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Cytokines Analysis Combined with Machine Learning for Determining the Etiology of Inaccessible Infection

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Dedication

This thesis is dedicated to myself to having the power to face challenges through thesis journey. To my parents for supporting. To PRCS Hospital. To Al-Quds University. To all who believed in my abilities and supported me.

Declaration

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study has not been submitted for a higher degree to any other university or institution

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Abstract

Accurate diagnosis of viral and bacterial infections in patients with fever is crucial in clinical diagnosis to ensure appropriate treatment. It requires obtaining a high-quality sample from the patient for laboratory testing. In some cases, obtaining a sample from the patient is difficult, especially in cases of inaccessible infections which may require invasive procedures. Routine laboratory tests, such as complete blood cell (CBC) count, C-reactive protein (CRP), etc. lack the high sensitivity to identify the primary cause of the infection associated with fever. This can lead to misdiagnosis in some cases which can impact patients' health. The goal of this study is to combine cytokine results with machine learning models to develop a unique diagnostic tool that can separate and differentiate between types of infections.

We focused on three cytokine indicators (IP-10, IL-6, and TRAIL) and combined them with advanced machine learning techniques such as Principal Component Analysis, Linear Discriminant Analysis, and Support Vector Machine (SVM). Our study included 268 pediatric patients, 245 of them met the inclusion criteria. Blood samples were drawn from the participants after obtaining ethical approval. 97 samples were selected for testing, based on our capabilities. 22 cases were confirmed as having a bacterial infection in the diagnostic microbiological laboratory, 55 were diagnosed with a viral infection (using PCR), 10 were diagnosed with fever of unknown origin, and 10 were diagnosed with an inaccessible infection. 87 controls participated in the study. Cytokine tests were performed using ELISA technique. We initially found the cut-off values for cytokine measurements, but the cut-off values failed to differentiate between bacterial and viral samples due to the high overlap between the results. This finding prompted us to move from using cytokine biomarkers alone to correlating cytokine values with more advanced techniques. PCA and LDA demonstrated the ability of cytokines to separate different infection groups, achieving success rates of up to 90%. However, when WBC and CRP variables were added to the cytokine biomarkers, our results showed lower success rates, dropping to 65%, and the accuracy of the model was reduced. Despite the high success rates of the PCA and LDA models, our data was abnormally distributed. For this reason, we turned to use a more advanced classification technique based on machine learning algorithms. The results of the three cytokines were integrated with the Support Vector Machine (SVM) technique, showing a sensitivity of 87.5% and a specificity of 73.6%, with an AUC of 0.94 for classifying viral infections from bacterial infections.

In conclusion, our study demonstrates that using cytokines combined with machine learning algorithms is a faster, newer, and less painful tool for detecting the cause of infection. Thus, directly treating the patient with the correct treatment, as well as improving clinical diagnosis and reducing the excessive consumption of unnecessary antibiotics, which reduces the spread of antibiotic-resistant strains of bacteria and promote effective antibiotic stewardship.

Keywords: cytokines, viral infection, bacterial infection, IP-10, IL-6, TRAIL, machine learning, SVM, PCA, LDA, diagnosis, fever, pediatric, ELISA, CRP, antibiotic resistance.

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Abbreviations

Abbreviate	Term in English
ADV	*Adenovirus*
AUC	Area Under Curve
CSF	Cerebrospinal Fluid
CV	Coefficient Of Variation
CBC	Complete Blood Cell Count
CRP	C-Reactive Protein
CXCR3	C-X-C Motif Chemokine Receptor 3
COX2	Cyclooxygenase-2
DR	Death Receptor
DNA	Deoxyribonucleic Acid
ED	Emergency Department
ELISA	Enzyme Linked Immunosorbent Assay
E. COLI	*Escherichia Coli*
EDTA	Ethylenediaminetetraacetic Acid
ESR	Erythrocyte Sedimentation Rate
FN	False Negative
FP	False Positive
FWF	Fever Without Focus
FWS	Fever Without Source
FLDA	Fisher's Linear Discriminant Analysis
GPCR	G protein-Coupled Receptor
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HRP	Horseradish Peroxidase
HIV/AIDS	*Human immunodeficiency Virus Infection*/ Acquired Immunodeficiency Syndrome
IFN-γ	Interferon Gamma

IP-10/ CXCR10	Interferon γ-induced protein 10 kDa/ C–X–C motif chemokine 10
IL-1	Interleukin-1
IL-1β	Interleukin-1-1 beta
IL-10	Interleukin-1-10
IL-12	Interleukin-1-12
IL-6	Interleukin-6
JAK-STAT	Janus Kinase/Signal Transducer and Activator of Transcription
LOO	Leave One out
LDA	Linear Discriminant Analysis
MRSA	*Methicillin-Resistant Staphylococcus Aureus*
NK	Natural killer Cell
NEC	Negative Extraction Control
NTC	Negative Template Control
NF-Kb	Nuclear Factor Kappa B
PRCS	Palestinian Red Crescent Society
PAMPs	Pathogen-Associated Molecular Patterns
PRR	Pattern Recognition Receptors
Pg	Picograms
PCR	Polymerase Chain Reaction
PCA	Principal Component Analysis
PCs	Principal Components
PCT	Procalcitonin
PGE2	Prostaglandin E2
RT-PCR	Real-Time Polymerase Chain Reaction
ROC curve	Receiver Operating Characteristic Curve
REC	Research Ethics Committee
RSV	*Respiratory Syncytial Virus*
RNA	Ribonucleic Acid
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
SIM	Sulfur Indol Motility

SVM	Support Vector Machine
3D	Three Dimensional
TLRs	Toll-Like Receptors
TMP	3,3′,5,5′-Tetramethylbenzidine
TSI	Triple Sugar Iron
TN	True Negative
TP	True Positive
TNF	Tumor Necrosis Factor
TRAIL	Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand
WBC	White Blood Cells
WHO	World Health Organization

Chapter One

Introduction

Fever is a prevalent symptom confronted in emergency scenarios within hospitals and health centers (Trapani et al., 2023). One must realize that fever can be a sign of many different medical diseases (Fernández Lopez et al., 2003). Infectious diseases caused by viruses and bacteria impact global human health, both in terms of morbidity and mortality. In European countries, as a direct result of an infection caused by bacteria resistant to antibiotics, over 33000 people die each year; the burden of these infections is approximately comparable to that of influenza, tuberculosis, and HIV/AIDS put together (Cassini et al., 2019). Globally, viral infections are responsible for a significant number of respiratory-related deaths, with fatalities ranging from 290,000 to 650,000 (Nair et al., 2011). On-time and accurate identification of the etiology of infections is important for appropriate treatment and medical decision-making (Lederberg, 2000). However, many challenges are met frequently in diagnosing the viral and bacterial infections due to the overlapping nature of their symptoms, such as fever, sore throat, cough, fatigue, respiratory issues, and body aches (Silterra et al., 2017). For patients' medical evaluation, clinical physicians depend on several practical points, including the patient's medical history. Besides the clinical examination, many cases need additional diagnostic procedures, such as radiological images, laboratory tests, including serological tests, and in some cases molecular diagnostics, to determine the causes leading to elevating the body temperature (Santana et al., 2019). In different cases, it is necessary to make an invasive procedure to diagnose the infection etiology to finally give the appropriate treatment (Lanks et al., 2019).

1.1 Routine Laboratory Tests and Their Limitations

Many procedures are performed to identify the causative agent of infections. In the laboratory, some routine tests such as complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin, and microbiological cultures

are providing good information about the patient's health in the cases of infection and inflammation (Massaro et al., 2007). However, these routine tests have some limitations. For example, the CBC test is not specific for indicating the infection causes. One of the important parameters in the CBC test is the white blood cell (WBC) count, which can give a good indication about the pneumococcal infections, but still less effective in *Staphylococcus* infections (Sarah S. Long, MD, n.d.). On the other hand, C-reactive protein (CRP) adds valuable clinical information as it is considered to be from the acute phase proteins secreted from the liver in infection and inflammation cases and in differentiating bacterial from viral infections. However, some studies have found that hospitalized patients in early stages of infections show low CRP levels similar to those of healthy individuals, leading the physicians to ignore the serious infections (Feigin et al., 2021). In another direction, regardless of the utilization of procalcitonin as a good lab biomarker for controlling antimicrobial therapy in bacterial infections, it may lose some of its sensitivity and specificity (Schuetz et al., 2011). Procalcitonin can be falsely elevated in major systemic inflammatory stress, such as severe trauma, surgery, burns, acute pancreatitis, and circulatory shock (Farooq & Colón-Franco, 2019), and cannot differentiate inflammatory disorders or viral infections from severe bacterial infections (Sager et al., 2017) . Furthermore, real-time polymerase chain reaction (RT-PCR) is effective in detecting the genetic material in the infectious samples. Obtaining high-quality samples is required; however, this is not feasible in the cases of inaccessible infections that need invasive procedures (Yang & Rothman, 2004). Although these lab tests help in confirming the presence of pathogens and aid in accurate diagnosis, they are often expensive, and time-consuming (sometimes requiring at least 48 hours for results (Finnell et al., 2011; Melendez & Harper, 2010; Rudinsky et al., 2009; Stoll & Rubin, 2004; Storch, 2000; Van den Bruel et al., 2011). Moreover, these routine tests are useless for inaccessible infections (Limmathurotsakul et al., 2010).

1.2 Immune System in Different Types of Infections

The immune system fights against the pathogens, leading to the release of several inflammatory molecules (Conti et al., 2004). Cytokines and chemokines are important inflammatory molecules secreted following the infections and help in the prediction of infectious diseases. Some studies mentioned the utilization of cytokines and chemokines as markers in lab studies (Frimpong et al., 2022; Kany et al., 2019). The immune system recognizes different pathogens using the pattern-recognition receptor (PRR), such as the existence of lipopolysaccharide on the surface of gram-negative bacteria, and the presence of a thick layer of peptidoglycan on gram-positive bacteria. Also, single and double-stranded RNA in RNA viruses (Smith & Fiddaman, 2022). Once PRR activated by each pathogen, a distinct pathway of immune response influences different scenarios of the production pathway of inflammatory molecules. Many studies have reported that different infections can induce the secretion of many inflammatory cytokines, including IL-1, IL-6, IL-10, and TNF-α (Rhedin et al., 2021; Tripathy et al., 2021; Yusa et al., 2017). Through the immune response to pathogens, pro-inflammatory cytokines such as interleukin 6 (IL-6) start to elevate and induce the CRP production from the liver, which signifies systemic inflammation, and influence the elevation of WBC counts which lead to the recruitment and activation of the immune cells (Slaats et al., 2016; Yan et al., 2021; Zarkesh et al., 2015). Moreover, IL-1, IL-6, and TNF are of the main cytokines that trigger fever, in which IL-6 activates cyclooxygenase-2 (COX2) to produce a major fever-inducing mediator called prostaglandin E2 PGE2 (Kulesza et al., 2023). Furthermore, some studies explained

the correlation between the cytokine levels such as IL-6 and IL-10, with the severity of sepsis as well as the survival rate of septic patients (C. Liu et al., 2020). All of these diagnostic tools show the importance of rapid and accurate identification of viral and bacterial infections using the detection of cytokine levels to improve patient health and manage the issues of antibiotic overuse.

1.3 Research Significance

The limits of popular diagnostic lab tests, including CBC, CRP, PCR, and microbiological tests, etc., are discussed in this research, including their unreliability, poor sensitivity, and low specificity in many clinical cases. The prolonged turnaround time of microbiological culture results can lead to a 30%–50% mistake rate in patients' diagnoses. This misdiagnosis could end with delayed or ineffective treatment and using unnecessary antibiotics, which contributes to the development of antibiotic resistance genes, leading to prolonged illnesses, increased mortality rates, and higher healthcare costs (G.-Y. Liu et al., 2024; McIsaac & Butler, 2000). The central focus of this study inquiry is the exploration of developed laboratory tests to enhance diagnostic accuracy and efficiency in individuals suffering from fever related to both accessible and inaccessible infections.

1.4 Aims & Objectives

1.4.1 Major Objectives

Investigate the potential of cytokine measurements in tandem with machine learning algorithms as biomarkers to differentiate between bacterial and viral infections. Additionally, evaluate whether these combined techniques can distinguish between Gram-positive and Gram-negative bacterial infections.

1.4.2 Minor Objectives

Detect distinct cytokine profiles linked to bacterial and viral infections. In addition, measure the diagnostic accuracy, sensitivity, and specificity of cytokine measurements as biomarkers for bacterial and viral infections.

Chapter Two

Literature Review

Cytokines are important signal molecules that guide the immune response and host defense against infections (Dinarello, 2007). Their function represents the communication and interaction between immune cells and orchestrating the body's response to various infections (C. Liu et al., 2021a). Cytokines are classified into subfamilies, interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), and chemokines. Each of them has a specific function in the immune regulation process (Monastero & Pentyala, 2017).

2.1 Cytokine Concentration Utilization in the Differentiation Between Viral and Bacterial Infections:

Many studies have shown the importance of cytokine concentrations in the differentiation between viral and bacterial infection cases (Caragheorgheopol et al., 2023; Hanna et al., 2015; Holub et al., 2013; K.-T. Liu et al., 2016a) as summarized in Table 1. The host's immune system responds in the production of deferent patterns of cytokines according to the type of infection. Several studies mentioned the reliance on cytokine profiles for determining the pattern of infection. Recent researches used the BV score as a tool combining between Interferon-gamma-induced protein 10 (IP-10), Tumor Necrosis Factor-related apoptosis-inducing ligand (TRAIL), and C-reactive protein (CRP) for the discrimination between bacterial and viral infections (Bachur et al., 2024; Fröhlich et al., 2023a; Halabi et al., 2023a; Mastboim et al., 2023; Papan et al., 2022; Stas et al., 2022) as summarized in Table 2. Furthermore, different studies explained the ability of cytokines in distinguishing between gram-positive and gram-negative bacterial infections (Hessle et al., 2005; K.-T. Liu et al., 2016b). For example, IL-6, IL-8, and IL-10 levels are elevated in the cases of gram-negative bacterium infections (Guan et al., 2020; Li et al., 2019), While IL-12, IFN-γ, TNF, and IL-1β are induced significantly in gram-positive bacterium infections (Dewi, 2024) (Keestra-Gounder & Nagao, 2023) (Keestra-Gounder & Nagao, 2023).

