The potential of using C reactive Protein point-of-care testing to detect bacterial infection in Paediatric emergency settings.

By

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Abstract

Sepsis is a life-threatening condition that can be caused by microbial infections, which can potentially lead to death of a patient in as less than 12 hours from admission. Paediatric sepsis is then further complicated by the patient's inability to succinctly communicate their symptoms to clinicians. Microbiological diagnosis of sepsis can take a minimum of 12 hours in giving an indication of a bacterial or viral infection. Therefore, this places clinicians in a challenging time critical window into commencing an effective treatment plan, commonly results in starting a course of antibiotics immediately. This clearly shows there is a need for rapid diagnostic testing to aid clinicians. The aim of this study was to investigate the potential of using Point of Care C-Reactive Protein to differentiate between bacterial and viral infections in paediatric patient.

POCT CRP was collected from the Paediatric department in Emergency Department of Chelsea and Westminster Hospital. The data was collected for 9 months in 2020 and included POCT CRP levels, haematological parameters, and clinical diagnosis. Statistical analysis of POCT CRP, Neutrophil-Lymphocyte ratio (NLR) and a novel sepsis index (SI) were analysed.

A total of 662 samples were collected however after the data cleaning process, 250 sample were removed leaving a total of 412 in the clinical diagnosis group. Through analysing hospital records, it was shown only 304 samples were confirmed in the laboratory, this group was defined as definitive diagnosis group. POCT CRP, NLR, SI were unable to differentiate between bacterial and viral infections. However, in the definitive bacteria groups with severe bacterial infections, the POCT CRP values and SI ratio was shown to be distinctive markers. Interestingly, the levels seen in COVID-19 samples made them indistinguishable to the levels seen in the bacterial groups. The effectiveness of the clinical biomarkers was analysed using the ROC curve, the findings have shown CRP (AUC: 0.892 95 % CI 0.802-0.982) to have a p-value of <0.0001 as well as SI to have a p-value of <0.0001 (AUC: 0.840 95 % CI 0.752-0.929). However, NLR (AUC: 0.696 95% CI 0.552-0.840) had limited discriminative ability producing a p-value of 0.0075.

The findings of this current study have shown the potential of CRP and SI as a diagnostic tool for paediatric sepsis however, due to the small sample size further investigation needs to be carried out.

Acknowledgements

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List of Abbreviations

AMR	Antimicrobial resistance
ARDS	Severe acute respiratory distress syndrome
AUC	Area under the curve
$Ca2^{+}$	Ionised Calcium
COVID-19	Coronavirus disease 2019
CRP	C-reactive Protein
CW-NHS	Chelsea and Westminster Hospital, Chelsea and Westminster Hospital NHS Foundation Trust
DNI	Delta Neutrophil Index
ED	Emergency department
EDTA	Ethylenediaminetetraacetic acid
ESR	Erythrocyte sedimentation rate
ICHNT	Imperial College Healthcare NHS Trust
Incal	Incalculable
IP-10	Interferon gamma-induced protein 10
ISO	International Organisation for Standardisation
IQR	Interquartile Range
N/A	Non-applicable results
NLR	Neutrophil-Lymphocyte ratio

LOS	Length of Stay
mCRP	Monomeric isoform of CRP
MIS-C	Multisystem inflammatory syndrome
PCh	Phosphocholine
PCIS	Paediatric Critical Illness Score
PCT	Procalcitonin
pCRP	Pentameric isoforms of CRP
PEWS	Paediatric Early Warning Score systems
PnC	C-polysaccharides
POCT	Point of Care Testing
PRISM III	Paediatric Risk of Mortality III
RLoS	Reducing Length of Stay
ROC	Receiver Operating Characteristics
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse transcription-polymerase chain reaction
SAP	Serum amyloid A protein
SARS	Severe Acute Respiratory Syndrome Coronavirus-2
SBI	Serious Bacterial infection
SLE	Systemic Lupus Erythematosus

SI	Sepsis Index
SOP	Standard Operating Procedure
TAT	Turnaround Time
TB Screen	Tuberculosis screening
TRAIL	TNF-related apoptosis inducing ligand
UK	United Kingdom
WHO	World Health Organisation

1. Introduction

1.1 Sepsis the condition

Sepsis is one of the predominant causes of paediatric mortality and morbidity nationally (WHO., 2020). Although a range of pathogens can cause sepsis, bacterial infections represent the majority of sepsis cases (Dolin, et al.,2019). This has led to concerted efforts and the development of initiatives and protocols to stem the sepsis incidences, leading to formulation and implementation of sepsis criterion (De Backer & Dorman, 2017).

Sepsis can lead to life-threatening organ dysfunction and deregulated physiological host responses to a wide variety of pro-inflammatory disease states. These might include bacterial stimuli, trauma, burns and pancreatitis (Singer et al., 2016). As a result of the physiological responses, the pathological changes observed include alterations in body temperature, tachycardia, tachypnea, and abnormalities in total and differential white blood cell count (Chakraborty & Burns, 2021). As a result of sepsis, the receptors largely trigger inflammation to the site of infection in the lining of blood vessels that detect components on pathogens' cell walls that stimulate physical response. The body detects initiates/inflammation, attempts to rectify the situation by mobilising leucocytes to the injury site to neutralise any pathogens present (Sugimoto, et al., 2016). According to the NICE (National Institute for Health and Care Excellence) guidelines “NG51, Sepsis: recognition, diagnosis and early management”, the risk factors for sepsis are to take into account people that are at a higher risk of developing sepsis. It was also estimated that there are around 123,000 cases of sepsis in England every year and subsequently leading to around 36,900 deaths (Razel-Potts, et al., 2020).

The sepsis incidence data published by the UK Sepsis Trust illustrated in figure 1 shows a significant increase in sepsis episodes each year in England with a startling percentage increase of 117.673% by the end of 2018. Thus, it further highlights the impact sepsis has in all the three different levels of healthcare settings (primary, secondary, and tertiary care) in the United Kingdom (UK) and reflects the same trends in other global healthcare settings. Therefore, there is a need for rapid diagnostic tools for earlier diagnosis and preventing disease progression.

The Global Burden of Disease team published a long-term study that monitored the global sepsis incidence and mortality from 1990-2017 (Rudd et al., 2020). It was concluded that although globally there was a decrease of 18.8% of sepsis cases, it was still estimated that high-income countries such as the UK are forecasted to see between 200 to 270 cases of Sepsis each year per 100,000 population (Rudd et al., 2020), which reflects the UK Sepsis Trust's findings of an 11.5% increase in sepsis incidence rates seen each year (see Figure 1) (Daniels, et al., 2017). Intriguingly, UK's counterparts, Australia, with a similar healthcare setting, have observed a significant reduction in paediatric sepsis mortality between 2002 and 2013 (Schlapbach et al., 2015) and in 2017 estimated only 8700 deaths (Harley, et al., 2021). Therefore, it shows that successful approaches can be implemented to potentially decrease the incidence rates of sepsis. Nevertheless, a study has concluded that sepsis remains the major cause of morbidity and mortality worldwide and is undeniably a worldwide severe health threat (O'Reilly, & Menon, 2021). In their study Watkins (2019) highlighted, studies previously conducted with paediatric sepsis as the focal point are merely observational or retrospective. Therefore, there needs to be a driving force in clinical research for more collaborative prospective studies with healthcare settings internationally, enabling rapid treatments for sepsis, decreasing disease progression, and improving clinical outcomes.

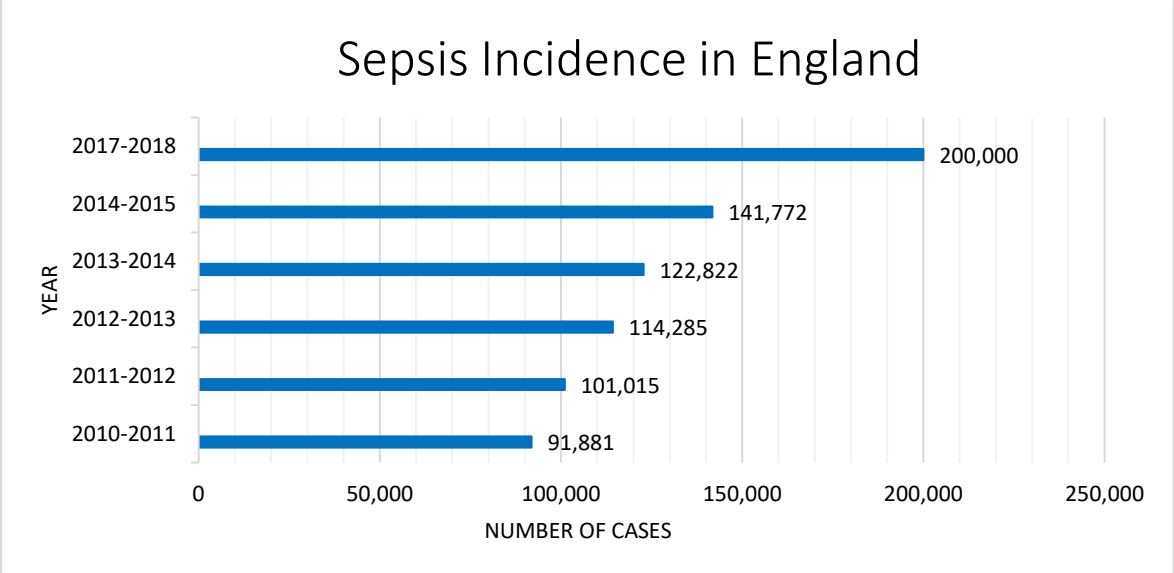

Figure 1: The Sepsis incidence rates in England from 2010-2018 (Daniels, et al, 2017).

1.1.2 Bacterial sepsis

Bacterial sepsis is known to present severe clinical complications in patients and an increased rate of patient morbidity internationally (Bullock and Benham, 2021). The pathogenicity of the Gram-negative and Gram-positive organisms can cause clinical differences and has specific antibiotic susceptibilities, with Gram-negative organisms has the propensity to induce a more clinically severe reaction in the human body as they produce an endotoxin that cause tissue destruction, septic shock, and death (Hotchkiss, et al., 2016). Gram-negative has through extensive research, been identified to be the most prevalent cause of bacterial sepsis due to its higher risk of morbidity and mortality and higher antibiotic resistance than Gram-positive bacteria (Oliveira & Reygaert, 2021) (Rottier, et al., 2021) Therefore, early bacterial identification is imperative in reducing the disease progression of bacterial sepsis, reduce unnecessary exposure to antibiotic and managing patient care.

1.1.2.1 The pathogenesis of bacterial sepsis in Paediatric cases.

Although a small percentage of bacteria are commonly associated with infections and disease, bacteraemia has a large impact on public health (Ferreras-Antolín et al., 2020). Bacteria cause both serious and minor diseases in children; as observed in Figure 2; it highlights the overall cases of paediatric mortality from 2013-2015, which are caused by bacterial infections (Ferreras-Antolín et al., 2020).

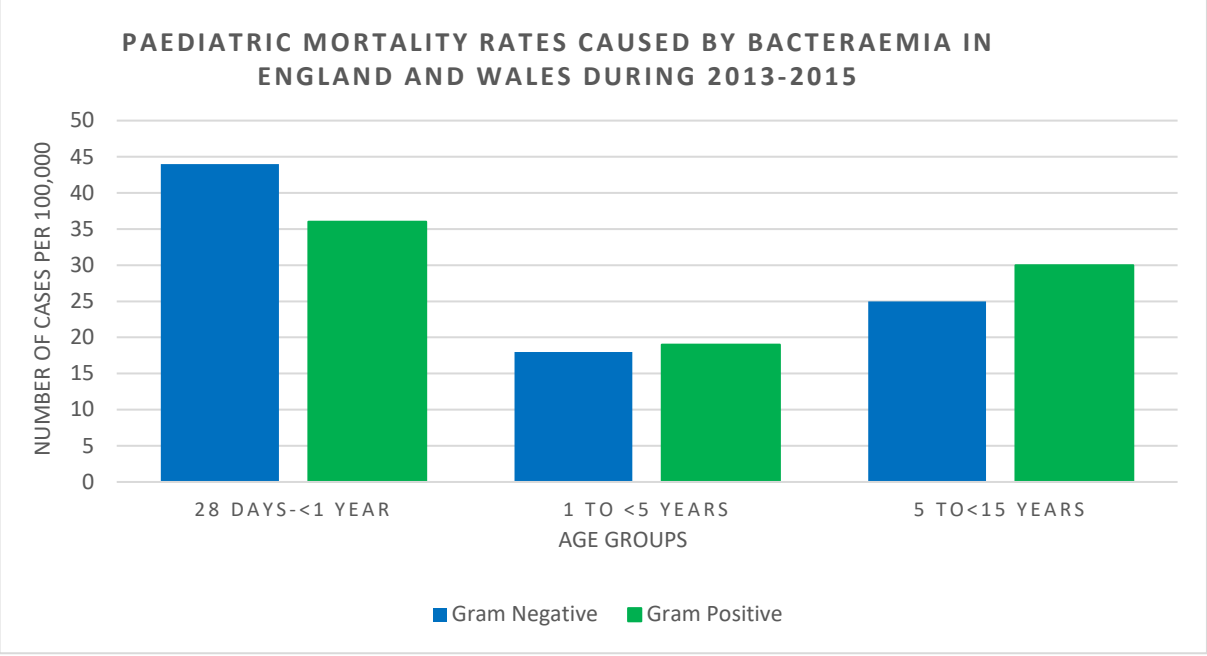

Figure 2: Graph illustrating paediatric mortality rates in the England and Wales between 2013-2015 (Ferreras-Antolin, et al., 2020).

Gram-negative organisms were associated with a higher rate of paediatric mortality than Gram-positive organisms during the neonatal period. This finding could be used to guide clinicians in the better-targeted use of antibiotics for different age ranges within paediatric cases. However, clinical biomarkers can be used for earlier diagnosis of bacterial infection, as seen in Liu and colleagues (2017), which concluded serum procalcitonin levels were higher in patients with Gram-negative rather than Gram-positive bacteraemia within 72 hours, which is a potentially beneficial finding for early diagnosis (Liu, et al., 2017). Although the overall paediatric mortality rates have declined after the neonatal period, the mortality rates are still high and require preventable measures.

1.1.3 Viral sepsis

Although, there is an assumption that the majority of sepsis cases are caused by bacterial infections, sepsis can be also caused by viral infections (Gupta, et al., 2018). Which can further be compounded by paediatric cases due having undeveloped immune systems, which can be made worse in cases were the patient is immunosuppressed (Simon, et al., 2015). A study conducted between 2003-2004 in the United States monitored the root causes of viral sepsis in paediatric intensive care unit (Bhat, et al., 2005). The findings highlighted influenza virus to have led to more incidences of viral sepsis in paediatric patients as well as respiratory syncytial virus (RSV) if left untreated in paediatric cases with underlying issues (Eiland, 2009). Therefore, the clinical presumptions made regarding sepsis can lead to unnecessary use of antibiotics, lack of antiviral therapy, lengthened hospitalisation, and disease progression. Therefore, there is an urgency to provide clinical biomarkers that can distinguish between bacterial and viral sepsis. Several biomarkers such as white blood cells, C-reactive Protein (CRP) and Procalcitonin (PCT) can highlight the attention to a viral infection (Nargis, et al., 2014).

The clinical presentations of viral sepsis in paediatric cases mimics the symptoms noticeable in bacterial sepsis, which places clinicians in a predicament to prevent disease progression. Furthermore, clinicians face the decision to either assume it is bacterial sepsis and starting a course of antibiotics or to wait for often delayed laboratory results which potentially may close the vital window for a definitive diagnosis and a treatment start. There are various factors that affect viral sepsis which include species of virus, patients age and underlying immune status and therefore, there is an importance to closely monitor the patient, review laboratory markers such as CRP (Meisner, et al., 1999).

1.2 Infection, Inflammation, and potential markers of sepsis: the role of CRP

1.2.1 Acute phase response of infections

The acute-phase response encompasses general pathophysiological and biochemical responses of mammals to most forms of tissue necrosis, infection, and inflammation. In particular, the acute phase proteins (APP) also possess the ability to distinguish the foreign markers and dead cells of the host and to mediate the elimination by activating the complement pathway and phagocytic cells (Sproston & Ashworth, 2018). Acute-phase proteins that are present in acute-phase response are defined as proteins that alter their serum concentration in direct response to inflammatory cytokines and arbitrating systemic effects as febrile infections, leucocytosis, increased cortisol levels and decreased serum iron (Kany, et al.,2019). APPs can either be considered as positive-increasing serum concentration or negative-decreasing serum concentration listed in Table 1.

Table 1: A table of illustrating the examples of positive and negative acute-phase proteins present (APPs).

POSITIVE ACUTE-PHASE PROTEINS	**NEGATIVE ACUTE-PHASE PROTEINS**
C-reactive protein	Albumin
Serum amyloid a	Transferrin
Haptoglobin	Transthyretin
Ceruloplasmin	Retinol-binding protein
a2-macroglobulin	Adiponectin
a1-acid glycoprotein	
Fibrinogen	
Complement C3, C4	

Additional positive and negative APPs include proteinase inhibitors and coagulation, complement and transport proteins, however, the one molecule that exhibits the required sensitivity and response rapidity like CRP is serum amyloid A protein (SAP).

1.2.2 The discovery of C-Reactive Protein (CRP)

CRP, the member of the phylogenetically ancient and highly conserved 'Pentraxin' family of proteins was named for its capabilities to react with Phosphocholine (PCh) residues of somatic C-polysaccharides (PnC) (Tillett & Francis, 1930). In the laboratory of Oswald T. Avery at the Rockefeller Institute in New York in 1930, William S. Tillett and Thomas Francis Jr. discovered CRP in patients' sera suffering from *Streptococcus pneumoniae* infections that interacted with pneumococcal cell wall residues (Tillett & Francis, 1930). Further progress in the investigation showed that this precipitation reaction was not exclusive to *Streptococcus pneumoniae* infections but has also been found in *Haemophilus influenzae* (Weiser et al., 1997), *Pseudomonas aeruginosa*, *Neisseria meningitides* and *Neisseria gonorrhoeae* (Serino & Virji, 2000), *Proteus morganii* (Potter, 1971), and Aspergillus *fumigatus* (Longbottom & Pepys, 1964). Accordingly, Theodore J. Abernethy and Oswald T. Avery distinguished CRP as an "acute phase protein" (APPs), an early indicator of infectious or inflammatory conditions such as haemolytic streptococci infection and rheumatic fever. This term was applied to the large cohort of other plasma proteins, the concentrations of which are raised in acute-phase sera. The acute phase nature of CRP observed in the early studies established that CRP is ordinarily present in trace levels in serum but increases rapidly due to an acute stimulus such as infections, inflammation states and tissue necrosis. It was one of the first acute phase proteins to become elevated in inflammatory disease and also the one exhibiting the most elevated increases in concentration.

Since its discovery, CRP has been studied as a diagnostic tool for inflammation responses such as vasculitis and rheumatoid arthritis, as well as a biomarker for disease activity that is extensively used in clinical practice (Centola, et al., 2013). A study conducted in 2007 presented that elevated baseline CRP values are a clinical biomarker of atherosclerotic vascular disease and may predict imminent cardiovascular events (Ridker, 2007) (Shrivastava, et al., 2015). Ridker (2007) also shown another advantageous role of CRP as a clinical biomarker of inflammation and is adapted to focus on mediating in inflammatory conditions. However, establishing the role of CRP as an effective clinical biomarker in inflammation is challenging due to conflicting findings found in literature. Nevertheless, recent studies highlighted that there is an existence of two conformations of the protein and the conformational change explains the contradictory data. The identification of the dissociation mechanism of the pentameric protein (pCRP) to its monomeric components has facilitated the localisation of injured necrotic and normal activated cells and platelets.

(Thiele, et al., 2014). This conformational change is accompanied by an alteration of the inflammatory profile of the protein. The proinflammatory properties of CRP are associated with the monomeric isoforms, and therefore the prime focus of the dissociation process is the anti-inflammatory therapeutic strategies (Thiele, et al., 2014). Importantly, the role of dissociation of pCRP mediates the pro-inflammatory potential of the monomeric protein (mCRP) in inflammatory responses such as neoplastic proliferation and surgical trauma (McFadyen, et al., 2018)

1.2.3 The structure, phylogeny, and physiology of CRP

CRP is a plasmatic member of this pentraxin family of calcium-dependent ligand-binding proteins and includes SAP. 'The pentraxin family aptly named for its electron micrographic image originates from the Greek terms Penta (five) and Ragos (berries) which remains a highly conserved evolutionary molecule illustrated in Figure 3'. The CRP structure is composed of a cyclical arrangement of five noncovalently linked subunits forming a discord molecule with each subunit being 23 kDa (Rajab, et al., 2020). The crystal structure has been determined with binding sites for two calcium ions and one Phosphocholine molecule as well as phosphatidylcholines such as lecithin and polyanions such as nucleic acids, and binding sites for C1q and Fc receptors (Du Clos, 2013).

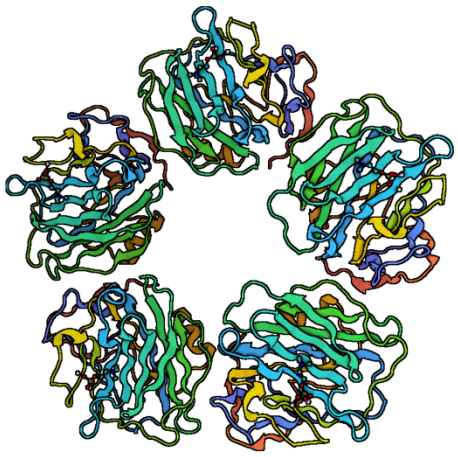

Figure 3: The structure of CRP adapted from ''C-reactive protein structure'' by Biorender.com (2021)

SAP is a non-acute phase plasma glycoprotein found in humans; phylogenetically distant arachnid Limulus *polyphemus,* the horseshoe crab. The structure is very similar to that of SAP, although certain unique features characterise each protein. The ligand-binding site,

composed of loops with two calcium ions bound 4 A apart by protein side-chains is located on the concave face. A single alpha-helix carries the other face.

However, after 500 million years of evolution, CRP remains a conserved protein expressing immunological reactions remarkably present in the blood in every organism where the presence of CRP has been located (Ansar & Ghosh, 2013). More importantly, the definition of CRP became distinctive through further progress in investigating the phylogeny of CRP. The initial investigation of the phylogeny of CRP followed the work of Abernethy who reported the presence of a precipitin comparable to CRP in acute phase monkey serum but had been unable to find any in mouse or rabbit sera (Pathak & Agrawal, 2019). Interestingly, 'Patterson and Mora reported the existence of CRP in chickens, dogs, horses, and mice based on results with anti-human CRP sera and calcium-dependent reactivity with CPS. Further progress in investigation of the phylogeny of CRP followed the work of Gotschlich & Stetson (1960) who demonstrated immunological cross-reactions between human, monkey, and rabbit CRP' (Pathak & Agrawal, 2019).

1.2.4 Ligand binding of CRP

The ligand-binding characteristic of human CRP structure has consequences for toxic and inflammatory conditions and throughout evolution the conservation of CRP is favoured. Additional ligand-binding property of CRP which aids host defence functions is the recognition of factor H in CRP in its non-native conformation (Pathak & Agrawal, 2019). 'The host defence functions of CRP evolved to expose a ligand-binding site only when needed, that is, an inflammatory microenvironment would have to be sensed by CRP first and that CRP would change its structure to execute a function' (Pepys & Hirschfield, 2003). Also, the range of autologous ligands categorised in Table 2 exceeds the spectrum found in antiphospholipid autoantibodies commonly autoimmune syndromes (Pepys & Hirschfield, 2003). Another important host defence function of CRP is potential to generate IgG antibody frequently, which mediates the development of an effective immune response. (Du Clos, 2000).

They also can bind to a variety of other autologous and extrinsic ligands addressed in table 2. A study was conducted in 1961 which concluded that CRP could bind to healthy cell membranes at sites of inflammation and TNF CRP was found in necrotic cell membrane (Kushner & Kaplan, 1961). In the situation of Calcium 2+ absence, CRP binds to polycations such as histones. The complexed CRP can activate the complement pathway starting at the

C1q binding site. CRP also initiates opsonisation, phagocytosis, lysis of invading organisms such as bacteria and viruses. CRP recognises toxic autogenous substances released from damaged tissue. They are detoxified and cleared from the blood and CRP itself is catabolised after opsonisation.

Table 2: A table highlighting the binding abilities of CRP (Shrivastavaa, et al., 2015).

CRP binding abilities		
Intrinsic ligands	**Autologous ligands**	**Extrinsic ligands**
Phosphocholine residues	Native plasma lipoproteins	Glycan
	Modified plasma lipoproteins	Phospholipids
	Damaged cell membranes	Histones
	Apoptotic cells	Chromatin
	Phospholipids such as Lecithin, lysolecithin, sphingomyelin.	Fibronectin
	Small nuclear ribonucleoprotein particles	Bacteria
		Fungi
		Protozoal parasites
		Virus
		Plant products

Ligand binding of CRP is an essential process that occurs before activating the complement pathway and the initiation of the inflammatory response seen in Figure 4. Therefore, CRP can have $Ca2^{+}$-dependent high-affinity binding to PCh as well as an affinity for autologous and extrinsic ligands highlighted in table 2. 'PCh is a component of many prokaryotes and is almost universally present in eukaryotes. A substantial proportion of germline-encoded, highly conserved natural antibodies resemble CRP in specifically recognising Phosphocholine; the constituent of many bacterial and fungal polysaccharides and most

biological cell membranes' (Volanakis & Kaplan, 1971). PCh is also present in most biological membranes' outer leaflet as the polar head group of lecithin and sphingomyelin. It remains crucial for both host defence and the handling of autologous constituents, including necrotic.

