Blood biomarkers differentiating viral versus bacterial pneumonia aetiology: a literature review

Student: Jithin Thomas
Supervisors: Lina Jankauskaitė MD, PhD
Co-supervisor: Assoc. Prof. Vaidotas Gurskis
# TABLE OF CONTENTS

| Section                                      | Pages |
|----------------------------------------------|-------|
| ABSTRACT                                    | 3     |
| ACKNOWLEDGEMENT                              | 4     |
| CONFLICTS OF INTEREST                       | 4     |
| ABBREVIATION                                 | 5-6   |
| INTRODUCTION                                 | 7     |
| AIM AND OBJECTIVES                           | 8     |
| 1.0 LITERATURE REVIEW                        | 9-15  |
| 1.1 AETIOLOGY                                | 9-10  |
| 1.2 CLINICAL FEATURES                        | 10-11 |
| 1.3 DIAGNOSTIC TESTING                       | 12-15 |
| 2.0 METHODOLOGY                              | 16-17 |
| 3.0 RESULTS                                  | 18-25 |
| 4.0 DISCUSSION                               | 26-32 |
| 4.1 C-REACTIVE PROTEIN (CRP)                 | 26-27 |
| 4.2 PROCALCITONIN                            | 27-28 |
| 4.3 WBC AND NEUTROPHILS                     | 28    |
| 4.4 MYXOMA RESISTANCE PROTEIN 1,            | 29    |
| 4.5 LIPOCALIN                                | 29    |
| 4.6 HIGH MOBILITY GROUP BOX1 PROTEIN, (HMGB1)| 30    |
| 4.7 COMBINATION OF MARKERS                   | 30    |
| 4.8 OTHERS                                   | 31    |
| 4.9 TABLE OF SENSITIVITY AND SPECIFICITY     | 31-32 |
| 5.0 STUDY LIMITATIONS                        | 32-33 |
| 6.0 CONCLUSION                               | 33    |
| 7.0 PRACTICAL RECOMMENDATIONS                | 34    |
| REFERENCES                                   | 35-43 |
ABSTRACT

Aim & Objectives: The goal of this literature review is to compare current studies regarding the accuracy of different serum markers in differentiating viral from bacterial pneumonia in the pediatric population with what is employed in the medical setting at present.

Methods: Literature search conducted on Medline using a combination of the following term "Community-acquired pneumonia" OR "CAP" OR "viral pneumonia" OR "virus-induced pneumonia" OR "bacterial pneumonia" AND "biomarker" OR "marker" OR "protein" OR "interleukin" OR "chemokine." Articles that were in English and within ten years of the search date were manually sorted according to inclusion and exclusion criteria.

Results: Initial search returned n=13405. After activating filters, n=137 were identified of which n=10 included for literature review. Markers that were investigated were C-reactive protein (CRP), Procalcitonin (PCT), White blood cell (WBC), Neutrophils, Myxoma resistance protein (MxA1), Lipocalin-2 (Lcn2), High mobility group box 1 protein (HMGB1) Syndecan4 (SYN4), Midregional Proadrenomedullin and Midregional proatrial natriuretic peptide.

Conclusion: Rise or drop in the concentration of a single marker is not accurate enough for predicting viral/bacterial CAP. This is because there is overlapping to a varying extent depending on the cut-off values, detection methods, analyses, the desired specificity, and sensitivity. Furthermore, the presence of mixed infection and makes almost all makers suboptimal to be used universally. New makers such as MxA1 and HMGB1 gave promising results. However, to replicate a similar testing condition in a clinical environment may not be practical. Another approach is to make use of more than one marker and combine with clinical signs and symptoms. This may not be cost-effective in many clinical settings; nevertheless, in many studies, using more than indicator greatly improved the predictive power.
ACKNOWLEDGMENT
I want to extend my sincere gratitude to my supervisor, Lina Jankauskaitė MD, Ph.D. for her support.

CONFLICTS OF INTEREST
There is no conflict of interest
ABBREVIATIONS

AV: Adenovirus
B: B Lymphocyte
CAP: Community-acquired pneumonia
CoV: Coronavirus
CRP/MPV: C-reactive protein-mean platelet volume ratio
CRP: C-reactive protein
EPIC: European Prospective Investigation into Cancer and Nutrition
GBS: Group B streptococcus
Hap: Haptoglobin
HMGB1: High mobility group box 1 protein
HRV: Human Rhinovirus
IFN-γ: Interferon-gamma
IgA: Immunoglobulin A
IgG: Immunoglobulin G
IgM: Immunoglobulin M
IL-10: Interleukin -10
IL-11: Interleukin -11
IL-12: Interleukin -12
IL-13: Interleukin 13
IL-15: Interleukin -15
IL-17A: Interleukin -17A
IL-2: Interleukin -2
IL-4: Interleukin -4
IL-5: Interleukin -5
IL-6: Interleukin -6
IL-7: Interleukin -7
IL-8: Interleukin -8
IL-9: Interleukin -9
IV: Influenza Virus
Lcn2: Lipocalin-2
mRNA: Messenger Ribonucleic acid
MR-proADM: Midregional proadrenomedullin
MR-proANP: Midregional proatrial natriuretic peptide
MxA1: Myxoma resistance protein 1
N/L, NLR: Neutrophil-Lymphocyte ratio
NK: Natural Killer
PCT: Procalcitonin
PIV3: Parainfluenza Virus 3
RSV: Respiratory syncytial virus
SYN4: Syndecan-4
Tc: Cytotoxic T- lymphocyte
Th: Hepler T- Lymphocyte
TNF-α: Tumour Necrosis Factor-alpha
UNICEF: United Nations Children’s Fund.
WBC: White blood cells
INTRODUCTION

Community-acquired pneumonia (CAP) is estimated to cause 31.1 per 100,000 deaths globally in the population under the age of 19 [1]. According to the epidemiological data, approximately 152 million cases of CAP are diagnosed every year in children under the age of five worldwide, of which, approximately 10-20 million are severe cases requiring in-patient treatment [2]. However, there has been a drop in the incidence and mortality of CAP with the introduction of vaccination against Streptococcus pneumoniae and Haemophilus influenza [3–5]. Thus, viral pathogens have become significant in causing CAP. It is estimated that approximately 50%-70% of cases of CAP are virally induced in children under the age of 5 [6,7].

The biggest challenge remains to differentiate common respiratory viral pathogens, such as respiratory syncytial virus (RSV), influenza virus (IV) or adenovirus (AV) from bacterial causes such as with S. pneumoniae. Clinical signs and symptoms of CAP of viral and bacterial origin overlap significantly [8]. The uncertainty is further exacerbated by the fact that direct isolation of possible causative agent from the lower respiratory tract is invasive and therefore rarely performed [9].

Consequently, indirect methods are utilized to isolate the organism. These include polymerase chain reaction (PCR) of throat swab, gram stain, and culture of nasopharyngeal aspirate, and blood cultures. However, interpretation can be difficult as children are found to be asymptomatic carriers of a range of organisms and a positive result on PCR may not be indicative of the cause of CAP [9,10]. Additionally, blood cultures are seldom positive on presentation, and the effect of co-infection can further complicate the interpretation [9,11,12]. C-reactive protein (CRP), and White blood cell count (WBC) are often part of the diagnostic workup in an inpatient setting. However, the changes observed are not specific to judge the causative agent.

Instrumental diagnostics, such as a chest X-ray is not sensitive or specific and is not recommended in the initial diagnosis of a suspected CAP [7]. Radiographic changes which show patchy bilateral involvement may suggest a viral aetiology; however, this is not specific [7]. Moreover, radiological findings may not correlate with the clinical situation, and the interpretation can be biased [11,12].

A great deal of attention, therefore, is given to quantitative changes in different serum markers to make better conclusions. Owning to the difference in the immunological and inflammatory response induced by bacteria and viruses, the disparity in the levels of specific markers may give an objective value that may equip us with better prediction power regarding aetiology. Many research studies have explored the different serum makers, but the conclusions are conflicting. Therefore, an intuitional review is vital to provide enough clarity to bridge the scientific gap. The underlying principle of this research is to summarise literature analysing different biomarkers and provide an overview.
AIMS AND OBJECTIVES

This literature review aims to compare current knowledge regarding the accuracy of different serum markers in differentiating viral from bacterial CAP in the paediatric population with what is employed in the medical setting at present.

Objectives that are raised:

- To review clinical, radiographic and laboratory clues that are practiced today to differentiate bacterial and viral CAP;
- To collect a list of current papers that compare serum makers in viral and bacterial CAP;
- To review the potential of those serum markers in differentiating viral and bacterial CAP.
1.0 LITERATURE REVIEW

Community-acquired pneumonia (CAP) is one of the significant causes of mortality amongst children. In 2013, CAP was responsible for almost 950,000 deaths of children under the age of 5, globally. However, this number has been decreasing ever since, and according to a report by UNICEF, the number dropped to 880,000 in 2016 [13–15]. The decline in mortality has been credited to the successful vaccination program against *Streptococcus pneumoniae* and *Haemophilus influenzae* infection [3–5]. Nevertheless, mortality rates in low-income countries are high as 72.3 deaths per 100,000 as compared to 1.7 per 100,000 in high-income regions [1]. This means that lower socioeconomic status is a critical factor in causing CAP-related deaths [16,17].

