphocyte counts.[239] Both elevated CRP levels[319 320] and reduced lymphocyte counts[328 329] are linked to impaired prognosis in COVID-19 patients compared to patients without these laboratory test derangements.[319 320 328 329] Therefore, LCR may exhibit prognostic value in COVID-19 patients in either a synergistic or additive manner.[236 239] From a biological viewpoint, lymphocytes are important mediators of the adaptive immunity[330] and CRP reflects changes in both the innate and adaptive immunity.[206] The combination of lymphocyte count and CRP may provide a more complete assessment of the inflammatory response in acute COVID-19.

Although a few retrospective studies have indicated that LCR could predict inpatient mortality and the severity of COVID-19 manifestation,[239-243 325] most of the studies included small

sample sizes.[239-242 325] Tonduangu et al.[243] demonstrated in 1035 patients that the lymphocyte and CRP ratio achieved fair diagnostic performance for predicting severe COVID-19 (AUC 0.679; cut-off 78.3; sensitivity 79%, specificity 47%) and mortality (AUC 0.607; cut-off 159.4; sensitivity 48%, specificity 70%).[243] The diagnostic performance of LCR in the work in this Chapter appears higher, which may be due to differences in the study populations.[243] A prospective study of LCR could address inter-study differences.

4.4.3 LCR: a marker of potential incremental value

The clinical value of the comparison between LCR and CRP is important. As an established biomarker, CRP has gained a firm foothold in daily clinical practice for assessing infections and inflammation.[242 319-322] It is familiar to most clinicians for this purpose.[242 319-322] Therefore, before any novel inflammatory marker should conceivably be introduced into the clinical arena, it needs to be compared against CRP as a benchmark. Practically, LCR is a slightly more "cumbersome" biomarker than CRP since the former needs to be derived from two tests whilst the latter requires only one. Therefore, to overcome any potential clinical inertia for introducing a potentially more complex test to derive, the relative efficacy of the new test (LCR) versus the existing, and possibly simpler, test (CRP) needs to be known.

The good prognostic value of LCR demonstrated by the work in this Chapter showed the usefulness of the "combination biomarker" concept for prognosticating COVID-19 patients.[236] Although CRP and lymphocyte counts performed similarly in predicting inpatient mortality, when the two biomarkers are included together as LCR, the subsequent prognostic value for inpatient mortality appeared to be slightly higher than each of its parts. It is entirely unclear whether a synergistic or additive effect has taken place, as the difference in AUC between CRP

and LCR was minimal. Further work is therefore required to better understand the mechanism underlying the combination effect in LCR.

LCR may also help evaluate the risk of COVID-19 patients experiencing fatal and non-fatal clinical outcomes. If prospectively validated, LCR could potentially categorise patients with low vs high risks for adverse clinical outcomes, which should be further investigated.

4.4.4 Limitations

As a retrospective study, LCR could not be used to guide clinical management decisions, whilst CRP would have influenced management. A future study that compares LCR and CRP would shed further light on their prognostic values. Lymphocyte subtypes were not separated, such as T-cells, B-cells and natural killer cells,[331] which could further inform about the effect of lymphopenia on prognosis in COVID-19 patients.[331] Further data on more specialised inflammatory biomarkers, such as interleukins,[332] could elucidate the host-versus-virus interplay in COVID-19 acute infections above and beyond what can be provided by routine laboratory tests. These would constitute areas of further research.

4.5 Conclusion

LCR appears comparable to CRP for assessing the prognosis of acute COVID-19 patients. Further studies are required to improve the diagnostic properties of LCR and facilitate its clinical implementation.

Chapter 5: Evaluation of the prognostic value of ferritin-lymphocyte ratio in COVID-19

The work in this Chapter has been published in the Biomedicines journal. I am the first author (see Appendix II). Of the 1043 patients in the original patient list, 650 patients were eligible to undergo data collection. Of these patients, 217 patients with FLR measured were included in the study.

5.1 Introduction

Developing novel inflammatory biomarkers that can predict adverse prognostic outcomes in COVID-19 patients is a clinical priority.[333] Ferritin levels in blood serum samples represent an inflammatory biomarker that becomes elevated during systemic infections, and ferritin itself has both host-protective and immuno-modulatory roles.[221] Whilst elevated ferritin levels have been linked to adverse clinical outcomes in COVID-19 patients in some reports,[226] others have failed to demonstrate a significant prognostic value.[227]

The limitation of the use of ferritin in COVID-19 lies in the fact that it is highly non-specific.[228] Ferritin is considered a non-specific acute phase reactant during inflammation and does not directly inform about the severity of viral infections.[228]

Patients with COVID-19 infections commonly exhibit lymphopenia.[328] Lymphocyte counts form part of the routine inflammatory cell count panel, typically included in the full blood count laboratory tests.[328] Although not specific to viral infection per se, the observation of lymphopenia appears in congruence with the presence of viral infection.[328]

The purpose of the work in this Chapter is to test the feasibility of combining two biomarkers, namely serum ferritin and lymphocyte counts, for assessing the severity of acute COVID-19.

The ferritin-lymphocyte ratio (FLR) is a novel inflammatory index[244] that can be calculated using routinely available blood tests.[244] In the presence of raised ferritin levels (suggestive of infection and/or inflammation) and lymphopenia (suggestive of viral infection) in patients presenting with acute COVID-19, FLR could potentially yield better prognostic value than either ferritin or lymphopenia on their own. An initial report suggested that FLR levels are related to COVID-19 disease severity and can predict mortality with extremely high diagnostic performance.[244] However, this finding has yet to be replicated.

A further knowledge gap exists in understanding the biomarker characteristics and prognostic value of FLR in relation to other established inflammatory markers already in clinical practice. These include C-reactive protein (CRP) and white cell count (WCC).

The specific objectives of the work in this Chapter are therefore to:

1. Establish the biomarker characteristics of FLR in an acute COVID-19 patient population in terms of its distribution and correlation with other inflammatory markers.
2. Establish the diagnostic value of FLR for predicting adverse prognostic outcomes in COVID-19, as compared to other inflammatory markers.

The primary hypothesis is that FLR can achieve a good prognostic value in acute COVID-19 patients. The second hypothesis is that FLR can outperform other inflammatory markers in prognosticating COVID-19 patients.

5.2 Methods

5.2.1 Study Subjects

The patient selection process took place as outlined previously in the Methods Chapter. In brief, adult patients (aged 18 years or older) admitted to hospital with acute COVID-19 between 3rd

February 2020 and 9th May 2020 were included. COVID-19 was diagnosed using real-time reverse transcriptase polymerase chain reaction (rt-PCR) testing of nasopharyngeal swabs. Serum ferritin and lymphocyte assessments were performed on admission to the hospital in all included patients.

Patients were excluded if they did not undergo assessment of other inflammatory markers such as CRP and total white cell count (1 patient); were lost to follow-up due to transfer to another hospital during the study period (1 patient); were treated at another hospital before admission (2 patients); had ferritin levels measured more than 24 hours after admission (2 patients).

5.2.2 Data collection

Data collection was performed as described in the Methods Chapter. A total of 217 patients were included for analysis.

5.2.3 Study Endpoints

The primary endpoint was inpatient mortality related to acute COVID-19.

The secondary endpoints were:

1. Requirement for non-invasive ventilation (NIV) related to acute COVID-19.
2. Critical illness, defined by a composite of the requirement for intubation, mechanical ventilation or intensive care unit (ICU) admission related to acute COVID-19.

FLR was calculated as ferritin (μg/L) ÷ lymphocyte count ($\times 10^9$/L).

5.2.4 Statistical Analysis

Statistical analyses were performed using methods outlined in the Methods Chapter. In brief, continuous variables were expressed as median (inter-quartile range).[290] Two groups of continuous variables were compared using the Mann-Whitney test.[290] Categorical variables

were compared using the Chi-square test.[334] Correlations between data groups were assessed using Pearson's correlation coefficient.[289 335] The diagnostic performance of variables for predicting clinical outcome endpoints was assessed using Receiver Operator Characteristics (ROC) analysis.[293] Inpatient survival was assessed using Kaplan-Meier curves and compared using the Log-rank test.[292] P values <0.05 denoted statistical significance. Statistical analysis was performed by the first author (MedCalc, Version 20.104, Ostend, Belgium) and independently validated by a medical statistician (Stata; Basic Edition version 17.0, Statacorp LLC, Texas USA).

5.3 Results

5.3.1 Baseline patient characteristics

The baseline characteristics of the 217 study patients (median age 69 years [55-82]; 60% males) are shown in Table 5.1. Non-survivors of acute COVID-19 were significantly older, but demonstrated a similar burden of presenting symptoms, as compared to survivors (Table 5.1). Non-survivors demonstrated a higher frequency of ischaemic heart disease, heart failure and chronic obstructive airway disease, as compared to survivors (Table 5.1). There was no significant difference in the prevalence of other co-morbidities between non-survivors and survivors (Table 5.1). A smaller proportion of non-survivors were taking regular beta-blockers and statins, as compared to survivors (Table 5.1).

Table 5.1 Baseline patient characteristics.

	All Patients (n = 217)	**Survivors (n = 159)**	**Non-survivors (n = 58)**	**P value**
Age (years)	69 (55-82)	64 (52-80)	75 (66-83)	0.001
Male (%)	130 (60)	95 (60)	35 (60)	1.000
BMI (kg/m^2)	26 (22-31)	26 (22-31)	26 (23-35)	0.478
Symptoms				
Chest pain	23 (11)	19 (12)	4 (7)	0.331

Cough	123 (57)	92 (58)	31 (53)	0.643
Dyspnoea	123 (57)	86 (54)	37 (64)	0.219
Fatigue	55 (25)	40 (25)	15 (26)	1.000
Fever	112 (52)	87 (55)	25 (43)	0.167
Comorbidities				
Atrial fibrillation	30 (14)	19 (12)	11 (19)	0.189
Ischaemic heart disease	33 (15)	15 (9)	18 (31)	<0.001
Heart failure	27 (12)	15 (9)	12 (21)	0.036
Hypertension	101 (47)	73 (46)	28 (48)	0.761
Diabetes	77 (35)	52 (33)	25 (43)	0.199
Dyslipidaemia	26 (12)	17 (11)	9 (16)	0.349
Current/Ex-Smoker	66/206 (32)	44/153 (29)	22/53 (42)	0.086
CKD	74 (34)	49 (31)	25 (43)	0.106
COPD	27 (12)	14 (9)	13 (22)	0.011
Asthma	23 (11)	16 (10)	7 (12)	0.628
CVA/TIA	24 (11)	15 (9)	9 (16)	0.225
Medications				
ACEi / ARB	57 (26)	40 (25)	17 (29)	0.602
Aspirin	40 (18)	26 (16)	14 (24)	0.235
Beta-Blockers	49 (23)	29 (18)	20 (34)	0.016
Statins	91 (42)	59 (37)	32 (55)	0.020

ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVA: cerebrovascular accident; TIA: transient ischaemic attack.

5.3.2 Clinical observations, blood results and complications

The clinical observations, laboratory blood results and clinical complications of the 217 patients are shown in Table 5.2. Non-survivors had significantly lower systolic and diastolic blood pressures compared to survivors (Table 5.2). Admission temperature and respiratory rate were similar between the two patient groups (Table 5.2). Non-survivors had higher FLR values (p=0.026), higher CRP levels (p=0.001) and lower lymphocyte counts (p=0.015) compared to survivors (Table 5.2). Haemoglobin levels were lower in non-survivors (Table 5.2). Although NIV requirement was higher in non-survivors, as compared to survivors, both patient groups exhibited a similar frequency of critical illness (Table 5.2).

Table 5.2 Patient observations, laboratory results and complications.

	All Patients (n = 217)	Survivors (n = 159)	Non-survivors (n = 58)	P value
Observations on admission				
Temperature (°C)	37.2 (36.6-37.9)	37.1 (36.6-38.0)	37.2 (36.7-37.9)	0.792
SBP (mmHg)	130 (116-146)	131 (117-147)	127 (111-138)	0.033
DBP (mmHg)	75 (66-84)	76 (69-84)	70 (57-83)	0.011
Respiratory Rate (/min)	22 (19-28)	20 (19-26)	23 (20-30)	0.053
Laboratory Results				
FLR	711 (272-1722)	662 (250-1543)	848 (447-2157)	0.026
Lymphocyte Count ($\times 10^9$/L)	0.87 (0.63-1.21)	0.90 (0.64-1.31)	0.73 (0.50-1.04)	0.015
CRP (mg/L)	123 (53-229)	111 (44-208)	159 (101-282)	0.001
Haemoglobin (g/L)	125 (109-141)	127 (109-146)	118 (109-134)	0.019
WCC (10^9/L)	8.1 (5.5-11.5)	7.9 (5.7-11.6)	8.6 (5.3-11.2)	0.815
Platelet Count (10^9/L)	234 (183-300)	238 (194-292)	221 (160-314)	0.344
Sodium (mmol/L)	138 (134-140)	137 (134-140)	138 (134-141)	0.635
Potassium (mmol/L)	4.3 (3.9-4.7)	4.3 (3.9-4.6)	4.5 (4.0-5.0)	0.028
Creatinine (μmol/L)	96 (75-185)	91 (72-153)	123 (83-241)	0.055
Complications				
NIV requirement	48 (22)	26 (16)	22 (38)	0.001
ICU admission	35 (16)	24 (15)	11 (19)	0.533
Intubation	18 (8)	13 (8)	5 (9)	1.000

SBP: systolic blood pressure; DBP: diastolic blood pressure; WCC: white cell count; CRP: C-reactive protein; LCR: lymphocyte to CRP ratio; NIV: non-invasive ventilation; ICU: intensive care unit.

5.3.3 FLR distribution

Figure 5.1 shows the distribution of FLR, CRP and WCC in the 217 COVID-19 patients. The FLR distribution (median 711 [272-1271]) exhibited a positive skew on the scatterplot in Figure 5.1, with over half of all FLR values (61%) being clustered under 1000 and the majority (93%) of FLR values falling under 5000 (Figure 5.1A). Similar positively skewed distributions were observed for CRP (Figure 5.1B) and WCC (Figure 5.1C) in the patient population.

