Childhood Pneumonia Diagnostics: A Narrative Review

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Abstract

Background: Childhood pneumonia remains the leading infectious cause of death in children with highest mortality figures in sub-Saharan Africa and Southeast Asia. The primary aetiologies are bacterial and viral; however, challenges in distinguishing bacterial and non-bacterial causes have culminated in antimicrobial overuse which has partly contributed to the rise in antimicrobial resistance, most notably among children in low- and middle-income countries.

Areas covered: Existing literature was reviewed regarding modalities available, including emerging radiological and laboratory techniques, to diagnose childhood pneumonia. We evaluated their strengths and limitations, and their ability to distinguish between bacterial and viral aetiologies.

Expert Opinion: The optimal modality to diagnose childhood pneumonia continues to be a challenge. This is a concern given its high disease burden and the importance of diagnostics for clinical care and antimicrobial stewardship, in the setting of rising antimicrobial resistance. Lung ultrasonography is a promising radiologic diagnostic modality. Combined serum biomarkers, micro-array-based whole genome expression arrays and metabolomic analysis are also emerging biochemical modalities for childhood pneumonia diagnosis. More research and further validation are required to evaluate the diagnostic strengths of these new and emerging modalities as well as their ability to discriminate between the major aetiologies of the disease.

Keywords: Bacterial, Childhood, Diagnosis, Pneumonia, Review, Viral

Article highlights:

1. The optimal modality to diagnose childhood pneumonia and the ideal test to discriminate between the major aetiologic groups remains a conundrum.
2. Radiological imaging like lung ultrasonography shows promise as a non-invasive sensitive bedside modality for the diagnosis of childhood pneumonia.
3. Several microbiological tests have high sensitivity for detecting potential aetiologic agent, but their ability to attribute causality is limited, particularly when examining upper respiratory tract samples like nasopharyngeal and oropharyngeal specimens.
4. The use of combined serum biomarkers, micro-array-based whole genome expression arrays and metabolomic analysis are emerging biochemical modalities for childhood pneumonia diagnosis.

Introduction

Pneumonia remains a major cause of morbidity and mortality in children [1], with about 100 million cases of the disease worldwide in 2017 [2]. It is also the single largest cause of mortality in children, causing approximately 15% of deaths among children less than 5 years of age in 2017 [1]. A child dies from the condition every 39 seconds [3]. The burden of the disease in children is particularly enormous in low- and middle-income countries (LMICs) in sub-Saharan Africa and southeast Asia [4]. Indeed, the global burden of childhood pneumonia is concentrated in 15 countries in these same regions of the world [5].

In clinical practice, the appropriate treatment for pneumonia is guided by clinical evaluation, assessment of disease severity, and rapid identification of the potential aetiologic agent [6]. While the gold standard for diagnosis of childhood pneumonia is chest x-ray, and lung aspirate for microbiological diagnosis, these can be a challenge in low resource settings. In most cases, the symptoms and physical signs, together with radiological evidence if available, have been the most utilised methods for diagnosing the disease [7]. However, over the past decade, new and emerging blood and radiological modalities have been studied to enhance prompt and accurate diagnosis [8].

This literature review aims to evaluate and provide a summary on the various modalities employed in current childhood pneumonia diagnosis based on clinical, laboratory, and radiological techniques, including a review of new and emerging approaches and assessment of their diagnostic strengths and limitations. In addition, the review also assesses the ability of these diagnostic modalities to discriminate between bacterial and

viral aetiology of disease given its implication on antimicrobial stewardship and prevention of antimicrobial resistance.

Clinical features

Children with pneumonia can present with fever, shortness of breath, cough, chest pain, and wheeze, however, these clinical features are nonspecific and vary with the age of the child [9,10]. In fact, a systematic review showed that no single clinical feature was sufficient to definitively diagnose pneumonia [11]. Although there was significant heterogeneity in the 18 studies reviewed, the features with the highest diagnostic accuracy included a respiratory rate greater than 50 breaths per minute (positive likelihood ratio of 1·90, 95% CI 1·45–2·48), grunting (1·78, 95% CI 1·10–2·88), lower chest wall indrawing (1·76, 95% CI 0·86–3·58), and nasal flaring (1·75, 95% CI 1·20–2·56). However, most of the surveys reported the symptoms and signs of the disease as being insensitive and nonspecific with cough and fever having low negative likelihood ratios of 0.30 (95% CI 0.09-0.96) and 0.53 (95% CI 0.41 -0.69) respectively [11]. Some reports have observed that the presence of crackles on lung auscultation increases the likelihood of radiographic pneumonia [12,13], but a review of 23 prospective cohort studies revealed that temperature >37.5°C and respiratory rate greater than 40 breaths per minute were not strongly associated with a childhood pneumonia diagnosis [14]. In addition, clinical symptoms and signs of pneumonia do not reliably discriminate between bacterial and viral childhood pneumonia [15,16].

Pulse oximetry during clinical examination has potential usefulness in diagnosing childhood pneumonia. A review of 147 children in three rural hospitals in Rwanda revealed that low oxygen saturations below 90% was a better clinical predictor of

radiologically diagnosed pneumonia when compared to increased respiratory rates (AUC of 0.67 versus 0.53) [17].