1.1 CAP Aetiology

Aetiology of CAP differs according to age. In general, neonates are more prone to organisms that are acquired by vertical transmission. These are mainly bacterial, such as Group B streptococcus (GBS). In 57% of neonatal CAP cases, GBS was the causative agent according to a study by Webber et al.[16]. With regards to the viral cause, disseminated Herpes Simplex virus (HSV) is the leading cause for concern in this age group. HSV infections are reported to have an estimated incidence between 3-18 per 100,000 live births, of which approximately 25% are in the disseminated form [17–21]. Other emerging organisms include *Escherichia coli*, *Klebsiella species*, and *Staphylococcus aureus*, but proportions differ according to different regions [22,23].

Outside the neonatal period and until the age of five, children are more prone to viral-induced pneumonia. According to various data, roughly 66% to 81% CAP’s are of viral aetiology, of which around 60% are comprised of RSV, IV and Human metapneumovirus (HMPV) [6,24]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study revealed that RSV is the most common respiratory pathogen in children under the age of five. However, from the age of 5 onwards, Human Rhinovirus (HRV) is encountered more often (Figure 1) [6]
In a study by Jain et al. [6] viruses were isolated from over 70% of children hospitalized with pneumonia. Bacteria were detected only in 15% of the cases. Another study by Rhedin et al. [24] isolated viruses in more than 80% of radiologically confirmed pneumonia cases in the paediatric population under the age of 5. A different study by Hammitt et al. [25] detected respiratory viruses in 53% of children less than five years old with pneumonia. Older children (>5 years) are affected by the same organisms as adults. In this age group *S. pneumoniae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* are the leading causative agents.

### 1.2 Clinical features

Pyrexia in addition to hypoxemia, together with signs indicative of increased work of breathing are reliable in diagnosing CAP [26]. It is suggested that CAP should be the top differential when confronted with a patient younger than two years of age, having a fever of more than 38°C and tachypnoea [26]. Tachypnoea is assessed according to the age specified breathing rate cut-off value by the World Health Organization (WHO) (Table 1). Studies have shown that respiratory rate alone is a poor indicator in discriminating radiographically positive and negative CAP cases [27,28]. Similarly, in an extensive systematic review, the breathing rate greater than 40 breaths per minute in 1-5-year-olds had a low specificity of only 51% for CAP [29].
**Table 1**: Age-specific cut off point for respiratory rate.

| Age              | Respiratory Rate |
|------------------|------------------|
| 0-12 months      | >50 Bpm          |
| 1-5 years old    | >40 Bpm          |
| >5 years old     | >30 Bpm          |

Bpm: Beats per minute.

Adapted from Saux et al. [30]

Fever is a common presentation of CAP and may be the only clinical feature irrespective of causative organism. Bachur et al.[31] found that over a quarter of children with CAP, regardless of pneumonia agent, had only fever as the presenting feature with no other respiratory findings. A fever higher than 38.5°C is one of the most shared features of bacterial CAP [7]. In a review by Tan et al. [32], 90% of children with *S. pneumoniae* induced CAP was pyrexial. Similarly, viral induced CAP may also present with fever; however this tends to be lower [7,33].

Cough is almost always present in bacterial and viral CAPs. 80% of *S. pneumoniae* cases had a cough in the review by Tan et al. [32], but in only 10% was it productive. Therefore, having a non-productive cough does not exclude bacterial CAP. A cough may not be evident in young children. In this age group fever, poor feeding and irritability are more common [34]. In older children, abdominal pain is commonly seen. In a study by Kirovski et al. [35] 8.5% of 3-14 years of age CAP cases were admitted with abdominal pain, leading to a delay in diagnosis.

Pleuritic chest pain is indicative of pleural effusion which is more suggestive of bacterial aetiology than viral [36]. The signs and symptoms may be more severe in addition to dullness and decreased breath sounds, and a pleural friction rub [37]. Parapneumonic pleural effusion is a common complication of CAP. A study by Krenke et al. [36] showed that 16.7% of paediatric CAP patients had pleural effusion on presentation. Roughly 50% of the cases with *S. pneumoniae* will develop parapneumonic pleural effusion leading to a diagnosis of complicated CAP if appropriate antibiotics are not initiated [38].

For unknown reasons, viral CAP’s are associated more with wheezing. In the study by Esposito et al. [39], 30% of all viral cases were wheezy as compared to 19.2% of bacterial CAPs. In a different study of 98 children with pneumonia, wheezing was present in 43% in patients with viral CAP and 16% bacterial CAP [40]. The likelihood of having a bacterial-induced CAP in a pre-school child with wheeze is low [7].
1.3 Diagnostic testing

X-ray and Ultrasound

There is an agreement between the Infectious Diseases Society of America (IDSA) and BTS with regards to when chest radiography should be performed. Both guidelines recommend not to routinely use imaging in outpatient or emergency settings if an uncomplicated CAP is suspected [7,41]. Some authors have argued the importance of a chest x-ray in giving valuable information such as presence and absence of lung infiltration, the pattern of infiltration and if there is any evidence of complication such as pleural effusion, which may point towards a bacterial cause [36,42,43].

In terms of the pattern of infiltration, a study published in 2014 by Guo et al. [44] revealed that out of 210 confirmed viral pneumonia cases, 133 (63.3%) had patchy bilateral consolidation (Figure 2), 33 (15.7%) had interstitial changes, 29 (39%) had diffuse alveolar consolidation and 15 (7%) had lobar consolidation (Figure 3). According to this study, 90% of the cases had infiltration in the lower lobes of the lung. In contrast, a previous study by Ashorn et al. [45] found that more than 80% of cases of pneumococcal pneumonia produced alveolar infiltrate and over 30% had a lobar or segmental pattern. In a different study, nearly half of the children with interstitial infiltrates on radiography had bacterial infection confirmed by bacterial antibody assays, giving a sensitivity and specificity of roughly 50% and 60% respectively. Figure 4 and 5 show the similarities in bilateral interstitial infiltration which is caused by S. pneumonia and AV [46].

A chest x-ray has several limitations. First, the radiological findings often lag the clinical features of CAP. Secondly, there is poor inter-reader agreement leading to differences in interpretations. In a recent study, 13% of cases with pleural effusion was missed, and 4 cases were classified as usual despite having lobar changes found on review [47]. In the same study, it was recognised that the variability was greatest when assessing chest X-rays of patients under the age of five [47]. Moreover, there is apparent exposure to radiation and the need for expensive equipment and trained staff, which may be an issue in financially limited settings. Finally, x-ray rarely changes the management as it cannot differentiate between bacterial and viral CAP.

Ultrasound has been employed in emergency settings. It is less prone to interference caused by movement and has excellent penetration in the paediatric population. Furthermore, this technique is devoid of ionizing radiation and is less expensive than a chest x-ray [48]. However, not having the entire lung field present in one view is a drawback of using ultrasound [48]. Also, one study which compared chest x-ray and ultrasound has found that chest x-ray had a better positive predictive value than ultrasound (71% and 61% respectively) [49].
Fig 2: An anterior-posterior X-ray signifying the patchy consolidation that is common for viral pneumonia [44].

Fig 3: Lobar consolidation in a paediatric patient with Influenza viral pneumonia [44].

Fig 4: Bilateral Interstitial infiltrates caused by *Streptococcus pneumonia* infection [46].

Fig 5: Bilateral Interstitial infiltrate caused by Adenoviral infection [44].
Laboratory Evaluation

Laboratory testing is not needed for a mild, uncomplicated CAP patients who is to be treated on an outpatient basis. In general, WBC count less then $15 \times 10^9$/L and increased lymphocyte levels as compared to neutrophil counts are indicative of viral pneumonia. However, studies have found that WBC count can be raised beyond $15 \times 10^9$/L in viral pneumonia [27,50].

Elevated CRP is indicative of bacterial aetiology. A meta-analysis of eight studies involving a total of 12340 paediatric CAP’s, highlighted a positive predictive value of 64% for bacterial aetiology when the CRP is greater than 40 to 60 mg/L [28]. On the other hand, Virkki et al. [46] suggested that CRP offered very low sensitivity in differentiating aetiologies to be used in clinical practice. Another commonly used marker is Procalcitonin (PCT), which was found to be better at ruling out bacterial infection. In a study by Stockman et al. [34] no child with PCT less than 0.1 ng/mL had bacterial pneumonia. The issue regarding both CRP and PCT is that no agreed cut-off point is present and sensitivity and specificity are suboptimal.

Point of care (POC) rapid antigen direct testing (RADTs) are commonly used in emergency settings and are recommended by BTS and IDS [7,41]. The main advantage of rapid testing is that it is relatively simple and can be carried out in an emergency setting. Another benefit (Table 2) is that the results are ready within 10-15 minutes with high specificity [51,52]. The major drawback is that although the specificity is high, the sensitivity is limited. In a study involving 267 Influenza positive cases confirmed by PCR, only 52 patients were found to be positive on rapid testing, giving an overall sensitivity of 19.5% [53]. Therefore, false negative cases are high and need additional confirmatory tests.