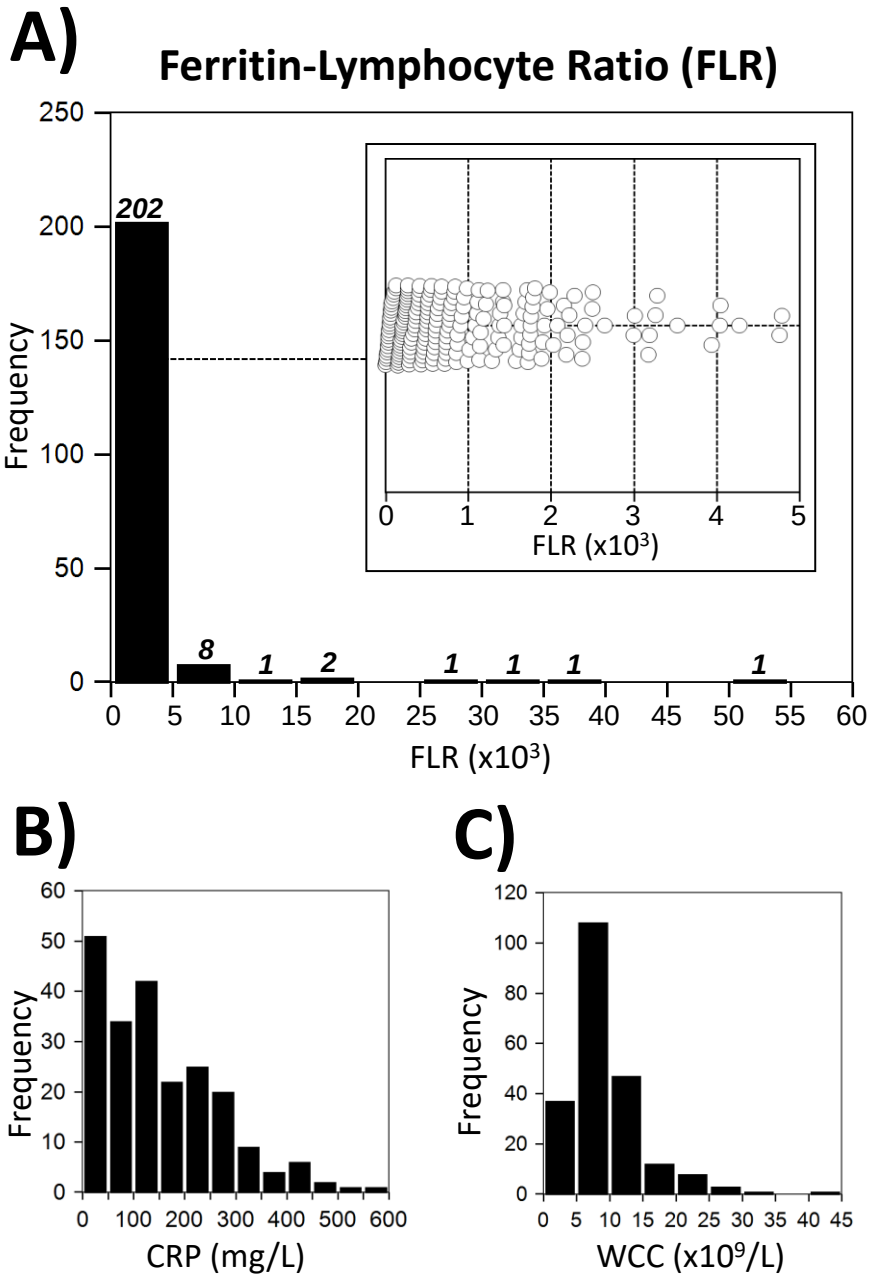


Figure 5.1. Distribution of ferritin-lymphocyte (FLR) and other inflammatory markers. In Panel A, the number of patients in each FLR range is indicated above each bar; the inset shows the distribution of FLR values between 0 and 5000. Panels B and C demonstrate the distribution of C-reactive protein (CRP) and white cell count (WCC) levels, respectively.

5.3.4 Relations of FLR with CRP and WCC

Figure 5.2 shows the distribution and correlation of FLR as compared with CRP and WCC in the 217 COVID-19 patients. FLR exhibited weak correlations with CRP (R=0.108, p=0.115) and WCC (R=-0.144; p=0.034, Figure 5.2).

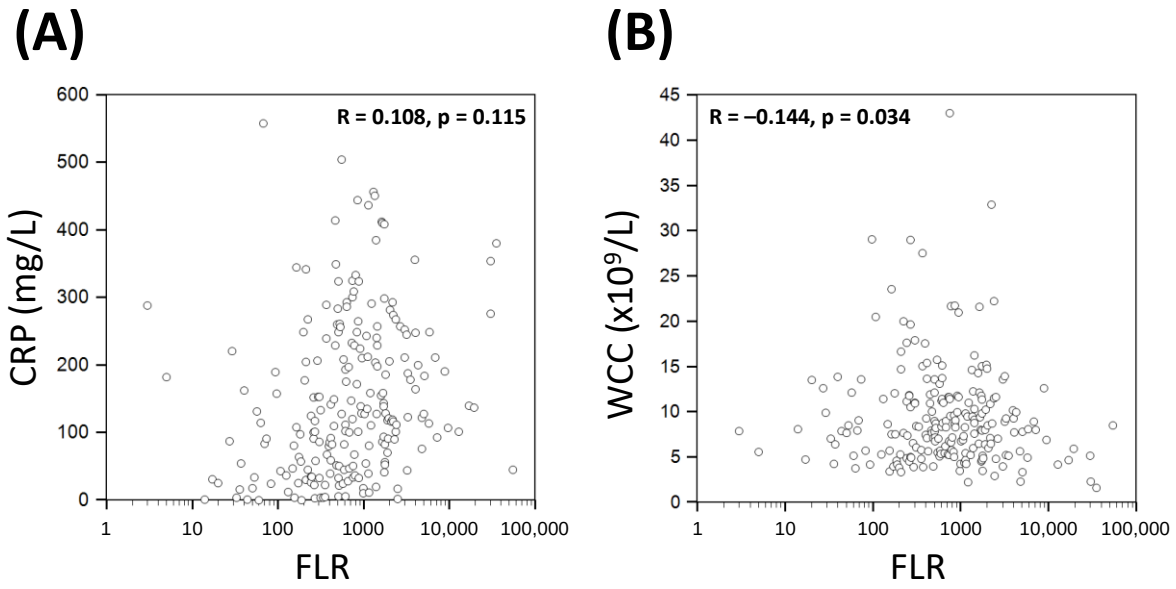


Figure 5.2. Correlations of Ferritin-Lymphocyte Ratio (FLR) with CRP (Panel A) and WCC (Panel B). Each point represents data from a single patient. Pearson's correlation coefficient (R) values are as indicated. CRP: C-reactive protein; WCC: white cell count.

5.3.5 Diagnostic value of FLR for predicting clinical outcomes

For predicting inpatient mortality in the 271 acute COVID-19 patients, FLR achieved an AUC of 0.60 (95% CI: 0.53-0.67; Figure 5.3) on ROC analysis, with a cut-off of 286 (defined by the Youden point) offering a sensitivity of 86% (95% CI: 75-94%) and a specificity of 30% (95% CI: 23-38%). FLR performed similarly to CRP (AUC 0.60 vs 0.64; p=0.375) and WCC (AUC 0.60 vs 0.51; p=0.115) for predicting mortality, despite an apparent visual separation between the three biomarkers on the comparative ROC curves shown below in Figure 5.3.

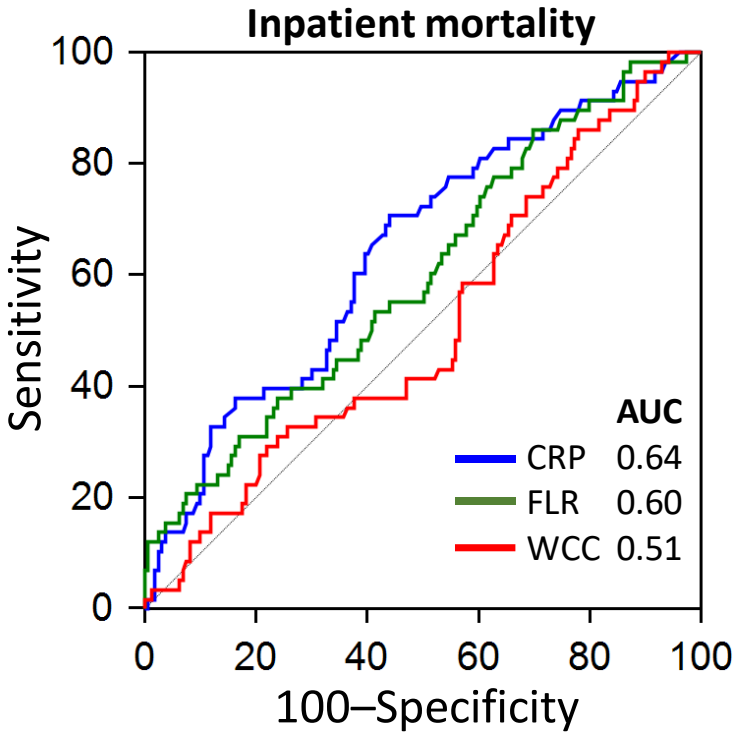


Figure 5.3. Receiver Operating Characteristics (ROC) curves of Ferritin-lymphocyte ratio (FLR) and other inflammatory markers for predicting inpatient mortality. AUC: area under the ROC curve; CRP: C-reactive protein; WCC: white cell count.

For predicting NIV requirement, FLR achieved an AUC of 0.55 (95% CI: 0.48-0.62; Figure 5.4) on ROC analysis, with a cut-off of 356 (Youden point) giving a sensitivity of 79% (95% CI: 65-90%) and a specificity of 33% (95% CI: 26-40%). CRP performed significantly better than both FLR (AUC CRP 0.73 vs FLR 0.55, $p<0.001$) and WCC (AUC CRP 0.73 vs WCC 0.56, $p=0.003$) for predicting requirements for NIV. FLR and WCC performed similarly (AUC 0.55 vs 0.56, $p=0.826$).

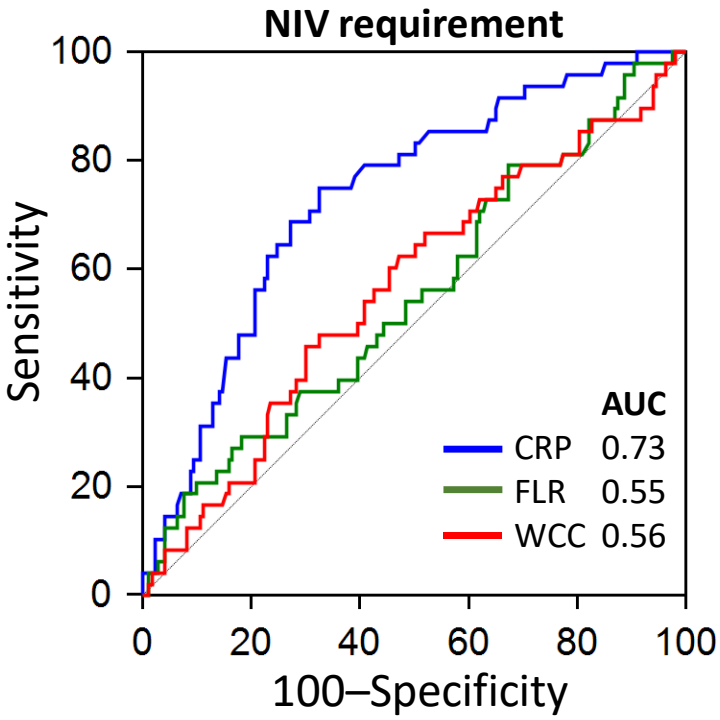


Figure 5.4. Receiver Operating Characteristics (ROC) curves of Ferritin-lymphocyte ratio (FLR) and other inflammatory markers for predicting non-invasive ventilation (NIV) requirement. AUC: area under the ROC curve; CRP: C-reactive protein; WCC: white cell count.

For predicting critical illness (defined as a composite of intubation and mechanical ventilation and/or ICU admission), FLR achieved an AUC of 0.58 (95% CI: 0.52-0.65; Figure 5.5) on ROC analysis, with a cut-off of 368 (Youden point) yielding a sensitivity of 86% (95% CI: 70-95%) and a specificity of 34% (95% CI: 27-41%). CRP outperformed FLR (AUC 0.72 vs 0.58; p=0.037), but not WCC (AUC 0.72 vs 0.65; p=0.375), for predicting the occurrence of critical illness in COVID-19 patients. FLR performed similarly to WCC (AUC 0.58 vs 0.65; p=0.328) for the same purpose.

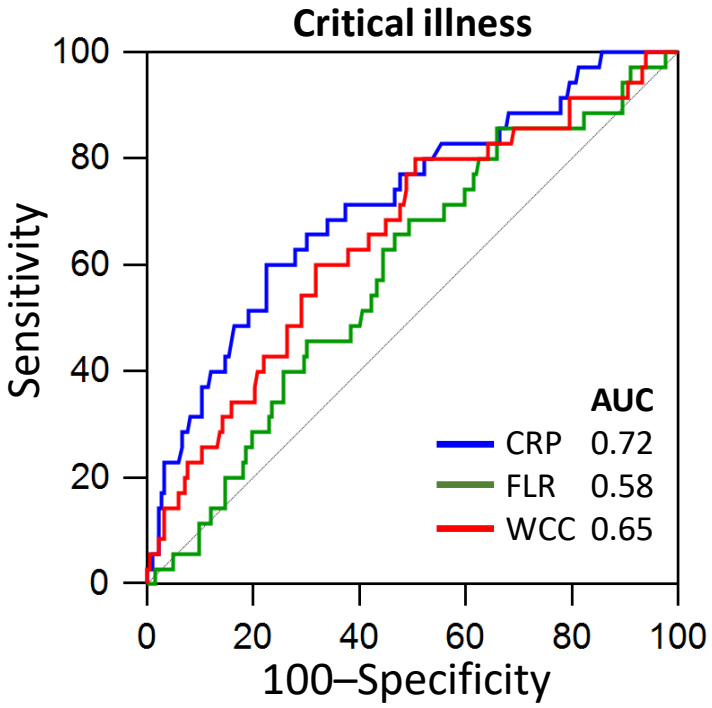


Figure 5.5. Receiver Operating Characteristics (ROC) curves of Ferritin-lymphocyte ratio (FLR) and other inflammatory markers for predicting critical illness. AUC: area under the ROC curve; CRP: C-reactive protein; WCC: white cell count.