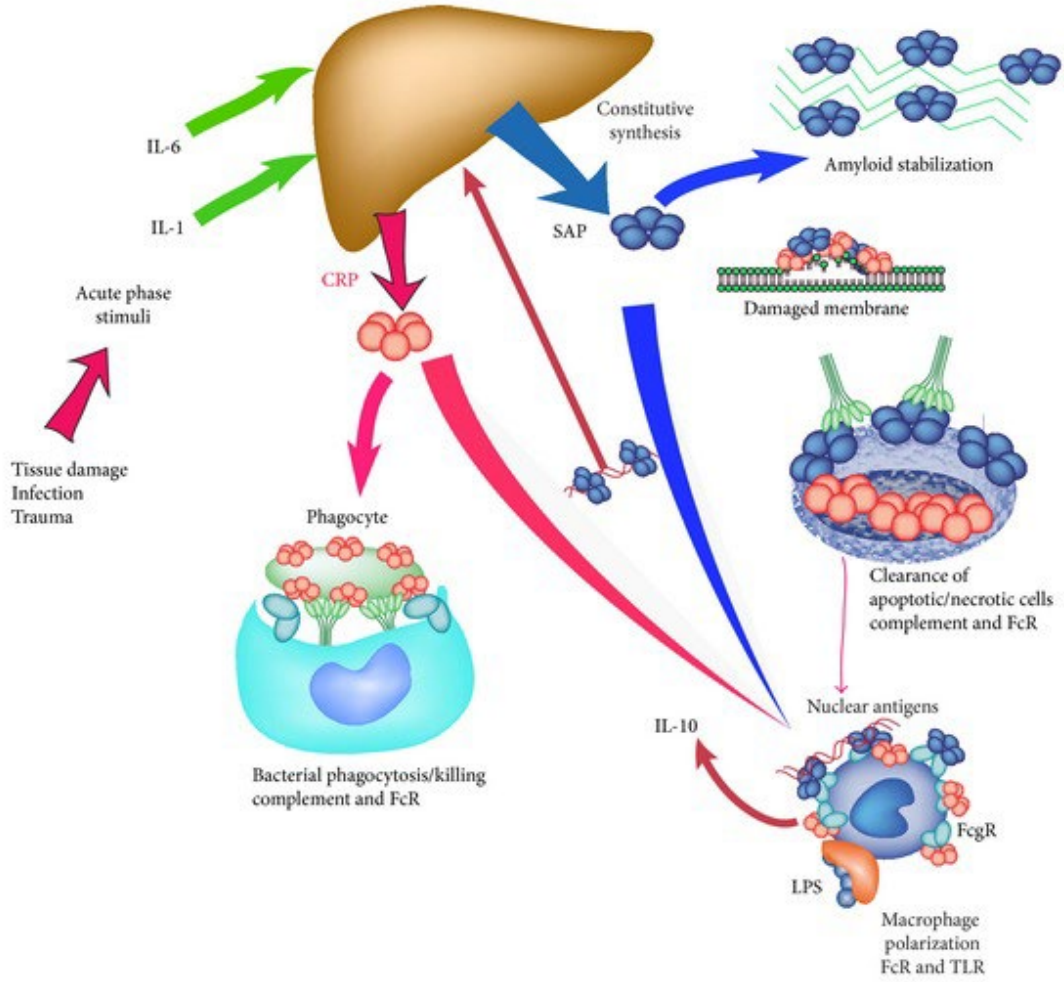

Figure 4: An illustration of the biological role of CRP *(Du Clos, 2013)*.

1.2.5 The response of CRP in infection and inflammation

CRP is secreted from the endothelial cells, hepatocytes, adipose tissues, and muscle cells under the stimulation of cytokines, IL-6, or the tumour necrosis factor (TNF) illustrated in Figure 4. The rapid and pronounced elevation in the serum concentration is displayed in response to an immunological response, bacterial infection, or tissue damage. Specifically, in *de novo* hepatic synthesis of CRP, an acute phase stimulus can cause within six hours for the CRP serum levels to elevate to pathological concentrations and, in course attaining the maximal serum levels within 48 hours (Axel-Shield & Presteg, 2012). It can also swiftly

elevate up to 1000-folds of basal levels which proves that it is the release rate that indicates a stimulus or immunological challenges other than infection and inflammation such as stress, trauma, surgery, or neoplastic proliferation.

Thus, CRP is a sensitive systemic biomarker of infection and non-infectious inflammatory diseases and appropriate direct therapy. Moreover, the feasibility of CRP is supported by unaffected CRP concentration levels after drug administration and consuming meals (Sproston & Ashworth, 2018). However, cord blood has low CRP concentrations, but in intrauterine infection, levels may be as high as 26,000 ug/dL. Levels in infancy rise for a few days after vaginal delivery, fall to deficient levels, and then gradually rise over several weeks to adult levels. Also, the presence of a raised CRP concentration is unequivocal evidence of inflammation and can be used to detect and assess organic diseases. The serum concentration of CRP provides a rough guide to the amount of tissue involved in inflammation and the inflammatory response's intensity. ‘Many chronic inflammatory diseases are challenging to monitor clinically, and CRP measurements can be costly in assessing the response to therapy and changes in disease activity’ such as rheumatoid arthritis, polymyalgia, giant cell arteritis, systemic vasculitis and inflammatory bowel disease noted in Table 3 (Pepys & Hirschfield, 2003).

Table 3: CRP responses in diseases (Pepys & Hirschfield, 2003).

CRP responses in disease Major CRP acute-phase response	
Infections	Bacterial Systemic Mycobacterial Viral
Allergic complications of infection	Rheumatic fever Erythema nodosum
Inflammatory disease	Rheumatoid arthritis Juvenile chronic arthritis Crohn disease
Necrosis	Myocardial infarction Tumour embolisation Acute pancreatitis
Trauma	Surgery Burns Fractures
Malignancy	Lymphoma Carcinoma Sarcoma
Modest or absent CRP acute-phase response	Systemic lupus erythematosus (SLE) Leukaemia Graft-vs-host disease

The CRP responses in disease differs with age as seen in many pilot studies, the behaviour of CRP in neonates rarely rising above ten mg/L, even in significant bacterial infection however there are higher cases of mortality (Brown, et al., 2020). When compared to the adult population which has on average a higher level of CRP and with the evidence of a slight age-related increase observed in population over sixty-five years of age. This can place a limit on the value of CRP in the assessment of infection in neonatal infants; it does put constraints on the assay used by a laboratory serving a neonatal unit (Hisamuddin, et al., 2015). This was later supported by the study completed by Brown and colleagues (2020), which concluded that the use of serum CRP for initial evaluation of neonatal infection is unlikely to be sufficiently accurate for early diagnosis. The persistent elevations of CRP can indicate a poor prognosis in both inflammatory and malignant disease. However, the 'CRP measurement may be useful in indicating bacterial tests for meningitis and pneumonia. In recent years, the increasing validity of data from CRP investigations has supported its use as a widely accepted' and appropriate point-of-care diagnostics tool of acute phase inflammation and assesses the extent of activity of the inflammation (Axis-Shield & Presteg, 2012). Nevertheless, the most potent activator of the acute phase response is a bacterial infection which causes CRP concentration levels to spike significantly and can be used as a clinical biomarker.

In all these cases, there is a risk of silent but severe sepsis especially with cases of viral infections cause little acute phase response and there expressing lower CRP concentration levels. This can unfortunately be missed by clinicians when diagnosing sepsis, therefore there is a need in clinical research to establish the thresholds in clinical biomarkers to aid rapid diagnostic parameter settings.

The median serum concentration of CRP is 800 ug/l, with 99% of normal subjects having concentrations below ten mg/L. Therefore, as a sensitive acute-phase protein any causes of inflammation listed in Table 3 can cause the liver synthesis of CRP to increase resulting in detectably raised serum concentrations above ten mg/L within six hours. As illustrated in Figure 5, the increase in CRP concentration levels can determine the severity of the disease. Typically, serum concentrations of between 10 to 40 mg/L represent mild inflammation but may be associated with severe viral infections. In the yellow stage in Figure 5, levels between 40 and 200 mg/L are associated with significant acute inflammation or bacterial infections. In burns or severe bacterial infections, concentrations may rise to more than 200 mg/L or

higher *(Vermeire, et al., 2006)*. The determination of CRP concentrations is clinically useful for screening for organic disease, assessing the activity of inflammatory diseases, detecting intercurrent infections in systemic lupus erythematosus (SLE) in Leukaemia, or after surgery and management of neonatal septicaemia and meningitis when specimen collections for bacteriological investigations may be difficult.

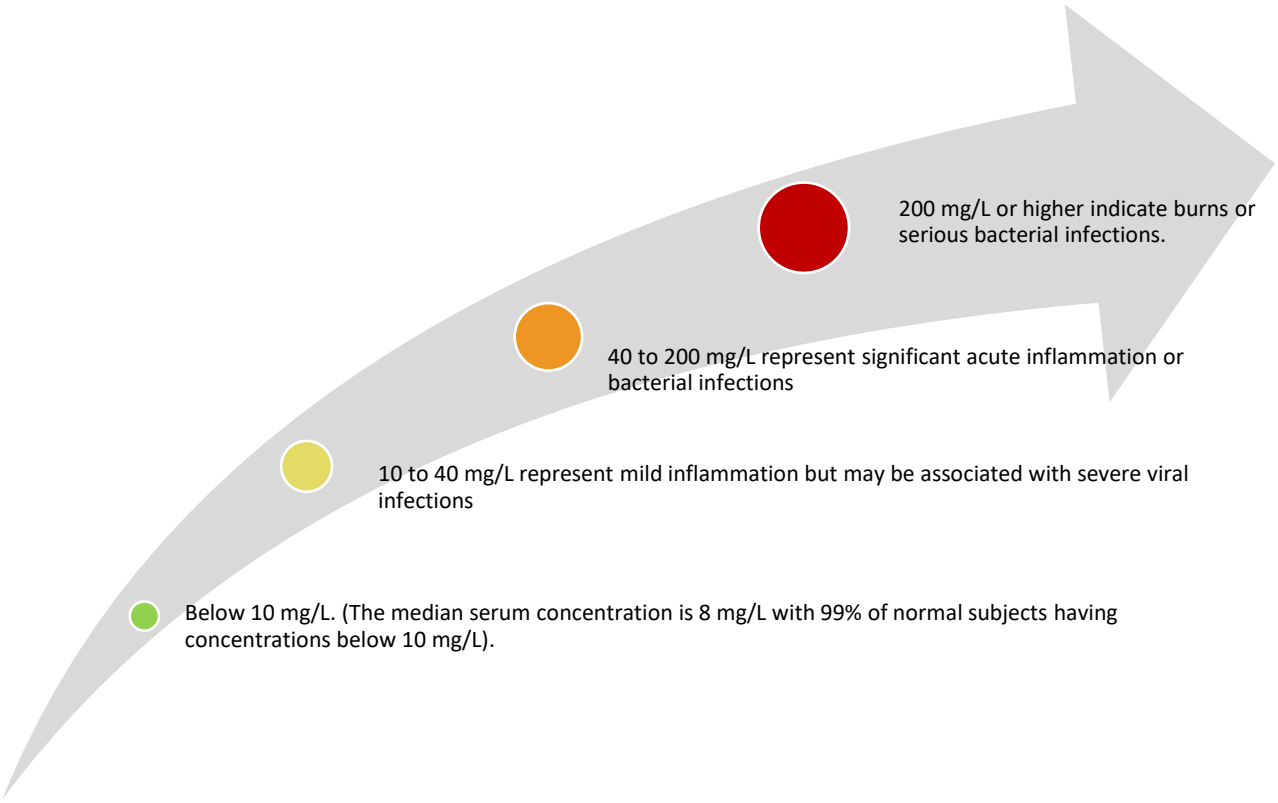

Figure 5: The concentration of CRP in human sera (Vermeire, et al., 2006).

Another clinical marker of inflammation, infection and tissue damage is Erythrocyte sedimentation rate (ESR). ESR was first noted by Dr. Edmund Faustyn Biernacki in 1897 through the measurement of the rate at which red blood cells fell within an hour in a Westergren tube of anticoagulated blood (Litao & Deepak, 2014). However, there are various differences between CRP and ESR, including the enhanced responsiveness and specificity of CRP test results (Landry, 2017). CRP levels rise more quickly than ESR and not as impacted by anaemia, pregnancy and elevated protein levels (Brigden, 1999) Therefore, it is appropriate to use CRP for monitoring acute disease activity such as acute infection rather than ESR (Harrison, 2015) Nevertheless, ESR is beneficial in assisting in the detection of certain inflammatory conditions such as SLE, polymyalgia rheumatica or polyarteritis (Litao & Deepak, 2014). As ESR and CRP are known to lack specificity and sensitivity and advised that neither should be used solely for clinical diagnosis (Litao &

Deepak, 2014) CRP is useful in monitoring the manifestation of neonatal sepsis and help determine the need for starting antibiotics. ‘Overall, the vital aim of the immune response is the first line of defence, working parallel to the adaptive immune system to clear invading pathogens or microbes’ (Axel-Shield & Presteg, 2012).

1.3 The use of Neutrophil-lymphocyte ratio as clinical biomarkers for systemic inflammation

The neutrophil-lymphocyte ratio (NLR) is an inflammatory biomarker found in the peripheral blood that can be used as 'an indicator of systemic inflammation; the NLR is defined by the absolute number of neutrophils divided by the absolute number of lymphocytes' (Naess, et al., 2017). This inexpensive and simple quantitative measure uses the parameters available in the full blood count examinations which are routinely performed by clinicians. The combination of NLR has been thought as better predictive factor than total WBC count or neutrophil count used separately. Several studies investigated the suggestion of NLR to be useful for the discrimination between these types of infection and also to predict the outcome of infection which was initially reported was found NLR to have a higher sensitivity of 83.9% compared to PCT, CRP and total white blood count and as well as a moderate sensitivity for diagnosing sepsis (Gürol, et al., 2015). Therefore, NLR can be used as a guide in prognosis of various diseases such as myeloid leukaemia, stroke, and more importantly early phase of sepsis. However, a study conducted by Gauchan & Adhikari (2016) explained that 'CRP is a better specific marker than NLR for differentiating bacterial and non-bacterial pneumonias in children of one to 60 months of age as the CRP values were significantly higher in the bacterial group compared to non-bacterial pneumonia group'. Another limitation of NLR was highlighted in a study which presented that in fact NLR can be elevated not only from sepsis but from other conditions such as trauma, surgery, acute pancreatitis, and atherosclerosis as well as a poor prognosis with cancer (Jeon & Park, 2017). Therefore, this limitation places a further emphasises for the need for a quantitative biomarker or index that can differentiate between infection and inflammation. In addition to above 'discussed laboratory parameters, which are currently used in clinical practice, novel biomarkers are potentially useful for screening, clinical management, and prevention of sepsis as well as better targeted use of antibiotics.' A study conducted in 2021, supported the use of NLR as a marker for detection for early onset neonatal sepsis (Mira, et al., 2021).

1.4. Clinical diagnosis of sepsis

Due to the rapidly deteriorating nature of sepsis and the wide range of pro-inflammatory stimuli, it is an underlying issue for clinicians in identifying patients with sepsis, especially in paediatrics. However, advancements have been made in clinical research with earlier diagnosis of sepsis (Gyawali et al., 2019).

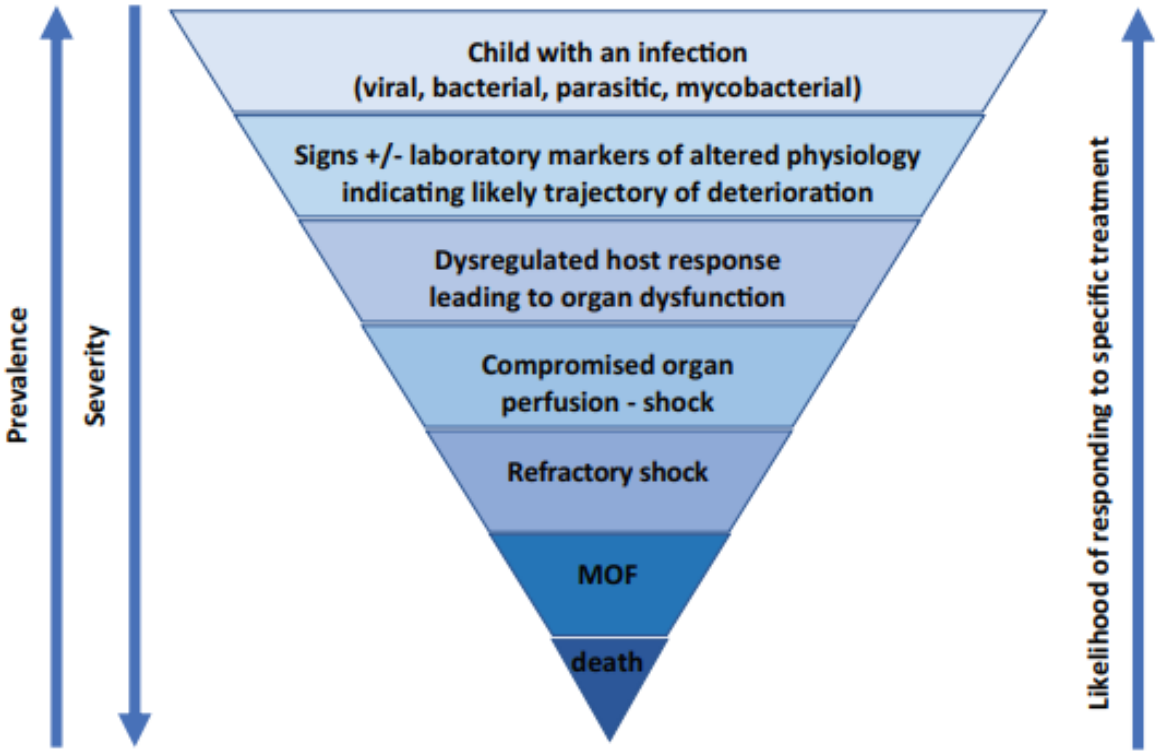

Figure 6: A sepsis model (Schlapbach, 2019).

The sepsis model in Figure 6 has established itself as a fundamental clinical ground to action potential septic cases (Schlapbach, 2019). The illustration succinctly highlights the increased severity of a sepsis case, the less likelihood of responding to specific treatment advised by the clinicians. Whilst there are issues in terms of the balance of antibiotics prescription and additional ordering, it remains the best example of global recognition of sepsis in clinical settings.

Hospital trusts across the UK have made a successful and proactive effort to establish their own qualitative sepsis index criterion for their clinicians to follow to diagnose sepsis faster. Paediatric Early Warning Score systems (PEWS) were established in the UK to provide a structured reproducible clinical framework for clinicians to follow when diagnosing and monitoring paediatric sepsis; many Local Trusts have continued to use PEWS charts to clinically assess patients triaged with suspected sepsis(Chapman & Maconochie, 2019).With the limited research available regarding PEWS, a study was conducted in 2015 to explore

the effectiveness of the PEWS charts in a paediatric emergency department (Gold et al., 2015). The study concluded that PEWS provides clinicians instant clinical data of the patient, improving the quality of patient care and reliability for nurses in the emergency department (Gold, et al., 2015). As expected, an elevated PEWS chart attributed to a transfer to intensive care unit and longer length of hospitalisation. However, a recent study in October 2021 concluded that PEWS has the poor discriminative ability and cannot be recommended solely to predict serious bacterial infections (SBI) in paediatric cases (Gardiner, et al., 2021). This further emphasises how early sepsis clinical signs can become vague, subtle, or challenging; for instance, a mild tachycardia or fever can be left open to interpretation by the clinicians and the patients (Vincent, 2016). Therefore, the use of PEWS raises questions on its effectiveness due to the lack of incorporating an analysis of the laboratory medicine; there is an absolute need for a more thorough quantitative model. In 2021; a novel nomogram was created that has performed better than the sepsis scoring systems such as PEWS, Paediatric Risk of Mortality III (PRISM III) and Paediatric Critical Illness Score (PCIS), which has incorporated the use of first positivity of blood cultures, serum albumin and lactate dehydrogenase was established (Liu, et al., 2021). The fact that it has used parameters found in the laboratory provides a more accurate and quantitative result for clinicians to act on as it there is no room for interpretation.

Therefore, the sepsis models must use haematological and biochemical parameters such as clinical biomarkers and full blood counts to provide valuable information, primarily focusing on the total and differential white blood cells counts. Clinical biomarkers are also observed as they have an important place in routine lab requests from Accident and Emergency departments such as the popularly requested CRP; it is one of the acute phase proteins that elevates in conditions such as chronic and acute inflammation (Ticinesi, et al., 2017). The sudden rise in CRP concentration may be a useful pointer to intercurrent sepsis, particularly in diseases where the acute phase response kinetics are known, such as following major surgery or with little intrinsic response Leukaemia, SLE and during peritoneal dialysis, therefore should be utilised when creating a sepsis model. Though the specificity of CRP has been challenged, it remains an important tool used by clinicians for guidance in the clinical diagnosis of sepsis. Therefore, it is paramount to combine and analyse clinical biomarkers relevant to infection and inflammation to provide a clearer clinical picture in acute ambulatory cares settings. Moreover, other beneficial uses of biomarkers include roles in guiding antibiotic therapy, evaluating the response of therapy and recovery from sepsis.

1.4.1 Index for diagnosing sepsis

Due to the demand for earlier recognition of sepsis, there have been efforts made to identify a suitable quantitative marker. Figure 7 demonstrated a study completed in South Korea which investigated the effectiveness of Delta neutrophil index (DNI) with detecting Sepsis (Lee, et al., 2014). DNI represents the proportion of circulating immature granulocytes in the peripheral blood, which has been shown to correlate with disease progression in patients diagnosed with sepsis. The long incubation time required for blood culture in the diagnosis of sepsis can lead to worse outcomes of sepsis and the possible misuse of antibiotics.

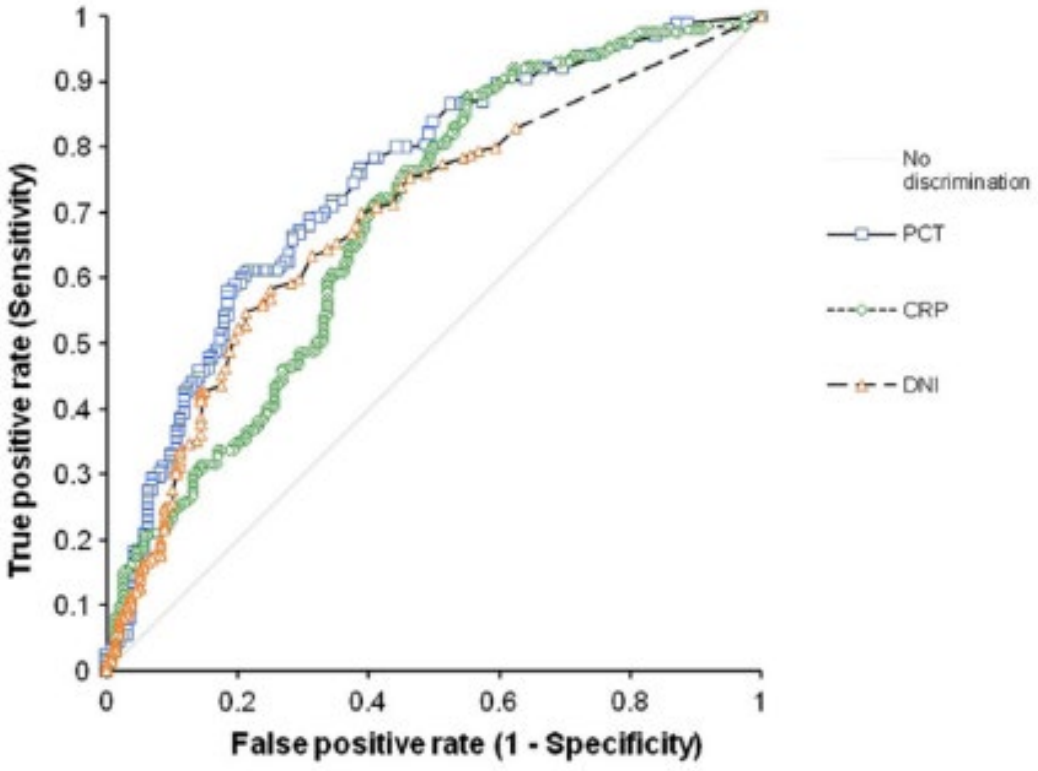

Figure 7: ROC curve illustrating the effectiveness of the clinical biomarkers (Procalcitonin-PCT & C-Reactive Protein-CRP) and a novel delta neutrophil index (DNI) (Lee, et al., 2014).