Additionally, RADT is limited to diagnosing IV-A, B, and RSV. A lengthier test as compared to RADT is a direct fluorescent antibody (DFA). DFA is more sensitive in children and can test for AV, IV-A, IV-B, HMPV, Parainfluenza virus (PIV)-1, PIV-2, and PIV-3, and RSV [54,55].

Nuclear Acid amplification techniques (NAAT) have been widely employed and are recommended by IDSA and BTS in diagnosing paediatric pneumonia if the management is expected to change [7,41]. Instead of testing for antigen in the sample, NAAT amplifies the genetic material of viruses in the sample by using specific primers. Multiplex PCR, is an example of the process that amplifies genetic material and can detect viruses in previously negative cases [56,57]. Even though PCR is highly sensitive, its use should warrant a high degree of caution when interpreting results. Advani et al. isolated viruses in over 40% of asymptomatic children[10]. This means that PCR cannot differentiate between colonization, incubation period or infection. Therefore, a positive result for a virus, which may not be the cause of the disease can mislead the health professional, and real underlying pathology may be overlooked. Moreover, it is not always adaptable in every setting or each emergency room (ER) as it needs additional knowledge for sampling, testing and more funds than routinely used markers.
### Table 2: Comparison of 3 methods of viral testing [51–59].

| Method | Advantages | Disadvantages |
|--------|------------|---------------|
| RADT   | Quick turn round time  
Low cost  
Can be incorporated into primary care or emergency setting  
High specificity  
No additional expertise or training required | Low sensitivity  
Influenced by the sample collection  
Only tests from IV and RSV  
Cannot differentiate between different strains of IV | |
| DFA    | Greater sensitivity than RDTA  
Able to differentiate between different strains  
More viruses can be tested than in RADT | More time consuming than RADT  
Requires microscopy | |
| NAAT   | Highly sensitive and specific  
Specific strains can be identified  
Quantitative (viral load measurement) | Expensive  
Needs specialist laboratory and expertise  
Longer turn around than DFA and RDTA | |

RADT: Rapid antigen direct testing; DFA: Direct fluorescent antibody; NAAT: Nuclear Acid amplification techniques; IV: Influenza virus; RSV: Respiratory syncytial virus.

Nevertheless, some clinical clues do exist that could indicate that viral or bacterial aetiology. They are given in Table 3.

### Table 3: A broad overview of clues that may suggest the aetiology [7,41,91,92].

|         | Bacterial | Viral                  |
|---------|-----------|------------------------|
| **Clinical** | Age >5 years  
Quick onset  
Temperature consistently >38.5°C  
Cough  
Signs of sepsis | Age <5 years  
Slow onset  
Fever <38.5°C  
Wheeze  
Cough  
Rhinitis  
Diarrhoea  
Prominent chest recession |
| **Radiography** | Unilateral lobar consolidation  
Pleural effusion | Bilateral patchy involvement |
| **Laboratory** | CRP elevated  
WBC >15x10⁹/L  
Neutrophilia | CRP mild elevated  
WBC <15x10⁹/L  
Lymphocytosis |

CRP: C-reactive Protein, WBC: White blood cell
2.0 METHODOLOGY

Search strategy

Literature was identified by searching Medline with the combination of the following terms: "Community-acquired pneumonia" OR "CAP" OR "viral pneumonia" OR "virus-induced pneumonia" OR "virus-induced pneumonia" OR "bacterial pneumonia" AND "biomarker" OR "marker" OR "protein" OR "interleukin" OR "chemokine" (Table 4). Further, filters were applied as shown in table 5. Then, the resulting papers were included or excluded (inclusion/exclusion criteria shown in table 6) by reading the title and abstract.

Table 4. An extensive list of the terms that were used in the initial search.

| • Community-acquired pneumonia/pneumoniae                     |
| • CAP                                                        |
| • Bacterial pneumonia/pneumoniae                            |
| • Viral pneumonia/pneumoniae                                |
| • Virus-induced pneumonia/pneumoniae                        |
| • Virus-induced pneumonia/pneumoniae                        |
| **AND**                                                     |
| • Biomarker                                                 |
| • Marker                                                    |
| • Protein                                                   |
| • Interleukin                                               |
| • Chemokine                                                 |
| **NOT**                                                     |
| • Klebsiella                                                |
| • Mycoplasma                                                |
| • Chlamydia                                                 |
| • Microbiological                                          |
| • HIV                                                       |
| • Diagnostic imaging                                       |
| • Ultrasound                                                |
| • X-Ray                                                     |
| • Radiological                                              |
| • Adult                                                     |

CAP: community-acquired pneumonia, HIV: Human immunodeficiency virus
### Table 5. Filters that were applied after the initial search.

| Date range: Within ten years (until 21st of January, 2019) |
| Species: Human |
| Language: English |
| Age: Birth-18 Years |

### Table 6: Criteria for inclusion and exclusion.

| Inclusion | Exclusion |
|-----------|-----------|
| Free text (also an external link to other sites) | Adult |
| Viral and bacterial aetiologies | Pneumonia with other aetiologies than viral/bacterial (HIV, immunodeficiency, cancer, post-transplantation) |
| No concomitant diseases | Literature reviews |
| Age 0-18 years | Metanalysis |
|                      | Systematic reviews |
|                      | Abstracts only with no external link |

HIV: Human immunodeficiency virus.
3.0 RESULTS

The search was done by combining following terms: Community-acquired pneumonia” OR "CAP" OR "viral pneumonia" OR "virus-induced pneumonia" OR "virus-induced pneumonia" OR "bacterial pneumonia" AND "biomarker" OR "marker" OR "protein" OR "interleukin" OR "chemokine" (Table 4). This returned 13405 records which were narrowed to 137 after activating the filters. Afterward, the title and abstracts were manually sorted and matched according to the inclusion and exclusion criteria, resulting in 10 articles. Afterward, details were extracted for each article as follows; Name of the author, date, country, age range, type of study, number of CAP patients, number of bacterial, viral cases and identification modality, makers tested conclusion. This information is then summarised in table 7.

Abstracts identified after database search: n=13405

Records excluded (n=127)
- Metanalysis (n=16)
- Systematic reviews(n=23)
- Abstracts only with no external link(n=32)
- Bacterial CAP only(n=7)
- Viral CAP only (n=2)
- No attempt to isolate organism (n=4)
- Other concomitant diseases(n=43)

Adapted Prisma flow chart [93]. n: sample size , CAP: Community-acquired pneumonia
| **Author (REF)** | **Date** | **Country** | **Age range** | **Type of the study** | **CAP number** | **Number viral and bacterial cases** | **Markers/chemokines tested** | **Conclusion and Notes** |
|-----------------|----------|-------------|---------------|-----------------------|----------------|------------------------------------|-------------------------------|----------------------------|
| Esposito et al. [60] | 2016 | Italy | <14 years old | Prospective cohort | 110 Radiologically confirmed CAP | Total n=110; n=20 no aetiology identified; Bacterial n=74; Viral n=16; Confirmed with PCR from nasopharyngeal swab positive | Lcn2, SYN4, CRP and WBC | Lcn2 and SYN4 cannot predict aetiology. CRP together with WBC and clinical data, when combined, is the best predictor. |
| Study                  | Year | Country | Age | Study Design | Sample Size | Aetiology Identification | Test Methodology | Findings                                                                 |
|-----------------------|------|---------|-----|--------------|-------------|--------------------------|------------------|--------------------------------------------------------------------------|
| Valim et al. [61]     | 2016 | Mozambique | <10 years | Prospective Cohort | 117         | Bacterial n=23; Viral n=30; Bacteria isolated from blood or pleural fluid; Viruses identified with PCR. | CRP and PCT, MR-proANP, MR-proADM, WBC, neutrophil percentages, CRP, PCT | Fifty-six markers in a multiplex immunoassay. Hap, TNF receptor 2 or IL-10, and tissue inhibitor of metalloproteinases 1 provided the best tool to differentiate bacteria from the virus. Note: Malaria was also studied in this investigation. |
| Esposito et al. [39]  | 2016 | Italy   | 4 months-14 years | Prospective Cohort, Multicentre | 433 radiologically confirmed CAP | Bacterial n=235; One or more viruses n=111; Unknown n=87; real-time PCR tests on blood samples and nasopharyngeal | CRP and PCT are better at predicting viral and bacterial aetiology than others. PCT and MR-proANP helped to identify severe cases. | Combination of three proteins (Hap, TNF receptor 2 or IL-10, and tissue inhibitor of metalloproteinases 1) provided the best tool to differentiate bacteria from the virus. |

Note: Malaria was also studied in this investigation.
| Study                        | Year | Country  | Age Range | Study Design          | Methodology                                                                 | Findings                                                                                                                                 |
|------------------------------|------|----------|------------|-----------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|
| Naydenova et al. [62]        | 2016 | Gambia   | 2-59 months| Retrospective Case-control | Only in 84 cases, the aetiology was identified using blood culture. 22 bacterial and 62 viral. | Lcn2 values below 200 ng ml are suggestive of a viral cause. Note: cases with no bacterial growth in blood culture were diagnosed as viral pneumonia. This is not reliable, as blood cultures are not always positive in bacteria CAP. This can lead to diagnostic bias. |