The diagnostic performance of FLR for predicting adverse clinical outcomes in acute COVID-19 patients is summarised below in Table 5.3.

Table 5.3 Prognostic values of ferritin-lymphocyte ratio (FLR).

	Mortality	NIV requirement	Intubation/ICU
AUC	0.60	0.55	0.58
AUC 95% CI	0.53-0.67	0.48-0.62	0.52-0.65
AUC P-value	0.023	0.312	0.098
Optimal cut-off	286	356	368
Sensitivity (95% CI)	86% (75-94)	79% (65-90)	86% (70-95)
Specificity (95% CI)	30% (23-38)	33% (26-40)	34% (27-41)
Positive LR (95% CI)	1.2 (1.1-1.4)	1.2 (1.0-1.4)	1.3 (1.1-1.5)
Negative LR (95% CI)	0.5 (0.2-0.9)	0.6 (0.4-1.2)	0.4 (0.2-1.0)
PPV (95% CI)	31% (28-34)	25% (22-29)	20% (17-23)
NPV (95% CI)	86% (75-92)	85% (75-91)	93% (84-97)

AUC: area under the receiver operator characteristics (ROC) curve; CI: confidence interval; NIV: non-invasive ventilation; NPV: negative predictive value; PPV: positive predictive value.

5.3.6 Inpatient survival analysis

On Kaplan Meier curve analysis, COVID-19 patients with FLR above 286 (derived as the Youden point cut-off from ROC analysis) had significantly worse inpatient survival compared to patients with lower FLR values, p=0.041 (Figure 5.6).

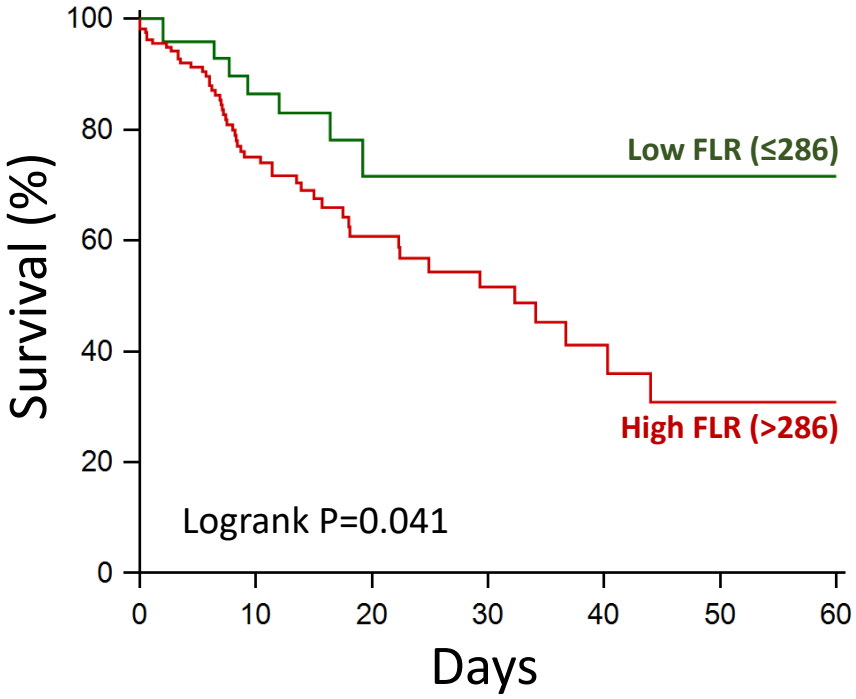


Figure 5.6 Kaplan Meier analysis of inpatient survival based on ferritin-lymphocyte ratio (FLR). The FLR threshold (286) was derived from Receiver Operating Characteristics analysis.

5.4 Discussion

The work in this Chapter evaluated the biomarker characteristics and prognostic value of ferritin-lymphocyte ratio (FLR) for assessing patients with acute COVID-19, compared to inflammatory markers already in routine clinical use. The main findings are as follows:

1. FLR has a positively skewed distribution within the study population.
2. FLR is weakly correlated to established inflammatory markers of CRP and WCC.
3. High FLR values are associated with impaired inpatient survival in COVID-19.
4. However, FLR is an inaccurate predictor of mortality on an individual patient basis.
5. FLR is inferior to CRP for predicting all adverse clinical outcomes in COVID-19.

The work in this Chapter produced overall neutral findings and the results *do not* suggest that FLR can be used in clinical practice for risk-stratifying COVID-19 patients at present. These findings are important since they provide a more realistic representation of the value of FLR amidst recent enthusiasm in the clinical literature.[244] Indeed, the previously reported high prognostic value of FLR in COVID-19[244] could not be replicated in this UK cohort of patients. Further work is required to improve the prognostic value of FLR in COVID-19.

5.4.1 FLR as a combination biomarker

Using the combination biomarker strategy, the prognostic value of FLR in COVID-19 derives from those of both ferritin and lymphopenia. The purpose was to develop a potentially unique surrogate marker of inflammatory disease severity that may distinctively surpass the diagnostic value of each of its constituents; the biomedical combination concept where the whole is greater than its parts.[236] Indeed FLR appears to be a standalone biomarker that demonstrated weak correlation with other commonly used inflammatory biomarkers, including CRP and WCC, which was shown in Figure 5.2. Moreover, the populational distribution of FLR values was also somewhat different to CRP and WCC by being clustered in the very extreme low end of the spectrum, despite a positive skew being observed in all three biomarkers. This is illustrated in Figure 5.1.

Perhaps the most important message from the work in this Chapter is a call for realistic expectations of the clinical value of FLR. The diagnostic performance of FLR for predicting adverse clinical outcomes was at best moderate and significantly inferior compared to the existing standard inflammatory biomarker (CRP).

Aygun et al. previously reported in 331 acute COVID-19 patients an AUC of 0.909 for FLR in predicting mortality,[244] which is significantly greater than the AUC of 0.60 demonstrated in the work in the Chapter using 217 patients. It seems unlikely, though not impossible, that such major differences in AUC could be solely explained by the differences in sample size or characteristic variations in the patient populations studied. Indeed, factors such as differences in selection criteria, follow-up methods and without the instigation of standardised FLR assessment may also contribute to inter-study differences in results. It cannot, at this stage, be reliably adjudicated whether this study or the study performed by Aygun et al.,[244] represents the true clinical value of FLR since both studies are relatively small and therefore vulnerable to selection bias. The only method it seems to find true adjudication is to perform larger, multi-centred and prospective studies to define the clinical usefulness of FLR. However, for now, the results of the work in this Chapter do cast doubt on the optimism shown for this novel biomarker.

5.4.2 Improving FLR – where do we start?

A strength of FLR observed in this Chapter lies in its high sensitivity and negative predictive value for foretelling adverse clinical outcomes in COVID-19 patients. This indicates that even in its current state, FLR may function as a rule-out test for mortality, which may be a substrate for further prospective validation. FLR below the ROC-derived threshold of 286 was associated with good inpatient survival. The current area for improvement for FLR appears to be its poor specificity and positive predictive value for adverse clinical outcomes in COVID-19 patients.

Improving FLR requires further developments in the prognostic values of both ferritin and lymphocyte counts. Ferritin has multiple mechanisms of action during infection and inflammation.[221] It may deprive pathogens of iron for replication or modulate host immune processes whilst possessing intrinsic anti-inflammatory properties.[221] However, its precise role in COVID-19 infections remains unclear and further work is required to elucidate the pattern and timing of ferritin elevation in viral infections.

Lymphopenia occurs in viral infections and is not specific to COVID-19.[336] Developing a specific lymphocyte-based marker for COVID-19 disease severity is a potential goal for improving the prognostic value of FLR. Certain lymphocyte subgroups, involving the CD4 and CD8 T-cell populations, may exhibit high specificity for COVID-19.[337] Surface marker expression, such as that involving CD38 and PD-1 on T-lymphocytes, has been linked to adverse clinical outcomes.[338] Recent evidence also suggests that the reduction of lymphocyte function observed in COVID-19 patients may lead to attenuated viral clearance.[338 339] Examination of the lymphocyte subgroups, function and surface marker expression, rather than performing lymphocyte cell counts alone, may offer greater prognostic specificity.[337-339] This is an interesting area of potential further work in biomarker development.

5.4.3 Limitations and Future Directions

The major limitation of this work is the single-centred nature of the study. The generalisation of the results is therefore not possible and requires a larger multi-centre study to confirm or refute. The effect of modern treatments such as steroid therapy or biological agents on FLR could not be tested and deserves further investigation. It is conceivable that immunosuppressive therapies could have important effects on the prognosis of COVID-19 patients and the predictive value of FLR for adverse clinical outcomes.

5.5 Conclusion

FLR has moderate prognostic value in COVID-19 patients and is not superior to existing inflammatory markers in this regard. Further work is needed to improve the specificity of FLR for predicting adverse clinical outcomes.

Chapter 6: CRB-65 for assessing the prognosis of acute COVID-19 patients with and without radiological evidence of pneumonia

The work in this Chapter has been written up as a scientific manuscript and published in a peer-reviewed journal (Biomedicines). I am the first author (see Appendix II).[190] Of the 1043 patients in the original patient list, 650 patients were eligible to undergo data collection. Of these patients, 589 patients with CRB-65 measured were included in the study.

6.1 Introduction

In patients with acute COVID-19, the development of clinical risk scoring systems can help to facilitate effective risk stratification.[277-280] CRB-65 is a clinical score used to predict the risk of mortality in patients diagnosed with community-acquired pneumonia (CAP).[199] It has been used to aid the physician in deciding whether patients with CAP should receive treatment at home or in the hospital.[199] The score itself is simple to calculate and is based on routinely collected observations in the initial assessment of patients.[199] These include confusion level, respiratory rate, blood pressure and age of the patient (CRB-65).[199]

Recent reports also suggested a role for CRB-65 in the risk stratification of patients with acute COVID-19 pneumonia.[277-280] Patients with an elevated CRB-65 score have a greater risk of suffering adverse clinical outcomes than patients with low CRB-65.[277-280] However, the role of CRB-65 for risk stratifying patients with COVID-19 but without concurrent pneumonia remains unclear.

Chest X-ray (CXR) abnormalities provide a radiological diagnosis of pneumonia[340] and are linked to an adverse prognosis in COVID-19.[341-344] Whilst CXR findings such as ground glass opacification and focal consolidation are commonly observed in COVID-19, patients can also

present without significant CXR abnormalities.[342 345 346] The relationship between CRB-65 and prognosis in COVID-19 patients without CXR evidence of pneumonia, as compared to COVID-19 patients with pneumonia, remains unknown

The work in this Chapter seeks to validate the prognostic value of CRB-65 in COVID-19 patients with normal and abnormal CXR, as compared to established serum inflammatory biomarkers.

6.2 Method

6.2.1 Study Subjects

The study inclusion and exclusion criteria are outlined in the Methods Chapter. Adult (18 years or older) COVID-19 patients were included if they were admitted to the hospital between 1st February 2020 and 9th May 2020 and had CXR evaluation as well as laboratory blood serum testing. COVID-19 was diagnosed as previously described. Patients were excluded if they had missing or inadequate follow-up information (n=8); no CRP or WCC assessment on serum blood tests (n=9); or were already treated at another hospital before admission which confounds the temporal relationship between the CXR and blood tests with prognosis (n=2). A total of 589 patients were eligible for the study and were included in the analysis.

6.2.2 Data Collection and Study Endpoints

Clinical information and demographics data, laboratory blood results and CXR findings were collected according to a standardised protocol as outlined in the Methods Chapter. The primary endpoint was the occurrence of inpatient mortality related to acute COVID-19. The secondary endpoints were:

1. Requirement for non-invasive ventilation (NIV); and
2. Critical illness related to acute COVID-19, defined as a composite of requirements for intubation, mechanical ventilation or intensive care unit (ICU) admission.

CRB-65 scores range between 0 to 4. The individual components of the CRB-65 are as previously described[200 277-280] and defined as follows:

1. Confusion (1 point)
2. Respiratory rate ≥30/min (1 point)
3. Systolic blood pressure ≤90mmHg or diastolic blood pressure ≤60mmHg (1 point)
4. Age ≥65 years (1 point)

CXR findings were reported by a radiologist as part of routine clinical care and blinded to this study.

6.2.3 Statistical Analysis

All continuous variables were assessed for normality using the Kolmogorov-Smirnov test and expressed as median (inter-quartile range).[290] Continuous variables were compared using the Mann-Whitney test.[290] Categorical variables were compared using the Chi-squared test.[334] The diagnostic performance for predicting clinical outcomes was assessed using the Receiver Operator Characteristics (ROC) analysis.[293] The area under the ROC curves was displayed with standard error of the mean (SEM) as appropriate. Inpatient survival from COVID-19 was assessed using Kaplan-Meier curves and compared using the Log-rank test.[292] P values <0.05 denote statistical significance. Statistical analysis was performed using MedCalc, Version 20.104, Ostend, Belgium.