Based on these reports, the presence of respiratory symptoms and signs have limited diagnostic value in detecting childhood pneumonia, and do not differentiate aetiology.

Serum studies

C- reactive protein (CRP)

CRP is an acute-phase plasma protein synthesised by hepatocytes and adipocytes in response to inflammatory cytokines and is an indicator of acute inflammation (Table 1) [18]. A rise in serum levels of this protein is commonly associated with bacterial infections and non-infectious inflammatory conditions [18].

This biomarker which first identified in patients diagnosed with pneumonia in the 1930s and has yielded different results when evaluated as a potential biomarker to differentiate between bacterial and viral infections [18]. Available data regarding the usefulness of CRP are contradictory and frequently difficult to interpret. A review of the usefulness of CRP as a biomarker of childhood pneumonia revealed that it does not provide significant diagnostic value in many studies, whereas, in others, it has been found to be an extremely useful tool to aid diagnosis and potentially predict the aetiology of the disease [19]. Although the mean CRP values of bacterial community acquired pneumonia (CAP) are generally higher than those of viral cases, a significant overlap has been found to occur which reduces its diagnostic strength [19]. Furthermore, there are different threshold

values for CRP in the identification of bacterial CAP, and in some cases, the thresholds are so high that they could only be quantified in a very small number of patients [19].

A study in Finland evaluated the usefulness of CRP as a potential biomarker in discriminating between bacterial and viral childhood pneumonia in 215 children with microbiologically- confirmed disease. A significant association was identified between CRP levels above 80mg/L and bacterial aetiology of childhood pneumonia (52%). Notably, CRP levels >80 mg/L were still found in 28% of children with viral disease (p=0.001) [20]. The CRP specificity was 72% with a lower sensitivity of 52%, indicating suboptimal levels for clinical use [20].

A meta-analysis of 8 studies that had significant heterogeneity and involved 1,230 children in the United States of America revealed that CRP levels >40–60 mg/L was a weak predictor of bacterial childhood pneumonia [21]. They also reported that with a pooled odds ratio of 2.58, the positive predictive value of CRP levels >40–60 mg/L in predicting bacterial aetiology was only 64%; further indicating its limited ability to predict childhood pneumonia of bacterial origin [21]. Furthermore, a more recent study in the United Kingdom found that in comparison to viral childhood pneumonia, bacterial infections had a higher median CRP level (165.5mg/L vs 40mg/L; p<0.001) [22]. Children with bacterial pneumonia were associated with higher CRP levels (>80 mg/L) when compared to viral infections (p=0.001), but levels <20 mg/L were not found to be discriminatory (p=0.254) [22].

Overall, there is a dearth of studies conducted in LMICs on the usefulness of CRP in the diagnosis of childhood pneumonia and its potential use in discriminating bacterial and non-bacterial aetiologies of the disease.

Procalcitonin (PCT)

PCT is a 116- amino acid peptide precursor of the hormone calcitonin produced by the C-cells in the thyroid gland as well as the neuroendocrine cells in the lung and intestine [23]. Usually, serum PCT concentrations are low or undetectable (<0.1 ng/mL) in healthy individuals but are usually markedly raised in patients with confirmed bacterial infection including those with pneumonia [23,24], compared to those with viral infections. However, PCT levels are also raised in severe trauma, burns, severe renal insufficiency as well as other bacterial infections like urinary tract infections [23].

The usefulness of the biomarker in discriminating between bacterial and viral childhood pneumonia has been evaluated in previous studies with conflicting reports. An initial study by Korppi et al identified no difference between pneumococcal, atypical bacterial pathogens (*Mycoplasma* and *Chlamydophila*), viral and infections of unknown etiology [25]. Another study by the same authors evaluating 101 children with radiologically confirmed pneumonia observed that PCT levels above 1ng/mL in those with WHO-defined mild to moderate disease was a reliable marker for CAP [16]; a finding which was in tandem with earlier research involving 126 children in Finland which found that PCT levels above 2ng/mL could correctly predict bacterial childhood pneumonia [26].

In comparison to CRP, PCT has been identified as a better predictor of bacterial CAP [27, 28] but findings are not consistent across studies [16, 25]. A more recent report in Italy demonstrated a marginal superiority of PCT in comparison to CRP in a study involving 433 otherwise healthy children with CAP [29]. It concluded that both biomarkers were suboptimal predictors of childhood pneumonia with the area under the curve (AUC) of

PCT and CRP being 0.69 (95% CI 0.63–0.75) and 0.66 (95% CI 0.61–0.71), respectively [29].

Lipocalin 2 (LIP -2)

LIP-2 is a protein found in human neutrophils granules. It is an acute-phase protein that rises in response to infection and is also a component of the innate immune system [30-32]. The biomarker also contributes to innate defence by interfering with bacterial iron uptake [32]. LIP-2 expression is significantly increased in bronchial epithelial cells and alveolar type 2 pneumocytes of animals following exposure to bacterial pathogens [30].