1 swabs were used to identify agents.
| Authors               | Year | Country | Age Range          | Study Design | Sample Size Description | Markers | Findings                                                                                                                                 |
|----------------------|------|---------|--------------------|--------------|--------------------------|---------|------------------------------------------------------------------------------------------------------------------------------------------|
| Zhu et al. [63]      | 2016 | China   | 10 months to 7 years | Prospective Cohort | 65 based on criteria provided IDSA and American Thoracic Society | 34 bacterial n=34; Non-bacterial n=32 | PCT | Bacterial pneumonia had far greater levels of PCT than non-bacterial. Also, statically significant changes in PCT level were noticed before, and after treatment. Therefore, PCT is an important marker. Note: The type of detection used to isolate the agents are not disclosed in this study. Moreover, there is a mismatch between the sample size given in abstract, methods and results. |
| Engelmann et al. [64] | 2015 | France  | 0-16 years         | Prospective cohort multicentre study | 41 | Viral clinically* diagnosed n=4. Viral microbiologically confirmed n=6. Bacterial clinically* diagnosed n=16 | MxA1 CRP | Over 200ng/ml of MxA has very high sensitivity and specificity in diagnosing a viral infection. NOTE: Not all cases were microbiologically confirmed as the study design did not accommodate this. The request for confirmation was based on the decision of the treating physician. |
| Studies                  | Countries | Age Groups | Study Design | Sample Size | Key Findings                                                                 |
|-------------------------|-----------|------------|--------------|-------------|-----------------------------------------------------------------------------|
| Lubell et al. [65]      | Cambodia, Laos, and, Thailand | Cambodia: <16 years Laos: 5–49 Thailand ≥5 years | Using stored samples from three prospective studies. | 1372 microbiologically confirmed mono-infections | Bacterial microbiologically confirmed n=6 No data n=9 *clinical diagnosis included signs and symptoms, and routine laboratory work up such as CRP. CRP is better in discriminating viral and bacterial infection in mono-infection than PCT. NOTE: studies did not single out CAP; however, Influenza/RSV were identified. |
| Hoshina et al. [66]     | Japan     | <15 years old | Retrospective cohort study | 31 | Bacterial n=21 Viral n=10 WBC, neutrophil PCT was a very useful marker to differentiate bacterial pneumonia. |
| Study                        | Year | Country | Age | Study Design | Sample Size | Diagnosis Method | Findings                                                                 |
|------------------------------|------|---------|-----|--------------|--------------|------------------|--------------------------------------------------------------------------|
| Elemraid et al. [67]         | 2014 | United Kingdom | ≤16 years | Two prospective aetiological studies | n=401 | Bacteria CAP was confirmed sputum culture, Viral CAP confirmed by nasopharyngeal aspirate, sputum or throat swab | CRP, PCT, Neutrophil count helped to discriminate bacterial bronchitis. |
| Zhou et al. [68]             | 2011 | China   | 0.11-2.57 years | Prospective cohort | 78            | Bacteria n=27 viruses n=25 Bacteria and viruses n=26 | CRP, WBC, IgA, IgG, IgM, percent of T, Tc, Th, B, NK, CD23+, and HMGB1 and WBC can differentiate between bacterial, viral and co-infected cases of bronchial pneumonia |
| CD25+cells and degree of expression of HMGB1 mRNA |
|--------------------------------------------------|

REF: Reference; n: Sample size; Lcn2: Lipocalin 2; SYN4: Syndecan 4; CRP: C-reactive protein; WBC: White blood cell; IL-10: Interleukin 10; MR-proADM: Midregional Proadrenomedullin; MR-proANP: Midregional proatrial natriuretic peptide; PCT: Procalcitonin; Hap: Haptoglobin; TNF: Tumor necrosis factor; MxA1: Myxoma resistance protein 1; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M; T: T-lymphocyte; Tc: Cytotoxic T Lymphocyte; Th: Helper T-Lymphocyte; B: B lymphocyte; NK: Natural Killer; CD: cluster of differentiation; HMGB1: High mobility group box 1 protein; mRNA: Messenger Ribonucleic Acid.
The initial recruitment of patient in most of the studies was based on clinical judgment and radiographic findings. Most of the studies involved isolating bacterial and viral organisms in the same population. The technique of isolation was either PCR of nasopharyngeal aspirate, throat swab or sputum for viruses and blood culture or PCR for bacterial organisms. The rate of positive bacterial culture was low and Naydenova et al. [62] categorized negative growth as viral CAP without isolating the viral organism. Markers that were investigated were C-reactive protein (CRP), Procalcitonin (PCT), White blood cell (WBC), Neutrophils, Myxoma resistance protein (MxA1) Lipocalin-2 (Lcn2). High mobility group box one protein (HMGB1) Syndecan4 (SYN4), Midregional Proadrenomedullin and Midregional proatrial natriuretic peptide. Two studies recommended using a combination of marker indicators (clinical and or markers) [61][67].

4.0 DISCUSSION

4.1 C-reactive protein (CRP)

A total of nine studies involved CRP [39],[60][62][64–68]. CRP is an acute phase protein which is produced in the liver and is released into serum following injury or inflammation. The induction of CRP depends on cytokines, chemokines and interleukins, especially IL-6 [69]. Previous studies have indicated the useful nature of CRP in ruling in or out bacterial CAP. A metanalysis by Flood et al. [28] suggested that bacterial pneumonia can be suspected when CRP is higher than 35-60 mg/L (OR 2.58, 95% CI 1.20–5.55). Likewise, Andreola et al. [70] suggested that a CRP greater than 80mg/L is an alarm sign referring to a serious bacterial infection, such as pneumonia. However, the relationship between CRP and the causative agent is more complex than expected. Even in viral CAP, CRP may rise significantly. Studies involving paediatric population during H1N1 in 2009 reported that CRP could rise over 180mg/L [39,65,71].

In all the studies analysing CRP as a diagnostic marker, the average CRP level was higher in the bacterial group than viral group [39,60,62,64–68]. In an investigation by Esposito et al. [60] the mean level of CRP was 32.2mg/L in 74 bacterial CAP cases as compared to 9.4mg/L from 16 viral CAP cases. Similarly, in a different study by Esposito et al. [39] with larger sample size, the average CRP was 21.3mg/L in 235 bacterial CAP patients and 8.0mg/L in 111 viral CAP patients. Although the mean levels of CRP were higher in bacterial CAP, there was overlap between viral and bacterial cases leading to issues in fixing a suitable cut-off point that is both sensitive and specific to differentiate between the two. In the study by Elemraid et al. [67] 25% of viral CAP cases had CRP over 80mg/L, and nearly 23% of bacterial cases had CRP less than 20mg/L [67]. This is consistent with a previously published study in which 40% of viral infections resulted in CRP concentrations above 20mg/L [72]. A previous paper speculated that there is a 15% risk of severe bacterial pneumonia with a CRP less than 80mg/L [70].
The overlap of CRP values has led to authors suggesting different cut-off values (Table 8). It is evident that a low reference point for CRP will diagnose almost all cases of bacterial aetiology but will include a significant number of false positive cases. On the contrary, a higher cut off value will give greater specificity but reduced sensitivity (Table 8) [65]. For example, with a cut-off value of 10mg/l, the sensitivity and specificity are 95% and 49% respectively. Doubling the cut-off point to 20mg/L led to a lower sensitivity of 85% and increased the specificity of 67%. However, a lower threshold value does not guarantee higher sensitivity. In a study by Esposito et al. [60] a cut-off value of 7.4mg/L only resulted in 64% sensitivity. Nevertheless, the disparity may be due to the differences in study setting as. Lubell et al. [65] involved patients with malaria, leptospirosis bacteremia, and Rickettsia infection, all of which can alter CRP concentration.

In a study by Naydenova et al. [62], the diagnostic value of respiratory rate, heart rate, oxygen saturation together with auscultation findings (presence or absence of grunting or crackles) was additionally analysed in association with CRP. The combination of these clinical parameters with CRP slightly improved the predictive power with a sensitivity of 64% and specificity of 88%. In the same study, the author added Lipocalin-2 (Lcn2) to CRP and clinical data which dramatically increased sensitivity to 81.8% and specificity to 90.6%.

A significant limitation of almost all the studies was the lack of inclusion of primary care patients. This meant that before hospital admission, many patients might have had exposure to antibiotics which may have altered the level of CRP [67]. Another drawback was that only one study investigated co-infection (viral-bacterial) and concluded that CRP level did not correlate with co-infection [68].