6.3 Results

6.3.1 Baseline patient characteristics

Baseline patient characteristics are shown in Table 6.1. Of the 589 patients (median age 71 [57-83]; 57% males), 186 patients (32%) had normal CXR findings, and 403 patients (68%) had abnormal CXR findings (Table 6.1). Patients with abnormal CXR findings had a higher symptomatic burden of dyspnoea and cough compared to patients with normal CXR. The two groups of patients had similar frequency of co-morbidities and medication history (Table 6.1).

Table 6.1 Baseline patient characteristics.

	All Patients (n = 589)	**Normal CXR (n = 186)**	**Abnormal CXR (n = 403)**	***p*-value**
Demographics				
Age (years)	71 (57-83)	73 (56-85)	71 (58-82)	0.512
Male (%)	337 (57)	101 (54)	236 (59)	0.370
BMI (kg/m^2)	26 (22-30)	25 (21-28)	27 (23-31)	<0.001
Symptoms				
Dyspnoea	328 (56)	80 (43)	248 (62)	<0.001
Cough	342 (58)	88 (47)	254 (63)	<0.001
Fever	289 (49)	84 (45)	205 (51)	0.215
Fatigue	143 (24)	39 (21)	104 (26)	0.216
Chest pain	67 (11)	23 (12)	44 (11)	0.676
Comorbidities				
Hypertension	272 (46)	79 (42)	193 (48)	0.248
Current/Ex-Smoker	174 (30)	51 (27)	123 (31)	0.497
Diabetes	168 (29)	49 (26)	119 (30)	0.492
CKD	142 (24)	41 (22)	101 (25)	0.469
Atrial fibrillation	98 (17)	26 (14)	72 (18)	0.284
Ischaemic heart disease	88 (15)	27 (15)	61 (15)	0.901
Asthma	73 (12)	24 (13)	49 (12)	0.789
COPD	71 (12)	17 (9)	54 (13)	0.173
Dyslipidaemia	69 (12)	22 (12)	47 (12)	1.000
Heart failure	65 (11)	19 (10)	46 (11)	0.777
CVA/TIA	65 (11)	25 (13)	40 (10)	0.206
Medications				
ACEi / ARB	153 (26)	40 (22)	113 (28)	0.106
Statins	196 (33)	52 (28)	144 (36)	0.074
Beta-Blockers	143 (24)	38 (20)	105 (26)	0.149
Aspirin	77 (13)	17 (9)	60 (15)	0.065

ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVA: cerebrovascular accident; TIA: transient ischaemic attack.

6.3.2 Clinical results and outcomes

The clinical results and outcomes of the patients are shown in Table 6.2. Ground glass opacification and interstitial opacification were the most frequently observed abnormal CXR findings (49%), followed by consolidation (28%), atelectasis (13%) and pleural effusions (11%; Table 6.2). Patients with abnormal CXR had significantly higher CRP levels (116mg/L [52-224] vs 64mg/L [23-143]; p<0.001) and lower lymphocyte counts (0.89×10^9/L [0.58-1.30] vs 0.98×10^9/L [0.69-1.39]; p=0.028, Table 6.2) compared to patients with normal CXR. Inpatient mortality, requirement for NIV and critical illness were more common in patients with abnormal CXR than in patients with normal CXR (all p<0.01, Table 6.2).

Table 6.2 Clinical results and outcomes

	All Patients (n = 589)	**Normal CXR (n = 186)**	**Abnormal CXR (n = 403)**	***p*-value**
Observations on admission				
Temperature (ºC)	37.1 (36.6-37.9)	36.9 (36.6-37.6)	37.1 (36.6-37.9)	0.031
SBP (mmHg)	128 (114-145)	129 (114-146)	128 (113-144)	0.739
DBP (mmHg)	74 (66-82)	74 (67-82)	74 (65-83)	0.930
Respiratory Rate (/min)	21 (18-25)	20 (18-22)	22 (19-28)	<0.001
Chest X-ray abnormalities				
Consolidation	113 (19)	-	113 (28)	-
GGO / Interstitial opacification	196 (33)	-	196 (49)	-
Atelectasis	52 (9)	-	52 (13)	-
Pleural effusions	43 (7)	-	43 (11)	-
Laboratory Results				
Haemoglobin (g/L)	125 (109-142)	127 (110-143)	125 (108-142)	0.354
WCC (10^9/L)	7.6 (5.6-10.5)	7.5 (5.7-11.2)	7.6 (5.5-10.3)	0.442
Lymphocyte Count ($\times10^9$/L)	0.90 (0.60-1.31)	0.98 (0.69-1.39)	0.89 (0.58-1.30)	0.028
Platelet Count (10^9/L)	224 (174-291)	221 (174-293)	226 (174-290)	0.742
CRP (mg/L)	101 (41-198)	64 (23-143)	116 (52-224)	<0.001
Sodium (mmol/L)	138 (134-140)	138 (135-140)	138 (134-140)	0.339
Potassium (mmol/L)	4.2 (3.8-4.6)	4.2 (3.8-4.5)	4.2 (3.9-4.6)	0.577
Creatinine (μmol/L)	88 (67-134)	86 (65-117)	89 (68-136)	0.206

Complications				
Inpatient mortality	153 (26)	27 (15)	126 (31)	<0.001
NIV requirement	83 (14)	9 (5)	74 (18)	<0.001
Intubation and ventilation	36 (6)	3 (2)	33 (8)	0.001
ICU admission	65 (11)	6 (3)	59 (15)	<0.001

CXR: chest X-ray; SBP: systolic blood pressure; DBP: diastolic blood pressure; WCC: white cell count; CRP: C-reactive protein; GGO: ground glass opacification; LCR: lymphocyte to CRP ratio; NIV: non-invasive ventilation; ICU: intensive care unit.

6.3.3 Distribution of CRB-65 scores

The distribution of CRB-65 scores in this patient population is illustrated in Figure 6.1. Most patients (96%) had CRB-65 scores ranging between 0 to 2. Normal CXR was observed in 39% of patients with a CRB-65 score of 0; in 25% of patients with a CRB-65 score of 1; in 36% of patients with a CRB score of 2; and 17% of patients with a CRB-65 score of 3. No patients with CRB-65 of 4 had normal CXR.

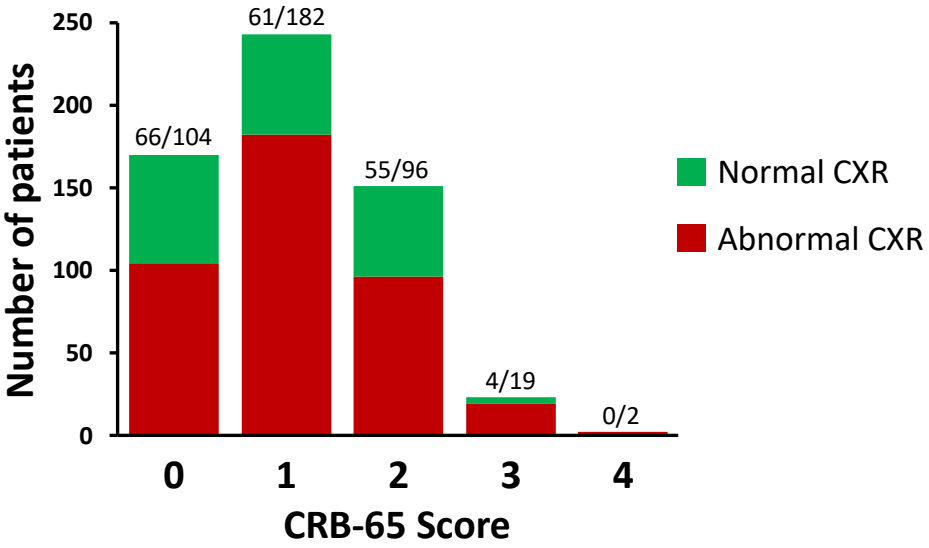


Figure 6.1. Distribution of CRB-65 scores in the patient population. The numbers of patients with normal and abnormal chest X-rays (CXR) are shown above each bar.

6.3.4 Prognostic value of CRB-65

For predicting inpatient mortality on ROC analysis (AUC 0.69±0.02, p<0.001, Figure 6.2), a cut-off CRB-65 score of 0 (Youden point) achieved a sensitivity of 92% (95% CI: 86-95) and a specificity of 36% (95% CI: 32-41). The diagnostic performance of CRB-65 for predicting inpatient mortality was similar in patients normal CXR (AUC 0.67±0.05, p=0.002; Figure 6.2B) and abnormal CXR (AUC 0.69±0.03, p<0.001; Figure 6.2C).

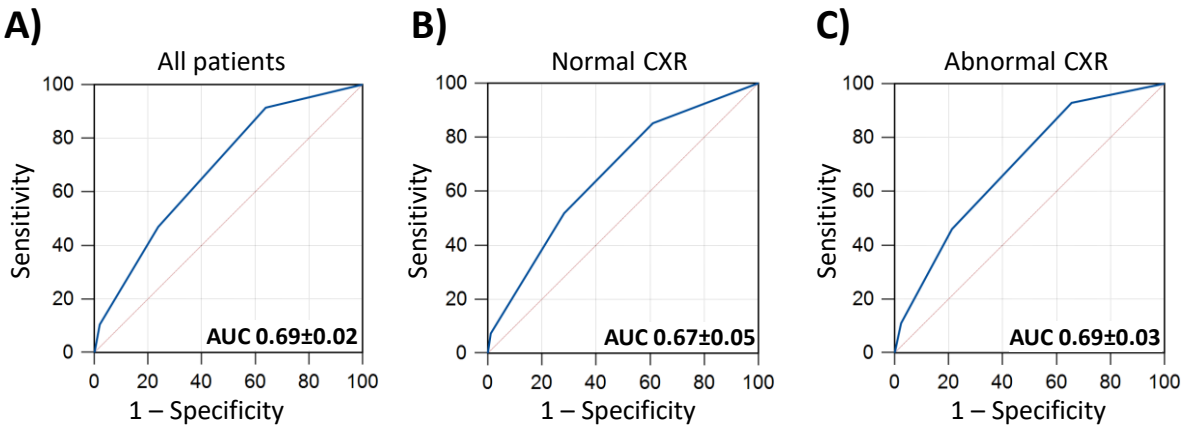


Figure 6.2. Performance of CRB-65 for predicting inpatient mortality. Panel A indicates all patients. Panel B indicates patients with normal chest X-rays (CXR). Panel C indicates patients with abnormal CXR. Areas under the ROC curves (AUC) are illustrated ± standard errors of the mean (SEM).

In the whole patient population, a CRB-65 score of 0 yielded a negative predictive value (NPV) of 92% for ruling out inpatient mortality; a NPV of 87% for ruling out NIV requirement; and a NPV of 90% for ruling out critical illness (Table 6.3).

In patients with normal CXR, a CRB-65 score of 0 achieved a NPV of 94% for ruling out mortality; a NPV of 98% for ruling out NIV requirement; and NPV of 99% for ruling out critical illness (Table 6.3). In patients with abnormal CXR, a CRB-65 score of 0 achieved a NPV of 91% for ruling out mortality; a NPV of 83% for ruling out NIV requirement; and a NPV of 86% for ruling out critical illness (Table 6.3).

Table 6.3. Diagnostic performance of low CRB-65 scores for predicting adverse clinical outcomes.

	N	AUC±SEM	*p*-value	CRB-65 Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	PPV (95% CI)	NPV (95% CI)
Inpatient mortality										
All patients	589	0.69±0.02	<0.001	0	92% (86-95)	36% (32-41)	1.4 (1.3-1.6)	0.2 (0.1-0.4)	33% (32-35)	92% (88-95)
Normal CXR	186	0.67±0.05	0.002	0	85% (66-96)	39% (31-47)	1.4 (1.1-1.7)	0.4 (0.2-1.0)	19% (16-23)	94% (86-98)
Abnormal CXR	403	0.69±0.03	<0.001	0	93% (87-97)	34% (29-40)	1.4 (1.3-1.6)	0.2 (0.1-0.4)	39% (37-42)	91% (85-95)
NIV requirement										
All patients	589	0.53±0.03	0.416	0	35% (25-46)	72% (68-76)	1.3 (0.9-1.7)	0.9 (0.8-1.1)	17% (13-22)	87% (85-89)
Normal CXR	186	0.68±0.08	0.026	0	67% (30-93)	66% (59-73)	2.0 (1.2-3.3)	0.5 (0.2-1.3)	9% (6-14)	98% (94-99)
Abnormal CXR	403	0.51±0.04	0.711	0	31% (21-43)	75% (70-80)	1.3 (0.9-1.9)	0.9 (0.8-1.1)	22% (16-30)	83% (81-85)
Intubation / ICU admission										
All patients	589	0.55±0.03	0.125	0	34% (23-47)	72% (68-76)	1.2 (0.8-1.7)	0.9 (0.8-1.1)	13% (9-18)	90% (88-91)
Normal CXR	186	0.72±0.11	0.047	0	83% (36-100)	66% (59-73)	2.5 (1.6-3.7)	0.3 (0.0-1.5)	8% (5-11)	99% (95-100)
Abnormal CXR	403	0.54±0.04	0.239	0	29% (18-42)	75% (70-79)	1.1 (0.7-1.8)	1.0 (0.8-1.1)	16% (11-23)	86% (84-88)

AUC: area under the receiver operator characteristics curve; CI: confidence interval; CXR: chest X-ray; ICU: intensive care unit; LR: likelihood ratio; N: number of acute COVID-19 patients; NIV: non-invasive ventilation; NPV: negative predictive value; PPV: positive predictive value; SEM: standard error of the mean.

A CRB-65 score of 1 achieved a NPV of 80% for ruling out mortality; a NPV of 86% for ruling out NIV requirement; and a NPV of 92% for ruling out critical illness (Table 6.4). A CRB-65 score of 2 or 3 had poor positive predictive values for predicting all adverse clinical outcomes (Table 6.4).