Serum LIP-2 was found to have a sensitivity of 77% (95% CI, 65.6%–89.9%) and higher specificity of 94.4% (95% CI, 86.8%–100%) for identifying children with probable bacterial pneumonia in a study in the Gambia evaluating 390 children with pneumonia who had malaria, acquired immunodeficiency infection and malnutrition [33]. The researchers concluded that serum LIP-2 had a superior diagnostic ability for bacterial pneumonia when compared with CRP and von Willebrand factor demonstrating a sensitivity of 77% (95% CI 0.65 -0.89) and specificity of 94.4% (95% CI 0.86 -1.00) [33].

Syndecan 4 (SYN4)

SYN4 is a heparin sulphate proteoglycan found on the surfaces of many cells, including epithelial cells, endothelial cells, macrophages, and fibroblasts [34]. The protein binds to and mediates the biological activity of several cytokines, chemokines, and growth factors.

SYN4 expression increases rapidly in response to bacterial infection but not in response to viral infection [34]. Although, significantly higher mean levels of SYN-4 were found in 30 adult patients with bacterial pneumonia when compared to 11 healthy volunteers (20.5ng/ml vs.15.1ng/ml; p=0.006) [35], no studies have yet been conducted in the paediatric population.

Myxoma Resistance Protein A (MxA)

The levels of MxA, a 662 amino acid peptide have been observed to be raised in viral infections: playing an important antiviral role against a wide variety of viruses such as influenza, parainfluenza, and measles virus [36]. This potential biomarker had an AUC of 0.89 with a 96.4% sensitivity and a lower specificity of 66.7% for differentiating bacterial and viral pneumonia in less than 16 years old children at a threshold of 200ng/ml [36]. The AUC for distinguishing 44 uninfected individuals from 77 virus-infected patients was 0.98, with a sensitivity of 96.4% and specificity of 85.4% [36]. However, additional studies are required before this marker can be validated and routinely used.

Soluble triggering receptor expressed on myeloid cells-1 (sTREM -1)

sTREM-1 is a transmembrane glycoprotein expressed on neutrophils, macrophages, and monocytes with increased levels in bacterial infections [37]. A multicentre study by Esposito et al in Italy found that this biomarker had very limited value in discriminating between bacterial and viral childhood pneumonia with an AUC of 0.50 and sensitivity of 31.8% and specificity of 73.7% in detecting cases of bacterial childhood pneumonia [29].

Mid regional pro-atrial natriuretic peptide (MR-proANP), and mid regional pro-adrenomedullin (MR-proADM)

MR-proANP and MR-proADM are peptides with primary biological effects on the cardiovascular system but their usefulness has also been evaluated in the setting of systemic infections given that levels increase in response to systemic inflammation and metabolic changes associated with critical illnesses [38][Yagmur, E., Sckaer, J.H., Koek, G.H. *et al.* Elevated MR-proANP plasma concentrations are associated with sepsis and predict mortality in critically ill patients. *J Transl Med* **17,** 415 (2019)]. The predictive value of these biomarkers has been observed to be limited in distinguishing between bacterial and viral childhood pneumonia in a multicentre study involving 433 children with radiologically confirmed pneumonia in Italy [29]. MR-proANP and MR-proADM had AUCs of 0.52 and 0.58 respectively with accompanying sensitivities of 76.1% and 78% [29].

High mobility group box one (HMGB1) protein

HMGB1 protein is a DNA-binding protein that promotes the transcription of several inflammatory markers [39]. The protein, which has been evaluated to be high in bacterial as well as bacterial-viral co-infections, has some extracellular functions, such as promoting migration and increasing the production of pro-inflammatory markers and cytokines like interleukin 6 (IL-6) and tumour necrosis factor (TNF) [39].

A study involving 78 patients and 34 healthy controls found that co-infection with viruses and bacteria can be concluded when HMGB1 protein expression is greater than 1.03 [40].

Furthermore, in the same study, HMGB1 protein expression of less than 1.03 and a WBC count of greater than 13×10^9/L had a positive predictive value of 92.3% for single bacterial pneumonia [40]. However, the need to isolate specific blood cells (monocytes) and the use of PCR potentially makes this method time-consuming and costly, particularly in LMICs [19].

Chitinase-3-like protein 1 (YKL-40)

YKL-40 is a 40 kilodalton glycoprotein secreted by macrophages, neutrophils, fibroblast-like synovial cells, chondrocytes, and vascular smooth muscles [41]. Although the biological function of YKL-40 remains unclear, its pattern of expression is related to tissue inflammation and extracellular tissue remodeling, with a rise in serum levels in response to inflammation in virtually all organs of the body [41].

Interestingly, this biomarker has also shown some promise in terms of potentially distinguishing between viral and bacterial childhood pneumonia. A prospective cohort study of 73 children in China revealed that YKL-40 levels in the bronchoalveolar lavage fluid specimens compared with serum levels of patients with bacterial pneumonia were significantly higher than in children with viral pneumonia (34.87 ± 5.42 vs. $26.45 \pm 3.65 \text{ng/ml}; p=0.02$) 42].

Combination of biomarkers

Attempts have also been made by previous researchers to evaluate a combination of biomarkers in discriminating between bacterial and viral childhood pneumonia with varying reports. A double-blind multicentre study involving 577 preschool children aged 2-60 months with childhood pneumonia or an unexplained febrile illness (71 identified as bacterial infection, 435 viral and 71 inconclusive) in Israel and the Netherlands tested the assay, 'ImmunoXpert' which had a combination of CRP, TNF-related apoptosis-inducing ligand (TRAIL), and plasma interferon-γ protein-10 (IP-10) in distinguishing between bacterial and viral infection [43]. The combination assay was able to discriminate bacterial from viral pneumonia at a sensitivity of 86.7% and higher specificity of 91.1% [43].