2.2 Combination the Cytokine Concentrations with Machine Learning Algorithms

Recently, the integration of cytokine concentrations with machine learning algorithms represents a promising advanced tool in infectious diseases field (Song et al., 2021) . The power of predictive models' creation can accurately identify different types of infections (Fröhlich et al., 2023b). Support vector machine and random forest are examples of machine learning algorithms that are used in the unique identification of the type of pathogens (Del Valle et al., 2020). which finally enhances patient care and facilitates early detection, and improves diagnosis accuracy (Atalan et al., 2022).

Table 1: Summarization of different studies in the Literature Review Part – 1

Study Title	Year	Result
Inflammatory molecules expression pattern for identifying pathogen species in febrile patient serum (K.-T. Liu et al., 2016b).	2016	Higher concentrations of interleukin (IL)-6 and IL-8 were detected in bacterial infection groups.viral infections: higher concentrations of IP-10.
Levels of 25 cytokines in the first seven days of life in newborn infants (Lusyati et al., 2013).	2020	Interleukins are stable in the first week of life in non-infected infants
Gram-Positive and Gram-Negative Bacteria Induce Different Patterns of Cytokine Production in Human Mononuclear Cells Irrespective of Taxonomic Relatedness (Hessle et al., 2005).	2010	Gram-positive bacteria-induced much more IL-12, IFN-γ, TNF, IL-1β IL-6, IL-8, IL-10 in Gram Negative
Cytokine Storm Syndromes in Pediatric Patients (Diorio et al., 2023).	2023	Soluble IL-2 receptor (sIL2R) was over 10-fold higher than the upper limit of normal.
Cytokines and chemokines expression pattern in herpes simplex virus type-1 encephalitis (Wang et al., 2021).	2021	IL-6
Cytokine Profiling in Influenza A Virus and Staphylococcal (Co-Infections) (Tölken et al., 2022).	2022	TNF-α, IL-1$^{\alpha}$, IL-1β
Potential of Interleukin (IL)-12 Group as Antivirals: Severe Viral Disease Prevention and Management (Rahman, 2023).	2023	Antiviral actions of the IL-12 family
Cytokines as Biomarkers and Their Respective Clinical Cutoff Levels (Monastero & Pentyala, 2017).	2017	Three cytokines, IL-6, TNF-α, and IL-1β, were investigated and strongly implicated as diagnostic tools in neonatal sepsis

Table 2: Summarization of Different Studies in the Literature Review – Part 2

Year of Publication	Research Title
2023	A point-of-need platform for rapid measurement of a host-protein score that differentiates bacterial from viral infection: Analytical evaluation.(Hainrichson et al., 2023).
2023	Validation of a multi-analyte immunoassay for distinguishing bacterial vs. viral infections in a pediatric cohort (Chokkalla et al., 2023).
2023	Host test based on tumor necrosis factor-related apoptosis-inducing ligand, interferon gamma-induced protein-10 and C-reactive protein for differentiating bacterial and viral respiratory tract infections in adults: diagnostic accuracy study (Halabi et al., 2023b).
2023	Bacterial vs viral etiology of fever: A prospective study of a host score for supporting etiologic accuracy of emergency department physicians (Mor et al., 2023).
2022	BV score differentiates viral from bacterial-viral co-infection in adenovirus PCR positive children (Stein et al., 2022).
2016	Diagnostic accuracy of a TRAIL, IP-10 and CRP combination for discriminating bacterial and viral etiologies at the Emergency Department (Eden et al., 2016).
2020	Differential Markers of Bacterial and Viral Infections in Children for Point-of-Care Testing (Tsao et al., 2020).
2023	Expression of TRAIL, IP-10, and CRP in children with suspected COVID-19 and real-life impact of a computational signature on clinical decision-making: a prospective cohort study (Fröhlich et al., 2023c).
2017	A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study (van Houten et al., 2017) .

2.3 IP-10, TRAIL, and IL-6 Importance in the Differentiation Between Viral and Bacterial Infections

2.3.1 IP-10

Interferon-gamma-induced protein 10 (IP-10), also known as CXCL10, is a cytokine that serves as a chemokine related to its function in the immune response, especially in viral infections. IP-10 is secreted by monocytes, endothelial cells, and fibroblasts in response to

interferon-gamma (IFN-γ). When IFN-γ binds to its receptor on these cells, the Janus kinase/signal transducers and activators of transcription (JAK-STAT) signaling pathway will be activated, leading to the transcription of the IP-10 gene (M. Liu et al., 2011; Platanias, 2005). After this process, the IP-10 interacts with CXC chemokine receptor-3 (CXCR3), resulting in inducing some physiological effects related to cell growth, development, and regulation, including tumor promotion, regulation, and angiostasis. Moreover, this integration activates the expression of G protein-coupled receptor (GPCR) on various cell types, such as activated T cells, natural killer cells, endothelial cells, and dendritic cells, leading to attract them to the site of infection (Madhurantakam et al., 2023). Furthermore, IP-10 can bind to different receptor subtypes, CXCR3-A and CXCR3-B. Each binding results in a distinct pathway. CXCR3-A is associated with tumor proliferation and migration, while CXCR3-B is associated with more apoptosis (M. Liu et al., 2011). In the cases of respiratory viruses such as rhinoviruses, respiratory syncytial virus (RSV), and SARS-CoV-2, IP-10 production is stimulated in the airway endothelial cells during the infection. Thus IP-10 serves as a biomarker for viral illness and progression (Coperchini et al., 2021; M. Liu et al., 2011; Mulla et al., 2022). In the bacterial infections, the role of IP-10 is less obvious but still important. IP-10 contributes to the recruitment of immune cells to the infection site, leading to the pathogen clearance (Abdel-Razik et al., 2015).

2.3.2 TRAIL

Tumor Necrosis Factor-related apoptosis-inducing ligand (TRAIL) is a cytokine that belongs to the TNF family and can induce apoptosis in the tumor cells (Ashkenazi, 2002a). During the infection, TRAIL secretion is expressed on the surface of different immune cells such as T cells, NK cells, and dendritic cells (Falschlehner et al., 2007). In viral infections, TRAIL aids in the elimination of viral-infected cells through the apoptosis process and inducing the death of infected cell (Griffith & Lynch, 1998; Shepard & Badley, 2009). By activating the death receptors (DR4 and DR5) on target cells, leading to the activation of the caspase cascade (Ashkenazi, 2002b). In bacterial infections, the TRAIL role depends on the pathogen and targets the bacterial-infected cell cases. For example, in the case of Mycobacterium tuberculosis infection, TRAIL-mediated the apoptosis of infected macrophages to control bacterial spread (Bocchino et al., 2005).

2.3.3 IL-6

Interleukin-6 (IL-6) is a cytokine that is elevated in cases of inflammation and infections, and is involved in B-cell maturation. IL-6 is secreted from different cells, including T cells, macrophages, and endothelial cells, in response to infections and tissue injuries (Tanaka et al., 2014). NF-κB and JAK-STAT pathways are signaling pathways that are activated in response to pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs). This results in the regulation of IL-6 secretions (Aliyu et al., 2022; C. Liu et al., 2020). IL-6 acts as a pro-inflammatory cytokine, elevating the body temperature and inducing the production of acute-phase proteins in viral infections, such as in the cases of influenza and COVID-19 (Zhang et al., 2020). IL-6 also plays a role in stimulating the production of acute-phase proteins by the liver and enhancing the differentiation of T-helper cells in bacterial infections, leading to start the immune response and eradicate the infection (Gabay & Kushner, 1999).

Chapter Three

Methodology

3.1 Ethical Approval

Ethical approval was obtained from Palestinian Red Crescent Society (PRCS) Hospital in September 2023 to start collecting patient samples and from the research ethical committee (REC) at al-Quds University in November 2023. In February and March 2024, ethical approval was obtained from Dora Hospital and Alia Hospital, respectively (see the appendix section pages 68-73). Blood samples were collected in the period from September 2023 until July 2024; ethical approval was obtained from each patient's parents to collect the blood sample and the required information using a form that includes the patient's information and the signed approval from the parents (see the appendix section page 67).

3.2 Samples Collection

Blood samples were collected from candidate patients who met the inclusion criteria after obtaining ethical approval from patients' parents using a form that includes the patient's information and the signed approval from the parents (see the appendix section page 71). Patients who had confirmed viral infections by clinical diagnosis and lab diagnosis using the gold standard PCR at PRCS's molecular biology lab or patients who were suspected of viral infection according to clinical judge (nasopharyngeal swabs were taken for the patients who were suspected of upper respiratory tract infections, tonsillitis, rhinitis, sinusitis, pharyngitis, and sore throat). Patients who had bacterial infections that were confirmed at PRCS's microbiology lab, patients who had inaccessible infections, patients who had FWF, and the control group from September 2023 to May 2024. EDTA tubes were filled with blood after sterilizing the venipuncture site using alcohol and withdrawing the blood by professional nurses or lab technicians. The control group blood samples were collected through a community service of free-laboratory tests campaigns for children

during three days in the Mohammed Eid Shaheen Center in the old city, Hebron. Plasma samples were separated by centrifugation EDTA tubes using [MIKRO 200] at 1000xg for 10 minutes at a temperature of 4 C (C. Liu et al., 2021b), then saved in no-additives tubes at -80 C (de Jager et al., 2009) until ELISA analysis of cytokines.

3.3 Patients' Selection

3.3.1 Inclusion Criteria

Pediatric patients aged < 18, who had clinically diagnosed to have suspected viral or bacterial infections or fever with unknown source (FWS) after doctor examination, with their body temperature being measured at or above 38.0°C at the Emergency Department (ED) or Pediatric ward, and having a history of illness lasting less than 7 days were included in the study.

3.3.2 Exclusion Criteria

Patients who met one or more of the following criteria were excluded. Patients who experienced another febrile episode during the preceding 2 weeks, or received antibiotic treatment for more than 48 hours, had a proven or suspected HIV/HBV/HCV infection, had primary immunodeficiency, had an active malignancy, received immunosuppressive or immunomodulatory treatment, having severe psychomotor retardation, or had severe congenital metabolic disorders, and patients with rheumatic, autoimmune, or autoinflammatory disorders.

3.4 Microbiological Lab Confirmation for Suspected Bacterial Infection Samples

After the clinical examination of patients, ethical approval was obtained from the parents of individuals suspected of having bacterial infections to participate in the study. the physician ordered various laboratory tests including a complete blood cell count (CBC), C-reactive protein (CRP), and urine analysis in selected cases, as well as culture samples (e.g., blood, urine, fluids, throat, and swabs) based on the patient's case. A total of 22 patients were enrolled, and their culture samples were transferred and treated according to the PRCS microbiological lab's protocol.

Aerobic and anaerobic blood culture bottles were placed in an automated system (Render Biotech, China), which alerted for bacterial growth within 48 hours of incubation. Blood culture bottles with positive bacterial growth were then sub-cultured onto blood agar (HiMedia Laboratories, India (*Blood Agar Base (Infusion Agar)*, n.d.)). MacConkey agar (HiMedia Laboratories, India, n.d.,(Bagley et al., 1978), mannitol salt agar (HiMedia Laboratories, India, n.d., (Wormser & Hanna, 2004), and Slantez & Bartley medium (Oxoid, n.d., England) -used for enterococcus species- to promote bacterial growth (*Medical Microbiology*, 2020). Urine and other swab samples were immediately cultured on the same agar types. Throat swabs were cultured on blood agar media, and cerebrospinal fluid (CSF) samples were cultured on blood agar and chocolate agar (*Medical Microbiology*, 2020). All samples' agar plates were incubated at 37°C for 24 to 48 hours to monitor bacterial growth (*Medical Microbiology*, 2020).

For samples that exhibited bacterial growth, a Gram stain was performed (Beveridge, 2001). Gram-negative bacteria underwent biochemical testing (Mac Faddin, 1980),

including urea, triple sugar iron (TSI), sulfur indole motility (SIM), and citrate tests (from Oxoid, England (Mac Faddin, 1980). Additional tests, such as the Hyenetrotest tube ('Hy Enterotest', n.d.) and API 20 (*API® ID STRIPS*, n.d.), were used for the identification of lactose and non-lactose fermenter colonies, along with the oxidase test (Hylabs, n.d., Israel) (Gaby & FREE, 1958). Gram-positive bacteria were tested for catalase (Mac Faddin, 1980) and coagulase tests (Oxoid Staphylase test, Oxoid, n.d. UK) for Staphylococcus species identification (Weber & Wachowitz, 1989). For Streptococcus species identification, catalase tests and latex grouping reagents (Oxoid Streptococcal grouping kit, Oxoid, n.d., UK) were performed to identify serogroups A, B, C, D, F, and G (Petts, 1999).