Table 6.4 Diagnostic performance of intermediate to high CRB-65 scores for predicting adverse clinical outcomes

	CRB-65 Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Inpatient mortality					
All patients	1	47 (39-55)	76 (72-80)	41 (35-47)	80 (78-83)
	2	10 (6-16)	98 (96-99)	64 (45-80)	76 (75-77)
	3	1 (0-4)	100 (99-100)	50 (6-94)	74 (74-74)
Normal CXR	1	52 (32-71)	72 (64-79)	24 (17-33)	90 (85-93)
	2	7 (1-24)	99 (96-100)	50 (13-87)	86 (85-88)
	3	0 (0-13)	100 (98-100)	-	86 (86-86)
Abnormal CXR	1	46 (37-55)	79 (73-83)	50 (42-57)	76 (73-79)
	2	11 (6-18)	97 (95-99)	67 (45-83)	71 (69-72)
	3	1 (0-4)	100 (98-100)	50 (6-94)	69 (69-69)
NIV requirement					
All patients	1	71 (60-81)	30 (26-34)	14 (13-16)	86 (82-90)
	2	95 (88-99)	4 (3-6)	14 (13-15)	84 (65-94)
	3	99 (94-100)	0 (0-1)	14 (14-14)	50 (6-94)
Normal CXR	1	89 (52-100)	33 (26-40)	6 (5-8)	98 (90-100)
	2	100 (66-100)	2 (1-6)	5 (5-5)	-
	3	100 (66-100)	0 (0-2)	5 (5-5)	-
Abnormal CXR	1	69 (57-79)	29 (24-34)	18 (16-20)	80 (74-86)
	2	95 (87-99)	5 (3-8)	18 (17-19)	81 (60-93)
	3	99 (93-100)	0 (0-2)	18 (18-19)	50 (6-94)
Intubation / ICU admission					
All patients	1	77 (65-87)	31 (27-35)	12 (11-14)	92 (87-95)
	2	100 (95-100)	5 (3-7)	12 (11-12)	-
	3	-	-	-	-
Normal CXR	1	83 (36-100)	32 (26-40)	4 (3-6)	98 (91-100)
	2	100 (54-100)	2 (1-6)	3 (3-3)	-
	3	100 (54-100)	0 (0-2)	3 (3-3)	-
Abnormal CXR	1	76 (63-86)	30 (25-35)	16 (14-18)	88 (82-92)
	2	100 (94-100)	6 (4-9)	15 (15-16)	-
	3	-	-	-	-

CI: confidence interval; CXR: chest X-ray; ICU: intensive care unit; NIV: non-invasive ventilation; NPV: negative predictive value; PPV: positive predictive value.

6.3.5 Prognostic value of CBR-65 compared to inflammatory markers

In the whole patient population, CRB-65 performed similarly to CRP for predicting mortality; CRB-65 and CRP both outperformed WCC ($p<0.001$, Figure 6.3). For predicting NIV requirements and critical illness, CRP outperformed CRB-65 and WCC (Figure 6.3).

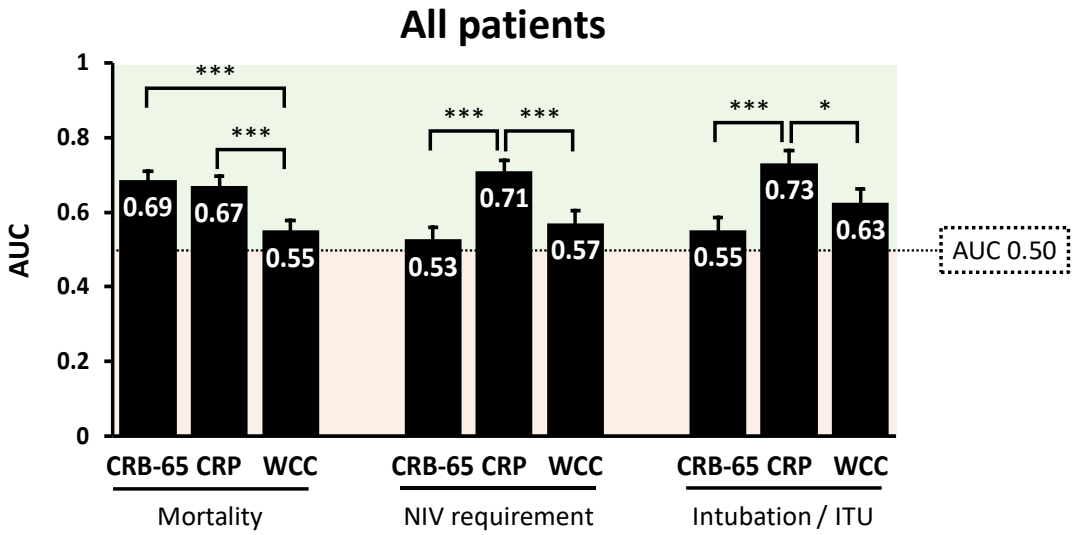


Figure 6.3. Diagnostic performance of CRB-65 and inflammatory markers for predicting inpatient mortality in COVID-19 patients. *p<0.05; **p<0.01; ***p<0.001.

In patients with normal CXR, CRP outperformed CRB-65 ($p<0.05$) and WCC ($p<0.01$) for predicting mortality (Figure 6.4).

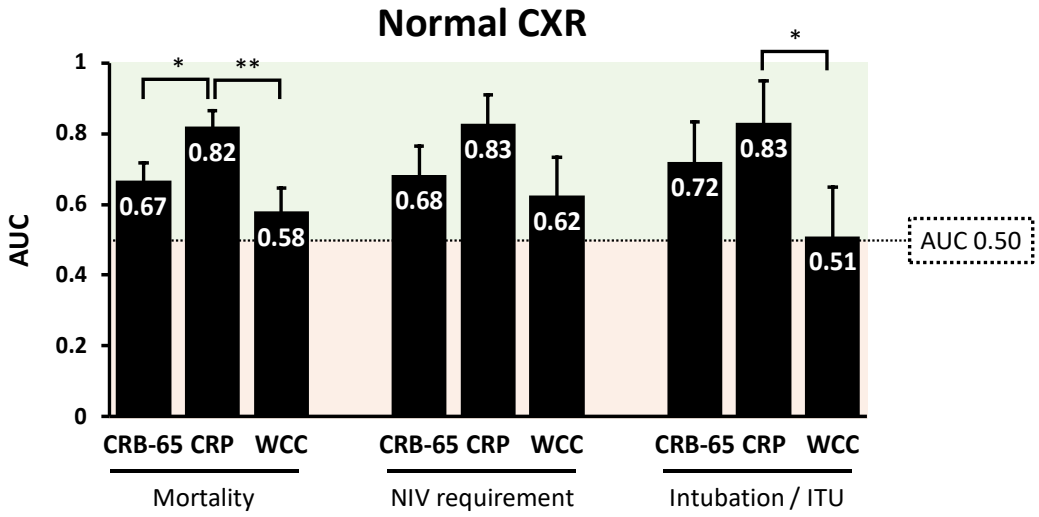


Figure 6.4. Performance of CRB-65 and inflammatory markers for predicting clinical outcomes in COVID-19 patients with normal CXR. *p<0.05; **p<0.01; ***p<0.001.

In patients with abnormal CXR, CRB-65 outperformed CRP and WCC for predicting mortality (all p<0.05; Figure 6.5). However, CRP outperformed CRB-65 and WCC for predicting NIV requirement (all p<0.05; Figure 6.5). Both CRP and WCC outperformed CRB-65 in predicting critical illness (Figure 6.5).

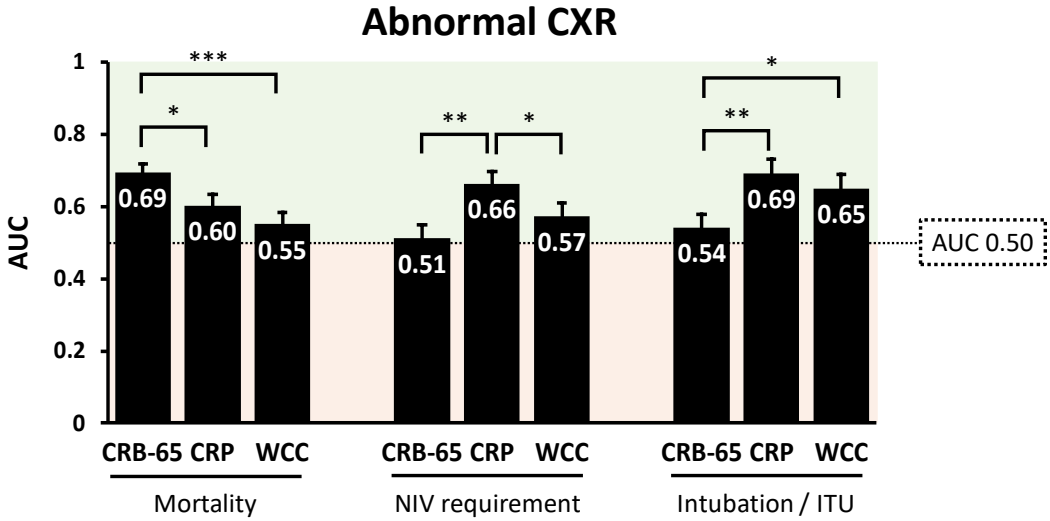


Figure 6.5. Performance of CRB-65 and inflammatory markers for predicting clinical outcomes in COVID-19 patients with abnormal CXR. *p<0.05; **p<0.01; ***p<0.001.

6.3.6 Survival analysis

On Kaplan Meier analysis, increased CRB-65 scores were associated with a stepwise reduction in inpatient survival within the whole study population (Figure 6.6A), in patients with normal CXR (Figure 6.6B) and patients with abnormal CXR (Figure 6.6C).

It should be clarified that after 42 days of follow-up, there were no patients with normal CXR and CRB-65 scores ≥2, which explains the apparent drop-off in the Kaplan Meier curve (Red line Figure 6.6B). This apparent drop-off in the Kaplan-Meier curve does not indicate that all patients died during follow-up.

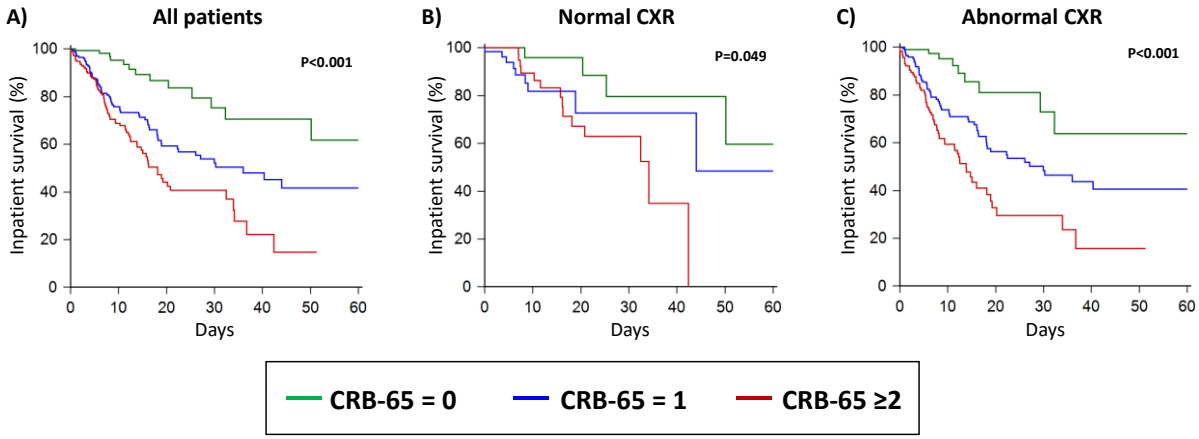


Figure 6.6. Inpatient survival of COVID-19 patients by CRB-65 score. CXR: chest X-ray.

6.4 Discussion

This Chapter examined the applicability of CRB-65 as a risk score in COVID-19 patients with and without CXR abnormalities suggestive of pneumonia, with comparisons made against established inflammatory markers. The main findings are:

1. A low CRB-65 score of 0 accurately ruled out inpatient mortality in COVID-19 patients without significant influence from the presence or absence of CXR abnormalities.
2. A CRB-65 score of 0 could also rule out NIV requirement and critical illness, but with greater confidence in patients with normal CXR than in patients with abnormal CXR.
3. A high CRB-65 score of 2 or 3 did not accurately predict clinical outcomes in this patient population.
4. CRB-65 risk scores outperformed established serum inflammatory markers of CRP and WCC for predicting inpatient mortality in patients with abnormal CXR.
5. CRB-65 was inferior to CRP for predicting NIV requirement and critical illness.
6. COVID-19 patients with low CRB-65 scores have better inpatient survival than patients with high CRB-65 scores, irrespective of the presence or absence of CXR abnormalities.

The findings of the work in this Chapter suggest that the potential usefulness of the CRB-65 may be as a rule-out test for adverse clinical outcomes in COVID-19 patients, which may help to identify patients in a safer prognostic group. The data also indicate that CRB-65 may be able to serve this purpose irrespective of CXR findings, i.e. in the presence or absence of radiological evidence of pneumonia.

In patients with normal CXR, a low CRB-65 score of 0 almost completely ruled out any adverse clinical outcomes, in this patient population. However, CRB-65 appears to be a poor positive predictor of adverse prognosis and the findings do not support the use of a high CRB-65 score in isolation for assessing a COVID-19 patient's clinical risk. The results in this Chapter pave the way for a prospective validation study to further investigate the use of CRB-65 for facilitating hospital admission avoidance and early safe discharge of clinically low-risk patients.

6.4.1 CRB-65 as a practical risk score

CRB-65 can be calculated using routine clinical information, which can be derived rapidly upon first assessment of a patient with COVID-19, thus offering a rapid assessment of clinical risk. CRB-65 has a further economic advantage in that it does not require any laboratory blood tests or imaging test results. CRB-65 therefore is an ideal on-the-spot assessment tool designed for the initial risk stratification of a patient.