Another study revealed 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 the diagnosis of bacterial childhood pneumonia [44]. A combination of age, CRP, and WCC together with neutrophils count had a 91.4% positive predictive value and 71.2% negative predictive value for bacterial childhood pneumonia in a study involving 401 children in the United Kingdom discriminating between bacterial and viral aetiologies [22]. A study evaluating the usefulness of a combination of biomarkers (CRP with lipopolysaccharide binding protein (LBP), PCT, IL-6, IL-18, or sTREM-1) found that the combined assay did not improve discrimination between patients with bacterial or viral childhood pneumonia, when compared with CRP alone [45].

Paired Serology

Rising titers in antibody complement fixation tests remain very useful diagnostic methods for identifying atypical bacterial pathogens like *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae, and Legionella pneumophilia* [9,46]. The serologic diagnosis of acute infection is made by detection of antibody conversion or a four-fold increase in immunoglobulin G levels in two consecutive serum samples collected a fortnight apart [47]. The length of time required to make this serologic diagnosis precludes clinicians from making management decisions in real time [47,48]. In addition, immunoglobulin M assays may take several days before antibody levels are detectable for a diagnosis to be made. These constraints have resulted in the use of alternative methods like PCR in well-resourced settings [47].

Newer innovations

Newer innovations, such as micro-array-based whole genome expression arrays and proteomics, are being investigated as potential biomarkers to determine the aetiology of pneumonia [19]. This is based on the fact that bacteria induce specific host responses that can be identified using blood leukocyte microarray analyses. The accuracy of RNA biosignatures in febrile infants within the age range of 0-60 days has been assessed, with 66 classifier genes identified to have a sensitivity of 94% and a specificity of 95% in differentiating children with bacteremia from those without bacterial infections [49].

In addition, metabolomics involves a comprehensive analysis of metabolites and biomarker discovery in body fluids, and has been explored in diagnosing diseases and monitoring therapeutic interventions [50,51]. The presence of metabolites such as L-histidine, L-tryptophan, and glutamic acid can indicate host response to an ongoing infection in the body [50]. A study of metabolites in urine samples (metabolomic studies) was carried out among 11 Gambian children and the authors reported that metabolites such as uric acid, hypoxanthine, and glutamic acid were higher in the plasma of children with pneumonia when compared to a control group [50]. Although there is relatively quick processing of samples; this technique is limited by the expertise required to analyse the complex data produced [52].

Radiology

Plain chest radiograph

Plain chest radiograph, commonly termed chest x-ray (CXR), is extremely important in pneumonia diagnosis as it not only provides structural evidence of disease but also reflects the extent of disease based on the number of lung lobes involved [48]. It is also very useful in the detection of complications like pleural effusions or lung abscesses [48]. In clinical practice, alveolar infiltration is commonly attributed to a bacterial cause, while bilateral diffuse interstitial infiltrates are deemed to occur due to atypical bacterial or viral infections. However, these approaches at not adequately sensitive [9].

CXRs are generally not advocated in children with pneumonia who are well enough to be treated as outpatients. This is based on the recommended guidelines of various national and international organisations like the Pediatrics Infectious Disease Society, British Thoracic Society, and the Infectious Disease Society of America [9,53]. However, they are generally required in cases of severe disease requiring hospitalisation, hypoxic children, or those suspected to have complications such as pleural effusions or lung abscesses [54].

Abnormal CXRs in the Pneumonia Etiology Research in Child Health (PERCH) project were reported to be significantly associated with high respiratory rates, as well as the presence of hypoxaemia and crackles on lung auscultation [13]. In addition, the presence of lung consolidations was associated with a higher 30 – day case fatality when compared to normal chest radiograph findings [13].

However, it is important to emphasise that the use of CXRs in pneumonia diagnostics has its drawbacks. This is because findings may lag behind clinical symptoms and signs, radiological findings can be masked by anatomical structures such as the heart and other mediastinal structures as well as the presence of inter-reader variability during interpretation or reporting [54,55]. Also, there is also a risk of tissue damage from exposure to low dose ionizing radiation from standardised chest radiographs particularly in children who have more rapidly dividing cells [56]. In addition, many primary healthcare

facilities in LMICs still lack access to basic plain chest radiograph facilities with an associated paucity of trained staff and irregular electricity supply [54].

Courtoy et al in 1989 evaluated 36 chest films from patients with pneumonia who had a laboratory-proven etiologic diagnosis. The sensitivity of plain chest radiographs in diagnosing bacterial pneumonia ranged from 42-58% [57]. When clinical and laboratory data were provided, the sensitivity range widened to 42-92%. The study concluded that plain chest radiograph was insensitive in distinguishing patients with bacterial and non-bacterial pneumonia [57]. Toikka et al reviewed 126 patients with childhood pneumonia all of whom had plain chest radiographs. It was concluded that changes on CXRs failed to discriminate between confirmed bacterial and viral pneumonia [26]. Another study by Virkki et al revealed that in children with alveolar infiltrates on chest X-ray, the sensitivity for bacterial infection was 72%, and the specificity was 51% [20]. The sensitivity and specificity for viral pneumonia with interstitial infiltrates were 49% and 72%, respectively [20]. Other surveys have also shown that plain chest radiographs are not accurate in discriminating between bacterial and viral childhood pneumonia [58,59].