As shown in figure 7, the diagnostic performance of DNI is comparable to the other routinely used clinical biomarkers, PCT and CRP. Although the area under the curve (AUC) of PCT has better performance, DNI still showed the ability to differentiate true bacteraemia. Since the parameters required to calculate DNI are already included in the complete blood count results, it proves to be an effective diagnostic and prognostic marker of sepsis (Lee, et al., 2014). However, a study completed the following year showed the limitations of using DNI as a diagnostic tool for bacteraemia in immunosuppressed children (Ahn, et al., 2014). The finding suggested that in the immunocompromised group, the immature granulocytes increased due to the stimuli in the bone marrow, which deemed DNI not to be a useful

biomarker in this instance. However, this study has highlighted the need for a novel sepsis index that encompasses all populations irrespective of conditions and uses clinical biomarkers for a more powerful and effective prognosis of sepsis.

1.5 The potential use of Point of Care Testing (POCT) for earlier clinical diagnosis of Sepsis.

Though various sepsis models have incorporated clinical biomarkers and laboratory parameters in recent times, the turnaround time (TAT) for the results to be available for clinicians must be improved. With the average turnaround time for laboratory results being two hours, due to the vast number of samples processed in a centralised laboratory, there is an urgency to resolve this matter. Certain local Trusts have made consolidations for their pathology services by creating 'Hot Labs' which are coined for being satellites laboratories located away from the main laboratory. These labs are design to be located inside the accident and emergency departments and have accredited biomedical scientists to book, process and analyse samples only from the E.Ds. The design of 'Hot Labs' were to combat the vital window that clinicians would like to use to reduce the chance of disease progression. A popular alternative and a convenient solution have been incorporating point of care testing (POCT) devices, which offer the accessibility of a wide range of devices covering different laboratory medicine disciplines in patient-focused care settings. POCT has become one of the fastest-growing sectors in clinical pathology seen in recent years due to the scope POCT provides easy accessibility for trained caregivers and rapid TAT. However, due to the distance from the centralised laboratories, POCT devices are more prone to be accessed and performed by non-laboratory caregivers, which increases the incidences of analytical and operator errors. Therefore, vendors have provided sophisticated informatics such as middleware to provide remote surveillance for laboratory staff to review. Local hospital trusts have been ensuring the quality of the POCT devices through clinical audits, regulatory compliance of the POCT devices, maintaining suitable training for non-laboratory clinicians, and ensuring adherence to the local hospital Trust policies (Anderson, 2001).

POCT clinical biomarkers and haematological parameters such as CRP, PCT and NLR are useful predictive tools in assisting clinical diagnosis of probable causes of febrile infections and early-onset neonatal sepsis (Ding, et al., 2020). Another study reviewing the benefit of CRP POCT testing was able to conclude that POCT CRP can be used as a supplement in course with routine clinical examinations will likely reduce the use of antibiotics, especially in primary care settings with acute respiratory infections without affecting patient recovery rates or the "duration of illness" (Aabenhus, et al., 2014). Interestingly, POCT has also

contributed to reducing antimicrobial resistance (AMR); AMR has been an internationally recognised issue that has placed an ever-mounting force on clinical settings, with Vietnam having one of the highest AMR levels recorded due to there being no sanctions enforced to limit antibiotic use (Lubell, et al., 2018). The further expansion of new POCT devices, new testing parameters and improved performances allows it to remain at the forefront of clinical medicine (Epner, 2014).

1.6 Aims and Objectives

The aim of this current study is to see if a rapid point of care testing platform using CRP and any other ratio can be developed to differentiate between bacterial and viral sepsis in paediatric patients.

The objectives of the study are:

1. To determine whether the use of CRP can improve diagnostic capabilities.
2. To utilise the POCT device as a diagnostic tool for earlier diagnosis of paediatric sepsis.
3. To combine CRP and NL ratio to create a novel Sepsis index (SI) that can provide clinicians with quantitative results relating to Sepsis.
4. To investigate the effectiveness of CRP, NL ratio and SI to diagnosis paediatric sepsis as well as predict the length of hospitalisation stay in paediatrics and improve clinical planning.

2. Materials and Methods

2.1 The POCT device: Microsemi Horiba CRP LC-667G Haematology analyser

The POCT device used in this study was the Microsemi Horiba CRP LC-667G Haematology analyser (Horiba) which was conveniently situated in the Resuscitation area in the Accident and Emergency department, Chelsea and Westminster Hospital, Chelsea and Westminster Hospital NHS Foundation Trust, London (CW-NHS) (Figure 8 below). In addition, this device is intended for use of clinicians from A&E paediatric department to analyse paediatric samples.

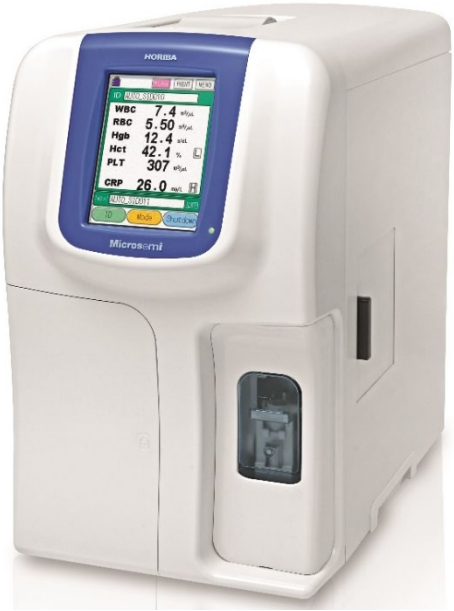

Figure 8: The POCT device: Microsemi Horiba CRP LC-667G Haematology analyser situated in the Accident and Emergency department, CW-NHS.

Following the POCT trust policy (NWLP), the analyser has passed the initial validation process, compulsory internal quality controls are preformed daily to monitor the quality of the analyser and in compliance with ISO 9001: 2015. Overall, the specifications of the analyser offer an acute setting as it has a rapid turnaround time of 4 minutes per sample and provides analysis of 19 parameters listed below (table 4).

Table 4: The wide range of parameters the Microsemi Horiba CRP LC-667G Haematology analyser (Horiba) offers at Accident and Emergency department, CW-NHS, London

Full blood count parameters	***Other parameters***
White Blood cells (WBC)	*C- reactive Protein (CRP)*
Red Blood Cells (RBC)	
Red Cell distribution width (RDW)	
Haemoglobin (HGB)	
Mean platelet volume (MPV)	
Haematocrit (HCT)	
Mean cell volume (MCV)	
Platelets (PLT)	
Mean corpuscular Haemoglobin (MCH)	
Mean corpuscular Haemoglobin concentration (MCHC)	
Lymphocyte count (LYM #)	
Lymphocyte percentage (LYM %)	
Monocyte count (MON #)	
Monocyte percentage (MON %)	
Granulocyte count (GRA #)	
Granulocyte percentage (GRA %)	
Platelet distribution width (PDW)	
PCT (Procalcitonin)	

Therefore, clinicians would collect a blood sample via venepuncture from admitted paediatric patients in a 10 ml BD Vacutainer EDTA sample tube. Before proceeding to analyse the sample, the clinician must ensure to invert the sample 20 times for more accurate results. The clinician will then use the interactive 'colour touch screen' panel on the analyser to select the type of sample whether it is an 'Adult' or 'Paediatric' samples. The analyser will request for the sample to be placed in the sample port area as an open tube and for the sample door to be closed. The analyser will use photometry for haemoglobin measurements and immunoturbidimetry technology for CRP measurement. Clinicians within this 4-minute time of analysis will also send a second set of the sample to the laboratory via the pneumatic tube. The laboratory turnaround time for producing results for urgent samples is within an hour which highlights the usefulness of POCT devices in acute settings.

2.2 Data selection and collation

The data collection phase of the thesis required the completion of three crucial components as highlighted in Figure 9.

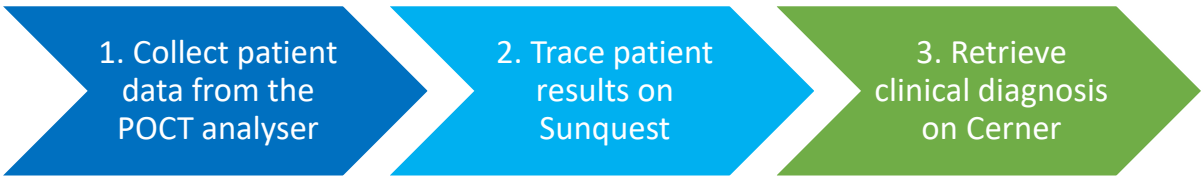

Figure 9: The data collation framework used to conduct this study.

The first stage of data collection involved collecting the patient data directly from the analyser via printing. The analyser possessed to ability to store 200 previous patient results; the data was collected starting from February 2020. The patient data collected from the analyser were then recorded on a master spreadsheet attached in the appendix. As the results had patient identifiers such as patient hospital number, this was used to progress to the second stage of the data collection.

The second stage involved in putting the hospital number into Sunquest (Laboratory information management systems) to trace the biochemistry, haematology, and serology results from the laboratory. As the results present on Sunquest showed a history of all the samples sent to the laboratory regarding the patients, it was important to filter the results were using the time stamp of when the sample was analysed on the POCT analyser in the ED for accuracy. The results were then recorded on the master spreadsheet in the appendix.

Third stage of the data collection involved using the hospital number to retrieve the clinical diagnosis made by the clinicians in the ED using Cerner (Hospital information management systems). The retrieval of the clinical diagnosis involved reviewing the entire patient record from initial ED admission, discharge notes to GP referral letters. Once the clinical diagnosis was retrieved, it was recorded in the master spreadsheet. The master spreadsheet incorporated the POCT results, laboratory results, clinical diagnosis and medication prescribed.

2.2.1 Exclusion and Inclusion criteria

Although the analyser was intended for the use of paediatric cases, it was situated in the adult resuscitation area of the A/E department. The convenience of the analyser in this area,

meant clinicians have been analysing both adult and paediatric samples. Therefore, the first stage of the data clean is to establish an exclusion and inclusion criteria to filter the data points (table 5).

Table 5: The exclusion and inclusion criteria required in this study

Exclusion Criteria	Inclusion Criteria
Adult samples analysed on the POCT device (17 years of age or older).	Paediatric samples (16 years of age or under).
Adult samples recorded on Sunquest (17 years of age or older)	Correct hospital number
Incorrect hospital number	Correct clinical diagnosis
Duplicate results	Inpatients
No results obtained in POCT device, Sunquest or no clinical diagnosis	

2.3 Data Entry

The data collected from the Horiba Microsemi POCT device, Sunquest and Cerner were collected in a master spreadsheet. This spreadsheet included the unique sample numbers populated, date collected, date tested, age, haematological and CRP parameters, length of hospitalisation, clinical diagnosis, medication prescribed, and a novel sepsis index.

2.4 The implementation of a Novel formula (Sepsis Index)

A novel sepsis index was devised which encompassed the use of a neutrophil and lymphocyte ratio and CRP value. The predicted outcome of this sepsis index was that a higher value should indicate disease severity and likelihood of sepsis and therefore could introduce a quantitative result for clinical guidance for sepsis in acute settings. To establish a neutrophil/lymphocyte ratio, the neutrophil values were divided over the lymphocyte values obtained from the POCT device. Also, to ensure accuracy, it was decided that CRP values less than 5 mg/L would be changed to 5 mg/l when calculating the sepsis index. Therefore, a separate column labelled changed CRP values were introduced into the

spreadsheet. Sepsis index was calculated using the formula (Equation 1) to provide a quantitative result.

$$\frac{(\frac{Neutrophil}{Lymphocyte} ratio \times CRP)}{10}$$

Equation 1:The novel sepsis Index formula

2.5 Statistical analysis

After the data clean stage, the data was statistically analysed within the Pathology department at CW-NHS, London using the statistical software package, Analyse-IT. To determine the appropriate statistical tool required, the data was tested for normal distribution. The statistical decision flowchart (see Figure 10) was used as a guide for statistically analysing the data.

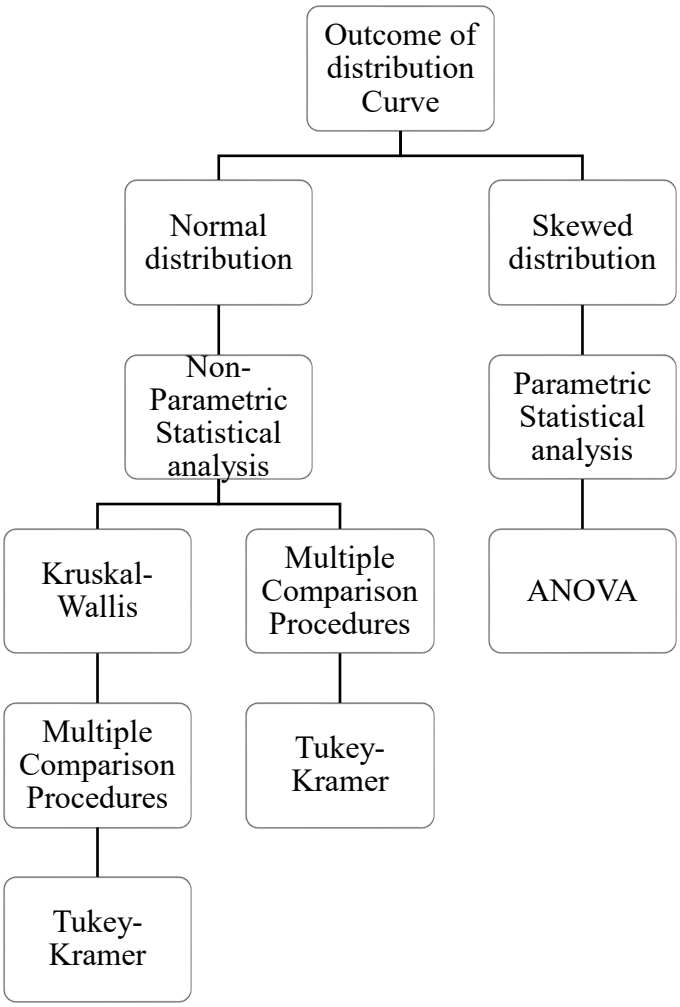

Figure 2:The statistical decision flowchart

The statistical tools that were required were normal distribution to determine whether further analysis required was parametric or non-parametric. Method comparison statistical analysis

was required to compare the performance of CRP from the POCT device and CRP from the biochemistry department using MULTIGENT CRP Vario which is a latex immunoassay on the Abbott Architect *c* analyser. Analyses were carried out in the statistical computer program (Analyse IT). Once the results from the normal distribution analysis were established, they were then further analysed using a selection of non-parametric tests (figure 10).

2.6 Ethics and General data Protection Regulation (GDPR)

The study was carried out in lines with the Chelsea and Westminster Hospital NHS Foundation Trust, ethics guidelines. as no samples were specifically being taken for this study and only data was used, the study was determined to be an audit and did not require NHS ethics. The primary analysis of all hospital data was carried out on secure NHS servers and the final spreadsheets that was produced had no patient identifiers listed therefore there is no way of tracing back the results to an individual patient. The working spreadsheet was stored on a secure file in box based on the university server, with only people directly involved in this study having access.

3.Results

3.1 Study Population

Over the course of nine months, patient data was manually collated from the Microsemi CRP haematology analyser set up in the Accident and Emergency department of Chelsea and Westminster Hospital, Chelsea and Westminster Hospital NHS Foundation Trust, London. (CW-NHS). The early phase of this study revealed a cohort of 662 patient data points however, only 412 patient data points were deemed viable and coincided with the inclusion criteria stipulated in this study. The excluded 250 patient data points as shown (see table 6) further details the reason for exemption from this study.

Table 6: Factors that are the reasons for exclusion from the study (n=250). n= Total number of patient data points excluded, %= Total percentage of patient data points excluded.

Reasons for exclusion from the Study	**n**	**%**
Adult samples	142	57
Incorrect hospital Number	70	28
No hospital Number	34	14
No patient results were found on Sunquest server	3	1
Duplicate samples	1	0.4

Although the POCT analyser was purchased for the sole intention to analyse samples from paediatric patients, Horiba has not introduced the safe operator sample type lockout like its counterparts. This resulted in 57 % of the excluded data points to be samples originated from adult patients upon reviewing their date of birth. Clinicians were still able to use the POCT analyser that was validated and approved by the Trust to be used for only paediatric patients. A further 25% were found to have incorrect patient number inputted by the user into the POCT device and therefore lost its traceability factor and to succeed to the next stage of this study of obtaining more clinical information. This study has also highlighted 14% of the excluded data points were breeching the local POCT Trust Policy and were not adhering to the POCT Microsemi Horiba Standard Operating Procedure (SOP), as there were no hospital number for the samples analysed on the device.

3.2 Paediatric ED rate of attendances in 2020

Paediatric A & E attendances at CW-NHS in 2020 has identified some potentially interesting trends which correspond to the national decisions enforced regarding Coronavirus-19 (COVID-19) and the public's reaction to COVID-19 (see Figure 11). Hospital Trusts were advised to concentrate their efforts in treating COVID-19, to redeploy hospital staff to newly constructed COVID-19 wards, reschedule non-urgent appointments and advise public to not attend the hospital with non-urgent symptoms. With this being public knowledge, this graph perfectly depicts the publics response to the pandemic and attending paediatric EDs with their children. Interestingly, this study has collected data from February 2020, before the World Health Organisation (WHO) had declared COVID-19 a global pandemic, the number of attendances to the Paediatric ED was greater than the consecutive month; March 2020 when national lockdown measures were enforced. In May 2020, the Paediatric ED saw a elevated number of paediatric cases which mimics the governments decision to begin easing lockdown restrictions in the same month. The following months in the summer period; June to July 2020 saw a decrease in hospital admissions which was expected as number of hospital admissions nationally decline in summer periods. In September 2020, the education secretary called for the decision for children to return to schools which the effects were seen in October 2020 with a significant increase in Paediatric ED attendances. This continued to increase until the end of the year which coincides with the governments acknowledgement of the increase in hospital admissions and rate of COVID-19 transmissions, this concluded the decision for the second lockdown to be enforced.

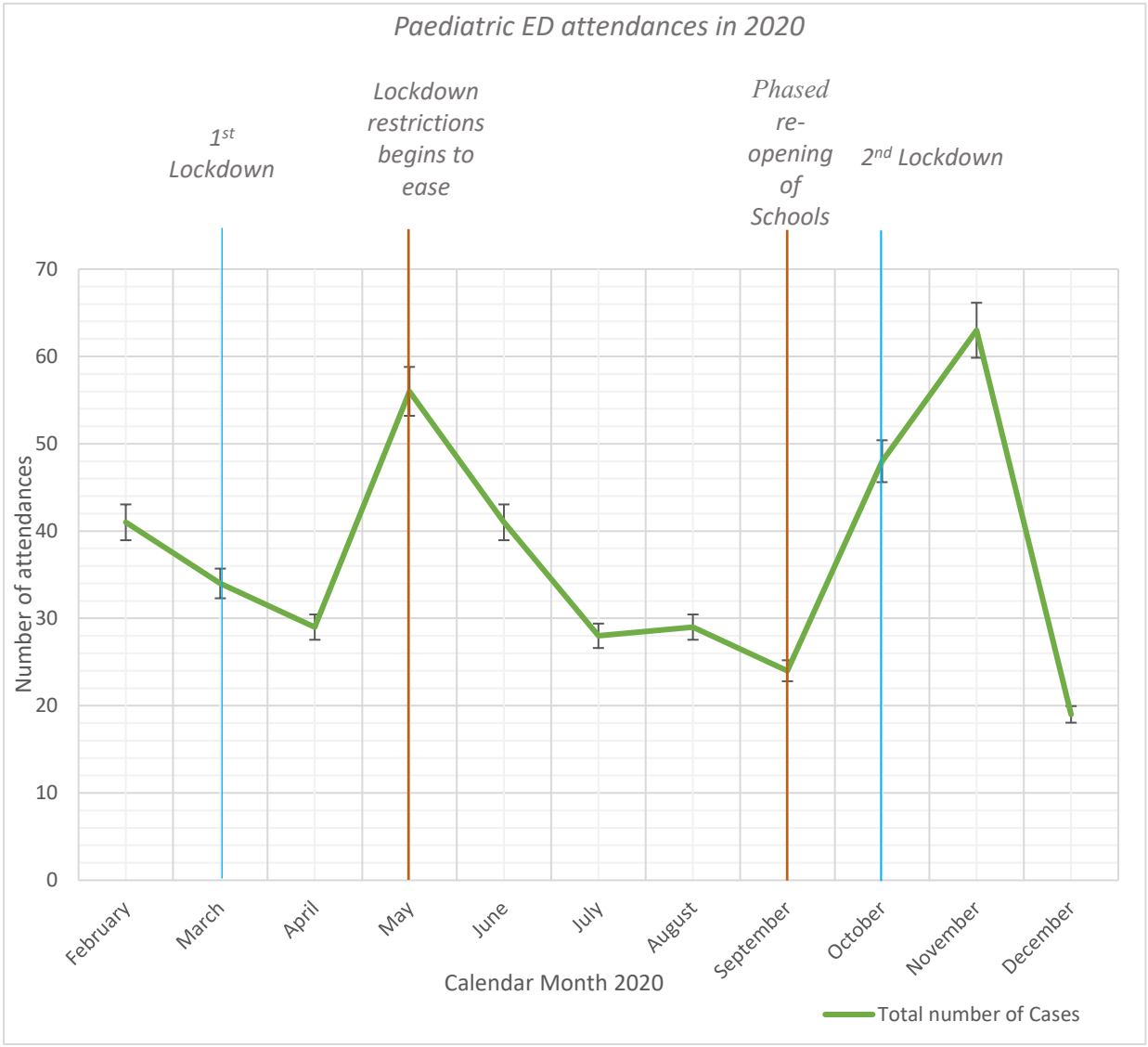

Figure 3:The total number of attendances in Paediatric ED during 2020, CW-NHS.

3.3 The findings from Paediatric ED admissions by age range groups in 2020.

In figure 12, demonstrate the age ranges for admissions at the Paediatric ED over the course of the study. The age group with the most admissions were those ranging from 6-year-olds to 12-year-olds, this was then followed by the 1-2 years old and finally by the 2–6-year-olds. Though the number of admissions for these groups varied across the months, but it can be seen the single largest intakes can be in 6-12 years olds in November 2020. The youngest age range groups 1-7 days demonstrated the least represented age range group in 2020, which is understandable with the lack of evidence published regarding the impact of COVID-19 in new-borns. Therefore, it could be suggested, parents/guardians of this age range groups were reluctant to attend the department during the pandemic. which poses the risk of severe clinical cases due to the delay in attending hospitals. Interestingly, in March 2020 when the COVID-19 pandemic was declared by WHO and the national lockdown imposed, the younger age range groups from 1 day to 3 months old did not attend the hospitals.

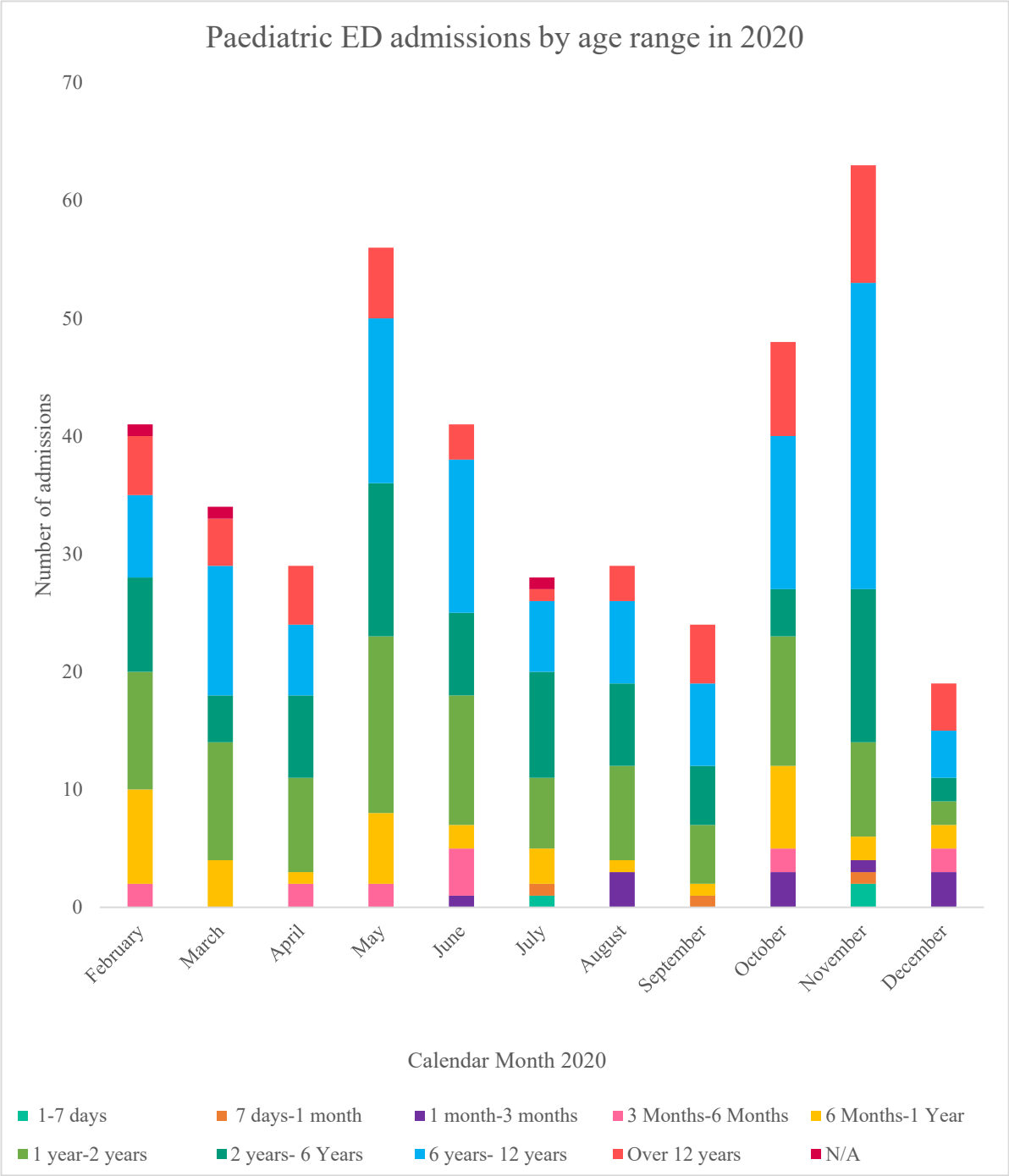

Figure 12: The total number of Paediatric ED admissions by age range groups in 2020, CW-NHS.