3.5 Molecular Biology Lab Confirmation for Suspected Viral Infection Samples

3.5.1 Nasopharyngeal Swab Collection

After obtaining ethical approval from each participant suspected of having an upper respiratory tract viral infection, a nasopharyngeal swab was obtained using a flexible flocked swab and inserted in universal transport medium (*UTM® Universal Transport Medium™ | COPAN*, n.d.). Then, the swabs were stored at −20°C for qualitative real-time PCR to detect viral genetic material (World Health Organization, 2011). The tested viruses included *Adenovirus, SARS-CoV-2 virus, Coronavirus NL63, Rhinovirus, Bocavirus,* Influenza A virus, Influenza B virus, *Respiratory Syncytial virus, and Herpes Simplex virus.*

3.5.2 Viral Nucleic Acid Purification

The viral nucleic acid purification procedure was proceeded following the manufacturer's protocol provided by Roche Diagnostics, Germany, using the ('High Pure Viral Nucleic Acid Kit', n.d.) [REF: 11858874001]. Working solutions were prepared, and all components were brought to room temperature before initiating this step for 50 nasopharyngeal swabs. 200 µl of the sample was added to a nuclease-free 1.5 ml microcentrifuge tube along with 200 µl of freshly prepared working solution, which was a carrier RNA-supplemented binding buffer. Then, 50 µl of Proteinase K solution was added and mixed immediately. The mixture was incubated for 10 minutes at +72°C. Following incubation, 100 µl of binding buffer was added and mixed to transfer the sample to a high pure filter tube. The high pure filter tube was inserted into a collection tube, and the entire sample was pipetted into the upper reservoir of the filter tube. The entire high pure filter tube assembly was placed into a standard tabletop centrifuge and centrifuged for 1 minute at 8,000 x g. After centrifugation, the filter tube was removed from the collection tube, and the flowthrough liquid along with the collection tube was discarded. The filter tube was combined with a new collection tube, and 500 µl of inhibitor removal buffer was added to the upper reservoir of the filter tube. The assembly was centrifuged again for 1 minute at 8,000 x g. post-centrifugation, the filter tube was removed from the collection tube, and the flowthrough liquid along with the collection tube was discarded. The filter tube was then combined with another new collection tube. 450 µl of wash buffer was added to the upper reservoir of the filter tube and centrifuged for 1 minute at 8,000 x g, after which the flowthrough was discarded to remove inhibitors. The filter tube was again combined with a new collection tube and another 450 µl of wash buffer was added to the upper reservoir of the filter tube. This was followed by centrifugation for 1 minute at 8,000 x g, and the flowthrough was discarded. The filter tube-collection tube assembly was left in the

centrifuge and spun for an additional 10 seconds at maximum speed (approximately 13,000 x g) to ensure the removal of any residual wash buffer. The collection tube was discarded, and the filter tube was inserted into a nuclease-free, sterile 1.5 ml microcentrifuge tube. Then, 50 µl of elution buffer was added to the upper reservoir of the filter tube to elute the viral nucleic acids, and the tube assembly was centrifuged for 1 minute at 8,000 x g. Finally, the eluted viral nucleic acids were stored at -20°C for analysis.

In this study, primers and probes for each virus were prepared at a 1:10 concentration, with working dilutions made at 1:100 from the stock solution. To enable sensitive and specific detection of respiratory viruses, primer and probe sets targeting conserved genomic regions of Influenza A (FLU A), Influenza B (FLU B), Respiratory Syncytial Virus (RSV), Herpes Simplex Virus (HSV), Adenovirus (ADV), COVID-19, Coronavirus NL63, Bocavirus, and Rhinovirus were employed in real-time PCR assays. Table 3 explains the primer pairs and probes (fluorescent reporters and quenchers) which synthesized commercially and used under optimized conditions in qPCR assays, with fluorescence signals monitored in real time to confirm target amplification. The master mix from (*GoTaq® Probe 1-Step RT-qPCR System Technical Manual*, n.d.) [Promega, USA, REF: A6121], had a total volume of 10 milliliters which contained DNA, RNA polymerase, nucleotides, and necessary additives, including the ligase enzyme. For each sample, 10 µl of the master mix was used. 1 µl of the prepared forward primer, 1 µl of the prepared reverse primer, 1 µl of the probe for each virus, and 0.4 µl of reverse transcriptase enzyme were added to the master mix. Nucleotide-free water was used to adjust the final reaction volume to 15 µl. All these components were added on ice within a safety cabinet, adhering to the manufacturer's protocol. The reaction mix, excluding the DNA template, was distributed into each PCR well of a PCR 8-strip tube optical-grade PCR plate. Then, 5 µl of each DNA template was added to the appropriate wells, while free-nucleotide water was added to the negative template control reactions. The wells were sealed and briefly centrifuged to ensure all components were collected at the bottom and evenly distributed. The prepared samples and a positive control for each virus, a negative extraction control (NEC), and a negative template control (NTC) were placed in the Bio-Rad CFX96 PCR System. Then, thermal cycling protocol was conducted within four phases: Reverse transcription at 45 °C for 15 minutes to convert the viral RNA into cDNA. Initial denaturation at 95°C for 2 minutes to separate DNA strands, annealing at 95°C for 15 seconds to allow primer binding, and extension at 60°C for 1 minute to synthesize new DNA strands repeated 39 cycles.

Table 3-A: Primer and Probe Sequences for Respiratory Viruses

Virus	**Forward Primer (5′-3′)**	**Reverse Primer (5′-3′)**	**Probe (5′–3′)**
Flu A	AGGGCATTYTGG ACAAAKCGTCTA	AGAAGATGGAGAAGG CAAAGCAGAACTAGC	6-FAM-TGCCATAGCATGAC ACAATGGCTCCT-BHQ-1
Flu B	TTCTTTCCCACCG AACCAAC	CAGAATATACATCCR GTCACAATTGGARAA	6-FAM-CTGGCCAGCACGTAT CCCAACAGCA-BHQ-1

Table 4-B: Primer and Probe Sequences for Respiratory Viruses

RSV	CGATTTTTATTGGATGCTGTACATTT	AACAGATGTAAGCAGCTCCGTTATC	6-FAM-CCGCCGAACTGAGCAGACACCCGCGC-BHQ-1
HSV	CATCACCGACCCGGAGAGGGAC	GGGCCAGGCGCTTGTTGGTGTA	Cy5-TGCACCAGACCCGGGCTCAGGTACTCCGA-BHQ-2
ADV	GCCCCAGTGGTCTTACATGCACATC	GCCACGGTGGGGTTTCTAAACTT	6-FAM-CTGGCCAGCACGTATCCCAACAGCA-BHQ-1
COVID-19	ACAGGTACGTTAATAGTTAATAGCGT	ATATTGCAGCAGTACGCACACA	6-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ-1
Coronavirus NL63	GCTGTGACTGCGGCAAACT	ACTTAGTGTGCGAGTTCTGAGTT	6-FAM-AGCCTCAGCACCAGCTGCTGAC-BHQ-1
Bocavirus	GAGCTCTGTAAGTACTATTAC	CTCTGTGTTGACTGAATACAG	6-FAM-AGCACCAGACGGAGCAGCTGAC-BHQ-1
Rhinovirus	CAAGCACTTCTGTTTCCC	ACGGACACCCAAAGTAG	6-FAM-TGGTGCCGGCCCCTGAATGYGGC-BHQ-1

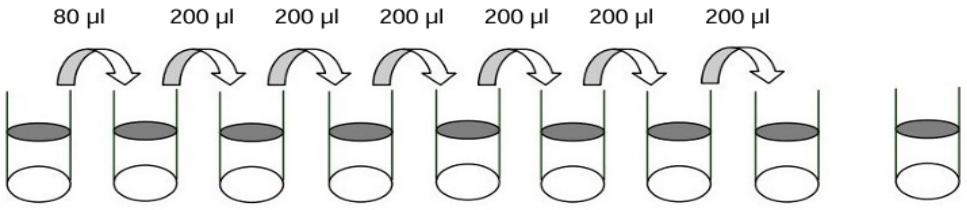
3.6 Cytokines Analysis Using Enzyme-linked Immunosorbent Assay (ELISA)

The ELISA technique from *Sigma-Aldrich, n.d.* company was used for cytokine measurements. Interleukin 6 (IL-6), IP-10, and TRAIL were analyzed following the procedure of each manufacturer's protocol for (*Human IL-6 ELISA Sigma*, n.d.) (*Human IP-10 ELISA Sigma*, n.d.; *Human TRAIL ELISA Kit for Cell Culture Supernatants, Plasma, and Serum Samples | Sigma-Aldrich*, n.d.). As shown in the literature review section, these cytokines are used to be the most common immunomodulatory molecules that can differentiate between different types of infections. ELISA kits components contained a pre-coated 96-well ELISA plate, biotinylated detection antibodies, protein standards, horseradish peroxidase (HRP)-streptavidin, wash buffer, TMB substrate, stop solution, and assay diluent buffers.

3.6.1 Human IP-10/CXCL10 ELISA Kit (RAB0119)

The assay included assay diluent buffer A (for serum/plasma) and 5x assay diluent buffer B (for cell culture supernatants). The assay diluent buffer B was diluted 5-fold with deionized or distilled water before use. Standards were prepared by reconstituting the lyophilized protein standard with 400 µl of assay diluent A to create a 50 ng/ml stock solution. Serial dilutions were performed as follows: 80 µl of the stock was added to 586.7 µl of the respective diluent to make a 6000 pg/ml standard. 1:3 serial dilutions was done to generate concentrations of 2000, 666.7, 222.2, 74.07, 24.69, and 8.23 pg/ml, and the diluent

as the zero standard as shown in Figure 1. Sensitivity was defined as 2.5 pg/ml. Recovery rates were measured by spiking the analyte into various matrices, yielding an average recovery rate of 93% (range: 85-100%). The coefficient of variation (CV) for intra- and inter-assay reproducibility was <10% and <12%, respectively.



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	586.7 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	50 ng/ml	6000 pg/ml	2000 pg/ml	666.7 pg/ml	222.2 pg/ml	74.07 pg/ml	24.69 pg/ml	8.23 pg/ml		0 pg/ml

Figure 1: Preparation of Serial Dilutions of IP-10 Standards

3.6.2 Human IL-6 ELISA Kit (RAB0306)

This kit provided Assay Diluent A (for serum/plasma) and 5x Assay Diluent B (for cell culture supernatants and urine). Assay Diluent B was diluted 5-fold with deionized water before use. The lyophilized IL-6 standard was reconstituted with 500 µl of Assay Diluent A to prepare a 12,000 pg/ml stock solution. A 1000 pg/ml working solution was prepared by mixing 40 µl of the stock with 440 µl of the respective diluent. Subsequent 1:3 dilutions were performed to create standards with concentrations of 333.3, 111.1, 37.04, 12.35, 4.12, and 1.37 pg/ml, with the diluent as the zero standard as shown in Figure 2. The kit's sensitivity was 3 pg/ml, and the average recovery was 92% (range: 83-105%). CVs for intra- and inter-assay variability were <10% and <12%, respectively.

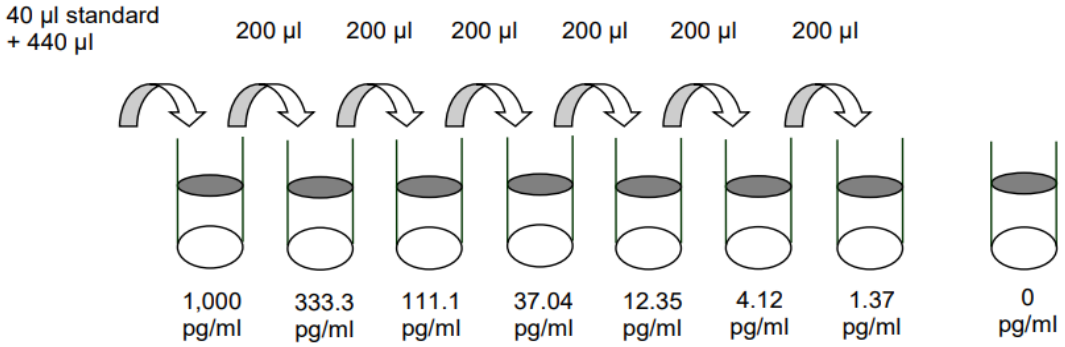
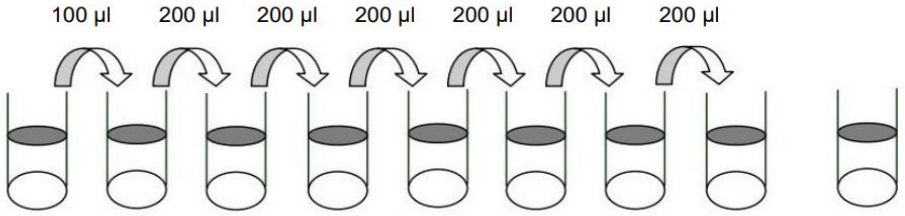


Figure 2: Preparation of Serial Dilutions of IL-6 Standards

3.6.3 Human TRAIL ELISA Kit (RAB1090)

The TRAIL kit included a single 5x Assay Diluent Buffer, which was diluted 5-fold with deionized water before use. The lyophilized TRAIL standard was reconstituted with 400 µl of 1x Diluent to prepare a 50 ng/ml stock solution. A 10,000 pg/ml working standard was created by mixing 100 µl of the stock solution with 400 µl of 1x Diluent. Serial 1:4 dilutions were made to produce standard concentrations of 10,000, 4000, 1600, 640, 256, 102.4, and 40.96 pg/ml, with the diluent serving as the zero standard as shown in Figure 3. The sensitivity of the assay was determined to be 40 pg/ml. Recovery studies demonstrated an average recovery of 94% (range: 85-105%). The CV for intra- and inter-assay reproducibility was <10% and <12%, respectively.



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	400 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl		300 µl
Conc.	50 ng/ml	10000 pg/ml	4000 pg/ml	1600 pg/ml	640 pg/ml	256 pg/ml	102.4 pg/ml	40.96 pg/ml		0 pg/ml

Figure 3: Preparation of Serial Dilutions of TRAIL Standards

The common assay procedure for all three kits has multiple shared steps. First, all reagents and samples were brought to room temperature (18–25°C) before use. Standards and samples (100 µl) were added to the appropriate wells and incubated for 2.5 hours at room temperature or overnight at 4°C with gentle shaking. After each step, Plates were washed four times with 1x wash buffer to ensure complete removal of liquids. 100 µl of the prepared biotinylated detection antibody was added to each well and incubated for 1 hour at room temperature, followed by another washing step. Then, 100 µl of the prepared HRP-streptavidin solution was added to each well and incubated for 45 minutes at room temperature. After another step of washing, 100 µl of TMB substrate solution was added and incubated for 30 minutes in the dark at room temperature. The reaction was finished with 50 µl of stop solution, and the absorbance was measured immediately at 450 nm by using the ELISA reader device EMP M201 (*M201 - ELISA Microplate Reader by CHENGDU EMPSUN MEDICAL CO.,LTD | MedicalExpo*, n.d.).