### 4.2 Procalcitonin

Four studies analysed the diagnostic value of procalcitonin (PCT) [39,63,65,66]. PCT is a precursor to calcitonin produced in the parafollicular cells of the thyroid gland by the transcription of as CALC-1 gene, During an infection CALC-1 gene, is activated and upregulated to increase the production of PCT in not only endocrine glands but also many parenchymal tissues [73]. The sudden and marked increase (over 2ng/ml) in PCT within four to six hours is a key indicator of bacterial infection [74]. It is hypothesized that viruses are not able to increase PCT to such a concentration as certain cytokines expressed during viral infection leads to decreased induction of PCT. This was reflected in the study by Esposito et al. [39] as the mean PCT was 1.1 ng/ml in viral CAP compared to 6.1 ng/ml in bacterial CAP cases. However, the study by Pavia et al. [71] on infected children with H1N1 found a significant rise in PCT concentration (>7ng/ml). This finding is consistent with other studies [75,76]. This may indicate that H1N1 strain differs in inducing inflammation or is more associated with bacterial co-infection.
Esposito et al. (40) showed that specificity to identify viral aetiology was higher of PCT compared to CRP. Authors reported that a PCT cut-off value of ≤0.07 ng/ml had the highest combined sensitivity (48.7%) and specificity (81.1%) for viral CAP. Hoshina et al. noted that PCT value higher than 0.2 ng/ml which had a sensitivity of 86%, the specificity of 80% [66]. This resembles an earlier study published in 2011 which concluded that PCT value greater than 0.1ng/ml is more sensitive and specific and has greater positive and negative predictive values than CRP [72]. In this investigation, area under the curve (AUC) of the receiver operated curve characteristic (ROC) was 0.93 for PCT (95% CI 0.85- 0.97) and 0.84 for CRP (95% CI 0.73 to 0.91) [72].

4.3 White Blood Cell (WBC) and Neutrophils

Five papers focused on WBC and or neutrophil count [39,60,66–68]. The total WBC count fluctuates in the paediatric population, especially in the early period of life. Therefore, the reference values differ between the age groups (table 8) [77]. In general, a value greater than 11x10⁹/L is considered to be leucocytosis [77].

Viral pathogens have known to cause relative lymphocytosis but sometimes may cause absolute lymphocytosis [78,79]. The total WBC count does not accurately differentiate viral and bacterial causes [80,81]. Research by Elemraid et al. [67] showed that almost 40% of viral pneumonia cases presented with WBC >15x10⁹/L. Similarly, Esposito et al. [60] highlighted that WBC had the lowest positive predictive value compared to PCT and CRP. According to Zhu et al. [63], the percentage of neutrophils compared to a total WBC count was to some extent better at discriminating viral from bacterial infection. According to the literature, passing neutropenia (Neutrophils <1.5x10⁹/L) is likely to begin from day three and last until day eight in many viral infections, including RSV, IV and AV [82,83]. However, none of the studies reported neutropenia. The lack of rising in neutrophil count correlates well with viral causes. In the study by Elemraid et al., 80% of patients with viral pneumonia had neutrophils less than 10 x10⁹/L [67].

Table 8. The reference data of WBC and its constituents according to the age. Adapted from [77].

| Age    | Mean WBC(10⁹/L) | Mean Neutrophils (10⁹/L) |
|--------|-----------------|--------------------------|
| Birth  | 18.10           | 11.0                     |
| 1 month| 10.80           | 3.80                     |
| 6 months| 11.19        | 3.80                     |
| 1 year | 11.14           | 3.50                     |
| 2 years| 9.10            | 3.50                     |
| 4 years| 8.50            | 3.80                     |
| 6 years| 8.30            | 4.30                     |

WBC: White blood cell
4.4 Myxoma resistance protein (MxA1)

Several new markers have been studied in the above data. Of this, MxA1 has shown promising results. Compared to other makers, MxA1 protein tends to rise significantly during viral rather than a bacterial infection. Type I or III Interferon (IFN) can activate MxA1 but not type II IFN signaling pathway or the direct interaction of bacteria or viruses [84]. IFN is classified into three groups depending on the similarities in their amino acid sequence. Type I IFN is called alpha, beta, tau, and -omega and are produced in all cells in the body [85]. IFN is also elevated in autoimmune conditions and some hematological cancers. Therefore, the value of IFN induced MxA1 in this population may not be dependable [86].

The study by Engelmann et al. [64] was the largest prospective study analysing the role of MxA1. A cut off value of 200ng/mL was 96.4% sensitive and 66.7% specific for identifying patients with viral CAP [64]. The author also hypothesized that if a bacterial infection is diagnosed and high levels of MxA1 are detected, this is an indication that bacterial organism preceded a viral cause [64]. This is because MxA1 stays elevated for approximately ten days after a viral insult in comparison to IFN which has a very short half-life [87]. The authors also made a correlation with CRP. Low CRP (<40mg/l) and MxA1 >200ng/ml is highly indicative of viral aetiology [64].

The study sample size was the biggest drawback of this investigation. Out of 553 children who were enrolled, only 41 had CAP. Moreover, not all cases of CAP were microbiologically confirmed. Therefore, more studies are needed to confirm the diagnostic value of MxA1.

4.5 Lipocalin-2 (Lcn2)

Lcn2 is a protein stored and released by neutrophils which distorts iron transportation within bacteria. This marker is of high interest in diagnosing aetiological factor of CAP. However, the results are conflicting [88]. Two studies involving Lcn2 were carried out in very different settings which may have contributed to the different results of those studies. The study by Esposito et al. [60] concluded that Lcn2 was a poor predictor compared to CRP or WBC [60]. While Naydenova et al. (2016) found the use of Lcn2 to help discriminate bacterial and viral pneumonia. The latter study was set in a developing nation amongst children with malaria, which is known to affect the concentration of Lcn2 [89]. According to Huang et al. [89], a cut-off value of more than 130ng/ml strongly correlates with bacterial aetiology (sensitivity 83.67% and specificity 85.71%). Also, Lcn2 of more than 160ng/ml is highly indicative of a positive isolate from a blood culture [89].
4.6 High mobility group box one protein (HMGB1)

HMGB1 is a protein which binds to DNA and causes the transcription of several inflammatory markers. Furthermore, it has some extracellular roles such as promoting migration and enhancing the production of pro-inflammatory markers and cytokines such as Interleukin 6 (IL-6), Tumour necrosis factor (TNF) or interferon gamma (IFN-γ). This protein elevates during CAP, sepsis and viral-bacterial co-infections, especially bacterial and IV co-infection [90]. A study by Zhou et al. [68] evaluated changes in the expression of HMGB1 gene in peripheral monocytes as opposed to measuring the concentration of HMGB1 in the serum. By using the PCR technique, gene expression was quantified by comparing HMGB1 proteins density to an 18S ribosomal ribonucleotide acid. It was found that co-infection (virus and bacteria) can be concluded when HMGB1 expression is greater than 1.0256. Furthermore, in this study HMGB1 expression <1.0256 and a WBC >13x10⁹/L had 92.3% positive predictive value for single bacterial pneumonia [68]. Therefore, HMGB1 seems to be a good marker. However, the need to isolate specific blood cells (monocytes) and to adopt PCR makes this method prolonged and expensive. Moreover, measurement of RNA may not necessarily correlate with functional serum HMGB1 protein.

4.7 Marker combinations

Valim et al. [61], evaluated 56 plasma proteins in training set, validation set, and healthy controls in order to distinguish bacterial, viral, and malaria in children presenting with clinical signs and symptoms of pneumonia. The result of the study found that combining haptoglobin (Hap), tissue inhibitor of metalloproteinases-1, Interleukin 19 (IL-19) or TNF receptor 2 resulted in a sensitivity of 96% and specificity of 86% in bacterial diagnosis CAP.

Meanwhile, Elemraid et al. [67] advocated for a rather simple combination of age, CRP, and WBC together with neutrophils count. This discriminatory model had 91.4% positive predictive value and 71.2% negative predictive value for bacterial CAP in children under 16.

Utilizing an extended number of markers with clinical signs does not improve the sensitivity or specificity according to Naydenova et al. [62]. Sensitivity and specificity when combining respiratory rate, heart rate, and oxygen saturation with Lnc2 was 82% and 91%, respectively. Adding CRP or Hap to this did not improve sensitivity or specificity.
4.8 Others

Several new markers, such as Syndecan 4 (SYN4), were explored with poor reliability. Results from the study by Esposito et al. revealed that SYN4 had an AUC on ROC of only 0.54 (95% CI 0.40-0.69) compared to 0.67 (95% CI 0.53-0.80) for CRP [60]. Proteins that can affect the cardiovascular system: Midregional Poadrenomedullin and Midregional pro-atrial natriuretic peptide were found to be not useful in predicting aetiology; instead, these were indicative of CAP severity [39].