3.4 Clinical diagnosis results

The clinical diagnosis groups (table 7) reflect the clinical diagnosis that was recorded in the cloud based electronic health record systems; Cerner used by CW-NHS. The clinical diagnosis groups were further divided into four distinct classifications: Communicable diseases, non-Communicable diseases, Normal and Unknown. Within the communicable diseases, viral infections were the most prevalent clinical diagnosis made, followed by bacterial infections, this follows the consensus of viral infections being the most occurrent communicable diseases in paediatric health. As this study has interestingly been undertake during the pandemic in 2020, a total of 9 patients were classed by clinicians as suspected COVID-19. Although there was the introduction of the DNA nudge; a POCT device providing reverse transcription-polymerase chain reaction (RT-PCR) COVID-19 test, the patient samples were still sent to the infection and immunity department for confirmation. Fever without a source made up 20% of the non-communicable diseases category which highlights how vague this classification of diagnosis is. Some data within this classification were prescribed antibiotics and some were discharged immediately with no medication. Although the symptom of fever falls in many categories within the communicable diseases such as sepsis. However, other studies have highlighted the lack of literature present regarding targeted treatment of fever without a source, this can be seen in the results (Barbi, et al., 2017).

Table 7: The clinical diagnosis results collated from Cerner, Pathology department, CW-NHS.

Clinical diagnosis groups	n
Communicable diseases	**163**
Bacterial infections	49
COVID-19	9
Viral infections	83
Sepsis	22
Non-Communicable diseases	**224**
Fever without a source	44
Trauma	26
Other	154
Unknown	**9**
Normal	**16**

3.5 Definitive diagnosis results

Although, in Table 7, the clinical diagnosis was confirmed by clinicians on the patient records on Cerner, the definitive diagnosis group was created by comparing with the laboratory results and filtering non-confirmed diagnosis. Therefore, there has been a 42 % decrease in the communicable diseases category from clinical to definitive diagnosis. The difference may be due to a prolonged TAT for laboratory samples despite the urgency and therefore clinicians are pressured to make a clinician diagnosis without the laboratory results. Interestingly, the impact of the COVID-19 pandemic can be seen in both Table 7 and Table 8 as it had influenced clinicians to diagnose 9 paediatric patients swiftly clinically with COVID-19 however, once analysed by the virology department, only 5 patients were confirmed cases by the laboratory for COVID-19. However, the bacterial infections subcategory has seen an 10 % increase from clinical to definitive diagnosis, which is due to clinicians ordering blood and urine cultures as routine testing upon admissions which has surprisingly presented positive bacterial infection. Sepsis subcategory has seen a 50 % decrease from initial clinical diagnosis to definitive diagnosis which might have led to unnecessary antibiotic prescription or invasive treatment. This can all be prevented if there is a POCT device that can provide a rapid TAT, reduce inaccurate clinical diagnosis, reduce disease progression and better targeted use of antibiotics. Another concern observed in the definitive diagnosis groups was the misdiagnosis/not confirmed by the lab sub-category. After extensive tracing of patient data and evaluating against the initial clinical diagnosis,

the data belonging to this sub-category included samples not sent to the lab to confirm the clinical diagnosis and inaccurate clinical diagnosis made that did not reflect the laboratory results and the clinical presentation of the patient admitted. Therefore, this sub-category would have been significantly reduced if there was a POCT device in the acute ambulatory settings.

Table 8: The definitive diagnosis results collated from Cerner, Pathology department, CW-NHS

Definitive diagnosis groups	**n**
Communicable diseases	**95**
Bacterial infections	54
COVID-19	5
Viral infections	24
Sepsis	11
Non-Communicable diseases	**189**
Fever without a source	32
Trauma	26
Other	131
Unknown	**6**
Misdiagnosis/Not confirmed by lab	**112**
Normal	**14**

From the patient data points collated from the POCT device, it has provided beneficial analytics regarding the volume of bacterial culture tests the clinical laboratory turnover in this study. These analytics from the clinical laboratory can be integrated with the paediatric Accident and Emergency department to predict workloads, improve diagnostic test utilisation and clinical performance. In total 53 bacterial culture tests were ordered in this study with the 43 % of the bacterial culture tests ordered by clinicians being specifically for urine microscopy tests (see Table 9). Drain fluid, TB Screen, Pneumococcal Ab screen were only ordered once by clinicians in this study which shows the infrequency of certain bacterial culture tests to be ordered by clinicians in Paediatric Accident and Emergency department, CW-NHS. However, in comparison with the clinical diagnosis numbers in Table 7, it has shown more bacterial culture tests were ordered in this study than clinically diagnosed cases of bacterial infections. Therefore, this is reassuring the clinicians have been taking effective measures, to identify bacterial infections. Nevertheless, the introduction of using POCT devices would have tailored to the clinicians to a more informed decision and reduce unnecessary laboratory testing.

Table 9: The total of frequently ordered bacterial culture test ordered by the Paediatric Accident and Emergency department. Data collated from Sunquest, Pathology department, CW-NHS.

Bacterial Culture test	**n**
Blood Culture	7
Drain Fluid	1
Faecal Pathogens	2
Pus Swab/Aspirate	8
TB Screen	1
Throat Screen	2
Urine Microscopy	23
Wound Culture	5
Anti-Streptolysin Ab	3
Pneumococcal Ab	1

In this study, the findings from table 10 have shown the most common root of definitively diagnosed bacterial infections to be Gram-negative bacteria at 47%. Subsequently followed by Gram-positive bacteria at 43%. This provides a concerning finding as the Gram-negative rod infections in Paediatric cases which are associated with increased probability of morbidity and mortality (Oliveira & Reygaert, 2021). Therefore, it is important to improve the current method of diagnosing paediatric cases at acute ambulatory settings, there is an urgency to utilise the POCT devices and the clinical biomarkers available on the POCT device to prevent disease progression and ultimately sepsis.

Table 10: The root causes of definitive diagnosis of bacterial infections are listed and collated from Sunquest, Pathology department, CW-NHS (n=60). n=Total number of bacterial species.

Root causes of definitive diagnosis of bacterial infections	***n***	***%***
Gram Positive bacteria	**25**	**42**
Enterococcus sp.	*2*	
Coagulase negative staphylococci	*2*	
Gram positive Cocci	*2*	
Micrococcus luteus	*1*	
Streptococci Group A	*3*	
Streptococcus milleri	*4*	
*Streptococcus pneumoniae (*Pneumococcal Ab*)*	*2*	
Staphylococcus hominis	*2*	
Staphylococcus aureus	*7*	
Gram Negative bacteria	**29**	**48**
Escherichia coli	16	
Acinetobacter lwoffi	1	
Campylobacteriacae	2	
Coliform	3	
Pseudomonas aeruginosa	4	
Pseudomonas pudita	1	
Proteus mirabilis	1	
Serratia liquefaciens	1	
Other	**4**	**<0.1**
Mixed Bacterial Growth	4	
Mycobacterium tuberculosis	**1**	**<0.1**
Fungi	**1**	**<0.1**
Yeast	**1**	**<0.1**

Interestingly, definitive diagnosis of viral infections in this study have been shown to be caused a range of viral species in table 11. Unlike, bacterial species identified in table 10, the viral infections are not caused by a predominantly by a specific viral species, but rather there is no existing trend noticed. With 25% of the viral infections in this study was caused by the Epstein-Barr Virus followed by 17% from Rhinovirus. Therefore, clinicians must utilise the use of POCT devices to monitor the disease severity of viral infections and provide results of clinical biomarkers with a rapid turnaround time.

Table 11: Data collated from Sunquest, Pathology department, CW-NHS(n=36). n=Total number of virus species that are identified to be the root causes of definitive diagnosis of viral species.

Root causes of definitive diagnosis of viral infections	**n**
Adenovirus	2
Cytomegalovirus	3
Epstein-Barr virus	9
Enterovirus	6
Human Herpesvirus 6	1
Human Metapneumovirus	1
Herpes simplex virus	1
Influenza A	1
Measles	2
Respiratory Syncytial virus	1
Rhinovirus	6
Rubella	1
Varicella Zoster virus	2

3.5. Statistical analysis

3.5.1 Method Comparison-Passing-Bablok Regression

Instrument validity is imperative part of introducing a new POCT device in a clinical setting distanced from the central laboratory. Therefore, in 2018 the Microsemi Horiba CRP LC-667G Haematology analyser was successfully validated before the final installation of the device in the Accident and Emergency department, CW-NHS, London. However, to establish the integrity of this study, a method comparison analysis using Passing-Bablok regression was still conducted (see Figure 13). The method comparison involved the CRP values obtained from the Microsemi Horiba CRP LC-667G Haematology analyser and the CRP values obtained from the Abbott Architect i2000sr analyser in the Biochemistry department, CW-NHS, London which are the same analysers used in initial validation process in 2018. The Passing-Bablok regression line equation for this study in Figure 13 was y= 0.5399 + 1.103 which has reconfirmed that the two methods of obtaining CRP values are comparable and within the investigated concentration range. Although there are residuals from the plot have revealed the presence of more outliers the higher the CRP value, the plots are in good agreement. The reason for such outliers, could be due to the reduced sample size this study has obtained. A larger sample size would have improved the intercept and slope parameters of this analysis.

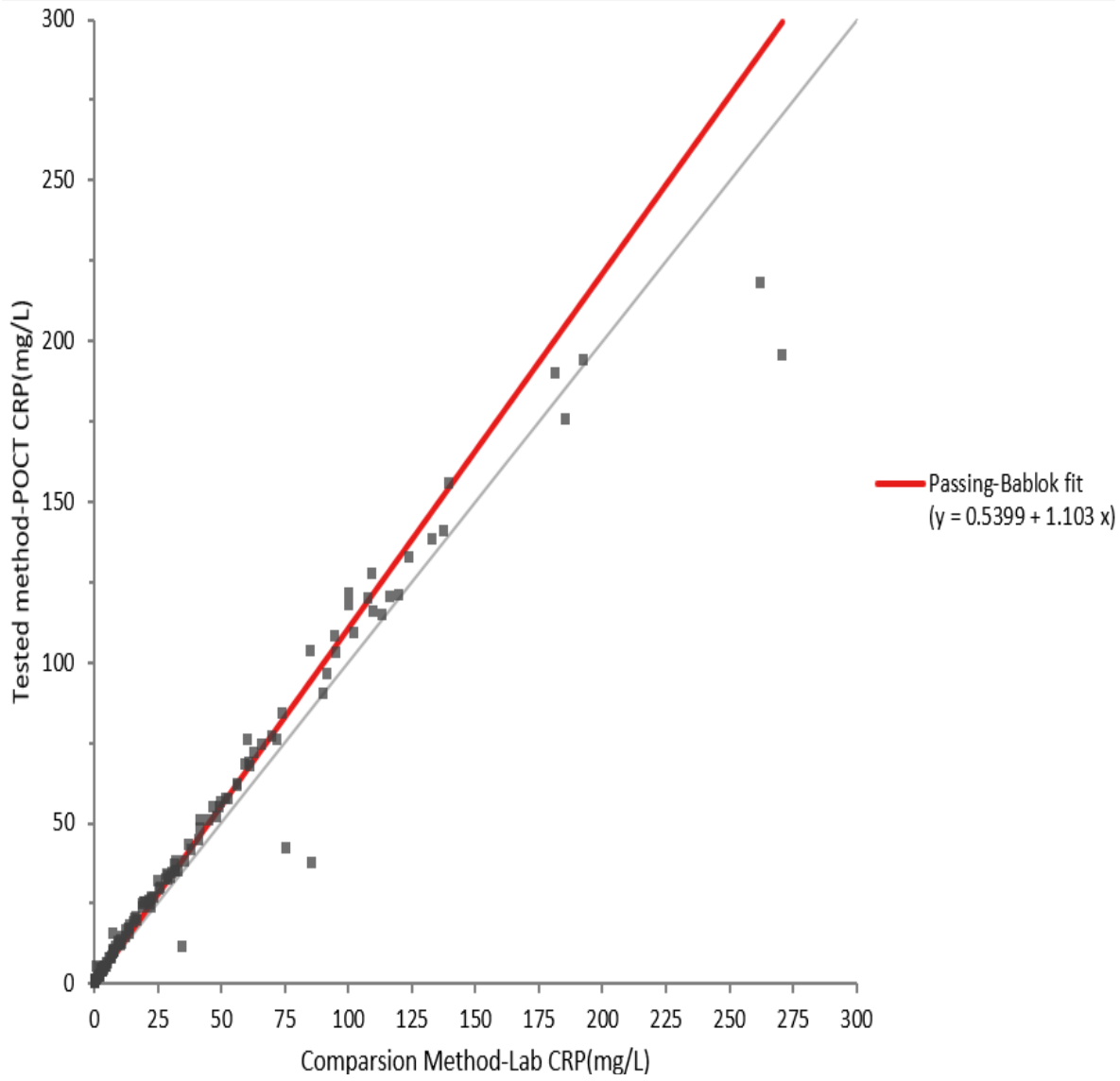

Figure 13: Passing-Bablok regression analysis of CRP values using Analyse-IT. Comparison Method-Lab CRP (mg/L) = C-reactive protein (mg/L) analysed using the Abbott Architect i2000sr analyser in the Biochemistry department, CW-NHS. Tested Method-POCT CRP (mg/L) = C-reactive protein (mg/L) analysed on the Horiba Microsemi CRP analyser at CW-NHS

Table 12: The parameters obtained from the Passing-Bablok regression analysis. Passing-Bablok Regression line equation: y= 0.5399 + 1.103 x; 95% Cl for intercept 0.2193-0.8835 and for slope 1.076 to 1.125 indicated overall good agreement.

Parameter	Estimate	Bootstrap 95% CI
Intercept	0.5399	0.2193 to 0.8835
Slope	1.103	1.076 to 1.125

3.5.2 POCT CRP by clinical diagnosis groups

To compare the effectiveness of POCT CRP (mg/L) in each clinical diagnosis sub-categories, Kruskal-Wallis statistical analysis was used (table 13). As expected, bacterial infections with the highest interquartile range of 107.84 mg/l, this explains the most concentration of the data points are of a higher CRP value which confirms the prediction that bacterial infections have higher CRP values seen previously in Figure 5. Viral infections have shown to have a significantly lower interquartile range of 29.76 mg/l and median of 12.90 mg/l compared to bacterial infections, attesting that CRP values are considerably lower in viral infections. Interestingly, COVID-19 has shown higher IQR of 63.34 mg/l and Median 35.90 mg/l, which was higher than what was seen in viral infections.

Although, COVID-19 is a viral infection, it does not a low IQR seen in other sub-groups and higher interquartile range of 63.34 mg/l and median of 35.90 mg/l. This can be explained due to COVID-19 induces a cytokine storm and therefore the CRP values elevate. Overall, these results have provided an insight into the potential usability of POCT CRP in differentiating communicable diseases.

Table 23: The Kruskal Wallis test determining the relation between clinical diagnosis groups collated from Cerner and POCT CRP obtained from the Horiba Microsemi CRP analyser.

POCT CRP (mg/L) by Clinical diagnosis groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
Bacterial	0.0	8.69	34.85	116.53	195.4	107.84
COVID	0.0	8.79	35.90	72.13	103.0	63.34
Normal	0.0	0.00	1.00	3.88	45.3	3.88
Sepsis	1.5	6.51	21.10	58.05	218.2	51.54
Unknown	0.0	5.47	32.50	85.17	176.9	79.7
Viral	0.0	4.27	12.90	34.03	189.9	29.76

3.5.3. Neutrophil-lymphocyte ratio (NLR) by clinical diagnosis groups

In the Appendix figure 21, the Kruskal-Wallis analysis explores the effectiveness of NLR at differentiating clinical diagnosis groups. Interestingly, viral infections presented the highest NLR value of 26.25 which could be due to the extreme levels of Neutrophils present during a viral infection. However, in Table 14 the sub-category of bacterial infections had the highest IQR of 4.383 as expected. Subsequently, followed by COVID-19 infections with an IQR of 3.038 which highlights the how COVID-19 differs with viral infections. Unexpectedly, sepsis has shown to have a lower IQR value of 2.333 compared to bacterial and viral infections despite its clinical severity, this may raise a question with the distinguishing ability to be a clinical biomarker for Sepsis. However, the 'Normal' sub-category consisted of only healthy patients, the NLR values remained low and presented the lowest IQR of 0.981 seen in the appendix. This highlights the prognostic value of NLR of diagnosing communicable diseases and with a larger sample pool, the statistics could be statistically significant.

Table 244: The Kruskal Wallis test determining the relation between clinical diagnosis groups collated from Cerner and NLR obtained from the Horiba Microsemi CRP analyser.

NLR by Clinical diagnosis groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
Bacterial infections	0.35	1.346	2.529	5.729	13.71	4.383
COVID	0.28	0.902	1.400	3.940	14.64	3.038
Normal	0.32	0.790	1.346	1.771	7.09	0.981
Sepsis	0.06	0.719	1.853	3.052	5.94	2.333
Unknown	0.43	0.429	1.183	1.938	1.94	1.509
Viral	0.13	0.628	1.556	3.046	26.25	2.418

3.5.4 SI by clinical diagnosis groups

Referring to Table 15, the novel SI was analysed against the clinical diagnosis groups and the graph available in the Appendix. Although, the intention for this SI was to detect sepsis by producing a significantly elevated result, the findings have shown quite opposite and in fact bacterial and viral infections have the maximal SI values of 124.93 and 157.47. Sepsis does in fact have a high IQR of 19.384 which does improve the performance of SI, but if the sample size for sepsis cases was significantly reduced in comparison to bacterial infections which may explain the findings. Bacterial infections have the highest IQR of 25.207 with Sepsis coming in second with 19.384, which does the performance of SI for Sepsis. But the sample must be increased.

Table 15: The Kruskal Wallis test determining the relation between clinical diagnosis groups collated from Cerner and SI obtained from the Horiba Microsemi CRP analyser.

SI ratio by Clinical diagnosis groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
Bacterial infections	0.22	3.940	12.958	29.147	124.93	25.207
COVID-19	0.23	0.810	5.760	11.790	67.69	10.98
Normal	0.16	0.395	0.673	0.886	7.30	0.491
Sepsis	0.04	0.740	2.326	20.124	72.91	19.384
Unknown	0.21	0.214	0.592	0.969	0.97	0.755
Other Viral infections	0.06	0.586	2.808	10.714	157.47	10.128

3.5.5 POCT CRP by Definitive diagnosis groups

Table 16 focuses on the impact of POCT CRP has with the definitive diagnosis groups, Table 16 highlighted the highest IQR values which was from the sub-category of Rhinovirus/Enterovirus with a bacterial infection of 135.67 mg/l, the elevated CRP level is primarily due to the presence of a secondary co-infection. Viral infections have also exhibited a high IQR value of 89.39 mg/l in comparison to Bacterial infections of 79.22 mg/l and sepsis of 36.10 mg/l cases. Therefore, this finding contradicts the raises concern regarding the ability of POCT CRP as a clinical biomarker.

Table 25: The Kruskal Wallis test determining the relation between definitive diagnosis groups collated from Cerner and POCT CRP analysed on the Horiba Microsemi CRP analyser.

	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) Normal	0.0	0.00	0.90	4.17	45.3	4.17
b) Sepsis	2.4	12.68	35.10	48.78	218.2	36.10
c) Bacterial	0.0	7.68	41.15	86.90	195.4	79.22
d) Viral	0.8	8.19	39.95	97.58	184.8	89.39
e) COVID	8.0	27.93	55.10	90.53	103.0	62.6
f) Rhinovirus/Enterovirus with a bacterial infection	37.7	39.49	78.45	175.16	218.2	135.67
g) Unknown	0.0	6.05	33.70	87.71	176.9	81.66

3.5.6 NLR by Definitive diagnosis groups

Bacterial infections presented the highest IQR on Table 17 of 5.554 which can be seen to have the highest NLR value of 26.50. Sepsis also has seen unexpectedly an IQR value of 1.983 which is considerably lower than bacterial, viral and COVID-19 sub-categories. The prediction for Figure 21 was to have higher NLR values for sepsis cases and Rhinovirus/Enterovirus with a bacterial infection case. The fact that Table 17 does not present a similar finding reaffirms that there is no statistical significance seen with NLR in definitive and clinical diagnosis in this study. However, the illustrated the usefulness of NLR on definitive diagnosis groups can be found in Appendix A.

Table 17: The Kruskal Wallis test determining the relation between definitive diagnosis groups collated from Cerner and NLR obtained from the Horiba Microsemi CRP analyser. NLR= Neutrophil/ lymphocyte ratio values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

NLR by Definitive diagnosis groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) Normal	0.32	0.712	0.897	1.678	7.09	0.966
b) Sepsis	0.71	1.244	2.396	3.227	5.94	1.983
c) Bacterial	0.20	1.259	2.529	6.813	26.50	5.554
d) Viral	0.20	0.964	2.527	4.133	11.20	3.169
e) COVID	0.28	0.730	1.922	3.940	6.57	3.210
f) Rhinovirus/ Enterovirus with a bacterial infection	0.35	0.512	1.514	2.431	2.53	1.919
g) Unknown	1.94	1.938	1.938	1.938	1.94	0.000

3.5.7 SI by Definitive diagnosis groups

For the data shown in Table 18, the viral infections exhibit the highest IQR of 156.16 and therefore presents a conflicting result for the effectiveness of the sepsis index. It was anticipated that Sepsis sub-category would have the highest maximum SI value and IQR but is found to be at 71.08 for the IQR. Interestingly, the COVID-19 sub-category presented the third highest IQR of 62.76 higher than bacterial infections of 40.851. This highlights the inflammatory nature of COVID-19 and can immunologically present as severe viral infection and possibly sepsis. Although, the findings from Table 18 were not expected, it has shown the possibility of using the Sepsis index formula to aid in detection of severe bacterial and viral infections in paediatric patients.

Table 18: The Kruskal Wallis test determining the relation between definitive diagnosis groups collated from Cerner and SI which its parameters obtained from the Horiba Microsemi CRP analyser.

SI by Definitive diagnosis groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) Bacterial	0.10	1.047	10.869	41.898	353.06	40.851
b) COVID-19	0.23	4.930	8.047	32.204	67.69	62.76
Normal	0.16	0.356	0.619	0.839	7.30	6.944
Rhinovirus/ Enterovirus with a bacterial infection	2.77	3.305	7.339	33.609	50.03	46.725
Sepsis	0.35	1.830	6.706	23.273	72.91	71.08
Unknown	0.97	0.969	0.969	0.969	0.97	0.001
Viral	0.10	1.309	8.928	26.199	157.47	156.16

3.5.8 The statistical significance of clinical biomarkers on the clinical and definitive diagnosis groups

Overall, Table 19 encompasses the statistical significance of all clinical biomarkers, clinical and definitive diagnosis sub-categories. The mean value for CRP clinical for bacterial infections is the highest at 61.10 mg/l and subsequently decreases to 57.6 mg/l when transitioning from CRP clinical to CRP definitive diagnosis. The mean value decrease is due to the study filtering clinical diagnosis bacterial infections into definitively diagnosed as bacterial infections with confirmation from the laboratory. However, both groups on Table 19 are classed as statistically non-significant due to the p-value being 0.400 for CRP clinical and 0.990 for CRP definitive. The p-value of 0.780 can be found in the NLR clinical diagnosis analysis which shows that solely using NLR as distinguishing between bacterial and viral infections is ineffective. The SI clinical and definitive parameters were also deemed as statistically non-significant with a p-value of 0.330 for SI clinical and an increase of 0.490 in SI definitive. Therefore, this finding may raise a question on the effectiveness of using clinical biomarkers to successfully distinguish between bacterial and viral infections. However, it can be used to highlight clinicians to conduct the appropriate tests for the patient in question.