3.7 Statistical Analysis

Using Excel 2019, the mean and standard deviation for all parameters including the age, WBC, CRP, IP-10, IL-6, and TRAIL concentrations were calculated to summarize the data distribution. The descriptive statistics provided the basis for determining the cut-off values for cytokines concentrations, which were visually illustrated in figures to demonstrate the threshold calculations and their application in differentiating between viral and bacterial infections using the average calculated for each group.

Cut-off = (Mean of Viral group + Mean of Bacterial group) / 2.

Different advanced computational methods are used to analyze the concentrations of cytokines. Multivariate analysis can be divided into supervised methods and unsupervised methods. Principal component analysis (PCA) is an example of an unsupervised method. Linear Discriminant Analysis (LDA) is an example of a Supervised method, which should be trained before the classification using GUI software. As well as the machine learning Support vector machine using MATLAB software.

3.8 Dimensionality Reduction and Discriminant Analysis (PCA & LDA)

Principal Component Analysis (PCA) was used as a first step in data preprocessing to manage the multidimensional cytokine data and highlight the most informative features. PCA reduces the dimensionality of the dataset by transforming the original correlated variables into a new set of uncorrelated variables (principal components) that capture the majority of the variance. This transformation is beneficial in the case of complex biological data, as it simplifies subsequent analyses while minimizing information loss (Jolliffe, 2002). Using Principal Component Analysis (PCA), we identify new directions (principal components or loadings) by mathematically computing the eigenvectors of the covariance matrix. These principal components (PCs) are ranked in descending order based on their corresponding eigenvalues. The larger the eigenvalue, the greater the variance it captures (Baker et al., 2014). Consequently, PC1 accounts for the highest variance, followed by PC2, and so forth. The cytokines concentrations can then be expressed as a linear combination of the leading PCs, which retain nearly all the variance.

Following PCA, Linear Discriminant Analysis (LDA) was applied to further enhance the separation between classes (viral versus bacterial infections). LDA finds linear combinations of features that best separate the predefined groups. This method is based on Fisher's criterion, which maximizes the ratio of between-class variance to within-class variance, thus ensuring that the separation between groups is as distinct as possible. The combination of PCA and LDA not only helps in visualizing the data in a reduced-dimensional space but also improves the robustness of the classification by focusing on the most discriminative features (Zhao et al., 2024). In our research, Linear Discriminant Analysis (LDA) was used to enhance class discrimination. The dataset was split into a training set and a validation set. The training set was used to develop linear discriminant functions for each cytokine category, optimizing the classification success rate on the validation data. To assess performance, we used the leave-one-out (LOO) approach. This process was used and repeated for each cytokine, allowing us to estimate the overall success rate (*Pattern Recognition and Machine Learning | SpringerLink*, n.d.).

3.9 Support Vector Machine (SVM) Classification

Support Vector Machine (SVM) was utilized to classify viral infected group and bacterial infected group based on cytokine concentrations. SVM is a supervised machine learning algorithm renowned for its effectiveness in high-dimensional spaces and its capacity to identify an optimal hyperplane that maximally separates the two classes. In this study, SVM was implemented using a kernel function (such as the radial basis function) to accommodate any nonlinear relationships in the cytokine profiles. SVM model was trained on the labeled dataset of cytokine concentrations, where hyperparameters were tuned through cross-validation to prevent overfitting and ensure generalizability. The model's performance was evaluated using metrics such as accuracy, sensitivity, specificity, and the area under the ROC curve to assess its effectiveness in differentiating the etiology of

infections (Noble, 2006). In this study sensitivity represents the percentage of true positive cases among all actual positive cases, and calculated using the equation: Sensitivity=TP/(TP+FN)(Nahm, 2022). Likewise, specificity measures the percentage of true negative cases among all actual negative cases, is determined by the equation: Specificity=TN/(TN+FP) (Nahm, 2022). TP cases characterize the number of true positive predictions, and FN is the number of false negatives. TN means the number of true negatives and FP the number of false positives. These metrics were important in assessing the model's ability to differentiate between viral and bacterial infections based on cytokine profiles.

Chapter Four

Results

4.1 Study Population and Sample Selection

A total of 268 patients were screened for eligibility, and 245 were enrolled. Participants were recruited from three hospitals: PRCS Hospital (n = 198), Alia Hospital (n = 65), and Dora Hospital (n = 5), as summarized in Figure 4. Among the 245 enrolled patients, 97 samples were selected for analysis according to various conditions related to limited resources. The enrolled samples consisted of 50 males [51.55%], and 47 females [48.45%], with a mean age of 4.9 ± 3.9 years. The etiological classification revealed 22 bacterial infections, 55 viral infections, 10 cases categorized as FWF, and 10 were inaccessible. A control group of 87 participants was also included for comparative analysis.

23 samples were excluded based on the exclusion criteria including missing or depleted blood samples, contamination, or patients with certain conditions (e.g., chronic UTI, metabolic disorders). There were no cases of suspected HIV/HBV/HCV infection, immunodeficiency, or autoimmune disorders among the excluded samples, Figure 4.

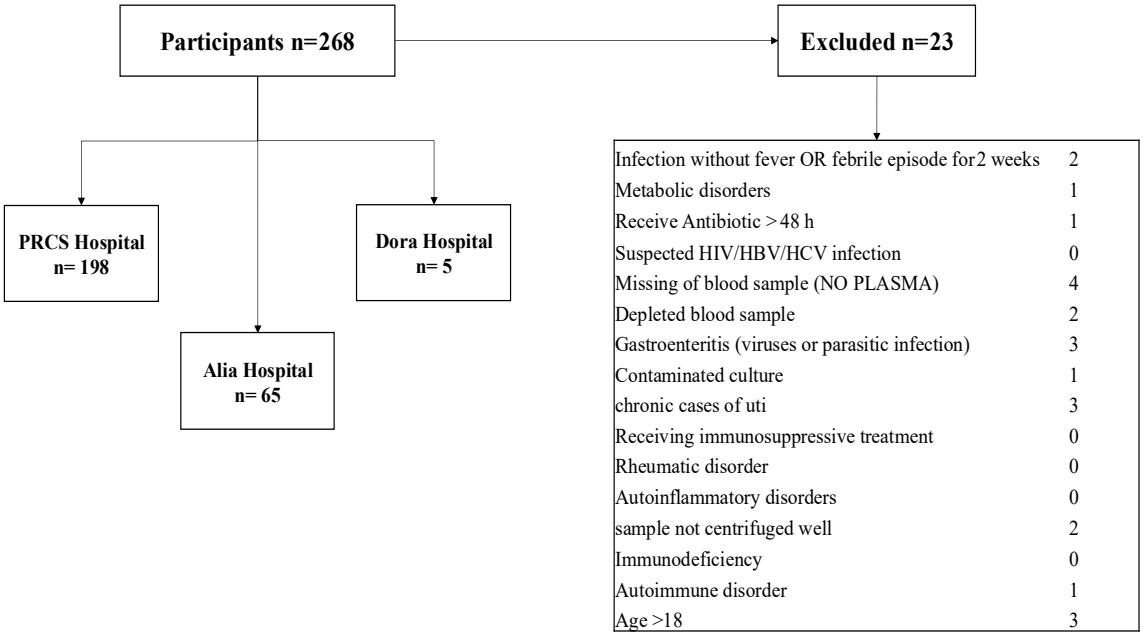


Figure 4: Study Population Chart Explains the Number of Included Participants in the Study and the Numbers and Causes of the Excluded Patients.

4.1.1 Bacterial-Infected Patients

22 participants were diagnosed with a bacterial infection by clinical diagnosis and laboratory examination. The causative agent was varied between samples [*Brucella* =1, *Streptococcus Pyogenes* = 12, MRSA =1, and *Staphylococcus Aureus* =1. *Proteus* =1, while *E. coli* =4 and, *Pseudomonas*=2]. As for the WBC the mean ± standard deviation was [15.6 ± 6.99 × 10^9/L], for the CRP, IP-10, IL-6, and TRAIL was [93.9 ± 65.4 mg/L], [808.9 ± 563.7], [4856.8 ± 3174.9], and [531.0 ± 687.5] respectively.

4.1.2 Viral-Infected Patients

55 participants were diagnosed with viral infection by clinical diagnosis and laboratory examination. 53 were tested for the viral panel using PCR in PRCS genetics labs, 2 of them were tested serologically for HAV IgM. 26 from the 53 participants had a confirmed positive viral infection. The causative virus was diverse between the infected samples [Hepatitis A virus = 2, *Herpes Simplex Virus* = 2, *Respiratory Syncytial Virus* (RSV) = 3, *Adenovirus* = 2, *Adenovirus* & Flu A = 1, *COVID-19* = 3, Flu A = 8, *Rhinovirus* = 5, *Adenovirus* & *Rhinovirus* = 1, *COVID-19* & *RSV* = 1, Flu B =0, *Bocavirus*=0, *Coronavirus NL63*= 0]. For the WBC, the mean ± standard deviation was [10.34 ± 5.02 × 10^9/L], and for the CRP, the IP-10, IL-6, and TRAIL was [22.96 ± 23.15 mg/L], [2050.18 ± 2585.80 pg/mL], [1291.67 ± 1629.58 pg/mL], [936.49 ± 676.63 pg/mL] respectively.

27 samples were negative for any virus when tested via PCR viral panel, they were clinically diagnosed as having a viral infection by experienced physicians' diagnosis. For WBC the mean ± standard deviation was [10.53 ± 4.47 × 10^9/L], and for CRP, IP-10, IL-6,

and TRAIL was [33.47 ± 36.57 mg/L], [1121.11 ± 1659.98 pg/mL], [2076.95 ± 2008.87 pg/mL], [919.12 ± 1106.72 pg/mL] respectively.

4.1.3 Fever Without Focus

10 infected patients were clinically diagnosed with fever without focus (FWF). For WBC the mean ± standard deviation was [9.11 ± 4.62× 10^9/L], and for CRP, IP-10, IL-6, and TRAIL was [21.64 ± 19.45 mg/L], [2186.91 ± 2245.02pg/mL], [277.48 ± 99.96pg/mL], [753.29 ± 453.57pg/mL] respectively.

4.1.4 Inaccessible Infection

10 infected patients were clinically diagnosed with an inaccessible infection. The mean ± standard deviation of WBC is [11.48 ± 4.22× 10^9/L], CRP [34.1 ± 33.43mg/L]. IP-10 [562.08 ± 555.89pg/mL], IL-6 [289.58 ± 130.93 pg/mL], and TRAIL [447.15 ± 176.94 pg/mL].

4.1.5 Control Group

87 healthy participants joined in the study, 32 males, 36.8%, and 55 females, 63.2%, with a mean age of 8.0 ± 2.7 years. The mean ± Standard Deviation of IP-10 [166.5± 59.0 pg/mL], IL-6 [148.8 ± 68.0pg/mL], and TRAIL [227.3± 86.9pg/mL].

4.2 The Utilization of Cut-Off for IP-10, IL-6, and TRAIL Concentrations in Infected Samples to Differentiate Between Viral and Bacterial Samples

In our study, we depended on the concentrations of the cytokines only as a promising tool to distinguish between viral and bacterial samples. Therefore, we classified the data into Viral-infected samples and Bacterial-infected samples after ELISA analysis. The value of the three cytokines for each sample was recorded in an Excel sheet (Version 2019), then we calculated the cut-off.

4.2.1 Cut-Off of the IP-10

As shown in Figure 5, the cut-off of the IP-10 was almost 2900 pg/ml. The values above this threshold are considered to have a viral infection, otherwise considered a bacterial infection. Viral infections generally trigger a strong interferon response, which leads to increased levels of IP-10 compared with bacterial infection. However, the number of samples above the cut-off was 8 samples from the 55 samples who were diagnosed with viral infections by clinical diagnosis and lab findings. Figure 5 shows that **91%** of viral infections fall below this threshold, making it insufficient for differentiation except in high concentrations. It lacks discrimination at lower concentrations.

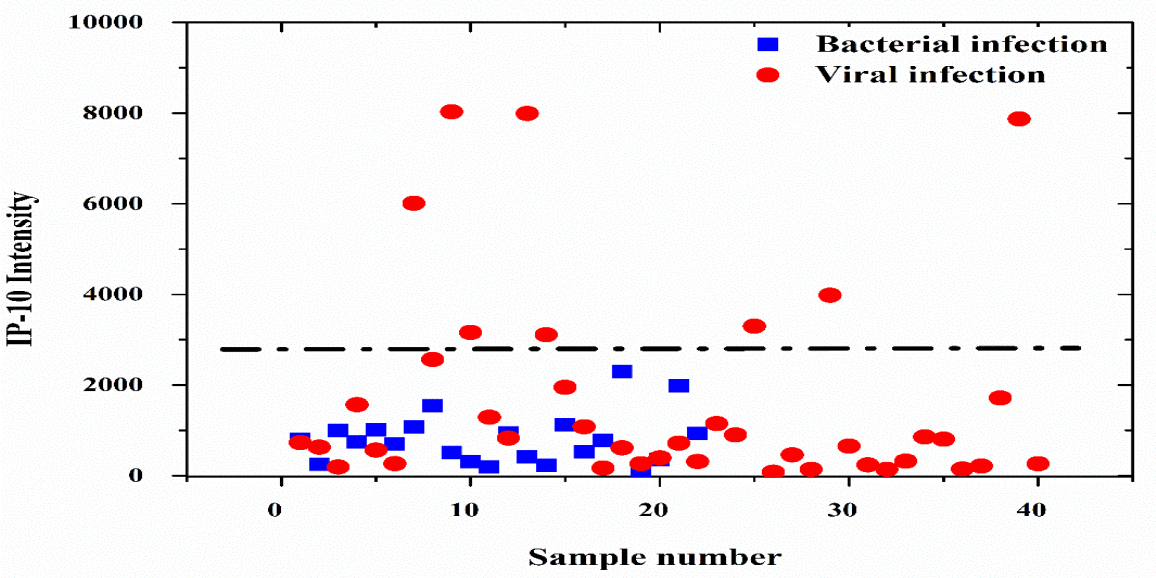


Figure 5: Cut-Off of IP-10 in Viral and Bacterial Infections.