Table 9: A summary of the markers with the cut-off values.

|          | Bacterial | Viral | Bacterial and Viral | Specificity | Sensitivity |
|----------|-----------|-------|---------------------|-------------|-------------|
| CRP [REF]|           |       |                     |             |             |
| [65] >10 mg/L |         |       |                     | 49%         | 95%         |
| [65] >20 mg/L |        |       |                     | 67%         | 86%         |
| [39] >7.98 mg/L |       |       |                     | 53.8%       | 63.5%       |
| [39] <7.5 mg/L |       |       |                     | 46.3%       | 88.2%       |
| [60] >7.4 mg/L |       |       |                     | 69.4%       | 64%         |
| [60] <5.2 mg/L |       |       |                     | 64.5%       | 75.0%       |
| PCT [39] >0.188 ng/ml | |       |                     | 65.1%       | 67.4%       |
| [39] <0.07 ng/ml |       |       |                     | 81.1%       | 48.7%       |
| [66] >0.2 ng/ml |       |       |                     | 80%         | 86%         |
| [65] >0.1 ng/mL |       |       |                     | 39%         | 90%         |
| [65] >0.5 ng/mL |       |       |                     | 76%         | 60%         |
| WBC [60] ≥13.500(S. pneumoni ae) | |       |                     | 68.6%       | 63.8%       |
| Neutrophils | Lcn2 | MR-proADM | MxA1 | HMGB1 (expression) | MxA1+CRP |
|------------|------|-----------|------|-------------------|---------|
| [39] >61.0% | [62] <200ng/ml | [39] >0.32nmol/L | [64] >200 ng/mL | [68] >1.0256 | [64] >200ng/ml + >40 mg/L |
| [39] <60.8% | ≥1,633ng/ml (S. pneumoniae) | ≤0.31nmol/L | ≥200 ng/mL | | |
|            | ≥1,633ng/ml | | | | |
|            | ≥896ng/ml | | | | |

REF: Reference, CRP: C-reactive protein, PCT: Procalcitonin; WBC: White blood cell; Lcn2: Lipocalin; MR-proADM: Midregional Proadrenomedullin; MxA1: Myxoma resistance protein; HMGB1: High mobility group box 1 protein; ng: nanograms; nmol: nanomolar; mL: milliliter; L: liter.

### 5.0 STUDY LIMITATIONS

Data collection was done by one member, and this could have had an impact on the articles that were selected. Furthermore, only one database was searched to provide a concise overview. Therefore, numerous...
articles on other respected databases, which may have been relevant, were not reviewed. Also, potential markers present in the urine were not reviewed. Moreover, the English language as the filter may have limited the number of articles during the search.

6.0 CONCLUSION

It is very challenging to accurately predict bacterial or viral pneumonia on clinical, radiological as well as on laboratory grounds. As far as the clinical picture is concerned, a child under the age of five, who is sub-febrile with lung field changes and is wheezing is most likely to present with a viral CAP. Radiological findings are less conclusive and widely overlap while quick and cheap RADT lacks sensitivity. Although DFA and NAAT were superior in terms of sensitivity, both were associated with additional cost and expertise.

With serum markers, the differences in cut-off values are related to the differences in detection methods, analyses, the desired specificity and sensitivity and the presence of mixed infection. From the results, almost all markers had a higher value in bacterial pneumonia. The only marker increased in viral pneumonia and not in bacterial pneumonia was MxA1. This is a promising development, and more studies need to be instituted, and if results are consistent, it may be an essential marker to rule in or out viral infections. Furthermore, co-infection was a constant dilemma in many studies. Although HMBG1 expression was vital in proving mixed infection, the need for PCR makes this test non-viable in clinical settings. Therefore, similar studies are needed to be conducted to measure the HMBG1 protein concentration in serum rather than the gene expression.

One approach is to make use of more than one marker and combine with clinical signs and symptoms. Lnc2, when combined with clinical features was 82% sensitive and 91% specific for bacterial CAP[62]. Lnc2 performed better than CRP, and therefore a solution is to include Lnc2 during laboratory work-up. When needing higher sensitivity and specificity, combining Haptoglobin (Hap), tissue inhibitor of metalloproteinases-1, Interleukin 19 (IL-19) or TNF receptor 2 could be a solution. However, this may not be cost-effective in many clinical settings.

7.0 PRACTICAL RECOMMENDATIONS

With regards to optimal cut-off value for different markers, more studies are needed to provide accurate results and associate it with patients or within the context of the clinical situation. Moreover, a reference value should be based on whether the aim is to diagnose bacterial CAP or viral CAP. Adding Lnc2 to clinical context together with CRP should be considered for better predictive power. Also, consider the combination Hap, tissue inhibitor of metalloproteinases-1, IL-19 or TNF receptor 2 if resources are available.
References

1. Kassebaum N, Kyu HH, Zoeckler L, Olsen HE, Thomas K, Pinho C, et al. Child and Adolescent Health From 1990 to 2015. JAMA Pediatr [Internet]. 2017 Jun 1;171(6):573. Available from: http://archpedi.jamanetwork.com/article.aspx?doi=10.1001/jamapediatrics.2017.0250

2. Rudan I. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ [Internet]. 2008 May 1;86(5):408–16. Available from: http://www.who.int/bulletin/volumes/86/5/07-048769.pdf

3. Petousis-Harris H, Howe AS, Janine P, Turner N, Griffin J. Pneumococcal conjugate vaccines turning the tide on inequity – a retrospective cohort study of New Zealand children born 2006–2015. Clin Infect Dis [Internet]. 2018 Jul 19; Available from: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciy570/5055833

4. Butler DF, Myers AL. Changing Epidemiology of Haemophilus influenzae in Children. Infect Dis Clin North Am. 2018;32(1):119–28.

5. Whittaker R, Economopoulou A, Dias JG, Bancroft E, Ramliden M, Celentano LP. Epidemiology of Invasive Haemophilus influenzae Disease, Europe, 2007–2014. Emerg Infect Dis [Internet]. 2017 Mar;23(3):396–404. Available from: http://wwwnc.cdc.gov/eid/article/23/3/16-1552_article.htm

6. Jain S, Williams DJ, Arnold SR, Ampofo K, Bramley AM, Reed C, et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Children. N Engl J Med [Internet]. 2015 Feb 26;372(9):835–45. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1405870

7. Harris M, Clark J, Coote N, Fletcher P, Harnden A, McKean M, et al. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax [Internet]. 2011 Oct 1;66(Suppl 2):ii1-ii23. Available from: http://thorax.bmj.com/cgi/doi/10.1136/thoraxjnl-2011-200598

8. Klig JE. Office pediatrics: current perspectives on the outpatient evaluation and management of lower respiratory infections in children. Curr Opin Pediatr [Internet]. 2006 Feb;18(1):71–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16470166

9. Rodrigues CMC, Groves H. Community-Acquired Pneumonia in Children: the Challenges of Microbiological Diagnosis. Kraft CS, editor. J Clin Microbiol [Internet]. 2017 Dec 13;56(3). Available from: http://jcm.asm.org/lookup/doi/10.1128/JCM.01318-17
10. Advani S, Sengupta A, Forman M, Valsamakis A, Milstone AM. Detecting Respiratory Viruses in Asymptomatic Children. Pediatr Infect Dis J [Internet]. 2012 Dec;31(12):1221–6. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006454-201212000-00002

11. Kramer MS, Roberts-Bräuer R, Williams RL. Bias and ‘overcall’ in interpreting chest radiographs in young febrile children. Pediatrics [Internet]. 1992 Jul;90(1 Pt 1):11–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1614759

12. Bruns AHW, Oosterheert JJ, El Moussaoui R, Opmeer BC, Hoepelman AIM, Prins JM. Pneumonia recovery: discrepancies in perspectives of the radiologist, physician and patient. J Gen Intern Med [Internet]. 2010 Mar;25(3):203–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19967464

13. UNICEF. Levels & trends in child mortality [Internet]. Newyork; 2107. Available from: https://data.unicef.org/topic/child-health/pneumonia/

14. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet [Internet]. 2015 Jan;385(9966):430–40. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673614616986

15. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet [Internet]. 2016 Dec;388(10063):3027–35. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673616315938

16. Webber S, Wilkinson AR, Lindsell D, Hope PL, Dobson SR, Isaacs D. Neonatal pneumonia. Arch Dis Child [Internet]. 1990 Feb;65(2):207–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2107797

17. Jones CA, Raynes-Greenow C, Isaacs D, Neonatal HSV Study Investigators and Contributors to the Australian Paediatric Surveillance Unit. Population-based surveillance of neonatal herpes simplex virus infection in Australia, 1997-2011. Clin Infect Dis [Internet]. 2014 Aug 15;59(4):525–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2484638

18. Mahnert N, Roberts SW, Laibl VR, Sheffield JS, Wendel GD. The incidence of neonatal herpes infection. Am J Obstet Gynecol [Internet]. 2007 May;196(5):e55-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17466681
19. Flagg EW, Weinstock H. Incidence of neonatal herpes simplex virus infections in the United States, 2006. Pediatrics [Internet]. 2011 Jan;127(1):e1-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21149432

20. Pinninti SG, Kimberlin DW. Maternal and neonatal herpes simplex virus infections. Am J Perinatol [Internet]. 2013 Feb;30(2):113–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23303485

21. Knezevic A, Martic J, Stanojevic M, Jankovic S, Nedeljkovic J, Nikolic L, et al. Disseminated neonatal herpes caused by herpes simplex virus types 1 and 2. Emerg Infect Dis [Internet]. 2007 Feb;13(2):302–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17479897

22. Shane AL, Stoll BJ. Neonatal sepsis: progress towards improved outcomes. J Infect [Internet]. 2014 Jan;68 Suppl 1:S24-32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24140138

23. Duke T. Neonatal pneumonia in developing countries. Arch Dis Child Fetal Neonatal Ed [Internet]. 2005 May;90(3):F211-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15846010