6.4.2 Clinical Advantages of CRB-65

Another facet of an effective non-invasive risk score lies in its relative clinical value compared to existing biomarkers. This retrospective study population showed that CRB-65 was overall comparable to CRP for predicting inpatient mortality in COVID-19 patients. The finding that

CRB-65 outperformed CRP for mortality prediction in patients with abnormal CXR is interesting. It may be that CRB-65 assesses a range of clinical manifestations of inflammatory response in patients with CXR changes, as compared to CRP which is a serum biomarker alone. This possibility deserves further investigation. In patients with normal CXR, CRP was superior to CRB-65 for predicting mortality. This finding is difficult to explain, though CRP may be able to detect underlying inflammatory responses which do not manifest in abnormal clinical parameters which CRB-65 relies on. The ability of a low CRB-65 to rule out adverse complications in COVID-19 patients may lie in its potential to identify low-risk patients, which appears an inherent property of risk stratification biomarkers such as cardiac troponin[296] or D-dimer.[347] Indeed, CRB-65 may be advantageous over serum biomarkers since the former does not require laboratory testing, thus avoiding potential delays and any added costs of blood sample testing.[200 279]

CRB-65 performed poorly in relation to CRP for predicting NIV requirements, intubation and ICU admission. This is a major disadvantage of CRB-65 and significantly limits its use as a biomarker to detect the occurrence of adverse complications. Although the underlying mechanism is unclear, one explanation may be that the selection of patients for NIV, intubation and ICU is not wholly based on changes in clinical parameters that make up the CRB-65 score. Parameters such as increased respiratory rate and hypotension at presentation do not necessarily lead to further clinical deterioration. Further, confusion can be multi-factorial rather than exclusively related to respiratory failure requiring NIV or intubation. Although older age is linked to clinical deterioration in COVID-19, it does not necessitate that all elderly patients would require NIV, intubation or ICU admission. Further work is required to elucidate the apparent disconnect between CRB-65 and non-mortality endpoints in COVID-19 patients.

6.4.3 Limitations and Future Directions

This study has several limitations. The retrospective and single centre nature of the study means that the results are vulnerable to selection bias, which drives the need for the next stage which is a prospective and multi-centre study to test the use of CRB-65 for identifying low-risk patient groups for admission avoidance and early discharge from hospital. The retrospective nature of the study also limited the completeness of certain datasets; we were unable to include more inflammatory markers and other clinical risk estimation systems, such as the 4C score, in the analysis. This limitation also drives the need for a prospective validation study. This study was conducted at a time before routine vaccination and many of the therapies for COVID-19 were widely used. Further work is required to test the performance of CRB-65 in a more contemporary COVID-19 population. Finally, all the patients who had CRB-65 assessments were admitted to the hospital. The clinical utility of CRB-65 as a rule-out test in a primary care setting in the GP surgery, before a patient attends the hospital, should also be tested in future studies.

6.5 Conclusion

In this retrospective study, a low CRB-65 score of 0 appears to be a good rule-out test for adverse clinical outcomes in acute COVID-19 patients irrespective of CXR abnormalities. This finding deserves further prospective validation with potential important values in admission avoidance and early hospital discharge.

Chapter 7: Biomarker Comparisons

In this chapter, all biomarkers of interest in the previous four data chapters are compared in a head-to-head manner to test their co-linearities and to see which biomarkers are the independent predictors of adverse clinical outcomes in COVID-19 patients. This is important to bring together the biomarkers tested in the project.

7.1 Introduction

Several biomarkers have been investigated in the project thus far for predicting adverse clinical outcomes in patients with COVID-19. These included: (i) hs-cTnT which examines myocardial injury; (ii) LCR and FLR which are combination inflammatory biomarkers; (iii) CRB-65 as a rapid access pneumonia risk score; and (iv) other conventional serum inflammation markers such as WCC, platelet counts, lymphocyte counts and CRP. Each biomarker has demonstrated different prognostic profiles in COVID-19 patients, which have been discussed in previous chapters. However, the co-linearities between these biomarkers and which biomarkers are the independent predictors of outcomes remain unclear. A further research question to answer in this chapter was whether combining the best-performing biomarkers was possible to develop a new risk score for prognosticating COVID-19 patients.

7.2 Statistical methods for biomarker comparisons

Continuous data were assessed for normality using the Kolmogorov-Smirnov test.[289] Parametric data were displayed as mean (SD).[48 246 290] Non-parametric data were displayed as median with inter-quartile range (IQR).[48 246 290] The diagnostic performance of biomarkers for predicting adverse clinical outcomes was assessed using Receiver-Operating Characteristics (ROC) curves. The area under the ROC curve (AUC) was displayed with 95% confidence intervals.[293] Multiple regression analysis was performed to assess the partial correlation

($R^{partial}$) between different biomarkers. Multi-variable Cox proportional-hazard regression analysis was used to assess the independent predictors of adverse clinical outcomes, with the hazard ratios (HR) displayed with 95% confidence intervals.[294] Statistical significance was denoted by $p<0.05$. Statistical analyses were performed using the commercially available MedCalc software (Version 12.7.8.0).

7.3 Results

A total of 121 patients had all the biomarkers performed and were eligible for further analysis and comparison. These biomarkers included hs-cTnT, LCR, FLR, CRB-65, CRP, ferritin, lymphocyte counts, WCC, and platelet counts. Data on significant oxygen requirement (defined using the established reference standard FiO_2 requirement >50%),[295] were available in 117/121 patients and were included in the analysis. The patient characteristics are shown in Table 7.1.

Table 7.1 Baseline patient characteristics.

	All Patients (n = 121)
Age (years)	67 (53 – 79)
Male (%)	76 (63)
BMI (kg/m^2)	28 (24 – 33)
Symptoms	
Chest pain	18 (15)
Dyspnoea	82 (68)
Palpitations	5 (4)
Fatigue	31 (26)
Cough	75 (62)
Fever	68 (56)
Diarrhoea	25 (21)
Comorbidities	
IHD	19/120 (16)
Heart failure	13/120 (11)
Hypertension	53 (44)
Diabetes	44 (36)
Dyslipidaemia	12/120 (10)
Current Smoker	6 (5)
Ex-Smoker	33 (27)
AF	14 (12)
CKD	38 (31)

COPD	13 (11)
Asthma	9 (7)
CVD	10 (8)
Dementia	12 (10)
Cancer	1/114 (1)
Medications	
ACEi / ARB	31/120 (26)
Beta-Blockers	29 (24)
CCB	29 (24)
Aspirin/Clopidogrel	18/119 (15)
Digoxin	0 (0)
Warfarin	2 (2)
DOAC	15 (12)
MRA	2 (2)
Nitrates	3 (2)
Statins	50 (41)

Hs-cTnT: high sensitivity cardiac troponin T; BMI: body mass index; IHD: ischaemic heart disease; AF: atrial fibrillation; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVD: cerebral vascular disease; ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker; DOAC: direct oral anticoagulant; and MRA: mineralocorticoid receptor agonist.

The results of the patients' observations, chest radiographs and biomarkers are shown in Table 7.2. Of the 121 patients, 30% required NIV during their admissions, 21% required ICU admissions, 8% underwent intubation and 30% suffered inpatient mortality.

Table 7.2 Observational, radiological and biomarker results.

	All Patients (n = 121)
Admission Observation	
Temperature (°C)	37.2 (36.6 – 37.9)
SBP (mmHg)	126 (115 – 143)
DBP (mmHg)	76 ± 15
Respiratory Rate (/min)	23 (20 – 28)
Significant Hypoxia*	31/117 (26)
Chest radiograph	
Consolidation	28/119 (24)
Opacification	56/119 (47)
Atelectasis	14/120 (12)
Pleural Effusion	8/119 (7)
Biomarker Results	
LCR	62 (32 – 175)

FLR	675 (291 – 1767)
CRP (mg/L)	137 (58 – 248)
WCC (10^9/L)	7.6 (5.3 – 10.2)
Lymphocyte Count (×10^9/L)	0.87 (0.63 – 1.15)
Ferritin (μg/L)	725 (285 – 1475)
Platelet Count (10^9/L)	239 ± 89
CRB-65	
Score 0	38 (31)
Score 1	47 (39)
Score 2	28 (23)
Score 3	8 (7)
Score 4	0 (0)
Complications	
NIV requirement	36 (30)
ICU admission	25 (21)
Intubation	10 (8)
Inpatient Mortality	36 (30)

CRP: C-reactive protein; FLR: ferritin lymphocyte ratio; LCR: lymphocyte CRP ratio; Hs-cTnT: high sensitivity cardiac troponin T; SBP: systolic blood pressure; DBP: diastolic blood pressure; ECG: electrocardiogram; WCC: white cell count; NIV: non-invasive ventilation; ICU: intensive care unit. *FiO_2 requirement >50%.[295]

7.3.1 Co-linearities between biomarkers

Using multiple regression analysis, co-linearities were detected between certain biomarkers, independent of other biomarkers within the analysis. The strongest correlations were between hs-cTnT and ferritin (partial correlation [$R^{partial}$] 0.42, p<0.001); between WCC and lymphocyte counts ($R^{partial}$ 0.45, p<0.001) and between WCC and platelet counts ($R^{partial}$ 0.38, p<0.001). Significant correlations between the biomarkers are shown in Table 7.3.

Table 7.3 Significant correlations between biomarkers

Hs-cTnT			LCR			FLR		
Versus	$R^{partial}$	P value	Versus	$R^{partial}$	P value	Versus	$R^{partial}$	P value
FLR	-0.19	0.034	CRP	-0.25	0.007	WCC	-0.26	0.004
Ferritin	0.42	<0.001	Platelet	0.25	0.005	Platelet	-0.26	0.005
CRP			Lymphocyte			WCC		
Versus	$R^{partial}$	P value	Versus	$R^{partial}$	P value	Versus	$R^{partial}$	P value
FLR	0.19	0.042	LCR	0.20	0.033	Platelet	0.38	<0.001
LCR	-0.23	0.011	WCC	0.45	<0.001			
WCC	0.25	0.006						

CRP: C-reactive protein; FLR: ferritin lymphocyte ratio; LCR: lymphocyte CRP ratio; Hs-cTnT: high sensitivity cardiac troponin T; Platelet: platelet counts; $R^{partial}$: partial correlation from multiple regression analysis; and WCC: white cell count.

7.3.2 Independent predictors of adverse clinical outcomes

Multivariable Cox proportional-hazard regression analysis was performed with a range of relevant clinical variables with prognostic implications in COVID-19. These included age, AF, CKD, COPD, diabetes, stroke / TIA, smoking, heart failure, hypertension, and ischaemic heart disease (IHD). The biomarkers included in the model included hs-cTnT, LCR, FLR, CRB-65, CRP, lymphocyte counts, ferritin, WCC, platelet counts and oxygen requirement (>50% FiO_2). The analysis was performed to predict inpatient mortality and a composite of non-fatal endpoints including NIV requirement, intubation and ventilation, and ICU admissions.

7.3.2.1 Independent predictors of inpatient mortality

There were 5 independent predictors of inpatient mortality in this sub-cohort of COVID-19 patients (Table 7.4). These included hs-cTnT (HR 5.58, 95% CI 1.23-25.4, p=0.026); oxygen requirement (HR 2.56, 95% CI 1.23-5.31, p=0.012); IHD (HR 2.53, 95% CI 1.10-5.82, p=0.029); CRB-65 (HR 1.92, 95% CI 1.22-3.01, p=0.005) and FLR (HR 1.00, 95% CI 1.00-1.00, p<0.001).

Table 7.4 Independent predictors of mortality in COVID-19.

	Hazard Ratio (95% CI)	P value
Hs-cTnT	5.58 (1.23-25.4)	0.026
Oxygen requirement	2.56 (1.23-5.31)	0.012
IHD	2.53 (1.10-5.82)	0.029
CRB-65	1.92 (1.22-3.01)	0.005
FLR	1.00 (1.00-1.00)	<0.001

CI: confidence interval; FLR: ferritin-lymphocyte ratio; Hs-cTnT: high sensitivity cardiac troponin T; and IHD: ischaemic heart disease.

7.3.2.2 Independent predictors of non-fatal endpoints

Using the same multivariable Cox proportional-hazard regression analysis, oxygen requirement was the only independent predictor of NIV requirement (HR 2.50; 95% CI: 1.16-5.37, p=0.019) and the composite of NIV requirement, intubation/ventilation and ICU admission (HR 2.42; 95% CI: 1.19-4.93, p=0.015).

7.3.2.3 Univariable regression values

The univariable regression values are shown for reference in Table 7.5 below.

Table 7.5 Univariate Cox Regression analysis of predictors of mortality in COVID-19.

	Inpatient Mortality			**NIV/Intubation/ICU**		
	HR	**(95% CI)**	**P value**	**HR**	**(95% CI)**	**P value**
Hs-cTnT	6.96	1.24 - 39.1	0.028	0.72	0.21 - 2.39	0.586
Oxygen requirement	2.90	1.12 - 7.52	0.029	1.58	0.61 - 4.09	0.347
FLR	1.00	1.00 - 1.00	0.001	1.00	1.00 - 1.00	0.077
IHD	2.17	0.66 - 7.19	0.204	10.6	2.01 - 56.0	0.005
CRB-65	1.43	0.74 - 2.79	0.291	1.31	0.55 - 3.13	0.540
LCR	1.00	1.00 - 1.00	0.964	1.00	1.00 - 1.01	0.356
CRP	1.00	1.00 - 1.01	0.907	1.00	0.99 - 1.00	0.352
Lymphocytes	0.83	0.49 - 1.41	0.497	0.90	0.64 - 1.27	0.551
Ferritin	1.00	1.00 - 1.00	0.150	1.00	1.00 - 1.00	0.262
Platelets	1.00	1.00 - 1.01	0.543	1.00	0.99 - 1.01	0.797
WCC	1.07	0.96 - 1.20	0.233	1.11	0.99 - 1.24	0.084
AF	0.52	0.16 - 1.67	0.270	0.05	0.00 - 0.69	0.026
Age	1.03	0.99 - 1.07	0.130	0.98	0.93 - 1.02	0.328
Stroke / TIA	4.23	0.90 - 19.8	0.067	1.80	0.29 - 11.3	0.529
CKD	0.44	0.14 - 1.40	0.166	0.44	0.17 - 1.64	0.221
COPD	1.27	0.38 - 4.23	0.700	1.82	0.36 - 9.22	0.467
DM	1.13	0.39 - 3.29	0.820	1.41	0.51 - 3.91	0.513
HF	1.58	0.37 - 6.75	0.539	0.38	0.03 - 5.53	0.482
HTN	0.76	0.30 - 1.95	0.571	2.94	1.02 - 8.50	0.046
Smoking	2.38	0.83 - 6.82	0.107	2.19	0.71 - 6.81	0.175

AF: atrial fibrillation; CI: confidence interval; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; DM: diabetes mellitus; FLR: ferritin-lymphocyte ratio; HF: heart failure; Hs-cTnT: high sensitivity cardiac troponin T; HTN:

hypertension; IHD: ischaemic heart disease; LCR: lymphocyte-CRP ratio; TIA: transient ischaemic attack; and WCC: white cell count.