4.2.2 Cut-Off of the IL-6

The cut-off of the IL-6 was 6000 pg/ml. The values above this threshold are considered to have a bacterial infection, otherwise considered a viral infection. The number of samples above the cut-off was 8 samples from the 22 samples which were confirmed by microbiological lab findings and physician clinical diagnosis as shown in Figure 6. The cut-off value in this cytokine insufficient to differentiate between viral and bacterial infections alone. Most of the infected samples are located under the detected cut-off of IL-6 cytokine**.**

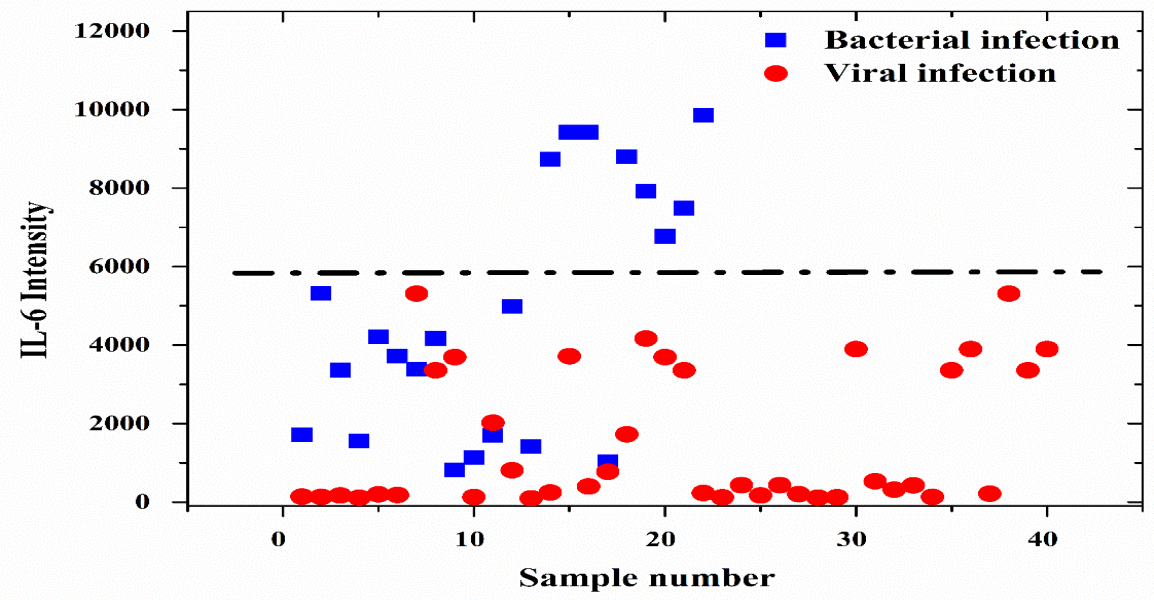


Figure 6: Cut-Off of IL-6 in Viral and Bacterial Infections.

4.2.3 Cut-Off of the TRAIL

The cut-off of the TRAIL was 2500 pg/ml. The values above this threshold are considered to have a viral infection, otherwise considered a bacterial infection. The number of samples above the cut-off was 2 samples from the 55 samples who were diagnosed with viral infections by clinical diagnosis and lab findings as shown in Figure 7. The cut-off value in this cytokine insufficient to differentiate between viral and bacterial infections. Most of the infected samples are located under the detected cut-off of TRAIL cytokine**.**

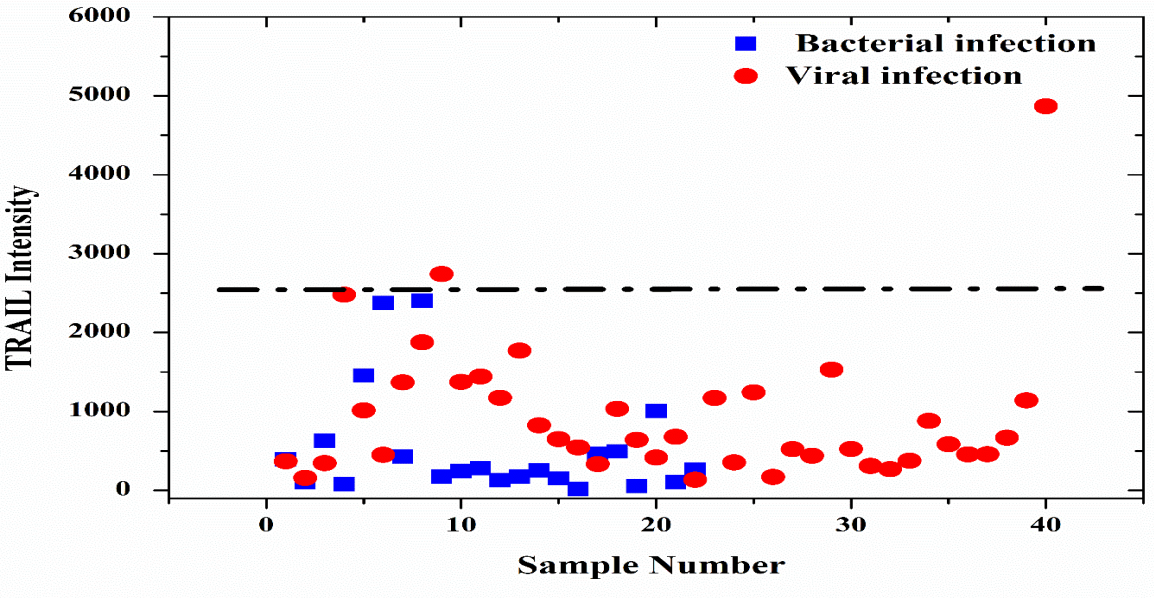


Figure 7: Cut-Off of TRAIL in Viral and Bacterial Infections.

4.3 Principal Component Analysis (PCA)

4.3.1 The Utilization of Principal Component Analysis (PCA) to Differentiate Between the Infected Samples and the Control Group

Principal Component Analysis (PCA) was applied to the cytokine concentration data to differentiate between the infected samples and the control group. By computing the eigenvectors of the covariance matrix, a set of principal components (PCs) was obtained and ranked according to their corresponding eigenvalues, which reflect the amount of variance each component captures. The first principal component (PC1) accounted for the greatest proportion of variance, followed by the second principal component (PC2), and so forth. Therefore, the cytokine concentrations can be represented as a linear combination of the leading PCs, preserving nearly all of the original variance.

We applied three-dimensional (3D) plots of PCs components for sample classification into two categories infected samples and control group using cytokines concentrations. Figures 8 (A &B) shows two views of 3D plots using the scores of three different PCs calculated using PCA. It is obvious in figure 8 that samples in each group are clustered together, but there is some mixing between them. The variable's numbers and the sample size are small in our study, thus the PCA does not visualize a clear differentiation between the infected samples and the control group.

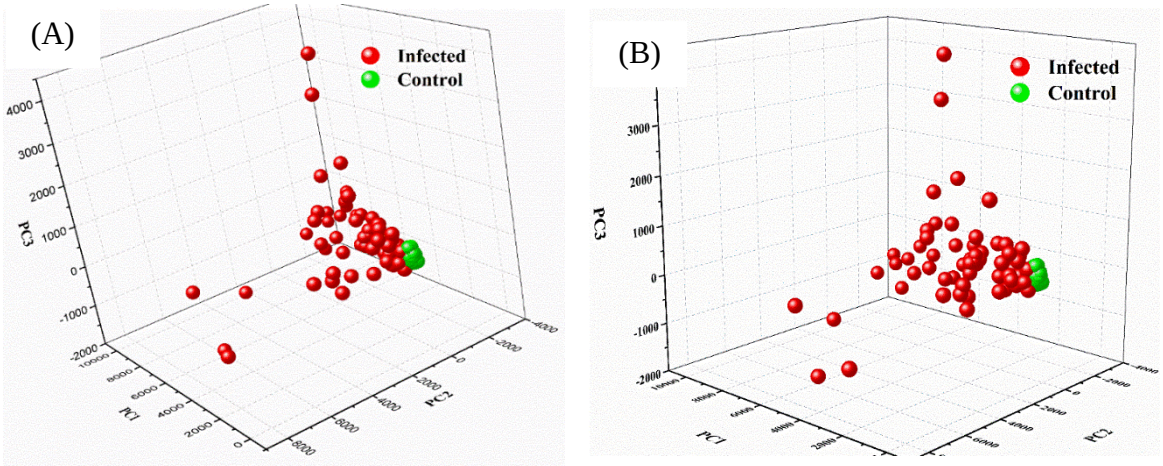


Figure 8 A&B: 3D Plots of Different PCs Scores Derived by PCA Calculation Using the Concentrations of the Three Cytokines (IP-10, IL-6, TRAIL) in the Infected Samples and Control Samples for the Classification of them into Infected and Control Group

4.3.2 The Utilization of Principal Component Analysis (PCA) to Differentiate Between the Bacterial Group and Viral Group by Cytokines Only

We explored different three-dimensional (3D) plots of PCs components for the classification purpose between the two categories of our sample: viral group or bacterial

group using the cytokines concentrations. Figure 9 shows 3D plots using the scores of three different PCs calculated using PCA to visualize the ability of cytokines concentrations to discriminate between the viral group and the bacterial group.

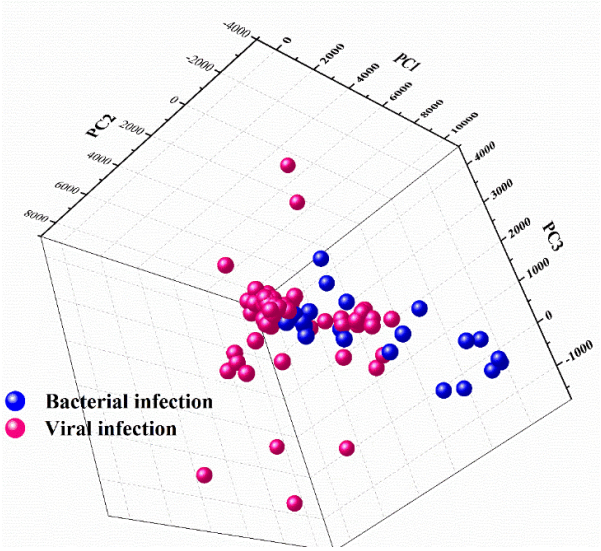


Figure 9: 3D Plots of Different PCs Scores Derived by PCA Calculation Using the Cytokines Concentrations of the Samples for the Classification of the Samples into Bacterial and Viral Groups.

4.3.3 Principal Component Analysis (PCA) Combines Cytokines Concentrations, WBC count, and CRP Concentrations to Differentiate Between the Bacterial and Viral Groups

We attempted to use five variables, the three cytokines (IP-10, IL-6, and TRAIL) with WBC count and CRP concentrations via different three-dimensional (3D) plots of PCs components for the classification purpose between the two categories of our sample: viral group or bacterial group using those five variables. Figure 10 shows 3D plots using the scores of three different PCs calculated using PCA to visualize the ability of cytokines concentrations, WBC count, and CRP to discriminate between viral group and bacterial group. The utilization of these variables (WBC, CRP) with the three cytokines demonstrates a less clear distinction between the viral and bacterial infection groups than the cytokines alone as seen in Figure 10.

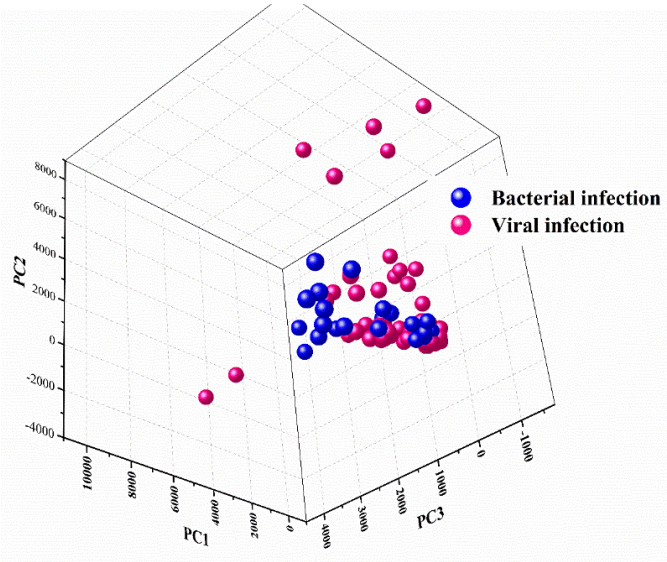


Figure 10: 3D Plots of Different PCs Scores Derived by PCA Calculation using the Cytokines, WBC, and CRP Concentrations of the Samples for the Classification of the Samples into Bacterial and Viral Groups.

It is clear from the previous figures that PCA cannot differentiate with high confidence between control and infected groups as well as between viral and bacterial. Therefore, we used another classification method based on Fisher's Linear Discriminant Analysis (FLDA). The leave-one-out (LOO) cross-validation strategy was applied to validate this approach which effectively distinguished between the classes.

4.4 The LDA Performances

In this study, we evaluated the classification performance of Linear Discriminant Analysis (LDA) in distinguishing between infected individuals and controls by examining the relationship between identification success and the number of principal components (PCs) used. In Figure 11, it can be seen how the number of principal components (PCs) relates to our success rate in classifying infected patients versus controls using LDA. Figure 11 revealed that using LDA the success rate to classify samples into two distinct groups infected and control is 90%.