24. Rhedin S, Lindstrand A, Hjelmgren A, Ryd-Rinder M, Öhrmalm L, Tolfvenstam T, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. Thorax [Internet]. 2015 Sep;70(9):847–53. Available from: http://thorax.bmj.com/lookup/doi/10.1136/thoraxjnl-2015-206933

25. Hammitt LL, Kazungu S, Morpeth SC, Gibson DG, Mvera B, Brent AJ, et al. A preliminary study of pneumonia etiology among hospitalized children in Kenya. Clin Infect Dis [Internet]. 2012 Apr;54 Suppl 2:S190-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22403235

26. Ostapchuk M, Roberts DM, Haddy R. Community-acquired pneumonia in infants and children. Am Fam Physician [Internet]. 2004 Sep 1;70(5):899–908. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15368729

27. Chen S-P, Huang Y-C, Chiu C-H, Wong K-S, Huang Y-L, Huang C-G, et al. Clinical features of radiologically confirmed pneumonia due to adenovirus in children. J Clin Virol [Internet]. 2013 Jan;56(1):7–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23021965

28. Flood RG, Badik J, Aronoff SC. The utility of serum C-reactive protein in differentiating bacterial from nonbacterial pneumonia in children: a meta-analysis of 1230 children. Pediatr Infect Dis J [Internet]. 2008 Feb;27(2):95–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18174874

29. Shah S, Bachur R, Kim D, Neuman MI. Lack of predictive value of tachypnea in the diagnosis of pneumonia in children. Pediatr Infect Dis J [Internet]. 2010 May;29(5):406–9. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/20032805

30. Le Saux N, Robinson J. Pneumonia in healthy Canadian children and youth: Practice points for management. Paediatr Child Health [Internet]. 2011 Aug;16(7):417–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22851898

31. Bachur R, Perry H, Harper MB. Occult pneumonias: empiric chest radiographs in febrile children with leukocytosis. Ann Emerg Med [Internet]. 1999 Feb;33(2):166–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9922412

32. Tan TQ, Mason EO, Barson WJ, Wald ER, Schutze GE, Bradley JS, et al. Clinical characteristics and outcome of children with pneumonia attributable to penicillin-susceptible and penicillin-nonsusceptible Streptococcus pneumoniae. Pediatrics [Internet]. 1998 Dec;102(6):1369–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9832571

33. Puljiz I, Kuzman I, Dakovic-Rode O, Schönwald N, Mise B. Chlamydia pneumoniae and Mycoplasma pneumoniae pneumonia: comparison of clinical, epidemiological characteristics and laboratory profiles. Epidemiol Infect [Internet]. 2006 Jun;134(3):548–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16316495

34. Stockmann C, Ampofo K, Killpack J, Williams DJ, Edwards KM, Grijalva CG, et al. Procalcitonin Accurately Identifies Hospitalized Children With Low Risk of Bacterial Community-Acquired Pneumonia. J Pediatric Infect Dis Soc [Internet]. 2018 Feb 19;7(1):46–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28158460

35. Kirovski I, Micevska V, Seckova L, Nikolovski L. Abdominal pain as a predictor of pneumonia in children. Eur Respir J [Internet]. 2011 Sep 1;38(Suppl 55):p1152. Available from: http://erj.ersjournals.com/content/38/Suppl_55/p1152.abstract

36. Krenke K, Urbankowska E, Urbankowski T, Lange J, Kulus M. Clinical characteristics of 323 children with parapneumonic pleural effusion and pleural empyema due to community acquired pneumonia. J Infect Chemother [Internet]. 2016 May;22(5):292–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26919911

37. McKee AJ, Ives A, Balfour-Lynn IM. Increased incidence of bronchopulmonary fistulas complicating pediatric pneumonia. Pediatr Pulmonol [Internet]. 2011 Jul;46(7):717–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21626711

38. Sahn SA. Diagnosis and management of parapneumonic effusions and empyema. Clin Infect Dis
39. Esposito S, Di Gangi M, Cardinale F, Baraldi E, Corsini I, Da Dalt L, et al. Sensitivity and Specificity of Soluble Triggering Receptor Expressed on Myeloid Cells-1, Midregional Proatrial Natriuretic Peptide and Midregional Proadrenomedullin for Distinguishing Etiology and to Assess Severity in Community-Acquired Pneumonia. PLoS One [Internet]. 2016;11(11):e0163262. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27846213

40. Turner RB, Lande AE, Chase P, Hilton N, Weinberg D. Pneumonia in pediatric outpatients: cause and clinical manifestations. J Pediatr [Internet]. 1987 Aug;111(2):194–200. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3612389

41. Bradley JS, Byington CL, Shah SS, Alverson B, Carter ER, Harrison C, et al. The Management of Community-Acquired Pneumonia in Infants and Children Older Than 3 Months of Age: Clinical Practice Guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis [Internet]. 2011 Oct 1;53(7):e25–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21880587

42. Sinaniotis CA, Sinaniotis AC. Community-acquired pneumonia in children. Curr Opin Pulm Med [Internet]. 2005 May;11(3):218–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15818183

43. Pelton SI, Hammerschlag MR. Overcoming Current Obstacles in the Management of Bacterial Community-Acquired Pneumonia in Ambulatory Children. Clin Pediatr (Phila) [Internet]. 2005 Jan 7;44(1):1–17. Available from: http://journals.sagepub.com/doi/10.1177/000992280504400101

44. Guo W, Wang J, Sheng M, Zhou M, Fang L. Radiological findings in 210 paediatric patients with viral pneumonia: a retrospective case study. Br J Radiol [Internet]. 2012 Oct;85(1018):1385–9. Available from: http://www.birpublications.org/doi/10.1259/bjr/20276974

45. Ashorn P. Bacteremic Pneumococcal Pneumonia in Children. Clin Infect Dis [Internet]. 1999 Sep;29(3):568–72. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/598635

46. Virkki R. Differentiation of bacterial and viral pneumonia in children. Thorax [Internet]. 2002 May 1;57(5):438–41. Available from: http://thorax.bmj.com/cgi/doi/10.1136/thorax.57.5.438

47. Elemraid MA, Muller M, Spencer DA, Rushton SP, Gorton R, Thomas MF, et al. Accuracy of the Interpretation of Chest Radiographs for the Diagnosis of Paediatric Pneumonia. Worgall S, editor. PLoS One [Internet]. 2014 Aug 22;9(8):e106051. Available from: http://dx.plos.org/10.1371/journal.pone.0106051
48. Zar HJ, Andronikou S, Nicol MP. Advances in the diagnosis of pneumonia in children. BMJ [Internet]. 2017 Jul 26;j2739. Available from: http://www.bmj.com/lookup/doi/10.1136/bmj.j2739

49. Ambroggio L, Sucharew H, Rattan MS, O’Hara SM, Babcock DS, Clohessy C, et al. Lung Ultrasonography: A Viable Alternative to Chest Radiography in Children with Suspected Pneumonia? J Pediatr [Internet]. 2016 Sep;176:93–98.e7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347616302414

50. Peltola V, Mertsola J, Ruuskanen O. Comparison of total white blood cell count and serum C-reactive protein levels in confirmed bacterial and viral infections. J Pediatr [Internet]. 2006 Nov;149(5):721–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17095353

51. Sandora TJ, Smole SC, Lee GM, Chung S, Williams L, McAdam AJ. Test characteristics of commercial influenza assays for detecting pandemic influenza A (H1N1) in children. Pediatr Infect Dis J [Internet]. 2010 Mar;29(3):261–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19935118

52. Kawachi S, Matsushita T, Sato T, Nunoi H, Noguchi H, Ota S, et al. Multicenter prospective evaluation of a novel rapid immunochromatographic diagnostic kit specifically detecting influenza A H1N1 2009 virus. J Clin Virol [Internet]. 2011 May;51(1):68–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21324735

53. Rouleau I, Charest H, Douville-Fradet M, Skowronski DM, De Serres G. Field Performance of a Rapid Diagnostic Test for Influenza in an Ambulatory Setting. J Clin Microbiol [Internet]. 2009 Sep 1;47(9):2699–703. Available from: http://jcm.asm.org/cgi/doi/10.1128/JCM.00762-09

54. Landry ML. Developments in Immunologic Assays for Respiratory Viruses. Clin Lab Med [Internet]. 2009 Dec;29(4):635–47. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0272271209000675

55. Lee JH, Shin SR, Cho JH. Evaluation of direct immunofluorescence test with PCR for detection of novel influenza A (H1N1) virus during 2009 pandemic. Yonsei Med J [Internet]. 2011 Jul;52(4):680–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21623613

56. Mayer LM, Kahlert C, Rassouli F, Vernazza P, Albrich WC. Impact of viral multiplex real-time PCR on management of respiratory tract infection: a retrospective cohort study. Pneumonia [Internet]. 2017 Dec 25;9(1):4. Available from: http://pneumonia.biomedcentral.com/articles/10.1186/s41479-017-0028-z

57. Schlaberg R, Queen K, Simmon K, Tardif K, Stockmann C, Flygare S, et al. Viral Pathogen Detection by Metagenomics and Pan-Viral Group Polymerase Chain Reaction in Children With Pneumonia Lacking
Identifiable Etiology. J Infect Dis [Internet]. 2017 May 1;215(9):1407–15. Available from: https://academic.oup.com/jid/article/215/9/1407/3090889