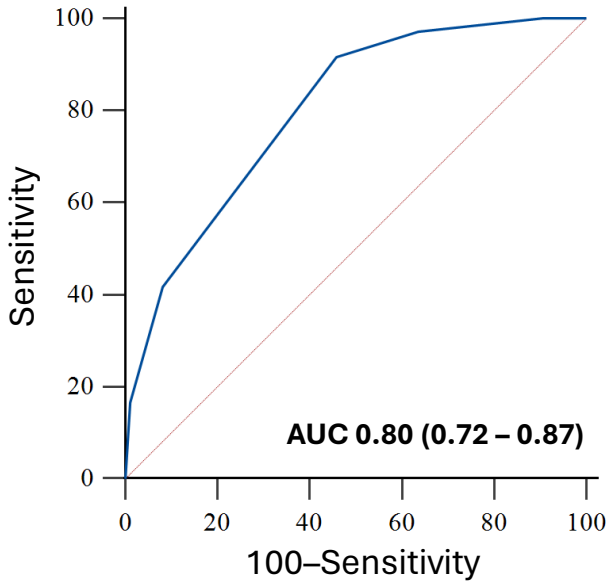
7.3.3 New risk score for predicting inpatient mortality

Using the 5 independent predictors of mortality in COVID-19 patients, cardiac troponin (Hs-cTnT), oxygen requirement, CRB-65, FLR and IHD, a risk score was assembled based on the cut-off values of each predictor.

- Hs-cTnT (cut-off 14ng/L)[296]
    - Score 0 (hs-cTnT >14ng/L)
    - Score 1 (hs-cTnT ≤14ng/L)
- Oxygen requirement[295]
    - Score 0 (≤50% FiO_2 requirement)
    - Score 1 (>50% FiO_2 requirement)
- CRB-65 (cut-off score 0)[190]
    - Score 0 (CRB-65 score 0)
    - Score 1 (CRB-65 score ≥1)
- FLR (cut-off 286)[348]
    - Score 0 (FLR ≤286)
    - Score 1 (FLR >286)
- IHD
    - Score 0 (no IHD history)
    - Score 1 (IHD history present)

Each predictor contributes either 1 or 0 points to the risk score. On ROC analysis, this risk score achieved an AUC of 0.80 (95% CI: 0.72-0.87) for predicting inpatient mortality in COVID-19 patients (Figure 7.1). A risk score of 1 achieved a negative predictive value of 97%

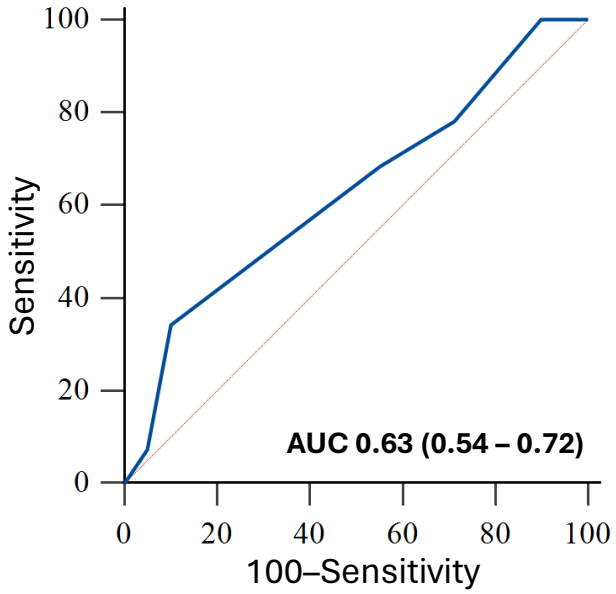
(95% CI: 82-100%) for ruling out inpatient mortality. A risk score of 4 achieved a positive predictive value of 86% (95% CI: 43-98%) for ruling in inpatient mortality.



Score	Sens (%)	Spec (%)	PPV (%)	NPV (%)
0	100	9	32	100
1	97	36	39	97
2	92	54	46	94
3	42	92	68	79
4	17	99	86	74

Figure 7.1: Receiver Operating Characteristics (ROC) curve showing the performance of the new risk score for predicting inpatient mortality in COVID-19 patients. AUC: area under the ROC curve; NPV: negative predictive value; PPV: positive predictive value; sens: sensitivity and spec: specificity.

On ROC analysis, this risk score achieved an AUC of 0.63 (95% CI: 0.54-0.72) for predicting a composite of NIV requirement, intubation/ventilation and ICU admission (Figure 7.2).



Score	Sens (%)	Spec (%)	PPV (%)	NPV (%)
0	100	10	36	100
1	78	29	36	72
2	68	45	39	74
3	34	90	64	73
4	7	95	43	67

Figure 7.2: Receiver Operating Characteristics (ROC) curve showing the performance of the new risk score for predicting a composite of non-fatal outcomes in COVID-19 patients. The non-fatal outcome was a composite of NIV requirement, intubation/ventilation and ICU admission. AUC: area under the ROC curve; NPV: negative predictive value; PPV: positive predictive value; sens: sensitivity; and spec: specificity.

7.3.4 Comparative performance of the risk score

On ROC analysis, the risk score (AUC 0.80) demonstrated the highest performance for predicting inpatient mortality, as compared to CRB-65 (AUC 0.74), hs-cTnT (AUC 0.73), LCR (AUC 0.68), oxygen requirement (AUC 0.66), IHD (AUC 0.63) and FLR (AUC 0.61; Table 7.6 shows the AUC with 95% confidence intervals).

Table 7.6 Comparison of biomarkers for predicting mortality.

	For predicting mortality	
	AUC	**95% CI**
Risk Score	0.80	0.72-0.87
CRB-65	0.74	0.65-0.82
Hs-cTnT	0.73	0.64-0.81
LCR	0.68	0.59-0.77
Oxygen requirement	0.66	0.56-0.74
IHD	0.63	0.53-0.71
FLR	0.61	0.51-0.70

Oxygen requirement was defined as FiO_2 requirement >50%.[295] CI: confidence interval; FLR: ferritin-lymphocyte ratio; Hs-cTnT: high sensitivity cardiac troponin T; IHD: ischaemic heart disease; and LCR: lymphocyte-CRP ratio.

For predicting the non-fatal endpoint (a composite of NIV requirement, intubation/ventilation, ICU admission), oxygen requirement alone performed the best (AUC 0.69), as compared to other biomarkers including LCR (AUC 0.68), the risk score (AUC 0.64), FLR (AUC 0.58), hs-cTnT (AUC 0.55), IHD (AUC 0.53) and CRB-65 (AUC 0.51; Table 7.7 shows the AUC with 95% confidence intervals).

Table 7.7 Comparison of biomarkers for predicting non-fatal endpoint.

	For non-fatal endpoint	
	AUC	**95% CI**
Oxygen requirement	0.69	0.59-0.77
LCR	0.68	0.59-0.76
Risk Score	0.64	0.54-0.72
FLR	0.58	0.48-0.67
Hs-cTnT	0.55	0.45-0.64
IHD	0.53	0.44-0.62
CRB-65	0.51	0.41-0.60

The non-fatal endpoint was a composite of non-invasive ventilation requirement, intubation/ventilation and ICU admission. Oxygen requirement was defined as FiO_2 requirement >50%.[295] CI: confidence interval; FLR: ferritin-lymphocyte ratio; Hs-cTnT: high sensitivity cardiac troponin T; IHD: ischaemic heart disease; LCR: lymphocyte-CRP ratio.

7.4 Discussion

This chapter provided the head-to-head comparisons of biomarkers validated in this project. The main findings are: (i) certain biomarkers demonstrate significant co-linearities, both within an inflammatory domain (e.g. WCC vs platelet counts) and across different domains (e.g. cardiac troponin vs ferritin); (ii) hs-cTnT, oxygen requirement (>50% FiO_2), CRB-65, FLR

and IHD were the 5 independent predictors of mortality in COVID-19 patients; (iii) a risk score developed based on the 5 independent predictors demonstrated good diagnostic performance for predicting mortality with an AUC of 0.80, better than other biomarkers in comparison; however (iv) for predicting non-fatal composite endpoint of NIV requirement, intubation/ventilation and ICU admission, oxygen requirement was the only independent predictor.

The results potentially give rise to a dual risk assessment strategy in patients presenting with COVID-19: (i) the new risk score may be used to assess the likelihood of a patient developing inpatient mortality; and (ii) oxygen requirement can be used to assess the risk of developing NIV/intubation/ventilation and ICU admission in line with existing practice. This approach needs to be further investigated in a validation patient cohort.

7.4.1. Risk Score for Mortality

The risk score developed in this chapter encompasses several aspects of COVID-19 disease pathophysiology that have prognostic implications. The inclusion of cardiac troponin in the risk model allows the assessment of myocardial injury which has a known prognostic impact in COVID-19 patients.[48] The use of oxygen requirement in the model assesses the severity of pulmonary infection and other potentially occult complications, e.g. ARDS or pulmonary embolism, which can affect the overall prognosis.[186] CRB-65 provides the model with a reflection of the clinical manifestations of sepsis and its severity, such as confusion, tachypnoea and hypotension.[279] FLR has come out as a somewhat surprising independent predictor of outcomes (when LCR and CRP both did not emerge as independent predictors in the Cox multivariable analysis). However, it does provide an assessment of both ferritin and lymphopenia which are known to have prognostic value in COVID-19 patients.[244] Finally, the risk model includes two non-modifiable risk factors, namely advanced age (>65 years old) and

ischaemic heart disease, both have a strong evidence base for prognosticating patients infected by SARS-CoV-2.[191] The coming together of these biomarkers appears to have improved the predictive performance for mortality in COVID-19 beyond the capabilities of each of the individual constituents.

Another interesting finding is that many of the tested biomarkers had co-linearities, despite being considered markers in different "classes". For instance, the partial correlation between cardiac troponin and ferritin potentially indicates that both biomarkers were activated by systemic inflammation, which is known to cause myocardial injury and the production of acute phase proteins.[34 349] The existence of inter-biomarker correlations called for the need to elucidate independent predictors of adverse outcomes using a multivariable model that included a wide range of predictors in COVID-19. I opted to include several non-modifiable co-morbidities such as diabetes and ischaemic heart disease, to provide a balanced assessment of the biomarkers and confounders. This is important since patients presenting to healthcare services with COVID-19 may have these co-morbidities.

7.4.2 Oxygen requirement for predicting non-fatal endpoints

The only independent predictor of escalation in ventilatory support and ICU admission was oxygen requirement. This observation is in line with clinical practice where hypoxia refractory to supplementary oxygen therapy calls for consideration of escalation to NIV and/or intubation.[191] The reference standard of oxygen requirement (set at FiO2 >50%) used in the analysis has been previously validated in the established APACHE II score.[295] Patients without significant oxygen requirements are often considered for early discharge with remote monitoring.[350] The results further support this approach with an added level of risk stratification using a new risk model which also assesses the likelihood of inpatient mortality.

7.4.3 Limitations

The total number of patients who had all the biomarkers performed was relatively small. Therefore, the findings in this Chapter are vulnerable to sampling bias. Further, in this relatively small sample size, the highest risk score value (5) was under-represented in the patient population, which might skew the positive predictive value and other diagnostic parameters of the test. Therefore, the findings in this Chapter need to be validated in a larger patient cohort, ideally on a multi-centre basis. It would have been ideal to include more data on oxygen saturation in this project. However, single time-point recordings of oxygen saturation readings on admission are often poor reflections of a patient's subsequent clinical course since oxygen saturation can be highly variable over time.[179] Patients presenting with normal oxygen saturations on admission (with symptoms such as dyspnoea) can later deteriorate during hospital stay (often at an unpredictable time point), and vice versa.[191] To "catch the desaturation" would require collecting a series of oxygen saturation readings throughout the patients' hospital stays which was highly labour-intensive for the data collection team and risked compromising data collection resources to answer other questions. The results in this chapter do drive the need for further investigation of the effect of oxygen saturation trends on new risk models and for predicting prognosis in COVID-19 patients.

7.4 Conclusions

A risk score model based on independent predictors, including hs-cTnT, oxygen requirement, CRB-65, FLR and IHD, demonstrated good performance in predicting inpatient mortality in COVID-19 patients. Oxygen requirement alone remained the optimal predictor for the requirement for non-invasive and invasive ventilation and ICU admission. Further work is required to validate the clinical applicability of these approaches for risk-stratifying COVID-19 patients.

Chapter 8: Synopsis and conclusions

The work in this project evaluated several biomarkers for assessing prognosis in patients with COVID-19. The cardiac biomarker hs-cTnT has shown potential as a rule-out test for inpatient mortality. Combination biomarkers including LCR and FLR are not superior to CRP for predicting adverse clinical outcomes in COVID-19. As a risk score, CRB-65 appears to be a good rule-out test for adverse clinical outcomes in COVID-19. Oxygen requirement remains the only independent predictor for a composite of requirements for NIV, intubation/ventilation and ICU admissions. A new risk score based on hs-cTnT, oxygen requirement, CRB-65, IHD and FLR performed well for predicting inpatient mortality in COVID-19. A selection of biomarkers studied in this project, including hs-cTnT, CRB-65 and the new risk score, have shown promise for further clinical validation in the journey towards clinical translation.