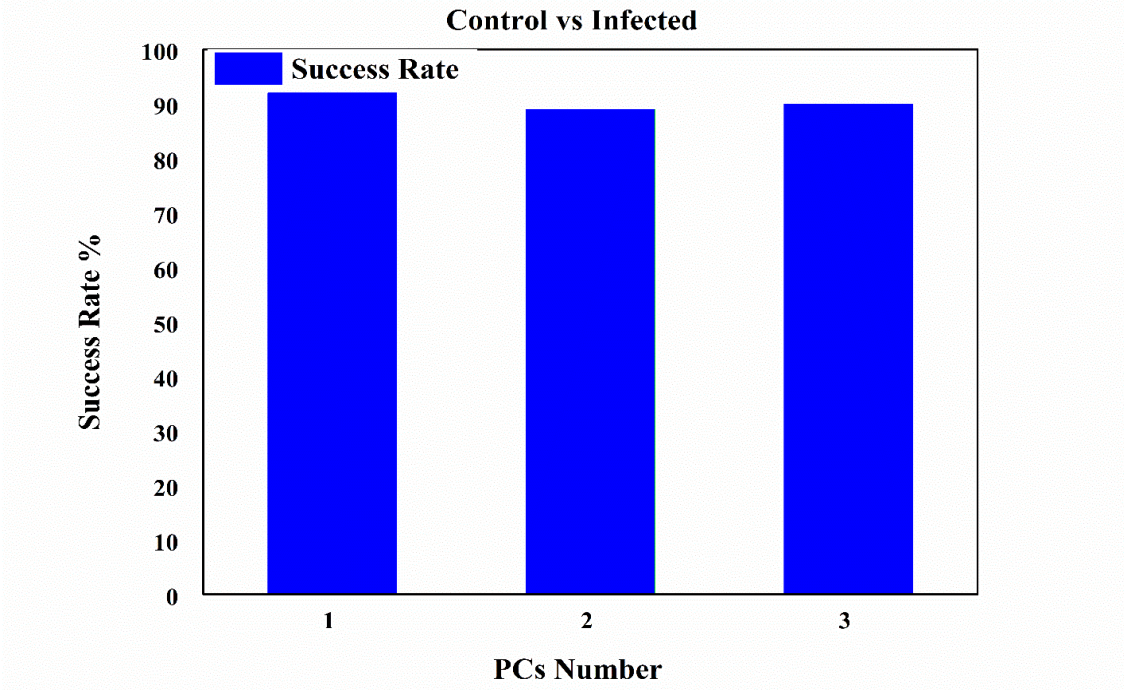


Figure 11: Success Rates Versus PC Number for the Distinction between the Infected Group and Control Group Depending on the three Cytokines Concentrations.

In Figure 12, it can be noticed how the number of principal components (PCs) influences our ability to differentiate between viral and bacterial groups based only on three cytokines concentration measurements (IP-10, IL-6, and TRAIL). Including additional PCs boosts the classification accuracy; however, after a certain point, the gains level off, terminating in an 85% success rate. This trend suggests that there's an ideal balance where enough informative variance is captured without complicating the model**.** However, when we add WBC and CRP into the mix, the accuracy drops to around 65% (see Figure 13). This proposes that including these additional markers might introduce noise, which can hinder the model's performance rather than enhance it.

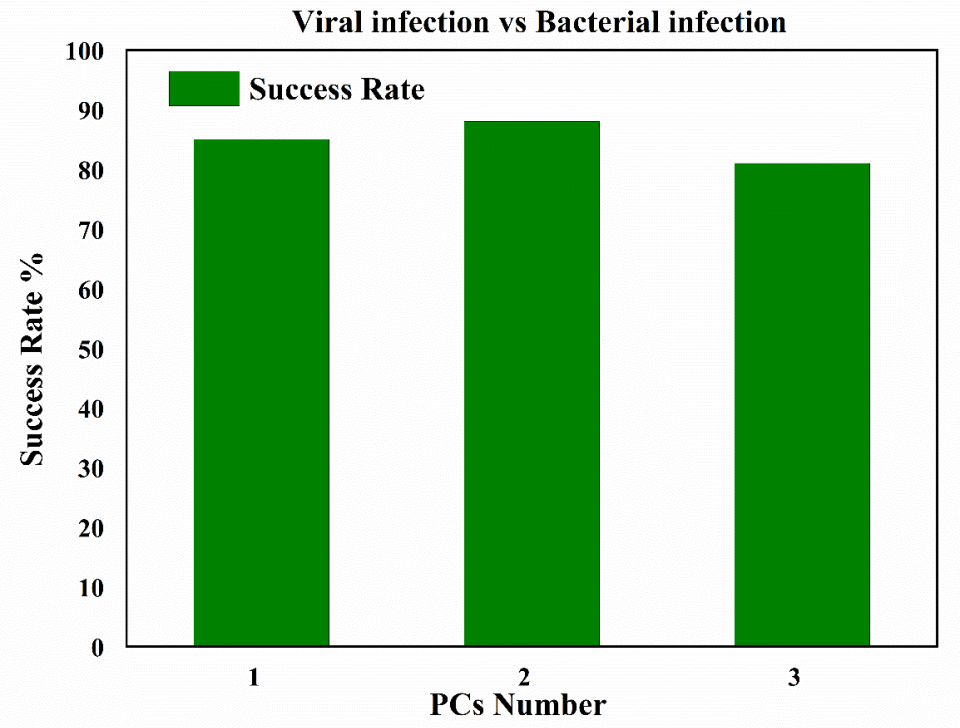


Figure 12: Success Rates Versus PC Number for the Distinction between the Viral and Bacterial Groups Depending on the three Cytokines Concentrations.

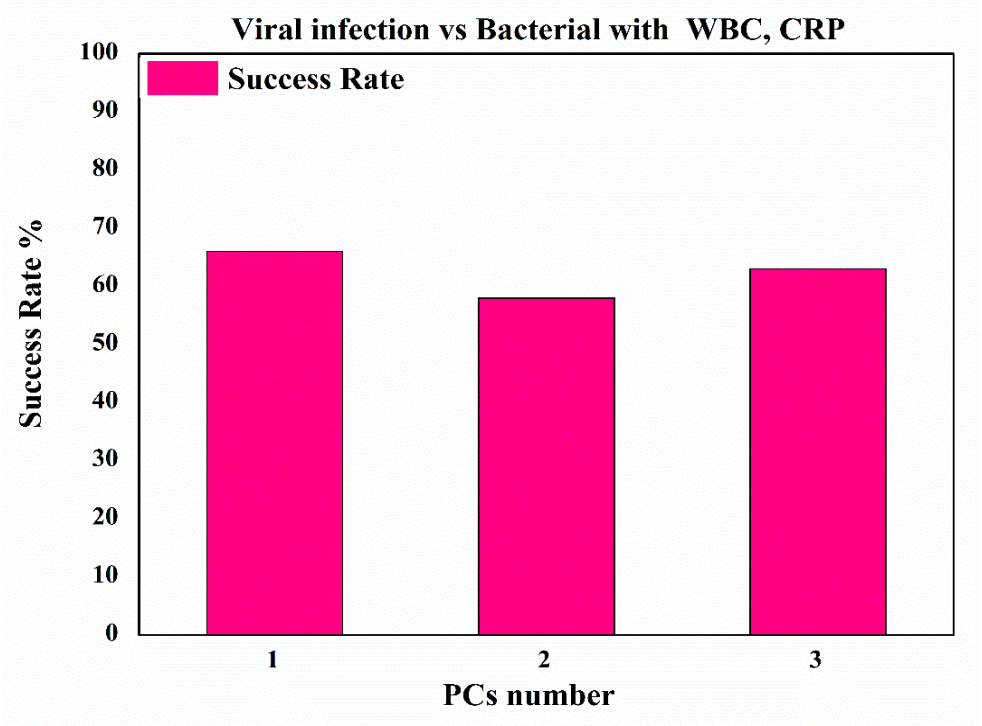


Figure 13: Success Rates Versus PC Number for the Distinction between the Viral and Bacterial Groups Depending on the Three Cytokines Concentrations with WBC and CRP.

In our study, although Linear Discriminant Analysis (LDA) achieved satisfactory classification results of up to 90%, it is considered a less effective method because our data was not normally distributed due to limited data availability. Therefore, Support Vector Machines (SVM), a more advanced supervised learning technique was applied for classification to achieve further accuracy. SVM is particularly creates classification boundaries that can separate bacterial and viral groups, even when the data is not normally distributed. Additionally, SVM performs well in the small sample sizes, making it an ideal choice for our analysis, also improves the accuracy and reliability of our results**.**

4.5 Support Vector Machine

We used SVM to differentiate between viral-infected and bacterial-infected data by using a dataset of cytokine concentrations from both groups. The dataset was divided into five folds, with four used for training and one for testing in each iteration. We labeled the viral group as (-1) and the bacterial group as (1) to train the model well. After training and testing, we visualized the classification results using a scatter plot to illustrate the model's performance in distinguishing between the two groups as seen in Figure 14.

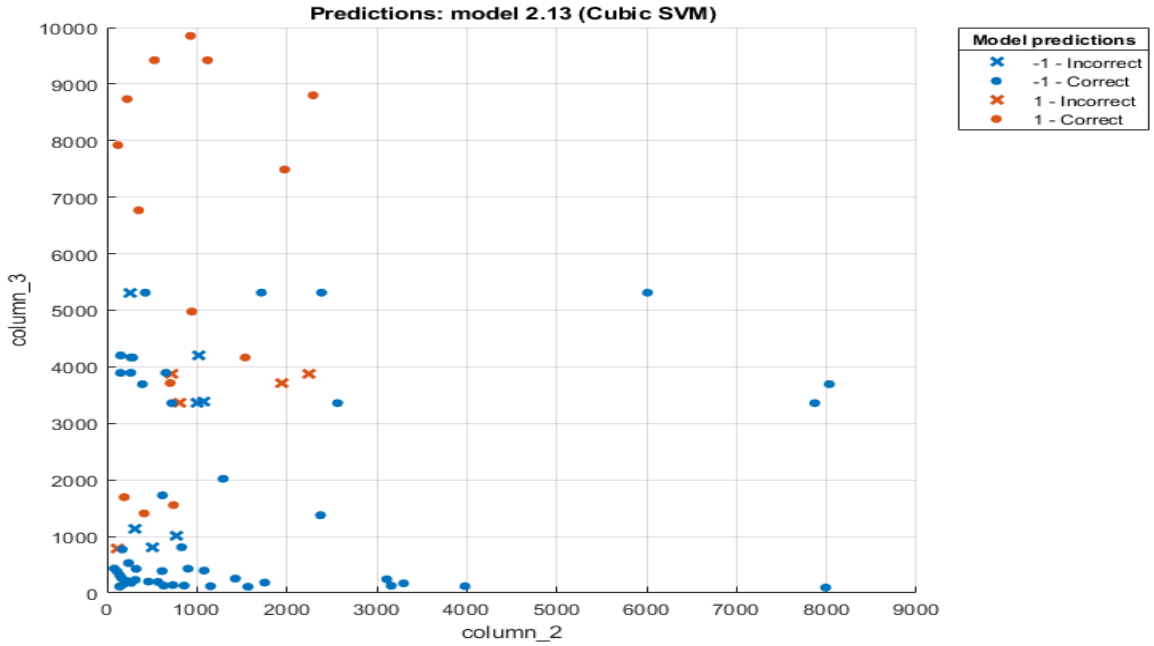


Figure 14: Scatter Plot SVM MATLAB used for classify samples into viral and bacterial infection groups.

To further analyze the model's performance, we constructed a confusion matrix to evaluate the correct and incorrect classifications for both the (-1) viral group and the (1) bacterial group. This matrix permitted us to quantify the true positives, false positives, true negatives, and false negatives, as seen in Figure 15, which then used to calculate the model's performances using the equations mentioned in the Methodology chapter / Support vector machine SVM section (Page 20)

True Positives (TP): Predicted bacterial (1) and actual bacterial (1) = 14

False Negatives (FN): Predicted viral (-1) but actual bacterial (1) = 5

False Positives (FP): Predicted bacterial (1) but actual viral (-1) = 7

True Negatives (TN): Predicted viral (-1) and actual viral (-1) = 49

Sensitivity =TP/(TP+FN) = 49/ (49+7) = 87.5%

Specificity =TN/(TN+FP) = 14/ (14+5) =73.6%

Accuracy =84 %

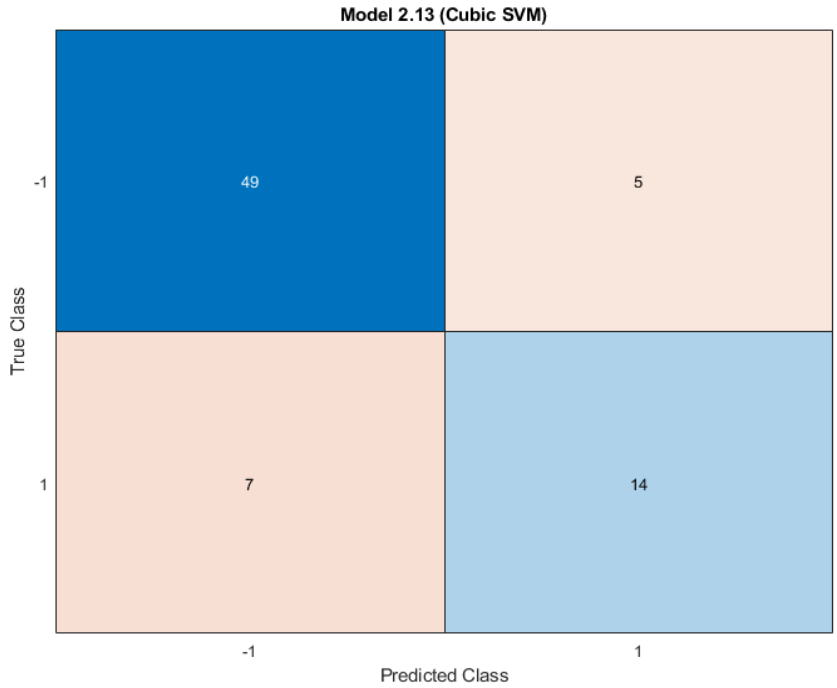


Figure 15: Confusion Matrix SVM MATLAB used for classify samples into viral and bacterial infection groups.