Lung Ultrasound (LUS)

An attractive alternative approach for pneumonia diagnosis is LUS, which is well suited for use in resource-limited settings. When compared to plain chest radiography, LUS can be performed at the patient's bedside, is less affected by crying or movements, and does not require expensive radiation-proof facilities [54, 60,61]. In addition, portable, battery-

powered devices suitable for use in community health facilities in LMICs with intermittent electrical power supplies are becoming more affordable [62]. With skilled operators, thoracic ultrasound has comparable specificity (94.0 vs. 90.4%) and superior sensitivity (81.4% vs.64.3%) to chest radiography to detect lung consolidations [62].

The sonographic signs of pneumonia include the presence of hyperechoic spots of variable size (air bronchograms) with a subpleural hypoechoic region, confluent B-lines, superficial fluid alveologram, a vascular tree-shaped pattern, and irregular borders of the pleural line [63,64]. Anechoic or hypoechoic fluid in the pleural space may also indicate a pleural effusion with a significant degree of accuracy [64].

Some previous studies have demonstrated considerable accuracy using LUS with superior sensitivity (94 - 96.4%) and specificity (95.6% -96%) when compared to plain chest radiographs in diagnosing childhood pneumonia [65,66]. LUS has been demonstrated to have a pooled sensitivity and specificity of 96% and 93% respectively in diagnosing childhood pneumonia when compared to plain chest radiograph alone or chest radiograph in combination with clinical and laboratory parameters [67].

LUS is easily repeatable in the context of monitoring of disease and can be performed by non-radiology clinicians who have had focused training. However, the fact that it is not widely available, the whole of the lungs cannot be visualised at once and the suboptimal technical know-how regarding its use in poor resource settings serve as major drawbacks

for its use [48,68]. Also, despite recognised sonographic signs of pneumonia, there is a need to develop a standardised interpretation method, similar to the WHO standard, to ensure consistent case definitions in studies evaluating the role of lung ultrasound in the diagnosis and management of childhood pneumonia [68].

Computed tomography (CT)

CT of the chest is not recommended as a first-line diagnostic tool for pneumonia. However, in facilities where it is readily available, it can be considered for detecting complications of pneumonia in the acute or subacute phase (for diagnosing a suppurative complication such as necrotising pneumonia, abscess, or empyema) and in the chronic phase (for diagnosing bronchopleural fistula or detecting and localising bronchiectasis) [48].

Magnetic Resonance Imaging (MRI)

Rapid sequence MRI of the lung can be considered when cross-sectional imaging is required for severe or complicated pneumonia in the context of reducing radiation risks from chest radiographs or computed tomography scans. However, the imaging of the lung by MRI is limited by low proton density in the organ as well as the fact that motion artefacts are produced by breathing movements [69,70]. It is also always a big challenge to get younger children to cooperate and stay still in the MRI scanner; hence bringing forth the need for sedation or anaesthesia [69].

Pneumonic changes appear as high-intensity signals that stand out against the low signal of the normally aerated lung [69]. Some studies have demonstrated good diagnostic performance when comparing the ability to detect pneumonic changes on MRI when compared to CT scan with sensitivities and specificities of over 90% [71,72].

Microbiology

Blood Cultures

Approximately 5-10% of blood cultures of suspected bacterial pneumonia cases are positive with a higher yield in children with severe pneumonia disease [73,74] and those living with HIV/AIDS [75]. The merit of using blood culture techniques is that it not only provides a cause but also enables antibiotic resistance testing and, in the case of pneumococcus and Hib, for serotyping, which is crucial for vaccination programmes [76]. It has also been observed that when blood samples of more than 4 mL are utilized for blood culture, there is a greater possibility of bacterial yield [77].

The major drawback of using blood culture as a tool to determine bacterial aetiology for childhood pneumonia is that results are available only 24 to 72 hours after presentation. In addition, its established low yield is further reduced by pre-treatment with antibiotics which is common practice prior to health facility presentation in LMICs [6,76].

Viral PCR

The advent of viral polymerase chain reaction techniques (PCR) on nasal or nasopharyngeal swabs has provided more information in the last two decades regarding the significant role of viral pathogens in the aetiology of childhood pneumonia globally; indicating their contribution beyond causing bronchiolitis or reactive airway disease [78,79]. The most commonly identified viruses include influenza virus, respiratory syncytial virus, adenovirus, parainfluenza virus, and human metapneumovirus [80]. In addition to detecting these known viral pathogens, PCR techniques have permitted increased identification of other viruses such as rhinovirus, bocavirus, and the HKU1 virus [81].

It is however very important to note that the isolation of adenovirus, human metapneumovirus or rhinovirus, even though associated with pneumonia, should be interpreted with caution, as healthy children, or those with upper respiratory tract infection (URTI) may also have a positive test result [82], and therefore detection may reflect carriage and not disease. A study by Cevey-Macherel et al found viral PCR of nasopharyngeal aspirates to be quite sensitive as two-thirds of the children evaluated had a viral isolate [73]. PCR has also been shown to be more sensitive than virus isolation in cell culture, viral antigen detection, and immunofluorescence testing, and it is now regarded as a standard technique for detecting respiratory viruses [83].