8.1. Key points

The findings in this project showed that there is no single "all-encompassing" biomarker capable of both accurately predicting and ruling out the development of adverse clinical outcomes patients with COVID-19. Chapter 7 showed that promising biomarkers investigated in this project may be better used as a combined risk score.

8.1.1. Timing is important

Aside from prognostic values, this project also showed that different biomarkers may have different logistical uses in the assessment of COVID-19 patients. For instance, CRB-65 can be performed within a few minutes of first meeting a patient since it is based on parameters that are readily available from the clinical history and examination alone.[190] This means that CRB-65 is particularly suited to be used early in a patient's journey, at first presentation to the hospital.[190] Cardiac troponins, LCR and FLR require blood tests to be drawn, transported to

the hospital laboratory, run on their respective assay machines and released by the biochemistry laboratory staff.[191 318 348] This time delay may be shortened by point-of-care testing, but with added cost implications.[351] Further, the combination biomarkers LCR and FLR each requires two different sets of blood test results to return, which may lead to further time delays.[318 348]

Whilst all the biomarkers tested in this project can be performed at any time in a patient's journey from admission to discharge, the order in which the biomarkers could be implemented is important. Figure 8.1 illustrates a summary of the potential stages of usefulness. As one moves from left (A&E and/or GP setting) to right (hospital admission), the pretest probability of the patient developing complications can change. Whilst the sensitivity and specificity of the diagnostic tests may remain similar moving from an early to late stage of a patient's journey through the healthcare system, the PPV is likely to increase and NPV is likely to fall. This model requires further evaluation.

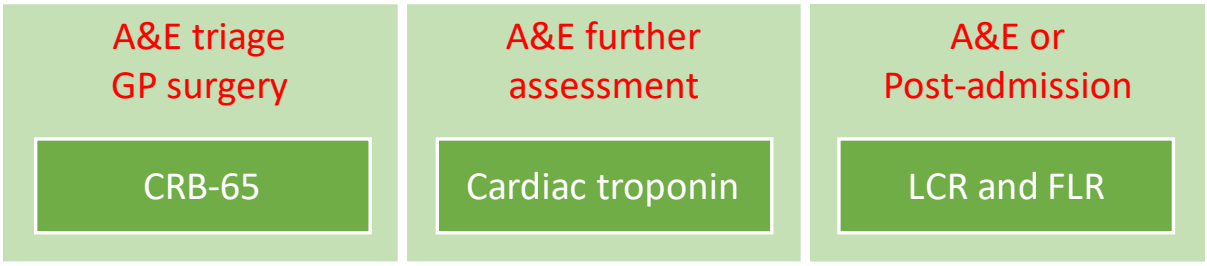


Figure 8.1: Possible order of biomarker assessment of a patient presenting with COVID-19. A&E: accident and emergency; GP: general practitioner; FLR: ferritin-lymphocyte ratio; LCR: lymphocyte-CRP ratio.

8.1.2 Ruling out is easier than ruling in

Most of the biomarkers tested in the project tend to be better at ruling out than ruling in adverse clinical outcomes in COVID-19 patients. This phenomenon can also be observed with biomarkers in other fields, such as the use of a low D-dimer level to rule out the presence of pulmonary emboli (PE; whilst a raised D-dimer is non-specific).[352] When elevated, the biomarkers in the project are not specific for detecting adverse clinical outcomes in COVID-19 patients. However, a negative test may identify patients at low risk. As seen with cardiac troponins, an elevated value did not necessarily mean that patients would suffer mortality.[296] Therefore, the results of the project suggest that biomarkers in COVID-19 may be better used to identify low-risk patients for safe discharge from the hospital. This would be the focus of further validation studies.

8.1.3 Choice of clinical outcome endpoints

With hs-cTnT, it was recognised that mortality was the hardest endpoint and the direct connection between hs-cTnT and other non-fatal endpoints was more indirect. Since the literature already existed on the use of cardiac troponins in predicting non-fatal endpoints, and the aim of the study was to explore the individual per-patient diagnostic performance of hs-cTnT in predicting the hardest endpoint, it was tested only for predicting mortality.[296]

For other biomarkers (e.g. CRB-65 which includes respiratory rate), it was appropriate to include the non-fatal respiratory-related complications as endpoints (in addition to mortality). Data for both the composite endpoints and the individual components of the composite endpoints were displayed in most cases.

8.1.4 Effect of chronic corticosteroid therapy

During the data collection process, the clinical trials showing the benefits of corticosteroid therapies were still in progress and were yet to be published.[353] Therefore the project did not

specifically examine the effect of steroid therapy on biomarker assessment of prognosis in COVID-19 patients. Since the project data collection was completed, there has been an emergence of evidence on the effect of steroid therapy on prognosis which has generated interesting results.[354] The implementation of steroid therapy has also been reported as non-universal.[355] Therefore future studies which examine the effect of steroid therapy on the use of biomarkers for predicting clinical outcomes in COVID-19 are required.

8.2 Combination risk score

Recognising the individual strengths and weaknesses of each biomarker in the project, the combined risk score demonstrated the best predictive values for inpatient mortality in COVID-19 patients. This risk score is potentially a useful risk stratification tool in the acute admissions setting since it is based on routinely performed tests and clinical characteristics of the patient. It could potentially offer a rapid access assessment of a patient's likelihood of inpatient mortality, to complement oxygen requirement as the marker of escalation in respiratory support. This combined approach derived from the MD project is potentially promising and deserves further investigation.

8.3 Conclusion

In COVID-19 patients, certain biomarkers, including cardiac troponin and the CRB-65 score, may be better used as rule-out tests for adverse outcomes. Combination biomarkers LCR and FLR do not appear to have significant incremental value over the existing inflammatory biomarker CRP. Oxygen requirement appears to be the only independent predictor of the requirement for higher-level respiratory support and ICU admissions. A risk model incorporating cardiac troponin, oxygen requirement, CRB-65, FLR and ischaemic heart disease performed well in predicting inpatient mortality in COVID-19 patients and deserves further validation for potential clinical translation.

Appendix I: Ethical approval

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr James Stirrup
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14 August 2020

Dear Dr Stirrup

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Retrospective Review of Clinical and Prognostic Markers in COVID**
IRAS project ID:	**287103**
Protocol number:	**N/A**
Sponsor	**The Royal Berkshire NHS Foundation Trust**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The *"After HRA Approval – guidance for sponsors and investigators"* document on the HRA website gives detailed guidance on reporting expectations for studies with HRA and HCRW Approval, including:

- Registration of Research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **287103**. Please quote this on all correspondence.

Yours sincerely,

Kevin Ahmed
Approvals Manager

Email: approvals@hra.nhs.uk

Appendix II: Published papers from MD

Chapter One (Introduction)

Liu A, et al. Effective prognostic and clinical risk stratification in COVID-19 using multimodality biomarkers. *J Intern Med.* 2023; 294: 21– 46. https://doi.org/10.1111/joim.13646

Chapter Three (Troponin)

Liu A, et al. (2023) Normal high-sensitivity cardiac troponin for ruling-out inpatient mortality in acute COVID-19. *PLOS ONE* 18(4): e0284523. https://doi.org/10.1371/journal.pone.0284523

Chapter Four (LCR)

Liu A, et al. Comparison of Lymphocyte–CRP Ratio to Conventional Inflammatory Markers for Predicting Clinical Outcomes in COVID-19. *Journal of Personalized Medicine.* 2023; 13(6):909. https://doi.org/10.3390/jpm13060909

Chapter Five (FLR)

Liu A, et al. Characterisation of Ferritin–Lymphocyte Ratio in COVID-19. *Biomedicines.* 2023; 11(10):2819. https://doi.org/10.3390/biomedicines11102819.

Chapter Six (CRB-65)

Liu A, et al. Low CRB-65 Scores Effectively Rule out Adverse Clinical Outcomes in COVID-19 Irrespective of Chest Radiographic Abnormalities. *Biomedicines.* 2023; 11(9):2423. https://doi.org/10.3390/biomedicines11092423

Appendix III: Data entry SOP

COVID Audit Data Entry Guide

Check that the SARS-CoV-2 swab result states "DETECTED", only collect data if this is the case.

- If swab "low level" or "inhibited/indeterminant", highlight the row red and indicate in comments column

Ethnicity: Patient information -> Patient demographics -> copy and paste as it is into spreadsheet

BMI: Results review -> Clinical Measurements OR Connected care sidebar -> GP records

- If BMI was not taken during the admission, enter in the height column the date it was taken.

Symptoms and Co-morbidities: Admission clerking (also double check post-take POD/ECPOD note)

- Longest symptom and duration gives indication of how far along the illness the patient presented

Cancer (after dementia): 0 if none. If present, please briefly note type, e.g. CLL or metastatic lung etc.

- This is current active cancer only, i.e. not previously cured cancer or those in long remission

Smoking: admission clerking OR Connected Care GP records

- Enter 1/0/Ex. 1=current; 0=never; Ex=ex-smoker

Rockwood score: Admission clerking OR work out from social history according to overleaf https://www.bgs.org.uk/sites/default/files/content/attachment/2018-07-05/rockwood_cfs.pdf

Medications: Admission clerking IF LACKING Connected care sidebar -> GP records

ECG: "ED CAS CARD" OR "IP Scanned D…" e.g. "GEN MED IP Scanned D…"

- If no ECG was done (after a good look!) – enter "NO ECG" in the "DATE PERFORMED" column

CXR Consistent with COVID?: enter format **"***1/0 (CVCX X)*" – 1 or 0 (yes or no) then (CVCX score) if in report

- Enter 1 if any of these are reported and are new: consolidation, ground glass changes, lung infiltrates or if the report indicates COVID status
- The report often compares with previous CXR, please include when pasting in the CXR report column
- If report states "…*COVID cannot be ruled out*", enter 1 for now and will be looked at later

Blood results: admission results where possible, if not enter next available values during this admission

- Highest valued troponin and CRP during this admission.

Observations: take the admission observation e.g. results review or Pre-Arrival Form or clerking

GCS: *Pre-Arrival Form* entry usually has admission GCS OR Admission clerking / Nurses entries

AKI?: check renal function trend in results review, beware, CKD can have AKI too if admission off baseline!

pH VBG: "ED CAS CARD" OR "IP Scanned D…", look for the VBG or ABG slip, leave blank if not present

ECHOCARDIOGRAM: Clinical documentation -> Diagnostic test tab, enter ECHO within last 5 years

- Also note if one was requested during the admission in Orders/CarePlans sidebar.

DNAR?: Orders/CarePlans sidebar -> Patient status -> Resuscitation status OR scanned RESPECT form

NIV? ICU? INTUBATION?: did the patient require NIV/ICU/INTUBATION during the admission?

- **NIV? ICU? INTUBATION?** Check clinical documentation for ICU entries and discharge summaries
- **NIV?:** Results review -> Clinical Measurements, are there entries like "CPAP"? Beware OSA pt.

DEATH?: for the date of death, check "verification of death" entries AND scanned death certificates

General Comments:

If something cannot be found after a good look, please leave the cells empty.

Use the codes as much as possible, this helps standardisation / analysis. Please leave comments as separate column at the far end of the spreadsheet, after "date of discharge" column and before the "End" columns.

Non-admission swabs: Some patients have positive swabs without records of admission to the Royal Berkshire Hospital. Please highlight the entire row in yellow and move on.

Community swabs: If a non-RBH swab was positive and the patient was admitted to RBH less than 14 days after the date of this positive swab, this admission data should be entered as normal.

If COVID swab positive >2 weeks after admission, for now take symptoms/observation/bloods close to positive swab date (look for EPR entries ?deterioration for re-swab to be taken?). For duration of longest symptom, enter the period between first COVID-related symptom to date of positive swab. Use closest ECG to swab, which may be on admission, make note of the date of this ECG and highlight it.

ROCKWOOD CFS

1 Very Fit – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.

2 Well – People who have no active disease symptoms but are less fit than category 1. Often, they exercise or are very active occasionally, e.g. seasonally.

3 Managing Well – People whose medical problems are well controlled, but are not regularly active beyond routine walking.

4 Vulnerable – While not dependent on others for daily help, often symptoms limit activities. A common complaint is being "slowed up", and/or being tired during the day.

5 Mildly Frail – These people often have more evident slowing, and need help in high order IADLs (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.

6 Moderately Frail – People need help with all outside activities and with keeping house. Inside, they often have problems with stairs and need help with bathing and might need minimal assistance (cuing, standby) with dressing.

7 Severely Frail – Completely dependent for personal care, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).

8 Very Severely Frail – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.

9 Terminally Ill – Approaching the end of life. This category applies to people with a life expectancy <6 months, who are not otherwise evidently frail.

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common **symptoms in mild dementia** include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In **moderate dementia,** recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In **severe dementia,** they cannot do personal care without help.

Appendix IV: STROBE statement

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page(s)
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	16
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	16
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	17-51
Objectives	3	State specific objectives, including any prespecified hypotheses	51
Methods			
Study design	4	Present key elements of study design early in the paper	52
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	55
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	55-58
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	61-62
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	58-60
Bias	9	Describe any efforts to address potential sources of bias	58-60
Study size	10	Explain how the study size was arrived at	63-67
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	61-63
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	61-63
		(*b*) Describe any methods used to examine subgroups and interactions	61-63
		(*c*) Explain how missing data were addressed	63-64
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	61-64
		(*e*) Describe any sensitivity analyses	61-63

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	63-67
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	89
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	65-67
		(b) Indicate number of participants with missing data for each variable of interest	63-67
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	66-67
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Within the results of data chapters
		(*b*) Report category boundaries when continuous variables were categorized	Within the results of data chapters
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Within the discussion of data chapters
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Within the discussion of data chapters
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Within the discussion of data chapters
Generalisability	21	Discuss the generalisability (external validity) of the study results	Within the discussion of data chapters
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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