Table 19: The statistical significance of the clinical biomarkers analysed in this study on the clinical and definitive diagnosis groups.

General statistics				
	Mean value		**P-value**	**Statistical significance**
	Bacterial	**Viral**		
CRP Clinical	61.1 mg/l	25.50 mg/l	0.400	Not Significant
CRP Definitive	57.6 mg/l	56.01 mg/l	0.990	Not Significant
NLR Clinical	3.96	3.41	0.780	Not Significant
NLR Definitive	5.35	3.31	0.546	Not Significant
SI Clinical	23.8	13.31	0.330	Not Significant
SI Definitive	40.8	22.60	0.490	Not Significant

3.5.9 POCT CRP by Length of Stay groups

In the data illustrated in Table 20 which monitored the potential of using POCT CRP to determine length of stay. The findings presented to be inconclusive to provide any solid form of hospital analytics however, there is potential for using POCT devices in analytics. The highest IQR of 143.10 mg/l was seen as predicted in the longest length of stay group; 14 days-4 weeks, however, only three data points were found to be appropriately classified for this group. This means a larger sample size was needed to provide a more conclusive finding. Subsequently, 2 days-7 days sub-group had the higher IQR of 96.14 mg/l than 7 days- 14 days which was 78.63 mg/l. This further emphasises the irregular trend found in Table 20 as it was expected that the POCT CRP values would increase, the longer the length of hospitalisation was for the patient.

Table 26: The Kruskal-Wallis test to determining the statistical significance between the Length of Stay groups and the POCT CRP obtained from the Horiba Microsemi CRP analyser.

POCT CRP (mg/L) by Length of Stay group	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) ED only	0.00	0.90	9.10	42.28	162.60	41.38
b) 24 hours-2 days	0.00	12.43	41.60	96.13	184.80	83.7 0
c) 2 Days-7 Days	0.80	11.68	37.80	107.82	218.20	96.14
d) 7 Days-14 Days	54.30	63.22	97.75	141.85	157.600	78.63
e) 14 Days-4 Weeks	5.50	5.50	77.05	148.60	148.60	143.10
f) N/A	2.80	23.87	55.10	111.07	176.90	87.20

3.5.10 NLR by Length of Stay groups

The Kruskal-Wallis testing used to determine the relationship between the Length of stay groups and their NLR has shown no overall statistical significance (see appendix). However, if this study were conducted with a much larger sample pool, this anticipates an improvement in the statistical analysis. Nevertheless, the findings as there are marginal differences with each Length of stay sub-category. Interestingly, the most extended Length of stay sub-category, as seen in this study, 14 days to 4 weeks, only saw 3 cases of hospitalisation meeting this sub-category; this sub-category has the highest IQR on NLR of 20.94. Shockingly, the non-applicable (N/A) sub-category which found to be inadequate to be categorised in any of the length of stay sub-groups presented with a much higher IQR of 18.74 than 7 days to 14 days which was at 8.32. This further highlighting the statistical non-significance this analysis has presented.

Table 21: The Kruskal-Wallis test to determining the statistical significance between the Length of Stay groups and the NLR obtained from the Horiba Microsemi CRP analyser.

NLR by Length of Stay group	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) ED only	0.20	0.80	1.43	2.35	26.50	1.85
b) 24 hours-2 days	0.20	1.04	2.58	5.08	11.20	4.01
c) 2 Days-7 Days	0.21	0.72	2.78	5.85	22.00	5.13
d) 7 Days-14 Days	1.54	1.69	3.04	10.01	14.17	8.32
e) 14 Days-4 Weeks	2.50	2.50	12.97	23.44	23.44	20.94
f) N/A	2.62	2.62	2.63	21.36	25.11	18.74

3.5.11 SI by Length of Stay groups

Table 22 has shown similar data as reported in Table 21 with an unexpected IQR result of 293.13 from the N/A sub-category which has a much higher IQR than 7 days-14 days group. Therefore, this finding has rendered the use of SI in determine the length of stay however, more extensive studies can be completed to ensure the reduced sample size in this current study has not impacted the findings in Table 22. The lengthiest hospitalisation stays (14days-4weeks) presented the highest IQR of 293.13 which may show the severity of their diagnosis, leading to higher SI which in result is longer hospital admissions. The investigation into the using SI as predicting hospitalisation rates may be useful adult intensive care units which are currently, pressurised by the vast number of patients with higher severity of disease and one of most long-term patient admissions.

Table 27: The Kruskal-Wallis test to determining the statistical significance between the Length of Stay groups and the SI which its parameters obtained from the Horiba Microsemi CRP analyser.

SI by Length of Stay group	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) ED only	0.10	0.40	0.85	10.44	288.59	10.04
b) 24 hours-2 days	0.10	1.36	11.68	52.39	157.47	51.03
c) 2 Days-7 Days	0.11	2.90	9.67	37.21	124.93	34.31
d) 7 Days-14 Days	11.68	16.24	44.27	72.33	76.93	56.09
e) 14 Days-4 Weeks	1.25	1.25	174.82	348.38	348.38	347.13
f) N/A	1.31	3.50	14.46	296.63	353.06	293.13

3.5.12 The statistical significance of Length of Stay

Although this pilot study had the intention of creating another clinical solution for ED seeing the highest influx of patients, the analysis is statistically significant for only CRP definitive and SI Definitive, However, it is not significant for NLR definitive as seen in Table 22. The highest mean value of 174.817 was found in SI definitive and in the 14 days-4 days sub-group. Upon reviewing this mean value, NLR was seen to the least effective in monitoring the length of stay and with SI being the more effective. Nevertheless, the p-value of CRP and SI definitive was <0.001 and NLR definitive was 0.0940 rendering the analysis as impracticable for clinical use at the moment.

Table 23: The overall statistical significance and mean-value of the clinical biomarkers by the length of Stay

Length of stay								
	Mean value						**P-value**	**Statistical significance**
	ED only	**24 Hours- 2 days**	**2 Days- 7 Days**	**7 Days- 14 Days**	**14 Days- 4 Weeks**	**N/A**		
CRP Definitive	21.25	45.16	55.17	61.68	77.05	53.10	<0.0001	Significant
NLR Definitive	2.703	3.567	4.196	5.445	12.972	10.117	0.0940	Not Significant
SI Definitive	10.960	24.757	30.192	31.530	174.817	62.600	<0.0001	Significant

3.5.13 POCT CRP by Age range groups

Age-range groups were analysed as against the CRP to determine a statistical pattern for better hospital management. In Table 24, the highest IQR of 141.91 mg/l was found in N/A category which has raise immediately raised a question on the practically of using CRP. As it was expected, the highest IQR result to be found in the older age rage sub groups of over 12 years of age. Nevertheless, the over 12 years category did have a higher IQR of 104.54 mg/l in comparison to the 6-12 years of 98.59 mg/l and 3-6 months of 26.41 mg/l. Interestingly, an IQR of 0.00 was found in the youngest age range categories in this study which were 1-7 days and 7days-1 month, this can be due to the reduced sample pool for these categories of the infancy of their immune system. Although, this finding does present a marginal trend with the CRP values progressing in age range groups it still remains insignificant for clinical judgements.

Table 24: The Kruskal-Wallis test to determining the statistical significance between the Age range groups and the POCT CRP obtained from the Horiba Microsemi CRP analyser.

POCT CRP (mg/L) by Age range groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) 1 Day-7 Days	6.9	6.90	6.90	6.90	6.9	0.00
b) 7 Days-1 Month	0.0	0.00	0.00	0.00	0.0	0.00
c) 1 Month-3 Months	0.0	0.00	0.45	0.90	0.9	0.90
d) 3 Months-6 Months	5.5	8.77	25.10	35.18	37.2	26.41
e) 6 Months-1 Year	0.0	7.99	30.75	55.63	218.2	47.64
f) 1 Year-2 Years	2.8	36.90	76.10	119.97	175.4	83.07
g) 2 Years-6 Years	0.0	13.30	45.30	104.97	184.8	91.67
h) 6 Years-12 Years	0.0	0.89	25.30	99.78	195.4	98.59
i) Over 12 Years	0.0	1.03	32.50	105.57	176.7	104.54
j) N/A	6.6	18.77	79.60	160.68	176.9	141.91

3.5.14 Neutrophil-Lymphocyte Ratio (NLR) by Age range groups

In Table 25, an IQR result of 0.00 was found for first three youngest age range groups (1-7 days, 7 days-1 month and 1-3 months) and therefore results are insignificant. The highest IQR of 8.388 was found in 2-6 years rather than over 12 years which was 4.553. Therefore, NLR cannot be used solely to present trends in age range groups and does not show an increase of NLR values with the older the patient was in this study. A lower IQR of 0.823 was found in 1-2 years compared to 3-6 months of 2.076. This irregularity presented in this finding shows how clinical biomarkers may not be useful in gathering demographic trends.

Table 25: The Kruskal-Wallis test to determining the statistical significance between the Age range groups and the NLR obtained from the Horiba Microsemi CRP analyser.

NLR by Age range groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) 1 Day-7 Days	0.897	0.897	0.897	0.897	0.897	0.00
b) 7 Days-1 Month	0.373	0.373	0.373	0.373	0.373	0.00
c) 1 Month-3 Months	0.797	0.797	0.797	0.797	0.797	0.00
d) 3 Months-6 Months	1.581	1.734	2.500	3.810	4.071	2.076
e) 6 Months-1 Year	0.197	0.423	0.867	2.428	4.031	2.005
f) 1 Year-2 Years	0.353	2.043	2.551	2.866	3.089	0.823
g) 2 Years-6 Years	0.564	1.452	4.810	9.840	26.500	8.388
h) 6 Years-12 Years	0.439	1.306	3.700	7.156	23.444	5.85
i) Over 12 Years	1.524	1.930	2.036	6.463	10.333	4.553
j) N/A	-	-	-	-	-	-

3.5.15 SI by Age range groups

Similar to Table 25, Table 26 captures the ineffectiveness of SI to use for demographic analysis. With an inconclusive IQR result of 0.00 found in 1-7 days, 7 day-1 month and 1-3 months. Although there is a general increase seen with the older the age range groups, the higher the IQR, 6-12 years seen to have the highest IQR of 57.74 than over 12 years of 47.71. Therefore, this finding cannot be used for clinicians to make quicker judgements. However, with an additional study completed focusing on demographic analysis and ensuring an increased sample size, it could lead to a conclusive finding.

Table 26: The Kruskal-Wallis test to determining the statistical significance between the Age range groups and the SI which its parameters obtained from the Horiba Microsemi CRP analyser.

SI by Age range groups	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range (IQR)
a) 1 Day-7 Days	0.62	0.62	0.62	0.62	0.62	0.00
b) 7 Days-1 Month	0.19	0.19	0.19	0.19	0.19	0.00
c) 1 Month-3 Months	0.40	0.40	0.40	0.40	0.40	0.00
d) 3 Months-6 Months	1.25	2.02	5.88	9.50	10.22	7.48
e) 6 Months-1 Year	0.10	0.33	2.39	10.62	50.03	10.29
f) 1 Year-2 Years	1.31	7.24	19.10	25.28	52.62	18.04
g) 2 Years-6 Years	0.28	6.00	11.68	52.94	353.06	46.94
h) 6 Years-12 Years	0.22	0.70	13.55	58.44	348.38	57.74
i) Over 12 Years	0.76	0.97	7.13	48.68	135.06	47.71
j) N/A	-	-	-	-	-	

3.5.16 The statistical significance of Age Range Groups

This study has investigated effectiveness of using clinical biomarker in demographic analysis and unfortunately, the statistical analysis is proved to be non-significant in Table 26. CRP definitive shows more promising results than NLR with mean value, gradually increasing as the older the patients are. The highest CRP mean value of 84.23 is seen in over 12 years groups and the lowest of 0.00 in 1-7 days, however, the p-value is 0.2459. NLR remains the least effective clinical biomarker with discrepancies in the results leading to incalculable (Incal) p-value. SI has shown to also present irregular mean values with the highest SI mean value found in the 6-12 years group not 12 years and furthermore the p-value of 0.0864 eliminates the use of SI.

Table 27: The overall statistical significance and mean values for clinical biomarkers against the age range groups.

Age range groups										
	Mean value								**P-value**	**Statistical significance**
	1 Day-7 Days	1 Month-3 Months	3 Months-6 Months	6 Months-1 Year	1 Year-2 Years	2 Years-6 Years	6 Years-12 Years	Over 12 Years		
CRP Definitive	0.00	0.00	0.00	36.84	94.35	56.46	52.24	84.23	0.2459	Not Significant
NL ratio Definitive	0.658	1.677	1.734	1.234	1.956	4.453	5.352	3.757	Incal	Not Significant
SI Definitive	0.414	4.236	3.394	6.036	9.341	26.057	33.316	15.607	0.0864	Not Significant

3.5.17 POCT CRP by gram-staining groups

Figure 14 as below has presented promising findings illustrating the distinguishing ability POCT CRP has with Gram-positive and Gram-negative bacteria. Due to the disease severity experienced in paediatric cases with Gram-negative, it was expected to have a higher CRP value. The Kruskal-Wallis analysis shows a gram-negative to have higher CRP values range from 80-200 mg/l in than Gram-positive of 60-160 mg/l. Therefore, CRP can be used by clinicians to as a semi-diagnostic tool and can prevent disease severity in bacterial infections. Interestingly, a mixed culture of Gram-negative and Gram-positive bacteria has a reduced CRP values ranging from 0-120 mg/l. The highest mean value can be seen in Gram-negative bacteria.

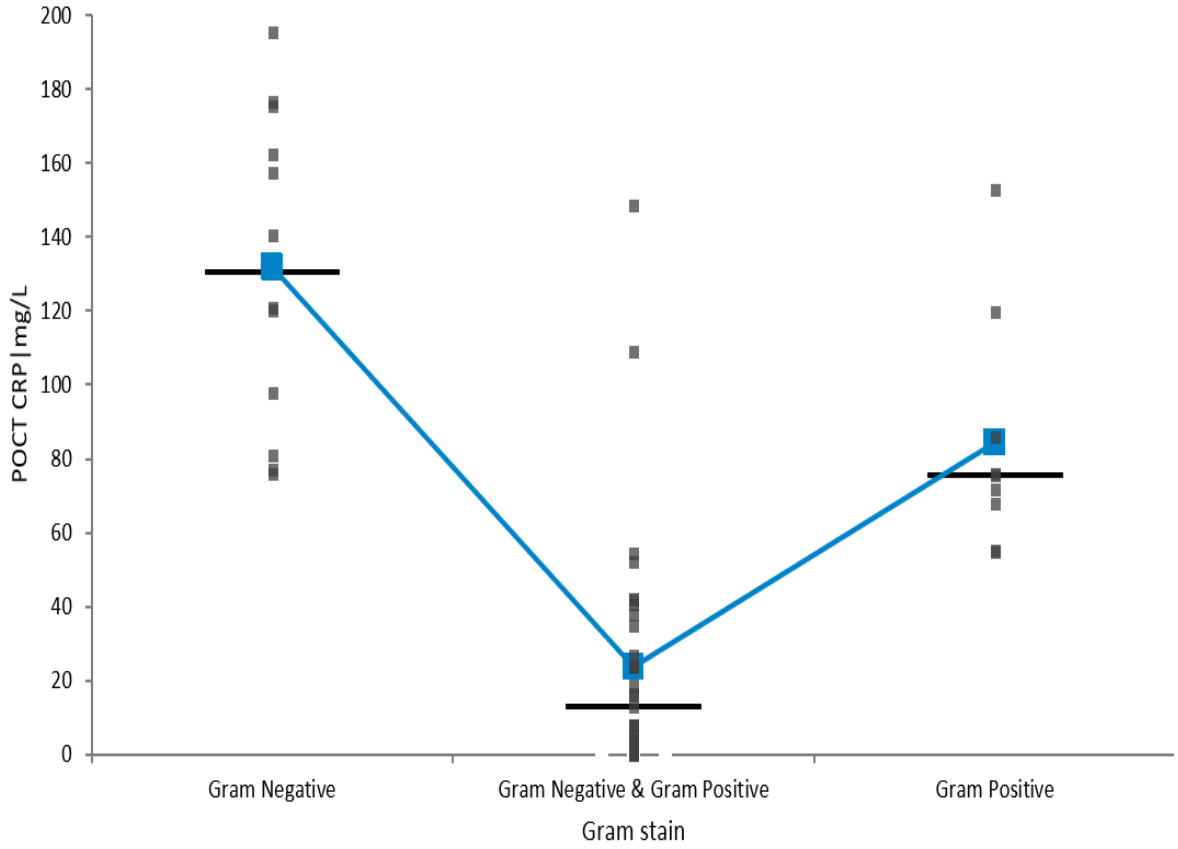

Figure 14: The Kruskal-Wallis test to determining the statistical significance between the Gram staining of bacterial species identified in this study and the POCT CRP obtained from the Horiba Microsemi CRP analyser. Gram stain: POCT CRP= C-reactive Protein values obtained from the POCT device in Paediatric ED, CW-NHS, London.

3.5.18 Neutrophil-Lymphocyte Ratio (NLR) by gram-staining groups

Figure 15 as below investigated the potential use of NLR in predicting gram staining of bacterial culture. The findings were inconclusive, with a mixed Gram-negative and Gram-positive bacterial culture group producing the highest NLR values. Gram-negative and Gram-positive groups presented similar range and NLR values rendering the effectiveness of NLR. Although, there might be an anomaly with a high value of 25 found in the gram-negative group, overall, there is no clear distinction as seen in figure 15.

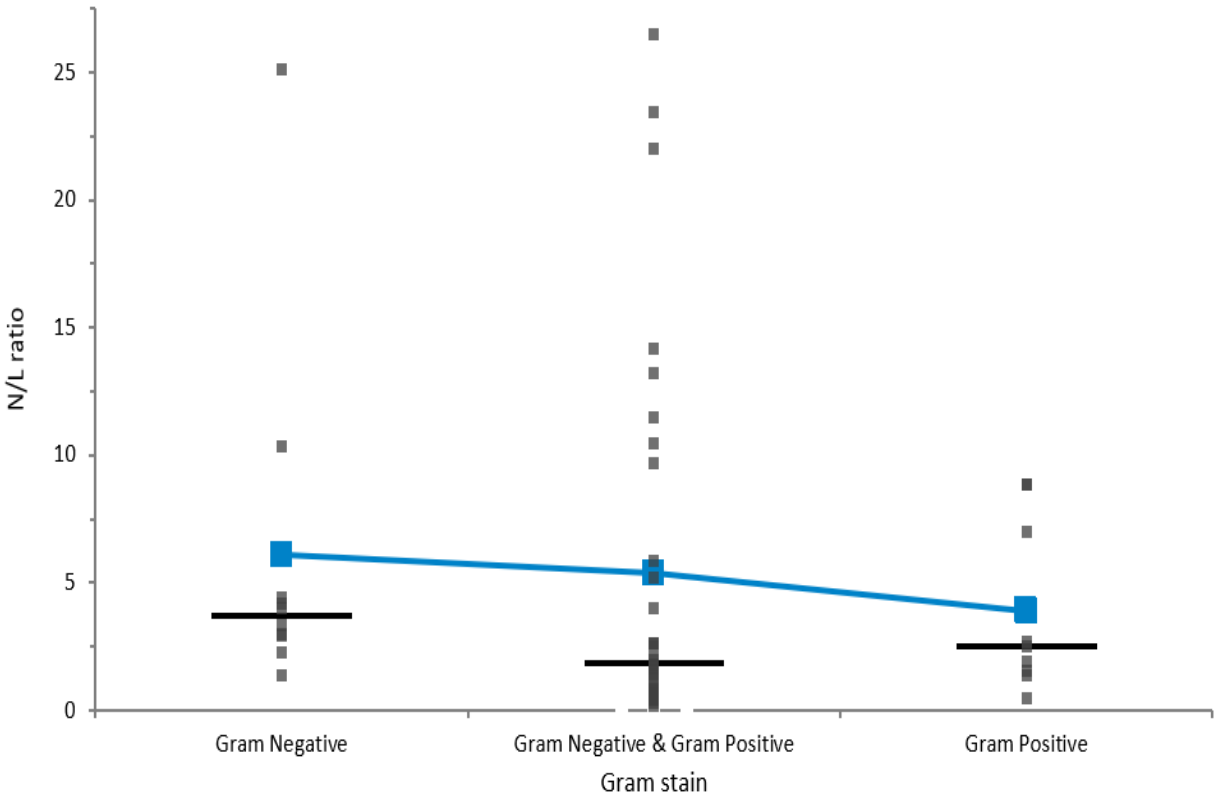

Figure 4:The Kruskal-Wallis test to determining the statistical significance between the Gram staining of bacterial species identified in this study and the NLR obtained from the Horiba Microsemi CRP analyser. Gram stain: NLR= Neutrophil/ lymphocyte ratio values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

3.5.19 SI by gram-staining groups

Figure 16 as below used the sepsis index created for this current study to investigate its discriminative abilities in gram-stain results, as it is important for SI to detect the possibility of severe bacterial infections and sepsis. Gram-negative bacteria can result in sepsis if left clinically untreated and theoretically should have a higher SI value. With a small sample size, SI still managed to produce a much higher SI range of 0 to 350 in comparison to gram-positive of 0-150. Therefore, SI has shown promise to be effective as another semi-diagnostic tool for clinicians for diagnosing bacterial infections and reducing sepsis.

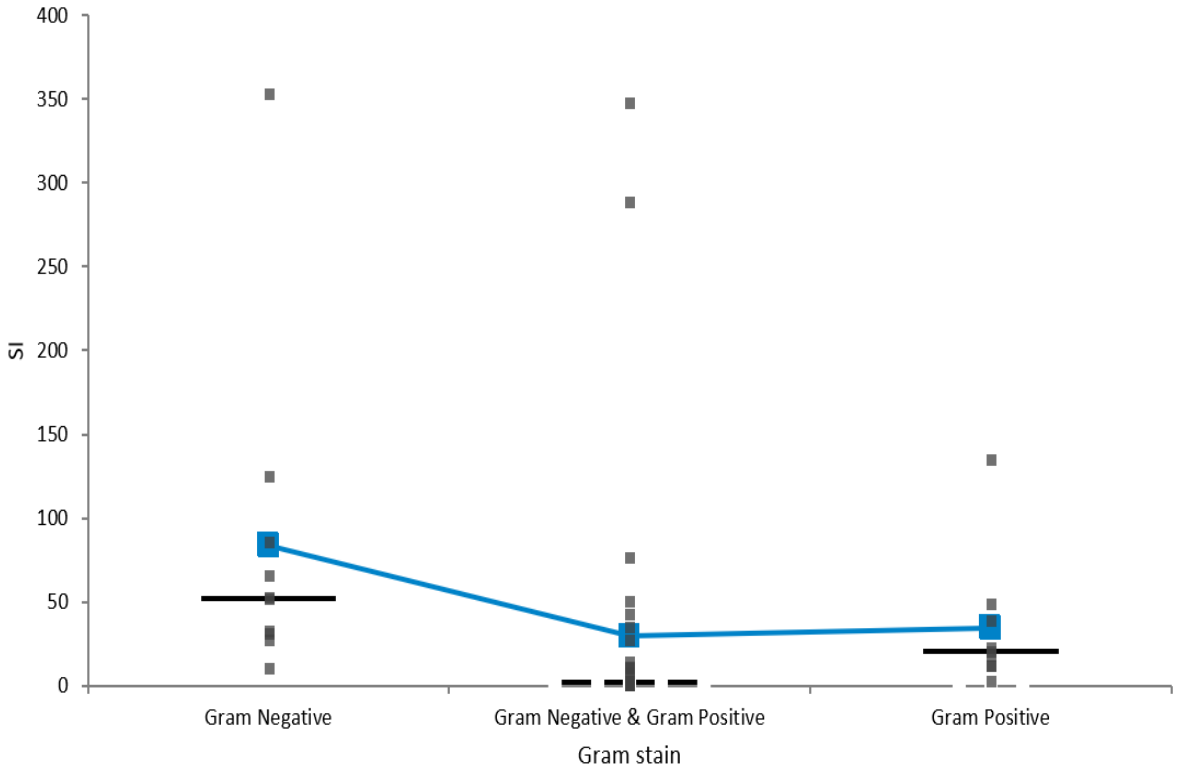

Figure 16: The Kruskal-Wallis test to determining the statistical significance between the Gram staining of bacterial species identified in this study and the SI obtained from the Horiba Microsemi CRP analyser. SI= Sepsis Index values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

3.5.20 The statistical significance of Bacteria gram staining

CRP and SI are shown in Figure 14 and 16 to be effective in distinguishing gram-staining of bacteria. However, CRP has proved to be more effective shown in Table 28 with P-value of <0.0001, therefore deeming CRP to be statistically significant. This finding does support the many studies and should be utilised by clinicians for better patient quality of care. Although, SI has shown distinguishing abilities, it is still statistically non-significant with a p-value of 0.002, this could be due to the reduced sample size, and therefore an additional study may improve the p-value. Unfortunately, NLR has the highest p-value of 0.2455 and can be seen in Figure 15 the ineffectiveness of discriminating between gram staining of bacteria. Overall, the findings found in Figure 14, 16 and table 28 is one of the more positive findings in this result chapter and provides promise for this study to be extended.