Multiplex PCR approaches is associated with increased diagnostic yield, can detect numerous pathogens simultaneously and the results are potentially available within a few hours [83,84]. A major drawback for its use in developing settings remains the high cost of the procedure and the fact that nonpathogenic colonisers may also be detected [84].

Sputum analysis

Expectorated or induced sputum samples in children can be utilised for bacterial culture as well as antigen or molecular detection of bacterial pathogens. It may however detect organisms that constitute normal flora colonizing the respiratory tract [48]. Murdoch et al [85] evaluated the diagnostic usefulness of induced sputum microscopy and culture in patients enrolled in the PERCH study, a large multinational study of severe and very severe community-acquired pneumonia in children aged 1–59 months. They reported that induced sputum microscopy and culture results were not associated with radiographic pneumonia, regardless of prior antibiotic use, stratification by specific bacteria, or the interpretative criteria used [85].

Urinary antigen detection

The most used urinary antigen tests which have been validated for use in pneumonia diagnostics are those for detection of *S. pneumoniae* and *Legionella pneumophilia* [86]. Although serotype-specific pneumococcal antigens in urine samples of adults have shown some diagnostic promise with a sensitivity of 70–97%, the immunochromatographic method has not demonstrated good diagnostic accuracy in

children due to their high pneumococcal carriage rate [9,87]. However, in detecting childhood pneumococcal pneumonia when compared with blood culture from previous studies [88,89], the sensitivity and specificity of the pneumococcal urine antigen test were 96–100% and 62–92%, respectively. Concomitant with a decrease in nasopharyngeal carriage of pneumococcus as children get older, so too does the negative predictive value of urinary antigen detection increase with age [9].

Pleural fluid analysis

Pleural fluid can be assessed for microscopy, culture, pneumococcal antigen by latex agglutination [48]. Bacterial growth in pleural fluid cultures is poor, with only 9% in a UK study evaluating 47 cultures in a UK study giving a bacterial yield. This can be explained by the fact that many children will have had antibiotics before pleural fluid aspiration [90]. However, when PCR was performed, 32 of the 47 cultures tested positive for pneumococcal DNA, while 12 tested positive for pneumococcal latex agglutination antigen. This demonstrated the superiority of PCR techniques on pleural fluid samples when compared to conventional bacterial culture techniques.

Additionally, in a study of 29 empyema samples, pneumococcal antigen detection gave a 90% positive yield with about 70% being serotype 1 [91]. This highlights the clinical usefulness of pneumococcal antigen detection in the diagnosis of pneumococcal childhood particularly as culture positive results from pleural fluid remain uncommon [91].

Lung aspirate fluid (LAF) culture

For several years, the isolation of a pathogen organism from lung aspirates served as the ideal diagnostic method for determining the aetiologic agent for childhood pneumonia. This was because the lungs were deemed to be a sterile organ and the identification of any organism was termed to be pathogenic [92]. However, it is now clear that the lung has a dynamic microbiome that can be affected by several factors [92]. A review of lung aspirates in childhood pneumonia before the year 2000 revealed that the procedure was still quite common up till the mid-nineties in LMICs. However, the use of the modality seemed to have reduced significantly by the 1970s in developed nations due to advancements in less invasive and more sophisticated diagnostic options [93].

A recent study of 95 children with radiologic evidence of pneumonia in Malawi revealed that while LAF culture yielded a bacterial pathogen in only 2 cases, LAF PCR detected bacteria in 36 cases with viruses also isolated singly or in combination in 24 cases [94].

Bronchoalveolar lavage (BAL) cultures

In settings where noninvasive samples from the lower respiratory tract are unavailable, flexible bronchoscopy with BAL is a viable alternative to obtaining sputum [95]. It involves introducing a measured amount of sterile fluid (up to 100millilitres) through the bronchoscope into the lower respiratory tract. The introduced fluid is then suctioned back into a sterile container and sent for analysis [95]. The procedure is safe even in very unwell children and can be utilised in improving the diagnostic yield of pathogens in

patients who are not responding to treatment [96]. A study in Taiwan of 90 children who were not responding to empirical antibiotic therapy for pneumonia revealed that in 55% of children with positive aerobic cultures, BAL results improved diagnostic yield and prompted a shift in antimicrobials, resulting in a high rate of effective therapy [95].

Although less likely to be contaminated by upper airway bacterial flora and more representative of the lower respiratory tract, BAL is invasive, expensive, and requires expertise to carry out [48].

Haematology

White blood cells (WBC) and neutrophil count

Total WBC and neutrophil counts have been evaluated in the past as potential discriminatory modalities in differentiating bacterial and viral pneumonia. However, it must be considered that the normal reference range for WBC count in children varies in the paediatric population based on age though levels more than 11x 10^9/L are generally considered abnormal [97]. A study in the United Kingdom found that total WBC and the neutrophil counts did not accurately discriminate between bacterial and viral childhood pneumonia [22]. They reported that a significant number of patients with viral disease had WBC counts > 15x 10^9/L which is normally expected in bacterial disease. In addition, 4 out of every 5 patients with viral pneumonia had neutrophils less than 10 × 10^9/L [22].