In Figure 16, it can be seen the ROC Curve of the SVM Classifier for Cytokine-Based Discrimination. ROC Curve is the best way to display the classifier performances. The ROC curve was generated using a Support Vector Machine (SVM) model that classified samples based on cytokine profiles. The x-axis represents the false positive rate, while the y-axis shows the true positive rate. An area under the curve (AUC) of 0.9392 indicates the excellent discriminatory performance of the SVM, suggesting that cytokine levels are strong predictors of the conditions under investigation and differentiate reliably between the viral and bacterial groups.

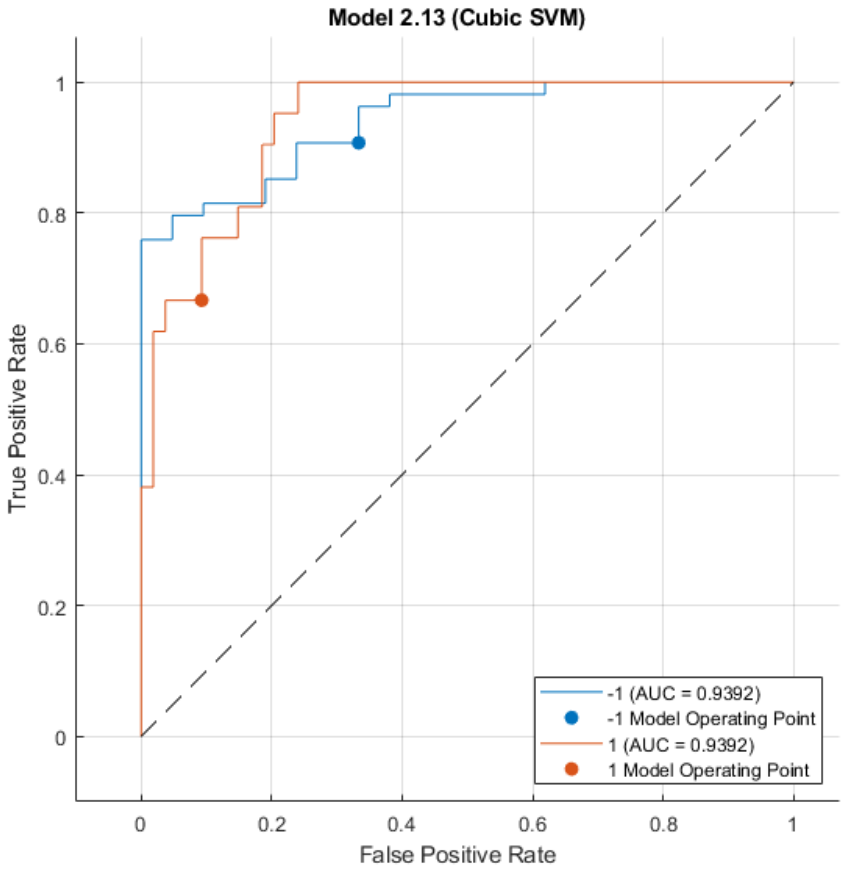


Figure 16: ROC Curve Evaluating Cytokine-Based Discrimination Using an SVM Model.

The ROC Curve Represents the Diagnostic Performance of a Support Vector Machine (SVM) Classifier Based on Cytokine Profiles The x-axis shows the false positive rate (1 – specificity), and the y-axis shows the true positive rate (sensitivity). Resulting ROC for classifying the Dataset to bacterial infected or viral infected using cytokines concentrations in tandem with the SVM machine learning that led to this classification.

Chapter Five

Discussion

In this study, the potential of cytokines concentrations in tandem with machine learning for investigating the etiology of infected samples was examined. Previous research papers have reported the challenges in detecting the infectious agents responsible for fever related to inaccessible infections or fever without source cases by routine procedures, especially in pediatric patients (Agbaria et al., 2020; Baraff, 2000; Kuzmanović et al., 2006). This study emphasizes that clinical diagnosis alone is insufficient to determine the etiology of infections. Routine laboratory examinations are still helpful but often lack the specificity to distinguish between different pathogens (Lien et al., 2022). Even the gold standard PCR can be deficient, particularly when in the cases of inaccessible infections where obtaining the required samples for lab examination is difficult or not feasible (Yang & Rothman, 2004) (Lanks et al., 2019). In some cases of the tested upper respiratory infections, the clinical diagnosis was incompatible with the confirmatory lab tests, including PCR, that we applied for each collected sample in our study. Despite the ability of pediatricians to identify the cause of fever in children through clinical diagnosis and routine laboratory tests such as CBC, particularly WBC counts and CRP levels, they have limited specificity (Feigin et al., 2021; Massaro et al., 2007). This limitation can lead to a percentage of misdiagnosis (C. Liu et al., 2020). According to these explanations, we worked on combining the immune response of the infected patients using ELISA-based cytokine measurements for samples with confirmed types of infections, with machine learning algorithms.

Elevated IL-6 levels were observed in bacterial cases, supporting previous findings that correlate IL-6 with systemic inflammation and bacterial sepsis (Remick et al., 2002, 2005). This supports the use of IL-6 as a sensitive indicator of bacterial infection. The increased expression of IP-10 and TRAIL in viral infections confirms their role in antiviral defense and apoptosis, as mentioned in different studies (Bachur et al., 2024; Fröhlich et al., 2023a;

Halabi et al., 2023a; Mastboim et al., 2023; Papan et al., 2022; Stas et al., 2022). But still not enough alone in our study as seen in the cut-off figures, therefore machine learning was combined for differentiation purpose.

The clinical diagnosis of viral infected cases suggests to us that the viral panel screening for these patients will be positive for the viruses tested. We had 55 upper respiratory tract infection cases that underwent molecular examination of the most common seasonal viruses (*RSV, SARS-COV2, Adenovirus, Bocavirus, Flu A&B, Coronavirus NL63*) to confirm their infections. Only 28 of them had a viral genetic material. The remaining samples may have had other types of viruses, or the samples collected were of low quality or the time at which the sample obtained was inappropriate, or the patients may have been misdiagnosed.

The variation between PCR-positive and PCR-negative viral cases shows our need for an accurate diagnostic tool. Cytokine profiling, when added to the clinical judgment, can enhance diagnostic accuracy, particularly when molecular techniques face limitations in detecting the pathogen.

In our study, we tried to define specific cut-off values for IL-6, IP-10, and TRAIL each alone to distinguish viral from bacterial infection groups. However, the cytokine levels overlapped significantly between the groups, without clear threshold limits to separate viral from bacterial infections (as shown in Figures 5,6,7). Our small sample size and the close clustering of cytokine values between the groups influenced the deficiency of the presence of distinct cut-off values. For this reason, we added multivariate analysis methods like PCA and LDA for resolving classification problems between groups. Likewise, (Rabbi et al., 2025) and (Jolliffe & Cadima, 2016) mentioned the benefits of PCA and LDA analysis in improving model strength and enhancing classification accuracy.

We used Principal Component Analysis (PCA) to visualize the multidimensional cytokine data, which allowed us to observe patterns that were not obvious through simple cut-off points. Following PCA, we applied Linear Discriminant Analysis (LDA) to quantify the success rate of each cytokine in differentiating between infected and control groups, as well as between viral and bacterial infections when using the three cytokines alone. In PCA calculations, we used different views to see the separation between the infected groups and the control group using cytokines concentrations with different principal components (PCs) as shown in Figure 8 A&B. Then we used the cytokines concentrations alone with different PCs to visualize the separation between the bacterial group and the viral group (as shown in Figure 9). LDA provided useful perceptions into the discrimination ability of the cytokines measurements with a success rate reaching about 90% between the infected group and the control group (as seen in Figure 11), and 85% between the viral infected group and bacterial infected group (as seen in Figure 12). We also extended our model by adding routine laboratory parameters including white blood cell (WBC) count and C-reactive protein (CRP) levels, together with the three cytokines. Interestingly, this addition resulted in dropped success rate of differentiation to about 65% (as seen in Figure 13). Several studies reported that routine biomarkers such as CRP and WBC count, when used alone, may not provide sufficient discrimination in some critical cases like sepsis (Wacker et al., 2013). LDA performance was limited by the non-normal distribution of our sample data. This limitation led us shift to the machine learning algorithms using Support Vector Machine (SVM) due to its ability to handle non-linear and high-dimensional data (Noble, 2006). We obtained accurate classification of infections based on cytokine profiles by applying SVM classifier to our data. We identified a hyperplane that separates the classes that allowing us to observe the precise differences in cytokine profiles between bacterial and viral infections groups. The SVM model provided accuracy of 84%, sensitivity 87.5%,

and specificity 73.6%. These percentages can be enhanced when we expand the sample size. Previous research mentioned that machine learning techniques can enhance diagnosis process in cases where routine methodologies fail (Raita et al., 2019). In our results, the ROC curve showed AUC =0.9392 indicating the excellent discriminatory performance of the SVM, suggesting that cytokine levels are strong predictors of the type of infection and in differentiate between the viral and bacterial groups.

One of our objectives in this study was to differentiate between Gram-positive and Gram-negative bacterial infections using cytokine profiling. However, this objective was not achieved due to the small sample size. Additionally, data from inaccessible infections and the fever without focus (FWF) group were not tested. The Support Vector Machine (SVM) model demonstrated high diagnostic performance, but we still cannot depend on it because the need for a larger dataset for proper training and validation to improve accuracy and reduce bias. Furthermore, combining additional cytokines variables is expected to enhance diagnostic accuracy and yield a higher area under the ROC curve (AUC). Although our study already achieved a high AUC, increasing the dataset size and the number of cytokines variables would further enhance the model and its predictive ability and improve its clinical applicability.

Chapter Six

Conclusion, Recommendations, Limitations, Budget and Funding

6.1 Conclusion

Our study focused on using cytokine concentrations associated with immune responses to infections as an alternative to routine diagnostic tests. Not only that, but we also combined machine learning models to these measurements, enabling them to differentiate between viral and bacterial infections with greater accuracy than using cytokine concentrations only. We initially used the cut-off for each cytokine, but this was insufficient to separate the different groups in this study. We then used the PCA and LDA as advanced models to separate the studied groups. PCA and LDA demonstrated success rates of up to 90% without the need to include routine tests in the model. The data was not normally distributed because the sample size of our study was small, so we switched to a model that had accurate results suitable to the study's sample size.

We conclude that the integration between cytokine levels (IP-10, TRAIL, and IL-6) with machine learning models provides a promising tool in contributing to the diagnosis of bacterial and viral infections. The model shows a sensitivity of 87.5% and a specificity of 73.6%. These are good results compared with the examined sample size. We achieved our goal of developing an alternative, faster, and less painful method for the patients by using a blood sample only. Compared to other methods, patients may require surgical procedures in cases of inaccessible infections, making diagnosis difficult. This benefits the patient in the early identification of the type of infection and reduces the unnecessary overuse of antibiotics, thus protecting the patient from antibiotic-resistant strains. Also, it reduces the financial burden as a result of treatment with medications that are not appropriate for their condition.

6.2 Recommendations

Our results revealed the ability of cytokine measurements (IP-10, TRAIL, and IL-6) in tandem with machine learning algorithms in the differentiation between viral and bacterial infections. Therefore, we recommend completing this research with a larger sample size to obtain higher levels of sensitivity and specificity. We will attempt to order other grants to purchase more ELISA kits to measure the cytokine profiles for the same used cytokines and test more types of cytokines to enhance the accuracy to achieve the big goal of detecting the etiology of the inaccessible infections.

6.3 Limitations

We faced several limitations while conducting this research. Firstly, our sample size was small due to difficulties obtaining eligible patients with confirmed infections via gold standard methods. This obstacle led us to order a grant to purchase a PCR requirement for viral panel testing in order to confirm the viral infections in the upper respiratory illness cases. Usually, these viral panel tests are not ordered from the pediatricians for all routine cases of viral illness. We also confirmed the pharyngitis cases by taking throat swabs culture for patients with sore throat symptoms and sending them to the microbiology lab for examination. Secondly, the grant was limited to buying just two kits from each type of cytokines; thus, a limited number of samples were tested. On the other hand, we had an objective that was not achieved in this study. Finally, we planned to test the ability of cytokine measurements integrated with machine learning algorithms to distinguish between gram-positive and gram-negative bacterial infections. The small sample size of bacterially infected cases (n = 22; 14 gram-positive and 8 gram-negative) prevented the good training of the model to differentiate between the bacterial subtypes.

6.4 Budget and Funding

This research was supported by the PRCS with a total grant of 24,500 NIS. Our need was 22,510 NIS. Of this amount, 15,000 NIS was paid for cytokine analyses, including ELISA assays for IP-10, IL-6, and TRAIL (SIGMA ALDRICH company), each receiving 2,500 NIS as shown in details in Table 9. The remaining funds were dedicated to procuring PCR extraction enzymes and primers as shown in detail in Table 10.

Table 5: The Budget Table of Cytokines ELISA Kits

Item	Quantity	Cost per Kit (NIS)	Total Cost (NIS)
Human IL-6 ELISA Kit (RAB0306)	2	2500	5000
Human IP-10 /CXCL10 ELISA Kit (RAB0119)	2	2500	5000
Human TRAIL ELISA Kit (RAB1090)	2	2500	5000
Total	6 Kits		15000

Table 6: The Budget Table of PCR Requirements

Customer 0090015
Red Crescent Hospital - hebron
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Item	Description	Unit	Price	Quantity	Total (NIS)
RGNT00072	HP Viral Nucleic Acid Kit 11858874001	KIT	1,210	1	1,210.00
0000A6102	GoTaq® Probe qPCR , A6102 , 10ml	KIT	4,500	1	4,500.00
0000A6120	GoTaq (R) Probe 1-Step RT-qPCR system, 2ml, A6120	KIT	1,800	1	1,800.00

Num. Of Item(s)	3	Total	7,510.00
Salesman	001 Salesman	Tax 0%	0.00
Net Total	SEVEN THOUSAND FIVE HUNDRED TEN New Sheqel Only	Net Total	**7,510.00**

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Appendices

Annex (1): Study Filling Form of Ethical Approval Obtained from the Parents of the Patients

Fever Assessment Questionnaire

Study Title: Cytokines analysis combined with machine learning to investigate the etiology of inaccessible infection.