Table 28: The overall statistical significance and mean values of the clinical biomarkers against gram staining of bacteria.

Gram staining of bacteria					
	Mean Value			**P-value**	**Statistical significance**
	Gram Positive	**Gram Negative and Gram positive**	**Gram Negative**		
CRP Definitive	84.411	23.467	131.775	<0.0001	Significant
NLR Definitive	3.905	5.370	6.094	0.2455	Not Significant
SI Definitive	34.419	29.382	83.550	0.0002	Not Significant

3.5.21 Receiver Operating Characteristics (ROC) CURVE on clinical biomarkers.

The performance of CRP, NLR and SI in differentiating the definitive diagnosis of bacterial, viral, and sepsis cases were evaluated in a ROC curve in Figure 17 and Table 29. The area under the ROC curve (AUC) has placed an effective way to understand the overall diagnostics capabilities of these clinical biomarkers. Overall, CRP, NLR and SI have correlated well with each other, and all show promise as effective clinical biomarkers, however, there is a significant difference in discriminating ability. The AUC was 0.892 (95% confidence interval CI; 0.802-0.982) for POCT CRP which suggests that the POCT CRP is very effective as clinical biomarker and has 89.2% chance of distinguishing infective cases, which is akin to the ROC curve in Figure 7. As the p-value for POCT CRP is <0.001, further indication that POCT CRP has an excellent discriminating ability, therefore, cementing the need to utilise POCT CRP in the diagnosis of sepsis. However, the AUC for NLR is considerably less at 0.696 (95% CI: 0.552-0.840) and a p-value of 0.0075 which means it is less effective and reduced discriminatory ability as a clinical biomarker when compared to POCT CRP and the Sepsis Index. The prediction for this ROC curve was it show SI to be overall the most discriminatory in diagnosing sepsis due to the formula combining both CRP values and NLR, however it has proved to be marginally less powerful than POCT CRP with an AUC of 0.840 (95% CI: 0.752-0.929). However, the p-value remains <0.001 and therefore still indicates SI has excellent discriminating ability and major contender for diagnosing sepsis. In comparison to the delta neutrophil index in Figure 7, the DNI has an AUC of 0.69 which is less discriminative compared to the novel sepsis index created during this study. Therefore, outlining the potential impact this novel sepsis index can have paediatric medicine.

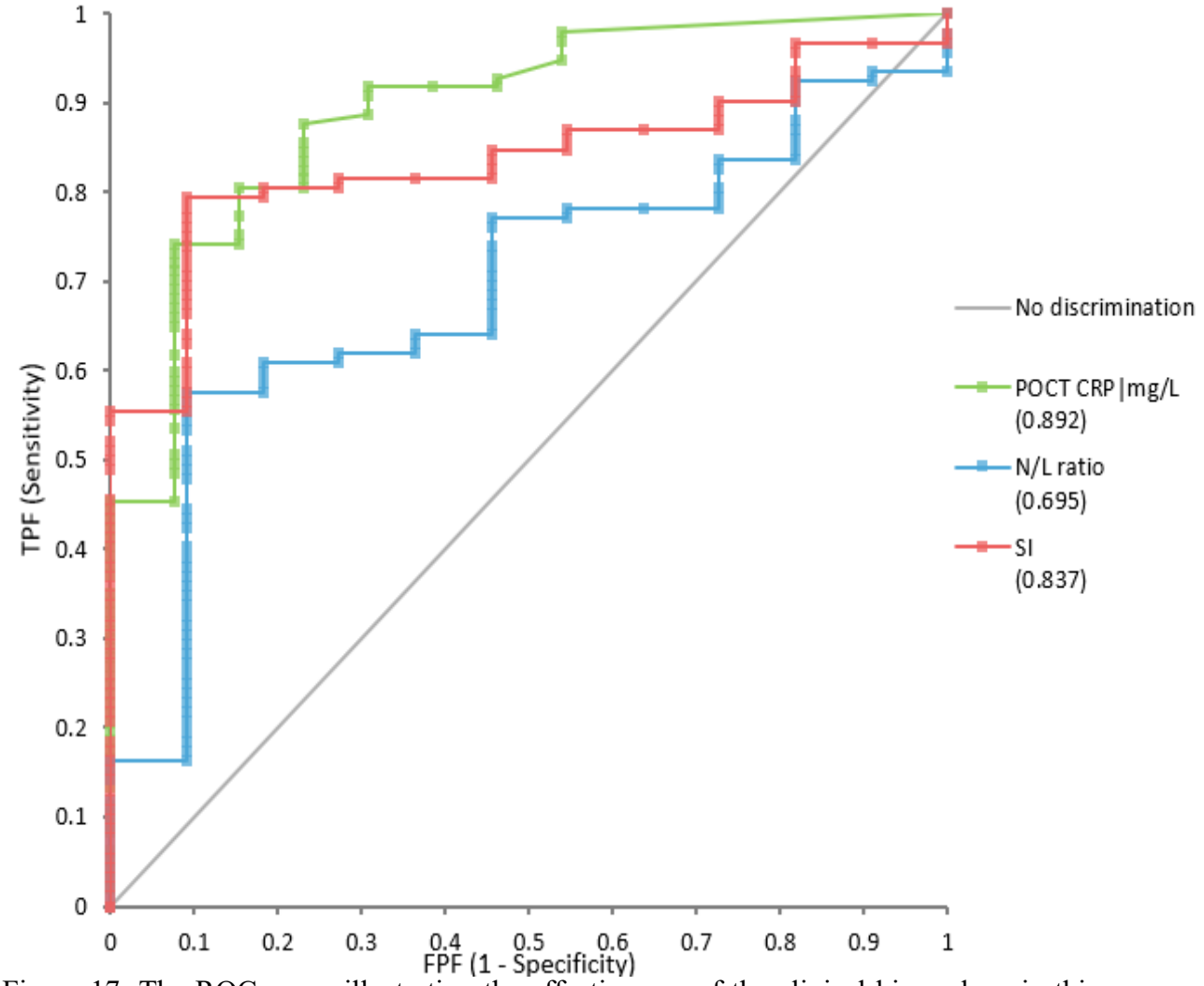

Figure 17: The ROC curve illustrating the effectiveness of the clinical biomarkers in this study. Axis-TPF(Sensitivity) (True Positive Fraction (Sensitivity)= The proportion of patients with a disease who tested positive), FPF (1-Specificity) ((False positive fraction) (1-Specificity) =The proportion of patients without a disease who tested positive). Clinical biomarkers tested -POCT CRP (Point of Care Testing C-reactive Protein), NLR (Neutrophil/Lymphocyte ratio) and SI (Sepsis Index).

Table 29: The parameters obtained from the ROC curve.

	AUC	95% CI	p-value
POCT CRP (mg/L)	0.892	0.802 to 0.982	<0.0001
NLR	0.696	0.552 to 0.840	0.0075
SI	0.840	0.752 to 0.929	<0.0001

Abbreviations: AUC, Area Under Curve, POCT CRP (mg/l); Point of Care Testing C-reactive protein values (mg/L); NLR, Neutrophil/Lymphocyte ratio; SI, Sepsis Index.

4. Discussion

The aims of this current study were to investigate the potential of rapid point care testing platforms using CRP, NLR and a novel sepsis index., to see if these tests can differentiate between bacterial and viral infections in sepsis. The data was collected from February 2020 to December 2020 at the Paediatric department in Emergency Department of Chelsea and Westminster Hospital.

4.1 The effectiveness of POCT Clinical biomarkers in Paediatric ambulatory care settings

The Passing-Bablok regression in Figure 17 has established the reliability and integrity of the POCT device in this study to reproduce comparable CRP values in the central laboratory. Therefore, clinicians can have the reassurance of using a POCT device in their department that can deliver the same quality of results as the central lab with a quicker turnaround time. This buys more clinical time to devise an effective plan in patient treatment, reducing length of stay and providing a tailored medication plan which will over time reduce antibiotic prescription and save the department as well as the hospital's budgets.

Although, this is a retrospective pilot, it has highlighted the use of POCT clinical biomarkers and incorporation of a novel sepsis index which has been devised in this study. In Figure 17, ROC curve concluded that the effective impact POCT CRP and SI has in diagnosing bacterial, viral infections and sepsis with significant p-values of <0.001. In 2009 a prospective study was completed investigating the effective of C-reactive protein as a clinical biomarker in diagnosing serious bacterial infections in paediatric cases, the ROC curve conducted in their study also highlighted CRP to have the most discriminative ability for detecting serious bacterial infections (Bilavsky, et al. 2009). Although, the findings in this study mimic countless of studies published in recent times, the utility of C-reactive protein are not promoted and implemented as the gold standard test to conduct with suspected bacterial, viral, septic, and paediatric cases (Escadafal, et al 2020) febrile as the concerns found in studies have not been rectified yet.

However, the non-parametric statistical analysis in Table 19 and 27 has shown the p-values for Length of stay, Age range groups, definitive and clinical diagnosis to be more than 0.001 and therefore, confirmed there is no statistical significance with the CRP, NLR, and SI. This can be due to the reduced sample size as there are studies supporting the effectiveness of CRP, NLR and devising novel sepsis indices in diagnosing bacterial, viral infections and sepsis. Therefore, the sample size should be increased to improve the statical significance.

4.2 The importance for POCT devices in Paediatric Ambulatory care settings

Due to the lack of POCT devices designed with the focal point on microbiology and immunology, many vendors have started competing in this niche sub-sector. Recently, MeMed Diagnostics Ltd have accomplished to create a cartridge based POCT device coined MeMed Key. This device incorporates the clinical biomarkers; POCT CRP, TNF-related apoptosis inducing ligand (TRAIL) and Interferon gamma-induced protein 10 (IP-10) which have all been confirmed to be effective in supporting the diagnosis of communicable diseases. MeMed Key unlike the Horiba Microsemi CRP analyser provides a scoring system, with the device explaining to the user that a low result may indicate viral infection and a significant high result is a bacterial infection. This device has animated the hypothesis in this study and has validated the importance for bridging the gap noticed in Paediatric emergency care settings. However, the main cause of concern is if this device has the ability to detect COVID-19 in a patient due to the strict scoring system with a definitive diagnosis given on either end of the scale. Recent studies have emphasised the ability of COVID-19 to produce a cytokine storm and therefore much higher CRP level in comparison to other viral infections study and also seen in Figure 20. However, as MeMed is in its infancy, the studies conducted on this device are small with only the Nordic and American markets included in the study, therefore more studies need to be conducted in the UK market. Although, the turnaround time of 14 minutes is significantly longer than the Horiba Microsemi CRP analyser of 4 minutes, it nevertheless provides a substantial reduced turnaround time for routine blood culture analysis. The development of this POCT device is important feat in emergency medicine and further highlights the importance of this study and impact using POCT devices with clinical biomarkers such as POCT CRP, NL ratios as well as the utilising the novel Sepsis Index can have for earlier diagnostics of sepsis.

4.3 Incorporating POCT devices in reducing length of stay.

One of the main issues faced by NHS trusts nationally is the storages of hospital beds and the difficulty of controlling influx of patient admissions especially during the winter periods indicating the need to analyse length of stay. Therefore, NHS England has rolled out the Reducing Length of Stay (RLoS) programme which aims to facilitate a clinical solution that local trusts can adhere to reduced length of hospitalisation. As increased length of stay remains an immense burden on hospitals, this has caused an increase in departmental costs, more workload for clinicians and in effect reducing quality of patient care promised. To alleviate hospitals from this burden one of the primary issues to resolve is the TAT for

laboratory results. The introduction of POCT devices in ambulatory care settings could reduce TAT and is more accessible for clinicians. Interestingly, as seen in this current study, patient data points collated from the POCT devices and using Cerner can provide key analysis of length of stay (Baek, et al., 2018). In Figure 26 , the effectiveness of POCT CRP with the length of stay in this study was illustrated. Although, with a reduced sample size and this being a pilot study it provides an overall trend, suggesting the higher the CRP value is, the increase length of stay will be. Therefore, clinicians can use the POCT device to obtain a result in four-minutes which clinicians can use to predict the outcome of the patient and communicate to the electronic bed management platform the expected length of stay. In the future, with the integration of POCT devices in departments and development of POCT analytics platforms, an effective and tailored hospital management plan can be disseminated counteract the increased length of stay.

4.4 The notable trends in this study

This retrospective study has not only established the potential of POCT clinical biomarkers but has also highlighted considerable trends for clinicians to utilise for effective hospital management and improving patient quality of care. As an international community, there is always a need for continuously improving quality of patient care and adapting to current climate. The main cause of concern in higher economically developed countries, is the sophisticating the bed management systems already in place. Currently, POCT devices can offer a solution by providing patient data points which hospital mapping can use to monitor average length of stay in severe bacterial and viral infections, the way COVID-19 is exacerbated in different age range groups and whether clinicians can devise initial care plans to improve the medical engagement and productivity.

In a study concluded in 2019 that higher serum CRP levels above 167 mg/l after post-operative care corresponded to an increased length of stay (Corsi, et al., 2019). Although, this study was conducted in an operative environment, the data points would have derived from patients with severe diagnosis and much higher CRP levels than expected. However, if their study was conducted in a paediatric acute setting with a high patient turnaround, their findings may not be as conclusive due to the array of clinical diagnosis presented. Therefore, may explain the findings from Table 19 as POCT CRP could not positively discriminate between the length of stay groups. Nevertheless, continual research into utilising POCT clinical biomarkers for improving healthcare processes is required. Continual research has

led to a study conducted in 2020, was able to conclude that POCT panels available on ambulatory care units might lead to faster discharge decisions (Goyder, et al., 2020). Also, with a similar outcome in Thailand, POCT was concluded to decrease the ED LOS and reduce crowding which is a main cause of concern in their study (Chaisirin, et al., 2020). To follow suit in identifying beneficial trends, the analysis of clinical biomarkers against age range groups was conducted in this current study. As the sample size was reduced in this study, it presented an interesting pattern in seen in Table 26 with an overall increase in the CRP mean values the older the age range groups were. Although, the findings were statistically non-significant with the p-values of 0.2459 and 0.0864, it does indeed show the need for POCT devices to be used for centralised data analysis.

The investigation of the clinical utility of POCT CRP in bacterial infections, has led to a key finding. As seen in Figure 14, the gram-negative bacteria expressed much higher CRP levels than gram-positive bacteria with a significant p-value of <0.0001 and therefore, POCT CRP can be used as a semi-diagnostic tool for clinicians to improve therapeutic monitoring.

4.5 The importance of COVID-19 in disease detection in paediatric patients.

COVID-19 is a Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection is the biggest public health crises of recent times. COVID-19 causes acute respiratory infection with disease severity on a spectrum depending on the age groups, the adult population are associated with increased mortality and severe acute respiratory distress syndrome (ARDS), whereas children have mild clinical presentations or remain uncomplicated (Dhochak, et al., 2020). Surveillance completed on the behaviour of COVID-19 early on showed the most common mode of transmission was via aerosol during face-to-face exposure or surface contamination. The viral loads appear in the upper respiratory tract leading to certain members of the public experiencing severe respiratory discomfort.

4.5.1 The pathogenesis of COVID-19 in paediatric health

The COVID-19 pandemic has significantly impacted paediatric medicine by affecting child development and mental health (Araujo, et al., 2021). In paediatric medicine, COVID-19 expresses itself as a multisystem inflammatory syndrome (MIS-C) or either asymptomatic or carriers of COVID-19. Due to asymptomatic nature, it expresses in children, clinicians misinterpreted clinical signs of COVID-19 as Kawasaki Disease and toxic shock-like syndrome in children (Kostik, et al., 2021). ‘Children of all ages are susceptible to COVID-

19, despite the higher incidence of COVID-19 in older children, infants' less than 12 months old seem to be the most vulnerable due to not establishing a stronger immune system (Han, et al., 2021). Therefore, further explaining only five COVID-19 cases were definitively diagnosed in this study. COVID-19 is only clinically exacerbated in paediatric cases if they are already diagnosed with an immunocompromised condition, pulmonary pathology, the less than 3 months of age of infants and patients' underlying diseases. With very scarce literature available regarding the effect of COVID-19 on paediatrics medicine, a prospective study was conducted in the UK providing necessary surveillance on how COVID-19 characteristics are exhibited in neonates (Gale, et al., 2021). It has revealed the incidence rate of neonatal COVID-19 infections in the UK is rare, but the long-term effects of COVID-19 are currently unknown. However, using the POCT devices in Paediatric emergency settings may provide ongoing data analytics for future investigations in COVID-19, connections, and novel treatment plans with all paediatric cases.

4.5.2 The Impact of COVID-19 on this study

Due to the aerosol transmission of COVID-19, it resulted in a very short time span; from the first case internationally identified in Wuhan, China in December 2019 to the reporting of the first cases seen in the United Kingdom on January 29th, 2020 (Lillie, et al., 2020), therefore leaving Local hospital Trusts in a predicament. Coincidently, this study was conducted in CW-NHS residing in the heart of the borough of Kensington and Chelsea, which sees the most tourism and international travelling residents in London and only 15 miles distance from London Heathrow; with mass international travel until unexpected closure in March 2020. Therefore, the hospital's prime location increased the chances of COVID-19 paediatric cases being admitted to Paediatric Accident and Emergency department. Despite there being strict lockdown regulations mandated in the UK, fascinatingly this study has found five definitively diagnosed paediatrics cases of COVID-19 (see Table 7). It has been predicted that figures of COVID-19 adult cases would be significantly exacerbated. COVID-19 in adult is considerably more severe in comparison to children (Zimmerman and Curtis, 2021). On the contrary, studies have mentioned the asymptomatic nature of COVID-19 in children has led to clinician's misdiagnosis COVID-19 early in the pandemic as it mimics the symptoms normally seen in upper respiratory virus as well as bacterial infections (Borrelli, et al. 2021). Therefore, clinicians may have misinterpreted the symptoms described by the paediatric patients or their carer. Therefore, this raises an interesting question of the misdiagnosis/not confirmed by lab subcategory in the definitive diagnosis groups (see Table 8). This subcategory was found have inaccurate clinical diagnosis of communicable diseases with no sample sent to the lab to prove this

diagnosis which can be argued due to the unavoidable pressures faced due to the pandemic. Nevertheless, the advancement and development of automated rapid molecular diagnostics and POCT devices such as DNA Nudge and Cephid Xpert® Xpress SARS-CoV-2 which both provide a rapid RT-PCR test for COVID-19, has ensured acute and ambulatory care settings to mandate the general public who arrive at the hospital to complete a COVID-19 test before further admittance to the hospital. Clinicians are now able to use DNA nudge at ambulatory care settings prior to admission to reduce the risk of COVID-19 transmission. The results obtained from the DNA nudge will provide a clinician a better stance in making clinical actions such as a positive result will obligate the patient to immediately discharge and complete a 14-day self-isolation following the government quarantining guidelines. This further establishes the importance of investing POCT devices in acute ambulatory settings, utilising the routine clinical biomarkers and the implementation of the novel sepsis index analysed in this study.

To understand the effect the pandemic has had on Paediatric emergency care, a study was conducted in Ireland in 2020 that has revealed a 73%-88% reduction in peadiatric Accident and Emergency department attendances during the pandemic (Dann et al., 2020). Illustrated better in Figure 18 below.

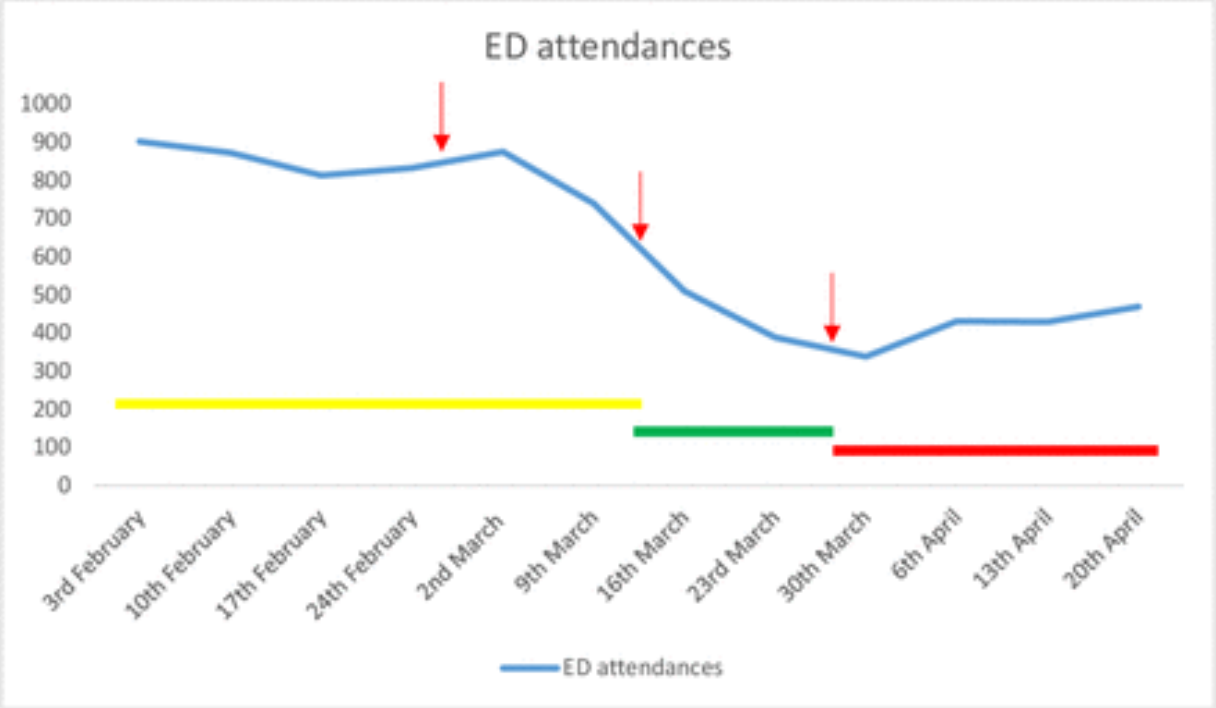

Figure 5: Presentations correlating with public health response phases. (Yellow = 'the containment phase'; green= 'delay phase'; and red= 'stay-at-home phase). 'Arrows indicate first case, school closure and commencement of stay-at-home phase' (Dann et al., 2020).

Although there have been several precautions and restrictions devised in regard to reducing COVID-19 transmission in Paediatric ambulatory care settings, this has seen a rise in more clinically severe cases witnessed in the later period of 2020. A study conducted by Macy and colleagues (2021) attested to this notion of the public's reluctance to visit the hospital for their clinical complaints and ultimately leading to an increase in more severe clinical cases which otherwise would have treatable if diagnosed at a much earlier stage. Thus, an incredible disadvantage the general public and healthcare trusts have faced in this pandemic. As best explained by the study completed in 2020 to understand the effect of COVID-19 on Parents and guardians of children, there had been hesitancy in regards to attending hospitals at the height of the pandemic and would prefer to nurse their childrens' clinical complaints at home to avoid contracting COVID-19 (Macy, et al., 2021).

Imperial College Healthcare NHS Trust has published a study in September 2021, comparing their Trusts' Emergency Department (ED) attendances to the national average during the pandemic (Vollmer, et al., 2021). These ED admissions graphs collated from the studies conducted in Ireland and England are all corresponding with the findings in Figure 12. However, interestingly this graph (see Figure 19) has captured ED admissions in January and February 2020, therefore highlighting the expected ED admissions figures that would have been seen pre-pandemic era. This raises the question on the impact the pandemic has had on this study and if conducted before the pandemic, there would have been more data points due to higher ED admissions nationally seen (see Figure 19).