In addition, Zhu et al reported that the percentage of neutrophils was slightly more accurate than WBC at distinguishing between bacterial and viral childhood pneumonia [98]. This was linked to the fact that a reduction in neutrophil count of less than 1.5×10^9/L (passing neutropenia) tends to occur between the third and eighth days of viral infections; especially with respiratory syncytial and influenza viruses [99,100].

Conclusion

Identifying the optimal modality to diagnose childhood pneumonia continues to be a challenge. The use of combined serum biomarkers, micro-array-based whole genome expression arrays and metabolomic analysis are emerging biochemical modalities for childhood pneumonia diagnosis. Radiologic diagnostic modalities such as lung ultrasonography hold promise, despite concerns regarding its general availability, expertise to perform at the bedside and its impact on clinical outcomes. Despite advances in radiological techniques and laboratory testing, there is a need for improved methods for diagnosing and identifying the aetiological pathogen, for which current tests still do not discriminate well. There is a need for more research and further validation to evaluate the diagnostic strengths and accuracy of these new and emerging modalities. Particularly in LMICs, diagnostics that can distinguish between bacterial and viral pneumonia are increasingly important given the impact of antimicrobial prescribing on the emergence of antimicrobial resistance.

Expert Opinion:

Pneumonia remains a significant cause of morbidity and mortality globally with enormous impact in LMICs. The effective management of the disease hinges on prompt and accurate diagnosis and identification of the aetiological agent. However, despite several radiological and laboratory modalities, the search for an optimal test remains a challenge.

Although the presence of opacities or infiltrates on plain chest radiographs have been commonly adopted for diagnosis of the disease for several years, the use of lung ultrasonography appears to be a promising noninvasive bedside radiologic modality for diagnosis with demonstrated sensitivities and specificities of over 90%. Also, lung MRI appears to be equally sensitive especially in complicated disease. However, the complexities involved, and the cost effectiveness of the test poses a challenge for its utilisation.

Furthermore, several microbiological tests including the use of PCR have high sensitivity for detecting potential aetiologic agent, but their ability to attribute causality is limited, particularly when examining upper respiratory tract samples like nasopharyngeal and oropharyngeal specimen.

In terms of biochemical tests, the use of combined serum biomarkers, micro-array-based whole genome expression arrays and metabolomic analysis appear to be useful emerging tests for childhood pneumonia diagnosis. However, further research and validation are needed to assess their diagnostic capabilities and accuracy of these new and developing modalities, as well as their capacity to distinguish between bacterial and viral aetiologies.

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References

1. World Health Organization (WHO). Fact Sheet on Pneumonia. Available at https://www.who.int/news-room/fact-sheets/detail/pneumonia.
2. Global Burden of Disease (GBD). 2017 Lower Respiratory Infections Collaborators. Quantifying risks and interventions that have affected the burden of lower respiratory infections among children younger than 5 years: An analysis for the global burden of disease study 2017. Lancet Infect Dis. 2020; 20:60-79.
3. United Nations Childrens Fund (UNICEF). Childhood pneumonia statistics: 2019. Available at https://data.unicef.org/topic/child-health/pneumonia/.

4. Marangu D, Zar HJ. Childhood pneumonia in Sub-Saharan Africa: Still a challenge. *JPATS, 2021; 2(1): 1-3.*

5. World Health Organization. (WHO). WHO-MCEE Estimates for child causes of death 2000-2017. Available at https://www.who.int/healthinfo/global_burden_disease/childcod_methods_2000_2017.pdf.

6. Izadnegahdar R, Cohen AL, Klugman KP, Qazi SA. Childhood pneumonia in developing countries. *Lancet Respir Med, 2013; 1(7):574-584.*

7. Lynch T, Bialy L, Kellner JD, Osmond MH, Klassen TP, Durec T, et al. A systematic review on the diagnosis of paediatric bacterial pneumonia: when gold is bronze. *PLoS One. 2010 6;5(8): e11989. doi: 10.1371/journal.pone.0011989.*

8. Thomas J, Pociute A, Kevalas R, Malinauskas M, Jankauskaite, L. Blood biomarkers differentiating viral versus bacterial pneumonia aetiology: a literature review. Ital J Pediatr, 2020; 46. *Available on https://ijponline.biomedcentral.com/articles/10.1186/s13052-020-0770-3.*

9. Harris M, Clark J, Coote, N, Fletcher P, Harnden A, McKean M, et al. British Thoracic Society Standards of Care Committee, et al. British Thoracic Society guidelines for the management of community acquired pneumonia in children. *Thorax 2011;66(Suppl 2): ii1-ii23.*

10. Iroh Tam PY. Approach to common bacterial infections: community-acquired pneumonia. *Pediatr Clin North Am. 2013, 60(2):437-453.*

11. Rambaud-Althaus C, Althaus F, Genton B, D'Acremont V. Clinical features for diagnosis of pneumonia in children younger than 5 years: a systematic review and meta-analysis. *Lancet Infect Dis. 2015; 15: 439-450.*