58. Ginocchio CC, McAdam AJ. Current Best Practices for Respiratory Virus Testing. J Clin Microbiol [Internet]. 2011 Sep 1;49(9 Supplement):S44–8. Available from: http://jcm.asm.org/cgi/doi/10.1128/JCM.00698-11

59. Chartrand C, Leeflang MMG, Minion J, Brewer T, Pai M. Accuracy of rapid influenza diagnostic tests: a meta-analysis. Ann Intern Med [Internet]. 2012 Apr 3;156(7):500–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22371850

60. Esposito S, Bianchini S, Gambino M, Madini B, Di Pietro G, Umbrello G, et al. Measurement of lipocalin-2 and syndecan-4 levels to differentiate bacterial from viral infection in children with community-acquired pneumonia. BMC Pulm Med [Internet]. 2016;16(1):103. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27439403

61. Valim C, Ahmad R, Lanaspa M, Tan Y, Acácio S, Gillette MA, et al. Responses to Bacteria, Virus, and Malaria Distinguish the Etiology of Pediatric Clinical Pneumonia. Am J Respir Crit Care Med [Internet]. 2016 Feb 15;193(4):448–59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26469764

62. Naydenova E, Tsanas A, Howie S, Casals-Pascual C, De Vos M. The power of data mining in diagnosis of childhood pneumonia. J R Soc Interface [Internet]. 2016;13(120). Available from: http://www.ncbi.nlm.nih.gov/pubmed/27466436

63. Zhu F, Jiang Z, Li WH, Wei HY, Su GD. Clinical significance of serum procalcitonin level monitoring on early diagnosis of severe pneumonia on children. Eur Rev Med Pharmacol Sci [Internet]. 2015 Nov;19(22):4300–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26636517

64. Engelmann I, Dubos F, Lobert P-E, Houssin C, Degas V, Sardet A, et al. Diagnosis of viral infections using myxovirus resistance protein A (MxA). Pediatrics [Internet]. 2015 Apr;135(4):e985-93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25802344

65. Lubell Y, Blacksell SD, Dunachie S, Tanganuchitcharanchai A, Althaus T, Watthanaworawit W, et al. Performance of C-reactive protein and procalcitonin to distinguish viral from bacterial and malarial causes of fever in Southeast Asia. BMC Infect Dis [Internet]. 2015 Nov 11;15:511. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26558692

66. Hoshina T, Nanishi E, Kanno S, Nishio H, Kusuhara K, Hara T. The utility of biomarkers in differentiating bacterial from non-bacterial lower respiratory tract infection in hospitalized children:
difference of the diagnostic performance between acute pneumonia and bronchitis. J Infect Chemother [Internet]. 2014 Oct;20(10):616–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25027057

67. Elemraid MA, Rushton SP, Thomas MF, Spencer DA, Gennery AR, Clark JE. Utility of inflammatory markers in predicting the aetiology of pneumonia in children. Diagn Microbiol Infect Dis [Internet]. 2014 Aug;79(4):458–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0732889314001667

68. Zhou WF, Chen Q, Jin MF, Ji ZH, Zhang MZ, Li HM, et al. The diagnostic accuracy of high-mobility group box 1 protein and twelve other markers in discriminating bacterial, viral and co-infected bronchial pneumonia in Han children. Microbiol Immunol [Internet]. 2011 Apr;55(4):279–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21223368

69. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci U S A [Internet]. 1987 Oct;84(20):7251–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2444978

70. Andreola B, Bressan S, Callegaro S, Liverani A, Plebani M, Da Dalt L. Procalcitonin and C-reactive protein as diagnostic markers of severe bacterial infections in febrile infants and children in the emergency department. Pediatr Infect Dis J [Internet]. 2007 Aug;26(8):672–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17848876

71. Paiva MBS, Botoni FA, Teixeira AL, Miranda AS de, Oliveira CRA de, Abrahão J de O, et al. The behavior and diagnostic utility of procalcitonin and five other inflammatory molecules in critically ill patients with respiratory distress and suspected 2009 influenza a H1N1 infection. Clinics (Sao Paulo) [Internet]. 2012;67(4):327–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22522757

72. Moulin F, Raymond J, Lorrot M, Marc E, Coste J, Iniguez JL, et al. Procalcitonin in children admitted to hospital with community acquired pneumonia. Arch Dis Child [Internet]. 2001 Apr;84(4):332–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11259234

73. Jin M, Khan AI. Procalcitonin: Uses in the Clinical Laboratory for the Diagnosis of Sepsis. Lab Med [Internet]. 2010 Mar 1;41(3):173–7. Available from: https://academic.oup.com/labmed/article-lookup/doi/10.1309/LMQ2GRR4QLFKHC9

74. Baumann P, Baer G, Bonhoeffer J, Fuchs A, Gotta V, Heininger U, et al. Procalcitonin for Diagnostics and Treatment Decisions in Pediatric Lower Respiratory Tract Infections. Front Pediatr [Internet].
75. Cunha BA, Syed U, Strollo S. Swine influenza (H1N1) pneumonia: elevated serum procalcitonin levels not due to superimposed bacterial pneumonia. Int J Antimicrob Agents [Internet]. 2010 May;35(5):515–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20188520

76. Guervilly C, Coisel Y, Botelho-Nevers E, Dizier S, Castanier M, Lepaul-Ercole R, et al. Significance of high levels of procalcitonin in patients with influenza A (H1N1) pneumonia. J Infect [Internet]. 2010 Oct;61(4):355–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20670651

77. Shapiro MF, Greenfield S. The complete blood count and leukocyte differential count. An approach to their rational application. Ann Intern Med [Internet]. 1987 Jan;106(1):65–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3538968

78. OLSON LC, MILLER G, HANSHAW JB. ACUTE INFECTIOUS LYMPHOCYTOSIS PRESENTING AS A PERTUSSIS-LIKE ILLNESS: ITS ASSOCIATION WITH ADENOVIRUS TYPE 12. Lancet (London, England) [Internet]. 1964 Jan 25;1(7326):200–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14074503

79. Cassuto JP, Schneider M, Bourg M, Bertrand A, Mariani R. Acute infectious lymphocytosis as a T-cell lymphoproliferative syndrome. Br Med J [Internet]. 1977 Nov 19;2(6098):1331–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/303926

80. Don M, Valent F, Korppi M, Canciani M. Differentiation of bacterial and viral community-acquired pneumonia in children. Pediatr Int [Internet]. 2009 Feb;51(1):91–6. Available from: http://doi.wiley.com/10.1111/j.1442-200X.2008.02678.x

81. Morens DM. WBC Count and Differential. Am J Dis Child [Internet]. 1979 Jan 1;133(1):25. Available from: http://archpedi.jamanetwork.com/article.aspx?doi=10.1001/archpedi.1979.02130010031003

82. Celkan T, Koç BŞ. Approach to the patient with neutropenia in childhood. Turk Pediatr Ars [Internet]. 2015 Sep;50(3):136–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26568688

83. Segel GB, Halterman JS. Neutropenia in pediatric practice. Pediatr Rev [Internet]. 2008 Jan;29(1):12–23; quiz 24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18166617

84. Holzinger D, Jorns C, Stertz S, Boisson-Dupuis S, Thimme R, Weidmann M, et al. Induction of MxA gene expression by influenza A virus requires type I or type III interferon signaling. J Virol [Internet]. 2007 Jul;81(14):7776–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1749406
85. Swiecki M, Colonna M. Type I interferons: diversity of sources, production pathways and effects on immune responses. Curr Opin Virol [Internet]. 2011 Dec;1(6):463–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22440910

86. Kalliolias GD, Ivashkiv LB. Overview of the biology of type I interferons. Arthritis Res Ther [Internet]. 2010;12 Suppl 1:S1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20392288

87. Haller O, Kochs G. Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res [Internet]. 2011 Jan;31(1):79–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21166595

88. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature [Internet]. 2004 Dec 16;432(7019):917–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15531878

89. Huang H, Ideh RC, Gitau E, Thézénas ML, Jallow M, Ebruke B, et al. Discovery and validation of biomarkers to guide clinical management of pneumonia in African children. Clin Infect Dis [Internet]. 2014 Jun;58(12):1707–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24696240

90. Angus DC, Yang L, Kong L, Kellum JA, Delude RL, Tracey KJ, et al. Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis. Crit Care Med [Internet]. 2007 Apr;35(4):1061–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17334246

91. Shah SN, Bachur RG, Simel DL, Neuman MI. Does This Child Have Pneumonia?: The Rational Clinical Examination Systematic Review. JAMA [Internet]. 2017 Aug 1;318(5):462–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28763554

92. Bradley JS. Management of community-acquired pediatric pneumonia in an era of increasing antibiotic resistance and conjugate vaccines. Pediatr Infect Dis J [Internet]. 2002 Jun;21(6):592-8; discussion 613-4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12182396

93. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med [Internet]. 2009 Jul 21;6(7):e1000097. Available from: https://dx.plos.org/10.1371/journal.pmed.1000097