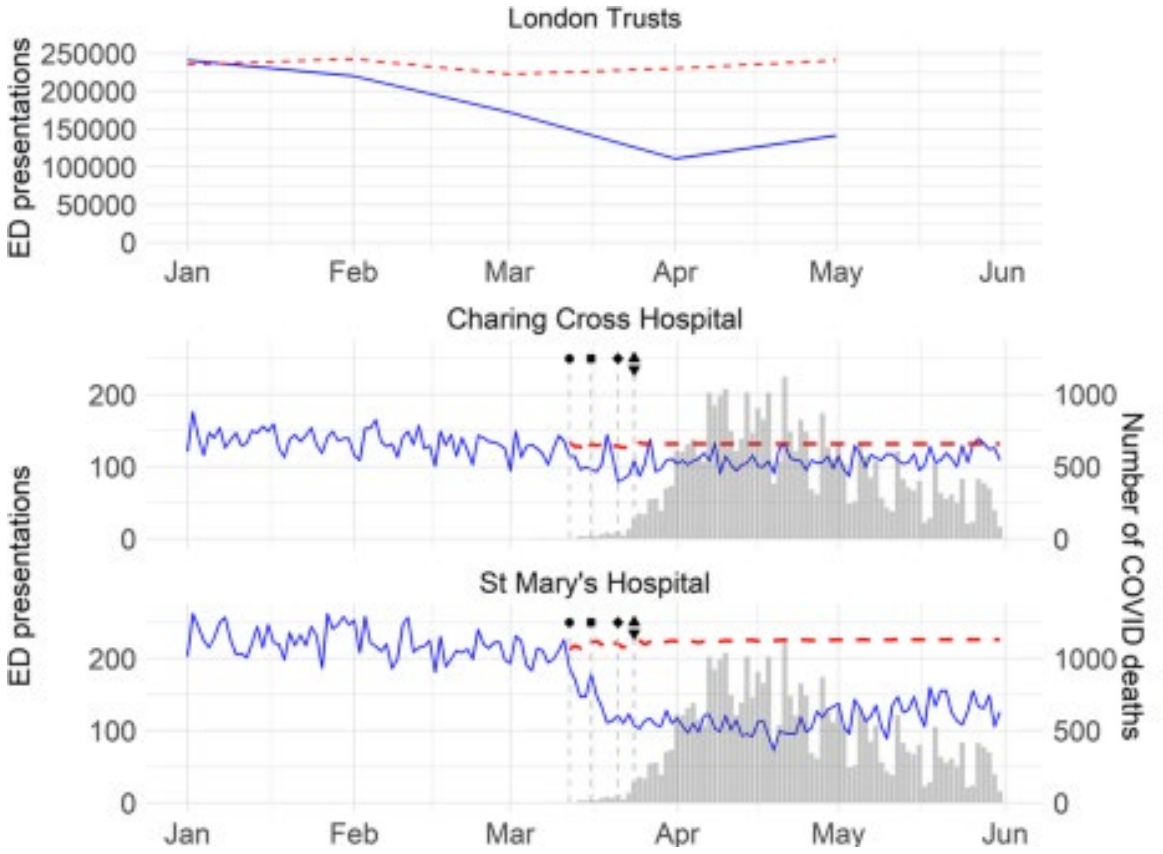

Figure 19: Time series of admissions to Emergency Department services at ICHNT in comparison to the local Eds decline in attendances. Data collated from Public Health daily for COVID-19 deaths in grey illustrated as a bar chart (Vollmer, et al., 2021).

4.7 The impact of the novel Sepsis index formula in this study.

The novel sepsis index in this study has also proved its diagnostic ability and studies internationally also have attempted to create a novel sepsis index incorporating the haematological parameters normally available such as monocyte distribution width (MDW) and mean monocyte volume (MMV) for request in the centralised laboratory (Agnello, et al 2021). In 2021, there have been concerted efforts in Palermo, Italy to devise a sepsis index for sepsis screening in the Accident and Emergency department (Agnello, et al 2021). Their sepsis index had a higher AUC value of 0.877 compared to this current study and therefore more distinguishing ability to diagnose infections. However, our study has only focused on paediatric cases which has limited the sample size, also their formula for sepsis index has not incorporated the C-reactive protein and Neutrophil/lymphocyte ratio which has been seen in many studies and used this current study. Therefore, the exclusion of clinical biomarkers in their formula could in effect limit its impact and usability when applied in acute paediatric settings. Notably, the haematological parameters used in their sepsis index

are not readily available in a POCT device and only exclusively found in new generation haematology analysers, DxH900 haematology analysers (Beckman Coulter, Inc., California, USA). Therefore, reducing the adaptability of their sepsis index and could incur hospitals additional costs to purchase these specialised analysers for a centralised laboratory and has no effect on reducing the TAT. In comparison, the sepsis index formula (see Equation 1) created for this pilot study has incorporated the use of POCT devices and produces an excellent discriminating ability that can compete with other studies. The impact of the novel sepsis index in this current study definitely is noteworthy and will provide a feasible solution to Paediatric Accident and Emergency care settings by reducing TAT, disease progression and departmental budget costs.

4.8 Does this study and recent literature facilitate the NHS Acute Ambulatory care needs?

Importantly, the main objectives in this present study were to provide a clinical solution with regards to improving the care in Paediatric ambulatory departments. The main causes of concern in these care settings are bed space, reducing length of stay, earlier diagnosis of sepsis, better targeted use of antibiotic to prevent antibiotic resistance and rapid TAT for laboratory tests. The primary solutions for these concerns which local trusts have considered is the regular use of POCT devices for more tailored laboratory requests. This study has identified the potential use of clinical biomarkers such CRP, NL ratio and the novel Sepsis index to counteract these issues consistently faced in EDs across the nation. Although, this was a pilot study with limited data points, it has significantly highlighted trends in clinical behaviours exhibited in age range groups, length of stay and differential of bacterial organisms which can be the focal point for local trusts to create statistical platforms with the POCT data always available due to the integrated and connected middleware.

4.9 Limitations

Due to the nature of this pilot study, there has been a multitude of factors that has limited the potential of this study. As the study was conducted throughout the pandemic, this has led to far less attendances at the hospital and therefore has significantly reduced the sample pool intended for this study. Another implication to this study was the Microsemi CRP device could only preserve 200 patient record history onboard, therefore there was a limited amount of data points collated for February 2020. This time period would have been beneficial in creating an additional novel comparative study focusing on the effect of COVID-19 in a paediatric ambulatory care setting. Nevertheless, a factor that hindered the further success

of this study is the frequent operator errors on device as well as patient samples analysed on the POCT device were not dispatched for comparative laboratory results.

The study could have also been undertaken at the higher patient turnover hospital in the trust (St. Mary's Hospital, Imperial Healthcare NHS Trust), this hospital is one of the major trauma centres in London. Hosting more patient admissions in their paediatric ambulatory care settings, specialistic paediatric departments. This could have resulted in the study having a higher number of patient and therefore considerably more data points, potential resulting in a more powerful data set. However, as this study was concluding, a second Microsemi CRP analyser in the Trust was established in the partnered West Middlesex Hospital, which would have improved the sample size if the installation period were much sooner. This further emphasises if the study were provided an extended timeframe and the involvement of more hospitals within the Trust and partnered Trusts, this would have produced more intriguing results and further establish the success of the novel sepsis index.

5. Future Work

As this was pilot study and conducted retrospectively, there is a promise for this study to expand to a much more extended prospective study. The aims of the study would remain, but an emphasis would be made of the proving the effectiveness of the novel sepsis index. This future expansion of this study may require more funding from governing bodies and the collaborated agreement from microbiology departments to complete a series of serological tests on each sample analysed and clinicians to ensure POCT CRP testing on every paediatric admission during the prospective study. Also, there is the potential to expand the study to include the newly installed Microsemi CRP device situated in Paediatric ED, West Middlesex Hospital.

Although this study has briefly explored the effectiveness of the Clinical Biomarkers (POCT CRP, SI and NLR) in the Length of hospitalisation stay, a principal study for this subject matter is required as it can potentially provide a poignant connection with the incorporation of Point of Care Testing devices in predictive modelling and clinical bioinformatics for Paediatric ED. Epidemiologically, the age range groups are also a fascinating focal point to research which will also require the concerted efforts and agreement from Paediatric ED clinical staff during the prospective study to collect patient sample for every Paediatric admission and to sent the laboratory and analysed on the POCT device available in the Paediatric ED.

6. Conclusion

This pilot study has highlighted the fundamental gaps in literature regarding the value of POCT devices in ambulatory care. Current literature has shown POCT CRP on its own cannot be used to differentiate between bacterial and viral sepsis. Most studies highlight the need for additional rapid diagnostic testing panels in ambulatory care settings (Goyder, et al., 2020). However, where possible, the implementation of other POCT devices such as the Accu-Chek Inform II, Abbott ISTAT analyser and Sysmex poCH-100i in paediatric ambulatory care has had a substantial benefit. In patient care, reduced clinical decision time, TAT and effective therapeutic monitoring (Larsson, et al., 2015). Furthermore, the development of the SI in this current study has demonstrated its potential for assistance for highlighting earlier stages of sepsis, for instance with higher SI score it demonstrates a strong correlation with a bacterial infection. However, the current systems cannot currently differentiate between bacterial and viral infections and these further confused due to COVID-19 having a high POCT CRP, NLR and SI values equivalent to bacterial infections. It must be noted that the results seen for COVID-19 in this current study is only based on five samples, therefore further studies are required in COVID-19 to see if high rates are seen in patients. However, considering the need for reducing length of stay (RLoS) in hospital Trusts, this study has noted trends in POCT CRP, NLR and SI, with promising results being found in definitive CRP and SI. Nevertheless, the utilisation of POCT devices in hospital data mapping is still in its infancy and therefore further work is required to confirm the effectiveness.

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Appendix

POCT CRP by clinical diagnosis groups

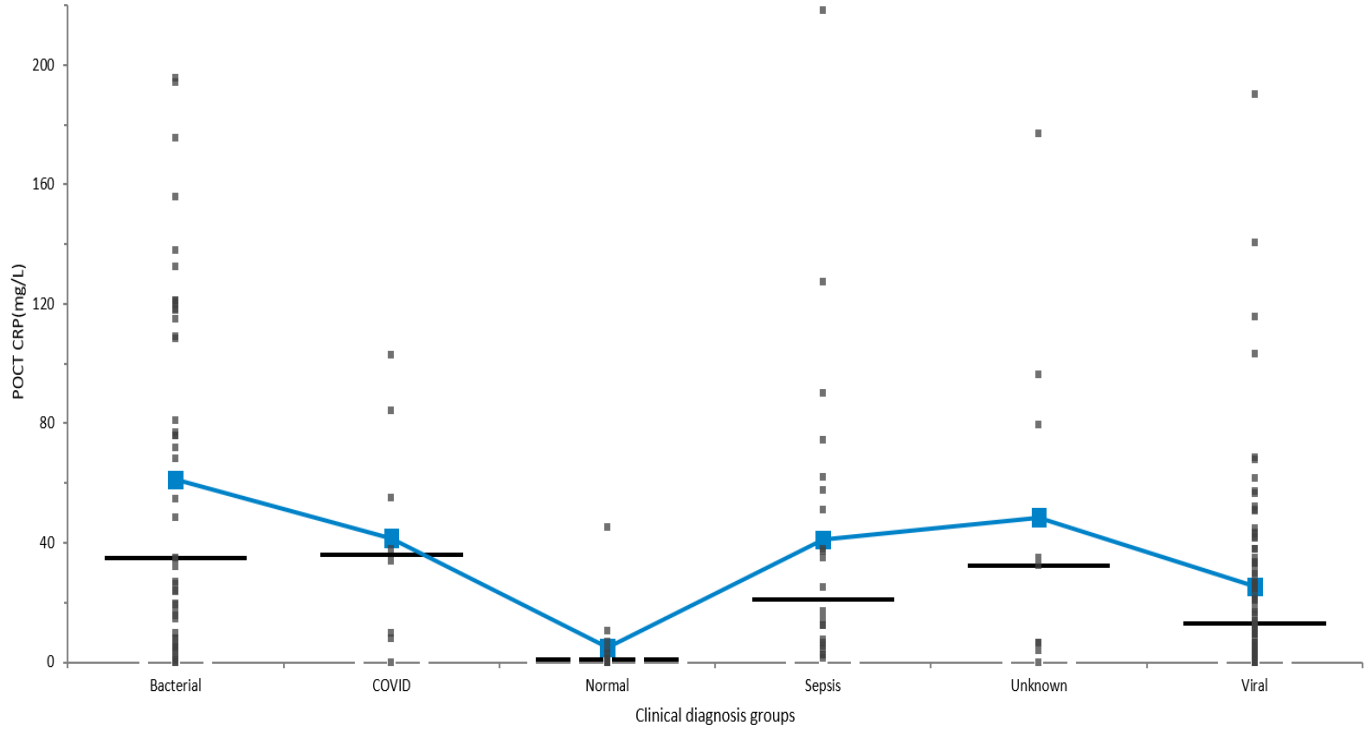

Figure 20: The Kruskal Wallis test determining the relation between clinical diagnosis groups collated from Cerner and POCT CRP obtained from the Horiba Microsemi CRP analyser. POCT CRP= CRP values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

NLR by clinical diagnosis groups

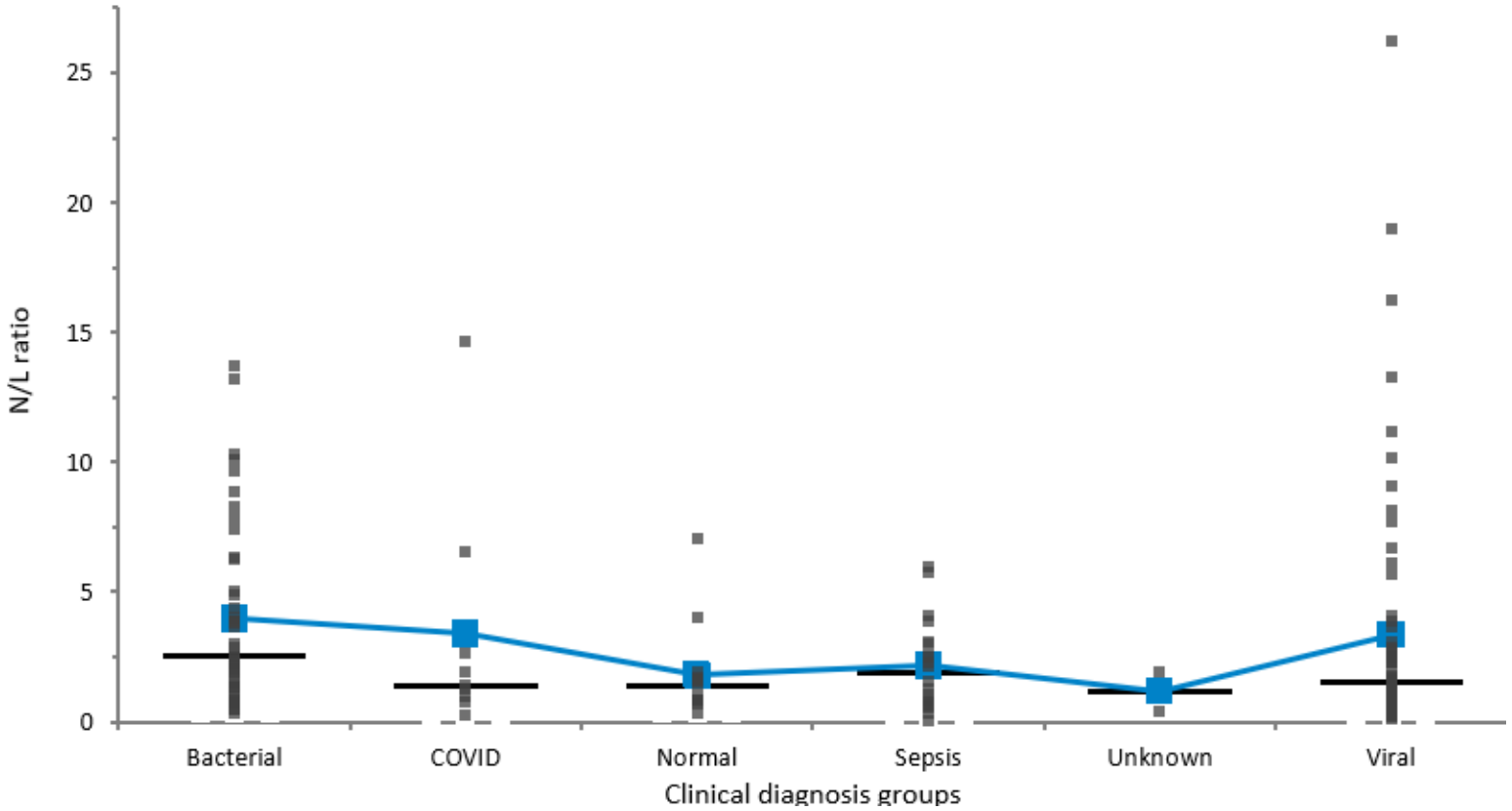

Figure 21: The Kruskal Wallis test determining the relation between clinical diagnosis groups collated from Cerner and NLR obtained from the Horiba Microsemi CRP analyser. NLR= Neutrophil/ lymphocyte ratio values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

SI by clinical diagnosis groups

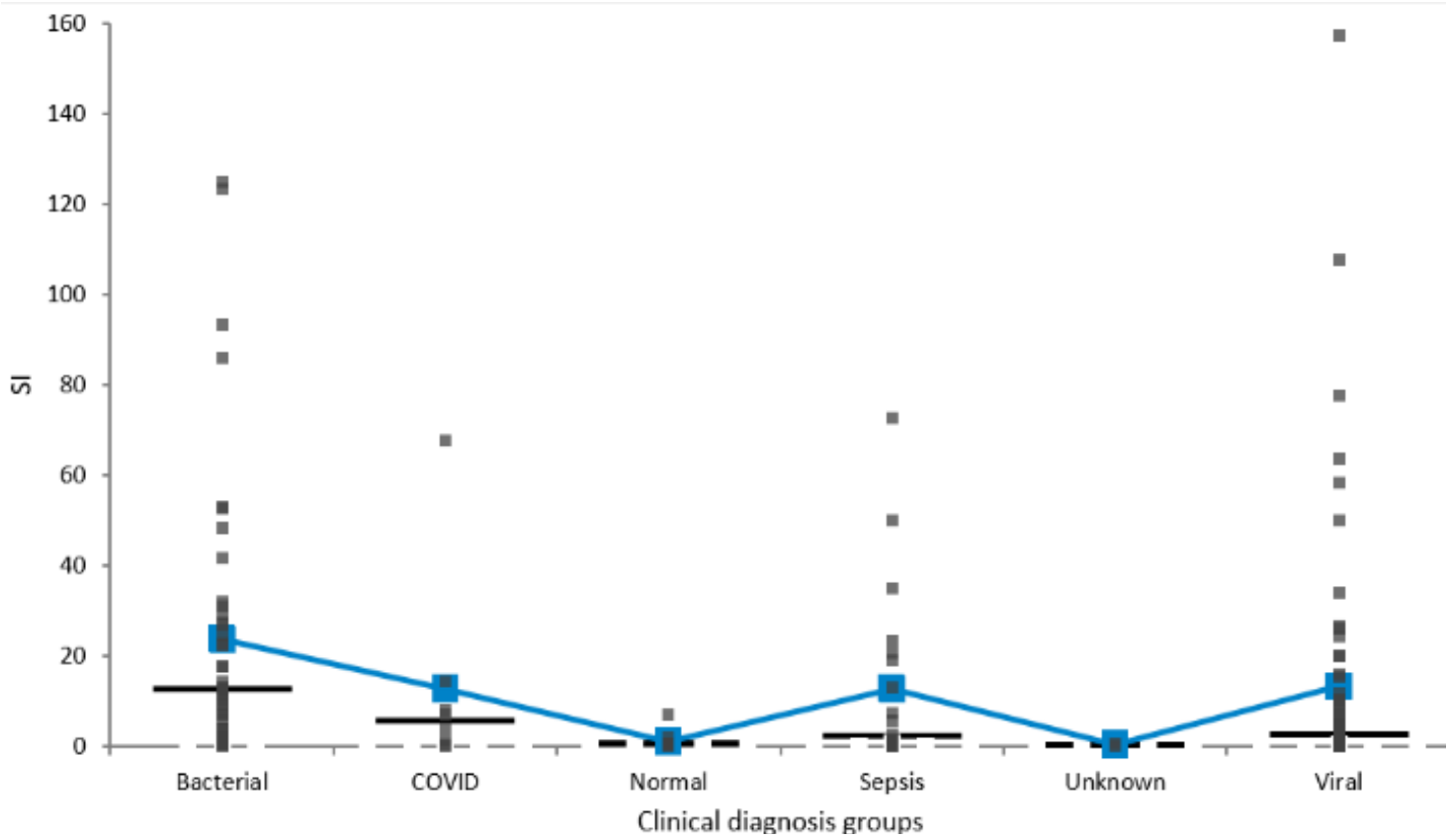

Figure 22: The Kruskal Wallis test determining the relation between clinical diagnosis groups collated from Cerner and SI obtained from the Horiba Microsemi CRP analyser. SI= Sepsis Index values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

POCT CRP by Definitive diagnosis groups

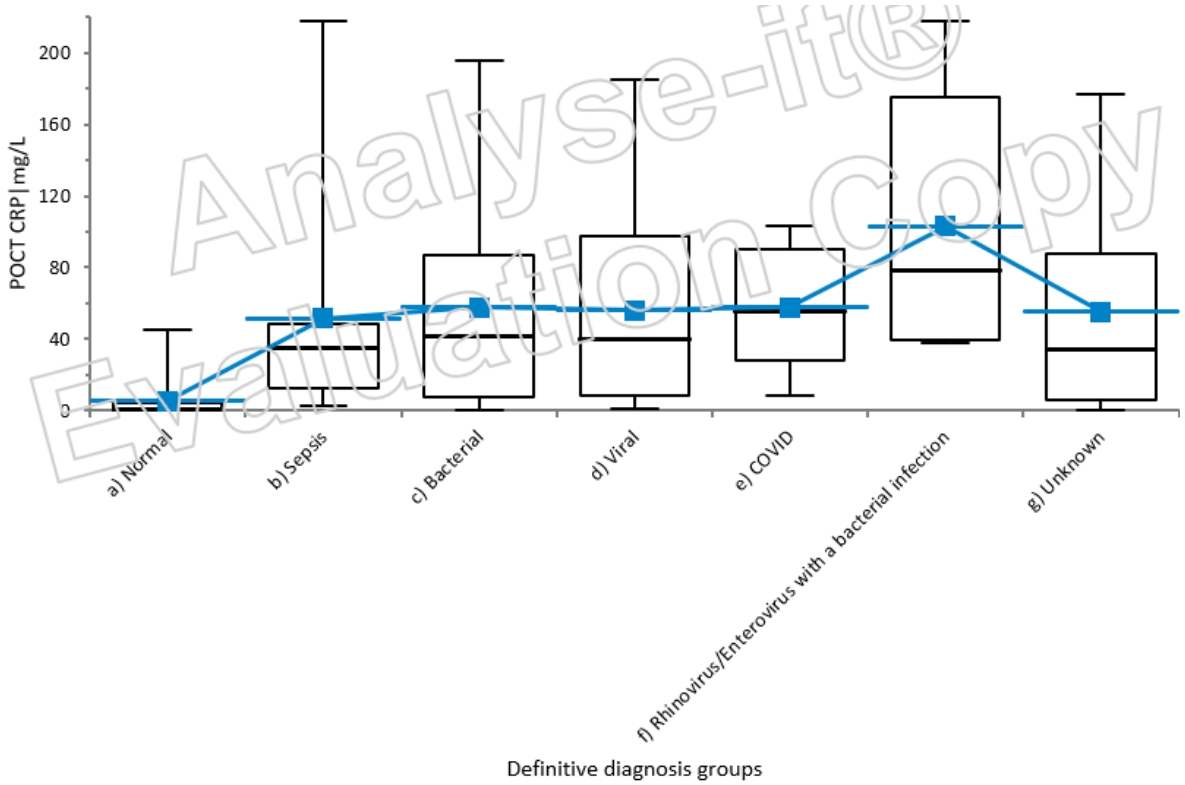

Figure 23: The Kruskal Wallis test determining the relation between definitive diagnosis groups collated from Cerner and POCT CRP analysed on the Horiba Microsemi CRP analyser. POCT CRP= C-reactive Protein values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

NLR by Definitive diagnosis groups

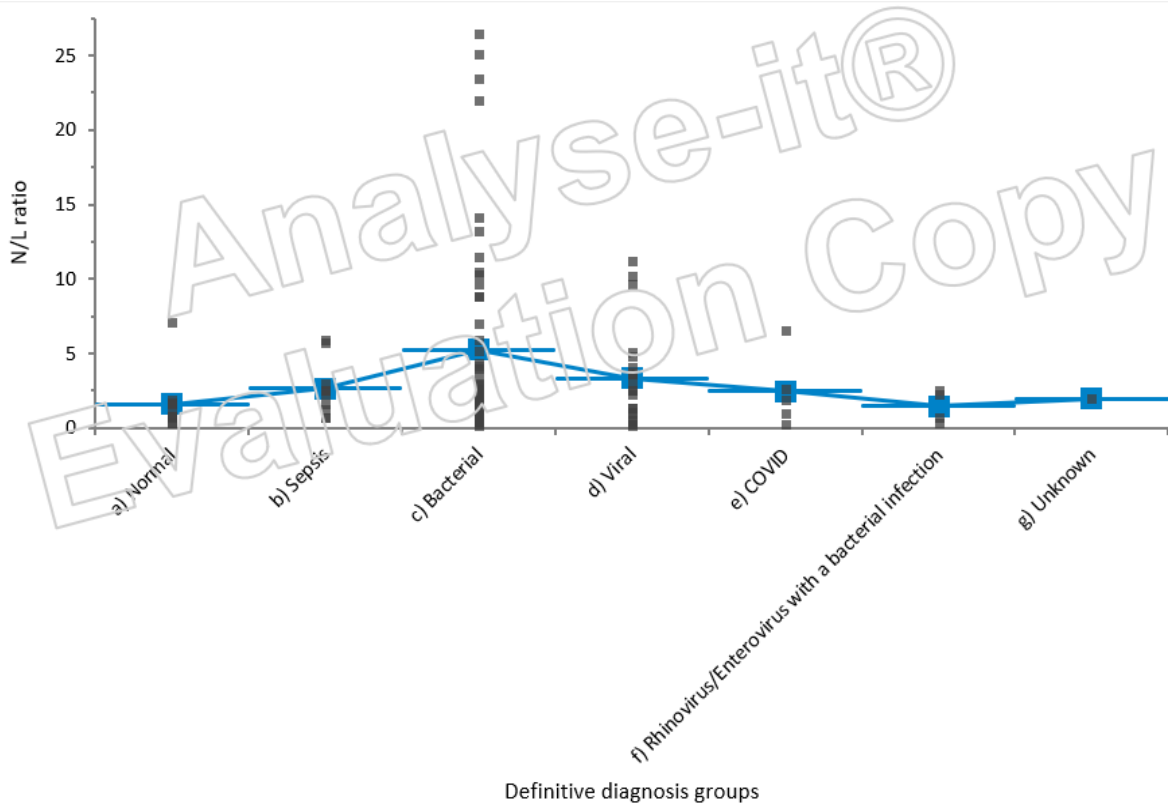

Figure 24: The Kruskal Wallis test determining the relation between definitive diagnosis groups collated from Cerner and NLR obtained from the Horiba Microsemi CRP analyser. NLR= Neutrophil/ lymphocyte ratio values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