BY: Amal M. Dofesh

✓ Inclusion Criteria:

- ✓ Patients with fever, clinically suspected viral or bacterial infections, or fever without source (FWS), exhibiting a body temperature above **38.0°C** in the Emergency Department (ED), Pediatric or Neonatal ICU.

✗ Exclusion Criteria:

- ☒ Patients who have febrile episode during the preceding 2 weeks.
- ☒ Patients who receive antibiotic treatment for more than 48 hours.
- ☒ Patients who have a proven or suspected HIV/HBV/HCV infection.
- ☒ Patients who have primary immunodeficiency, have an active malignancy, receiving immunosuppressive or immunomodulatory treatment, or congenital metabolic disorders.
- ☒ Patients with rheumatic, autoimmune, or autoinflammatory disorders.

Please fill out the following information:

1. **Name:** _____ **Age:** _____ **Gender:** ○ Male ○ Female
2. **Reason for Fever:**

Type of infection	○ Mostly bacterial infection	○ Mostly Viral infection	○ Fever Without Focus	
Please specify			○ Accessible site	○ Inaccessible site
Please write the Doctor decision				

Health Information Table:

#Case	Fever (°C)	WBC Count	CRP Level	Culture Result	Viral Lab Results

لا مانع لدي من المشاركة وسحب عينة الدم مع الحفاظ على معلوماتي واستخدامها فقط بغرض البحث العلمي؛
التوقيع _____

Annex (2): REC (Research Ethics Committee) Approval

Al-Quds University
Jerusalem
Deanship of Scientific Research

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: Jan 8, 2024
Ref No: 356/REC/2024

Deare Dr. Uraib Sharha, Ms. Amal Dofesh,

Thank you for submitting your application seeking approval for research ethics. After a thorough examination of your submission titled: "Cytokines analysis combined with machine learning to investigate the etiology of inaccessible infection", the Research Ethics Committee (REC) at Al-Quds University is pleased to confirm that your application is in accordance with our research ethics guidelines.

This ethical approval will remain valid as long as there are no alterations to the data collection procedure or modifications to any aspect of the research protocol. Please be aware that while this approval authorizes your research, however, please keep in mind that this approval does not substitute for any departmental or other approvals that may be necessary, including but not limited to sample shipment, data sharing permissions or administrative approval to distribute questionnaires.

In addition, we kindly request that you provide us with a copy of your final research report or publication once it becomes available.
Thank you once again for your commitment to conducting ethical research, and we extend our best wishes for a productive research endeavor that serves the best interests of your research subjects.

PS: Please note that this ethical approval letter will remain valid for a period of two years from the date of issuance. Should your research extend beyond this timeframe, a request for renewal will be necessary.

Sincerely,

Subeir Eregat, PhD
Associate Professor of Molecular Biology
Research Ethics Committee Chair

Cc. Prof. Imad Abu Kishek - President
Cc. Members of the committee
Cc. file

Abu-Dies, Jerusalem P.O.Box 20002
Tel-Fax: #970-02-2791293
research@admin.alquds.edu
ابوديس، القدس ص.ب. 20002
تلفاكس: #970-02-2791293

Annex (3): Palestinian Red Crescent Hospital Ethical Approval

Consent Form for Blood Sample Collection

Study Title: Cytokines analysis combined with machine learning to investigate the etiology of inaccessible infection.

Principal Investigator: Amal Dofesh

Hospital/Institution: Palestine Red Crescent Society Hospital

Introduction:

You are invited to participate in a research study conducted by Amal Dofesh, a master's student in the Microbiology & Immunology department at Al-Quds University, under the supervision of Dr. Uraib Sharha. The purpose of this study is to investigate the potential of cytokines, which are small proteins produced by the immune system, as biomarkers for identifying bacterial and viral infections in both accessible and inaccessible sites of infection among patients exhibiting signs of fever in tandem with machine learning algorithms. Before you decide whether to participate, it is important that you understand the study's objectives, procedures, and benefits. Please read this consent form carefully and feel free to ask any questions before making your decision.

Purpose of the Study: The primary purpose of this study is to advance our understanding of bacterial and viral infections and explore the potential of cytokines as diagnostic biomarkers in tandem with machine learning algorithms. To achieve this, we will collect blood samples from both outpatients and inpatients at Palestine Red Crescent Society Hospital who present with fever as a symptom.

Study Procedures: If you choose to participate in this study, you will be asked to:

1. Provide written consent for the collection of blood samples.
2. Provide EDTA blood samples through venipuncture, where needles will be inserted into the vein in patients' arms.

Consent: I have read and understood the information provided in this consent form, and I have had the opportunity to ask questions. I voluntarily consent to participate in the study "Cytokine Measurement as Biomarkers for Bacterial and Viral Infections" conducted by Amal Dofesh, and I agree to the collection and analysis of my blood samples for research purposes.

Participant's Name: Dr. AbdelRazzaq AbuMayaleh

Participant's Signature: Dr. AbdelRazzaq

Date: 23/9/2023

Annex (4): Dora Hospital Ethical Approval

State of Palestine
Ministry of Health
Education in Health and Scientific Research Unit

دولة فلسطين
وزارة الصحة
وحدة التعليم الصحي
والبحث العلمي

Ref.: ....
Date:..................

الرقم: ٢٠٢١/١٤٧/١٣٢٣
التاريخ: ٢٠٢٤/٢/١

عطوفة الوكيل المساعد لشؤون المستشفيات والطوارئ المحترم،،،

عطوفة الوكيل المساعد للمهن الصحية المساندة المحترم،،،

تحية واحترام...

الموضـــوع: تسهيل مهمة بحث

يرجى تسهيل مهمة **الطالبة: امل دوفش– ماجستير العلوم الطبية المخبرية/ جامعة القدس**، وباشراف د. عريب شرحة، في عمل بحث بعنوان:

'Cytokines analysis combined with machine learning to investigate the etiology of inaccessible infection'

من خلال السماح للطالبة بجمع عينات دم من الاطفال المرضى بعد اخذ موافقة الاهل (سحب العينة سيتم بالتعاون مع الطاقم الطبي او من بواسطة الطالبة نفسها حيث انها تحمل مزاولة مهنة سارية)، وذلك في:

– مستشفى دورا الحكومي

على ان يتم الالتزام باساليب واخلاقيات البحث العلمي، وعد التعرض للمعلومات التعريفية للمرضى.
على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على موافقة الوزارة على نتائج البحث.

مع الاحترام...

د. عبد الله القواسمي
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: منسق برنامج الماجستير دائرة العلوم الطبية المخبرية المحترم/ جامعة القدس

Annex (5): Queen Alia Hospital Ethical Approval

State of Palestine
Ministry of Health
Education in Health and Scientific
Research Unit

دولة فلسطين
وزارة الصحة
وحدة التعليم الصحي
والبحث العلمي

Ref.: .....
Date:.....

الرقم: ع.ص/٤٧٩/٢٠٢٤
التاريخ: ٢٠٢٤/١٢/٢٤

عطوفة الوكيل المساعد لشؤون المستشفيات والطوارئ المحترم،،،

عطوفة الوكيل المساعد للمهن الصحية المساندة المحترم،،،

تحية واحترام،،

الموضـــوع: تسهيل مهمة بحث

يرجى تسهيل مهمة الطالبة: امل دوفش- ماجستير العلوم الطبية المخبرية/ جامعة القدس، وبإشراف د. عريب شرحة، في عمل بحث بعنوان:

'Cytokines analysis combined with machine learning to investigate the etiology of inaccessible infection'

من خلال السماح للطالبة بجمع عينات دم من الاطفال المرضى بعد اخذ موافقة الاهل (سحب العينة سيتم بالتعاون مع الطاقم الطبي او من بواسطة الطالبة نفسها حيث انها تحمل مزاولة مهنة سارية)، وذلك في:

- مستشفى عالية الحكومي

على ان يتم الالتزام باساليب واخلاقيات البحث العلمي، وعد التعرض للمعلومات التعريفية للمرضى.
على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على موافقة الوزارة على نتائج البحث.

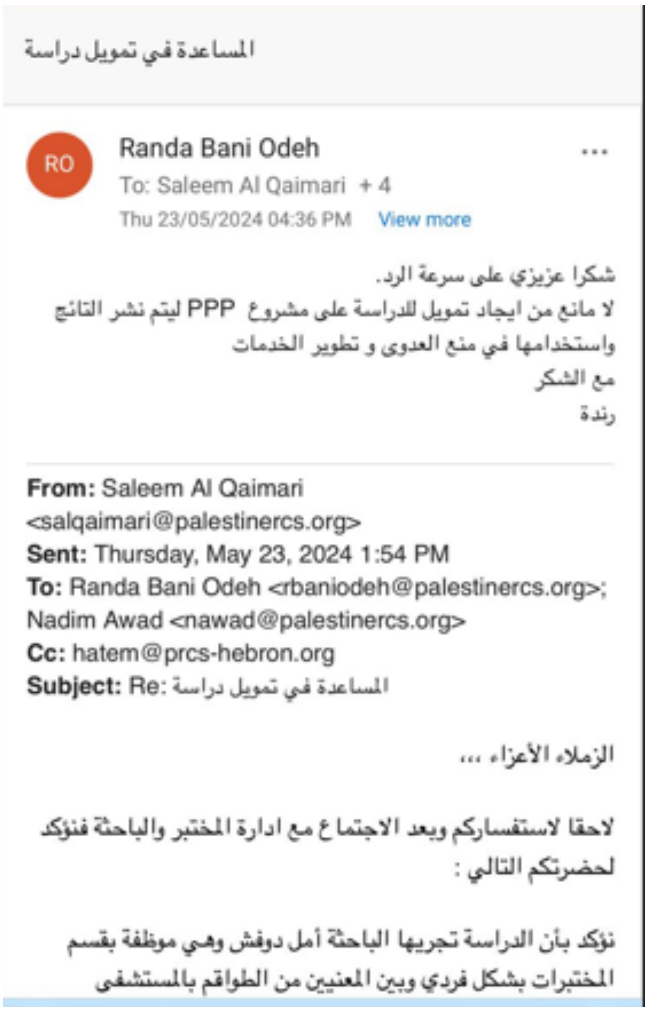
مع الاحترام،،

د. عبد الله القواسمي
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: منسق برنامج الماجستير دائرة العلوم الطبية المخبرية المحترم/ جامعة القدس

Telfax.:09-2333901 scientificresearch.dep@gmail.com 09-2333901 :تلفاكس

Annex (6): PRCS funding agreement E-mails



المساعدة في تمويل دراسة

RO

Randa Bani Odeh ...

To: Saleem Al Qaimari + 4

Thu 23/05/2024 04:36 PM View more

شكرا عزيزي على سرعة الرد.
لا مانع من ايجاد تمويل للدراسة على مشروع PPP ليتم نشر النائج واستخدامها في منع العدوى و تطوير الخدمات
مع الشكر
رندة

From: Saleem Al Qaimari <salqaimari@palestinercs.org>
Sent: Thursday, May 23, 2024 1:54 PM
To: Randa Bani Odeh <rbaniodeh@palestinercs.org>; Nadim Awad <nawad@palestinercs.org>
Cc: hatem@prcs-hebron.org
Subject: Re: المساعدة في تمويل دراسة

الزملاء الأعزاء ،،،

لاحقا لاستفساركم وبعد الاجتماع مع ادارة المختبر والباحثة فنؤكد لحضرتكم التالي :

نؤكد بأن الدراسة تجريها الباحثة أمل دوفش وهي موظفة بقسم المختبرات بشكل فردي وبين المعنيين من الطواقم بالمستشفى

الملخص

العنوان: ربط مؤشرات السيتوكينات مع نظام التعلم الآلي لتحديد مسببات العدوى التي يصعب الوصول إليها

اعداد: امل مازن دوفش

اشراف: د. عريب عيسى شرحه

يُعد التمييز الدقيق بين العدوى الفيروسية والبكتيرية لدى المرضى الذين يعانون من الحمى أمراً حاسماً لضمان تقديم العلاج المناسب وتفادي المضاعفات الناتجة عن التشخيص الخاطئ. تعاني الطرق التقليدية، مثل فحص تعداد كريات الدم البيضاء وCRP، من انخفاض الحساسية والدقة، بينما تتطلب طرق مثل PCR الحصول على عينات عالية الجودة، وقد تكون غير ممكنة في بعض الحالات، مثل التهابات الأعضاء الداخلية.

استجابة الجسم المناعية للعدوى تختلف حسب نوع المسبب؛ إذ تُفرز السيتوكينات بأنماط مميزة في العدوى الفيروسية مقارنة بالبكتيرية. في هذا السياق، تهدف دراستنا إلى استخدام ثلاثة مؤشرات سيتوكينية (IP−10, IL−6, TRAIL) وربطها بخوارزميات تعلم آلي مثلPCA ، LDA، وSVM، بهدف تحسين دقة التشخيص وتقليل الحاجة للإجراءات الجراحية.

شملت الدراسة 268 طفلاً، تم تحليل عينات من 97 منهم باستخدام .ELISA أظهرت العدوى البكتيرية ارتفاعًا ملحوظًا في IL−6 وCRP، بينما تميزت العدوى الفيروسية بارتفاع IP−10 و .TRAILلم تكن قيم الـ cut−offكافية للتمييز بين النوعين بسبب التداخل، لكن استخدام خوارزميات PCA و LDA حقق دقة تصنيف تصل إلى 90%. عند استخدام نموذجSVM ، ارتفعت حساسية التشخيص إلى 87.5% وخصوصية 73.6%، مع AUC بلغ 0.94.

تُظهر نتائجنا أن الجمع بين مؤشرات السيتوكينات وتقنيات الذكاء الاصطناعي يُعد أداة فعّالة، دقيقة، وغير جراحية لتشخيص أنواع العدوى المختلفة، ما يساهم في تحسين رعاية المرضى وتقليل الاستخدام غير الضروري للمضادات الحيوية، وبالتالي الحد من انتشار مقاومة البكتيريا للمضادات.