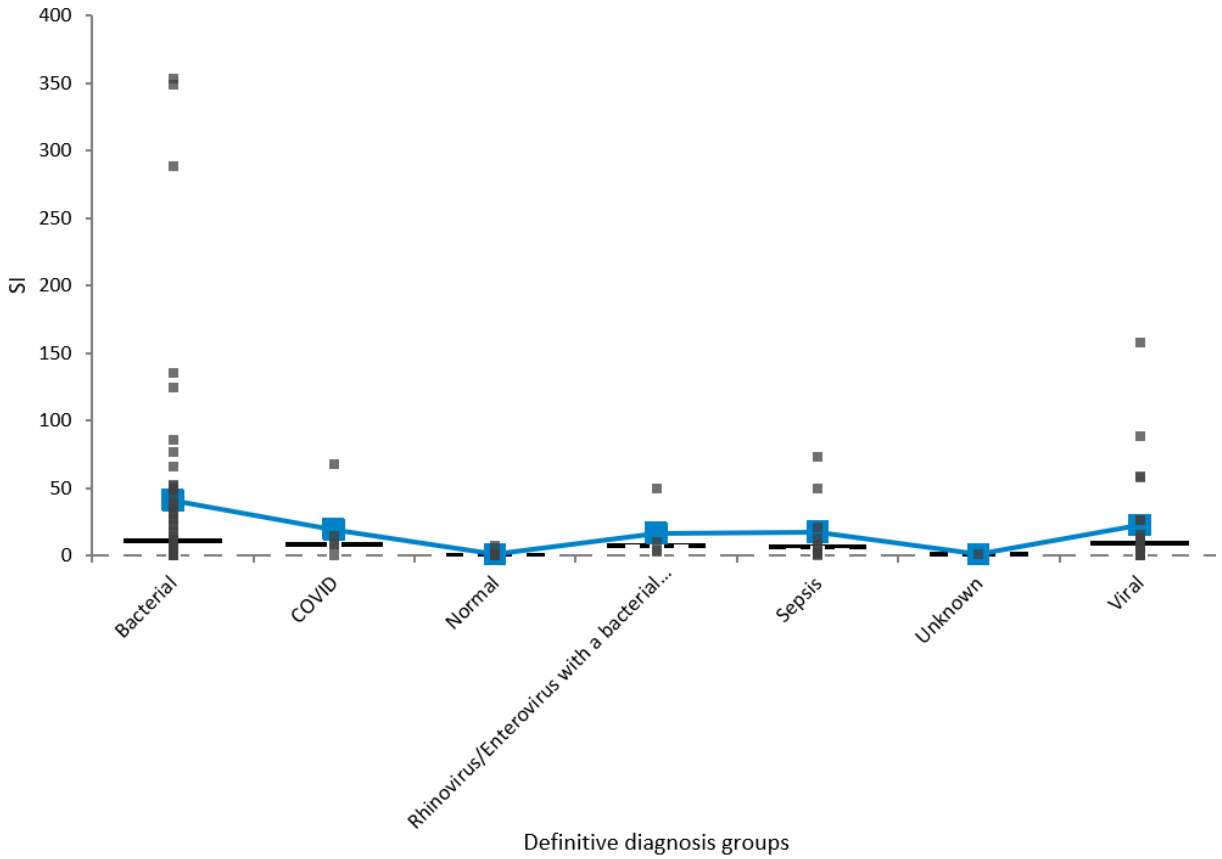

Figure 25: The Kruskal Wallis test determining the relation between definitive diagnosis groups collated from Cerner and SI which its parameters obtained from the Horiba Microsemi CRP analyser. SI =Sepsis Index values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

POCT CRP by Length of stay groups

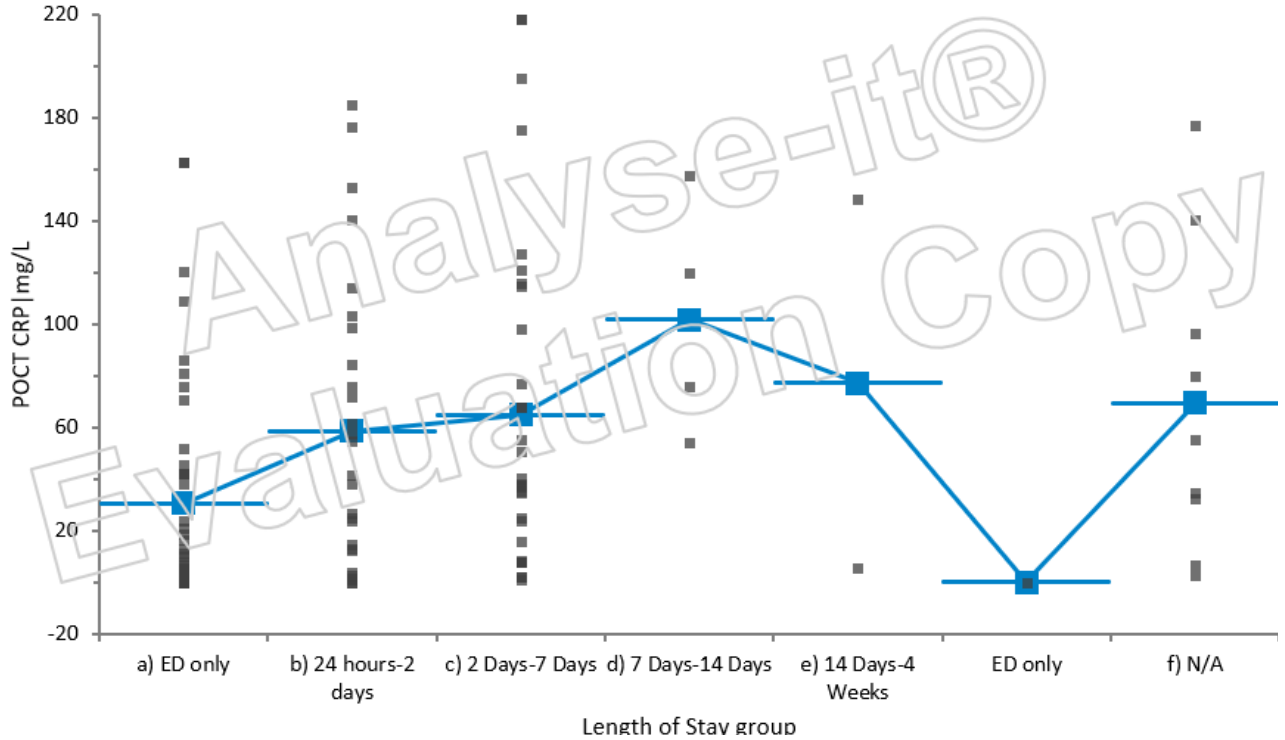

Figure 26: The Kruskal-Wallis test to determining the statistical significance between the Length of Stay groups and the POCT CRP obtained from the Horiba Microsemi CRP analyser. Length of Stay= The period of hospitalisation seen in paediatric cases in this study. POCT CRP= C-reactive Protein values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

NLR by Length of stay groups

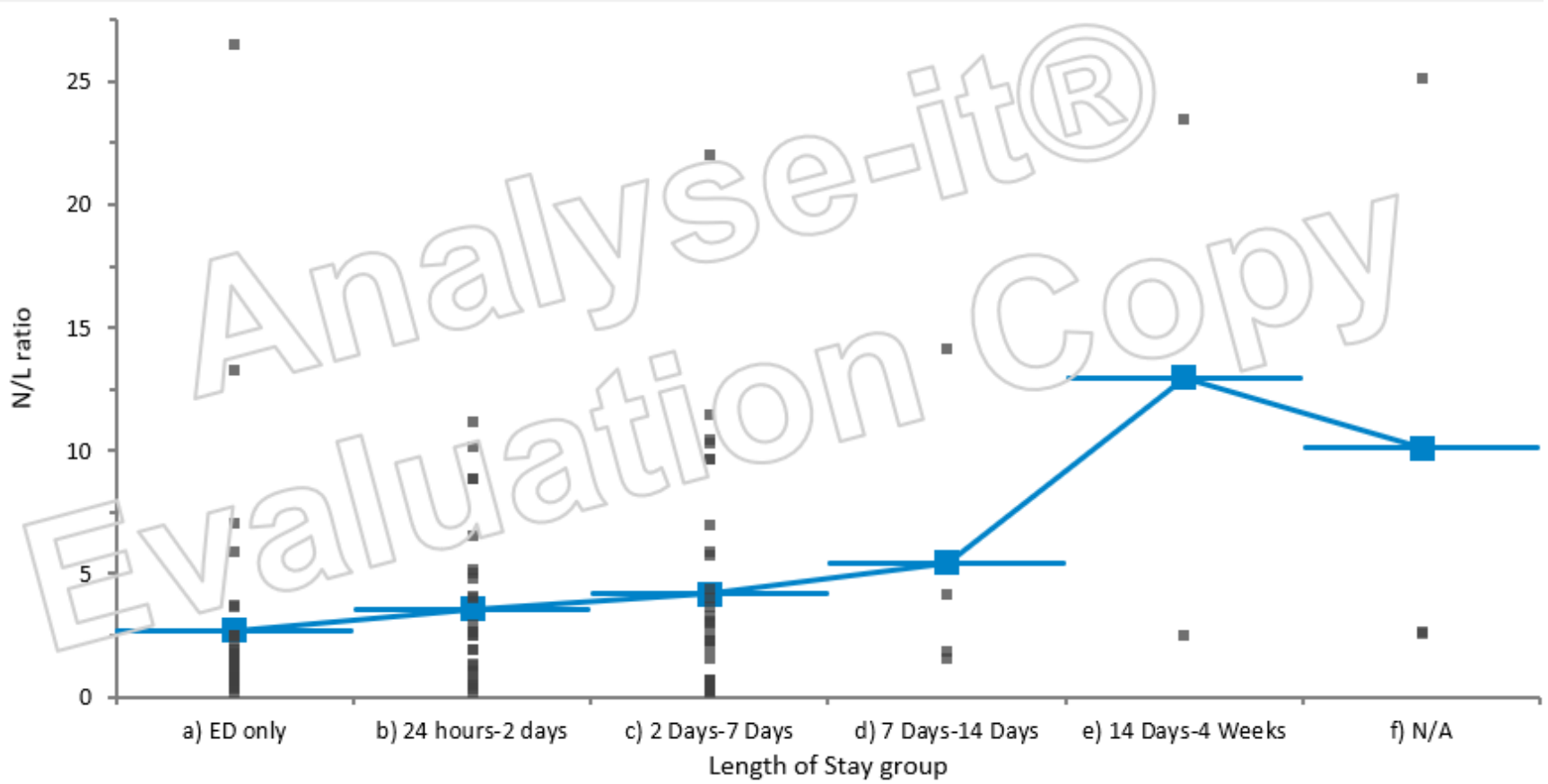

Figure 27: The Kruskal-Wallis test to determining the statistical significance between the Length of Stay groups and the NLR obtained from the Horiba Microsemi CRP analyser. Length of Stay= The period of hospitalisation seen in paediatric cases in this study. NLR= Neutrophil/ lymphocyte ratio values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

SI by Length of stay groups

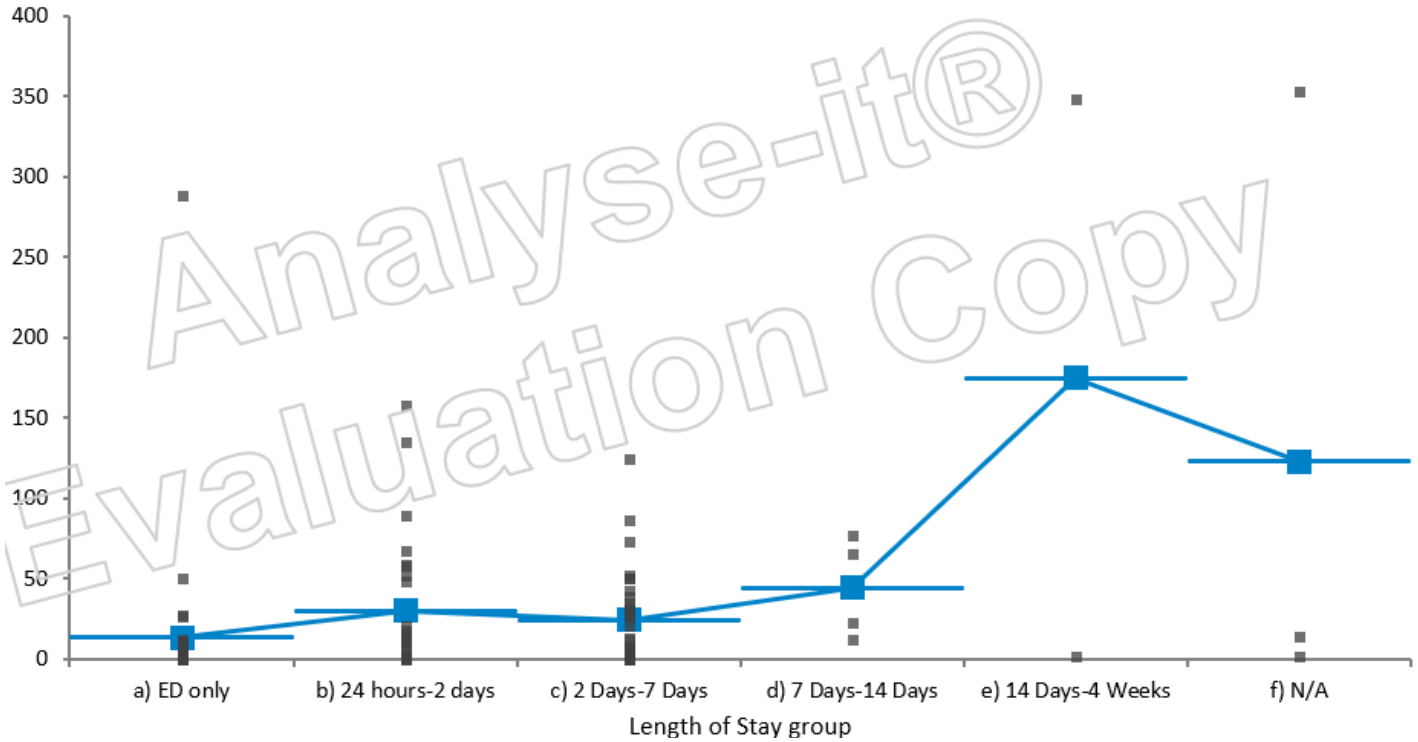

Figure 28: The Kruskal-Wallis test to determining the statistical significance between the Length of Stay groups and the SI obtained from the Horiba Microsemi CRP analyser. Length of Stay= The period of hospitalisation seen in paediatric cases in this study. SI =Sepsis Index values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

POCT CRP by Age range groups

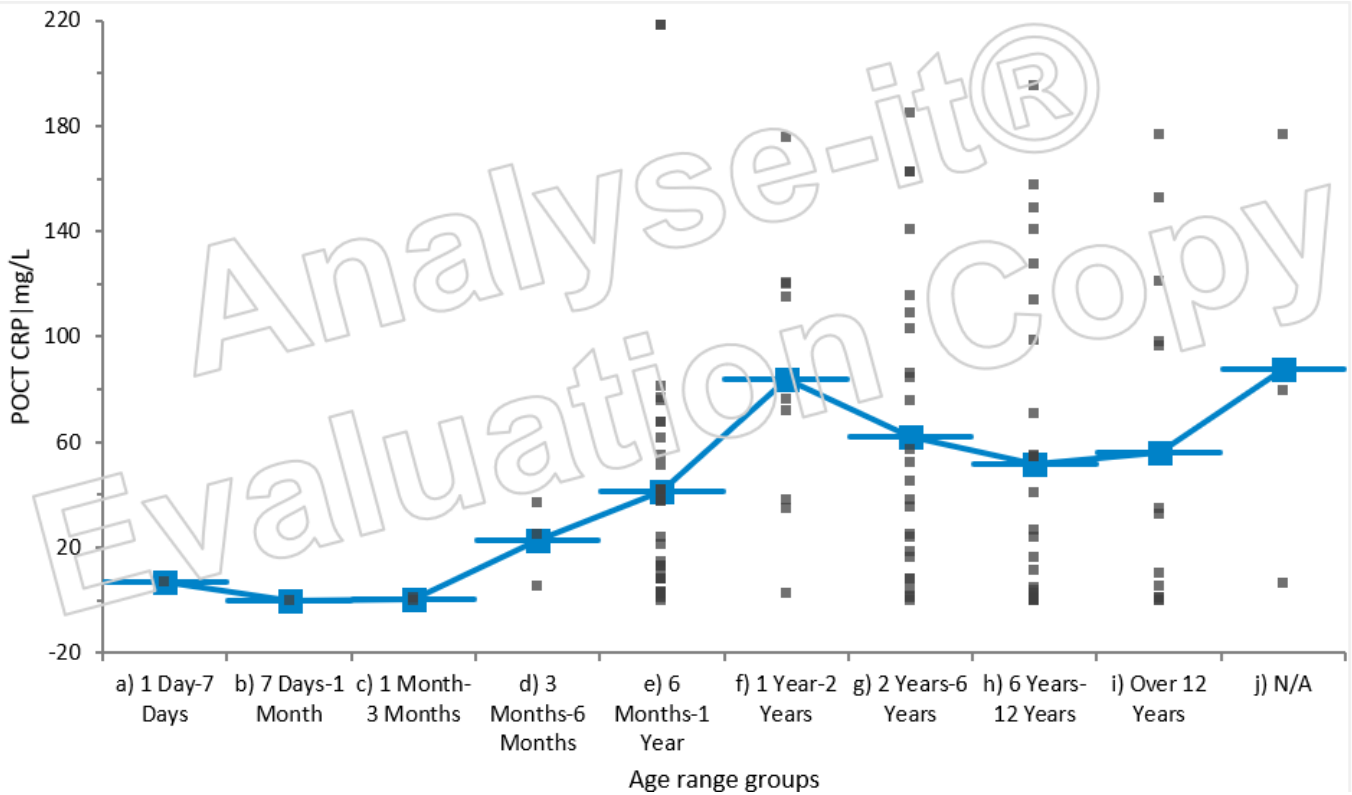

Figure 29: The Kruskal-Wallis test to determining the statistical significance between the Age range groups and the POCT CRP obtained from the Horiba Microsemi CRP analyser. Age range= The age range groups that have attended Paediatric ED in this study. POCT CRP= C-reactive Protein values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

NLR by Age range groups

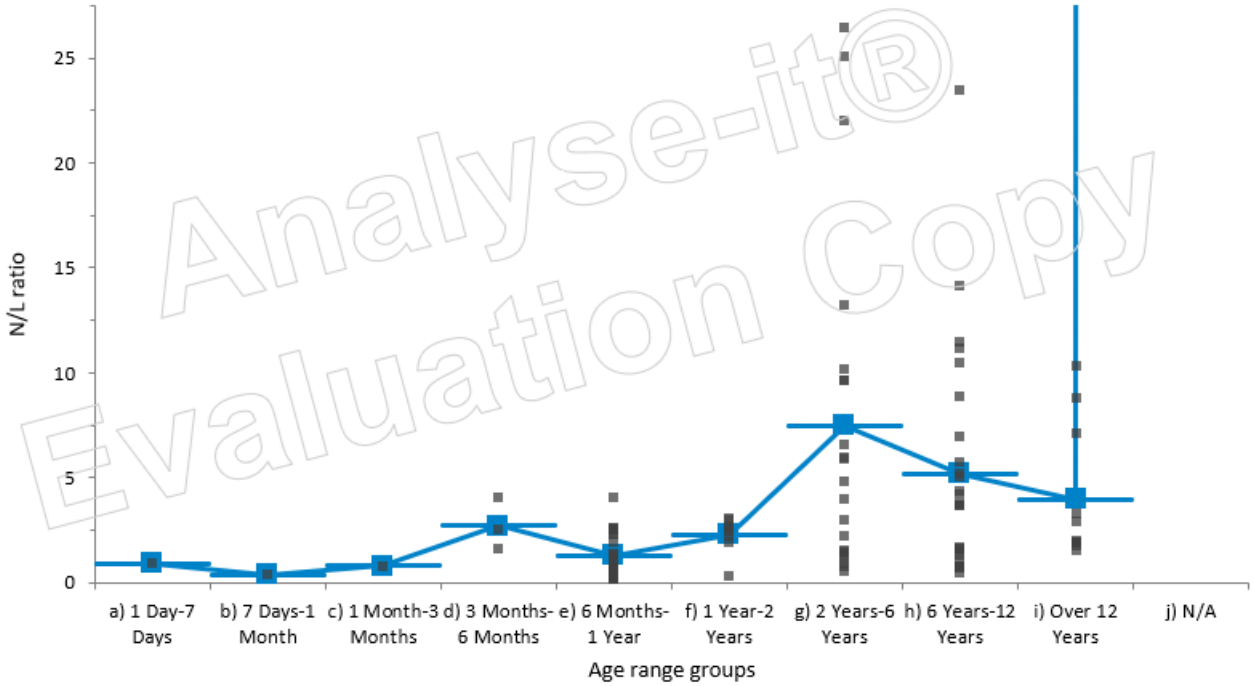

Figure 30: The Kruskal-Wallis test to determining the statistical significance between the Age range groups and the NLR obtained from the Horiba Microsemi CRP analyser. Age range= The age range groups that have attended Paediatric ED in this study. NLR= Neutrophil/ lymphocyte ratio values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.

SI by Age range groups

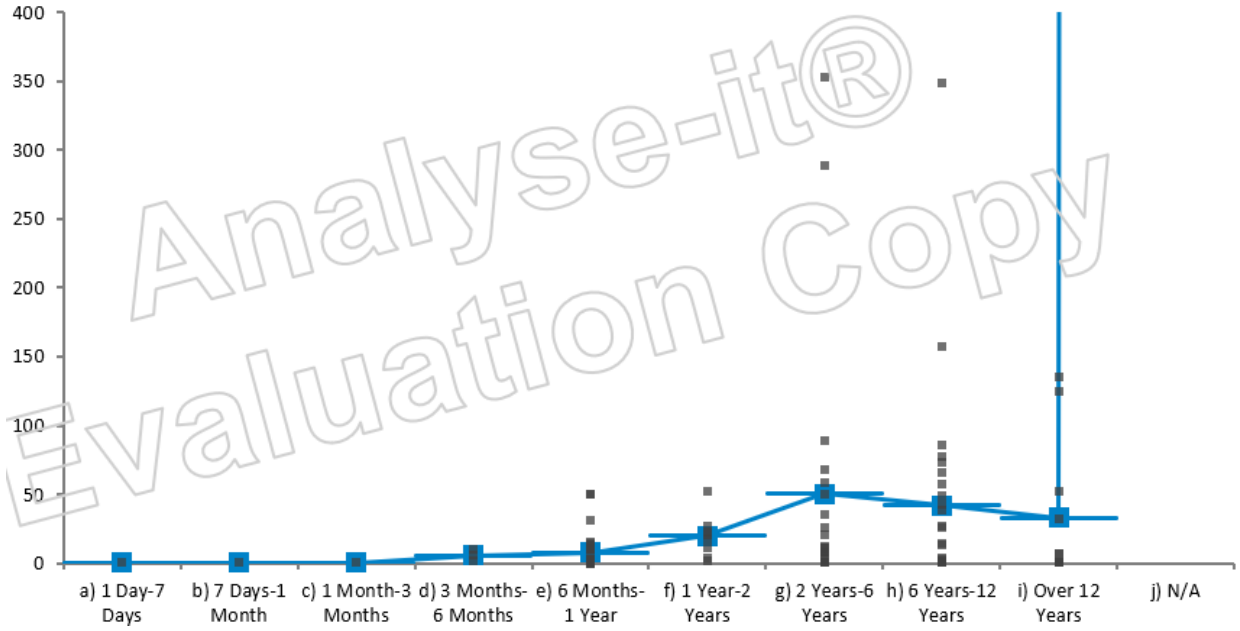

Figure 31: The Kruskal-Wallis test to determining the statistical significance between the Age range groups and the SI obtained from the Horiba Microsemi CRP analyser. Age range= The age range groups that have attended Paediatric ED in this study. SI =Sepsis Index values obtained from the POCT device in Paediatric Accident and Emergency department, CW-NHS, London.