12. Margolis P, Gadomski A. Does this infant have pneumonia? *JAMA. 1998; 279: 308-313.*

13. Fancourt N, Deloria Knoll M, Baggett HC, Brooks WA, Feikin DR, Hammitt LL, et al. Chest Radiograph Findings in Childhood Pneumonia Cases from the Multisite PERCH Study. *Clin Infect Dis. 2017, 15, 64 (suppl_3): S262-S270.*

14. Shah SN, Bachur RG, Simel DL, Neuman MI. Childhood Pneumonia. *JAMA. 2017, 1;318(5):490*

15. March M, Sant'Anna CC. Signs and symptoms indicative of community-acquired pneumonia in infants under six months. *Braz J Infect Dis. 2005; 9:150–155.*

16. Korppi M, Don M, Valent F, Canciani M. The value of clinical features in differentiating between viral, pneumococcal and atypical bacterial pneumonia in children. *Acta Paediatrica, 2008;* 97*:943–947.*

17. Modi P, Munyaneza RB, Goldberg E, Choy G, Shailam R, Sagar P, et al. Oxygen saturation can predict paediatric pneumonia in a resource-limited setting. *J Emerg Med, 2013; 45(5): 752-760.*

18. Pepys MB, Hirschfield G.M. C-reactive protein: a critical update. *J Clin Invest, 2003;* 111: *1805–1812.*

19. Principi N, Esposito S. Biomarkers in Paediatric Community-Acquired Pneumonia. *Int J Mol Sci. 2017; 18(2):447.*

20. Virkki R, Juven T, Rikalainen H, Svedström E, Mertsola J, Ruuskanen O. Differentiation of bacterial and viral pneumonia in children. *Thorax. 2002;* 57*: 438–441.*

21. 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. 2008;27(2):95-99.*

22. 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. 2015 ;79(4): 458–462.*

23. Reinhart K, Karzai W, Meisner M. Procalcitonin as a marker of the systemic inflammatory response to infection. *Intensive Care Med. 2000; 26:1193-1200*.

24. Becker KL, Nylén ES, White JC, Müller B, Snider R.H Jr. Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. *J Clin Endocrinol Metab. 2004; 89 (4): 1512-1525.*

25. Korppi M, Remes S. Serum procalcitonin in pneumococcal pneumonia in children. *Eur Respir J. 2001;* 17:623*–627*.

26. Toikka P, Irjala K, JuvÉn T, Virkki R, Mertsola J, Leinonen M, et al. Serum procalcitonin, C-reactive protein and interleukin-6 for distinguishing bacterial and viral pneumonia in children. *Pediatr Infect Dis J. 2000;* 19*: 598–602.*

27. Hatzistilianou M, Hitoglou S, Gougoustamou D, Rekliti A, Tzouvelekis G, Nanas C, et al. Serum procalcitonin, adenosine deaminase and its isoenzymes in the

aetiological diagnosis of pneumonia in children. *Int J Immunopathol Pharmacol. 2002; 15(2):119-127.*

28. Prat C, Domínguez J, Rodrigo C, Giménez M, Azuara M, Jiménez O, et al. Procalcitonin, C-reactive protein and leukocyte count in children with lower respiratory tract infection. *Pediatr Infect Dis J. 2003; 22(11): 963-968.*

29. 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 pro-atrial natriuretic peptide and midregional pro-adrenomedullin for distinguishing etiology and to assess severity in community-acquired pneumonia. *PLoS ONE. 2016, 11: e0163262.*

30. Borregaard N, Sorensen OE, Theilgaard-Monch K. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol. 2007;28(8):340–345*

31. Lim WS. Pneumonia—Overview. Reference Module in Biomedical Sciences, B978-0-12-801238-3.11636-8. Available at https://doi.org/10.1016/B978-0-12-801238-3.11636-8.

32. Abella V, Scotece M, Conde J, Gomes R, Lois A, Pino J, et al. The potential of lipocalin-2/NGAL as biomarker for inflammatory and metabolic diseases. *Biomarkers*. 2015;20(8):565-571.

33. 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. 2014, 58: 1707–1715*.

34. Esko JD, Lindahl U. Molecular diversity of heparan sulfate. *J Clin Invest. 2001;108(2):169-173.*

35. Nikaido T, Tanino Y, Wang X, Sato S, Misa K, Fukuhara N, Sato Y, Fukuhara A, Uematsu M, Suzuki Y, Kojima T, Tanino M, Endo Y, Tsuchiya K, Kawamura I, Frevert CW, Munakata M. Serum syndecan-4 as a possible biomarker in patients with acute pneumonia. *J Infect Dis. 2015; 212:1500–1508.*

36. Engelmann I, Dubos F, Lobert PE, Houssin C, Degas V, Sardet A, et al. Diagnosis of viral infections using myxovirus resistance protein A (MxA). *Paediatrics. 2015; 135, e985–e993.*

37. Siranović M, Kovac J, Gopcević S, Kelecić M, Kovac N, Rode B, et al. Human soluble TREM-1: lung and serum levels in patients with bacterial ventilator associated pneumonia. *Acta Clin Croat. 2011; 50:345–9.*

38. Kitamura K, Kangawa K, Eto T. Adrenomedullin and PAMP: Discovery, structures, and cardiovascular functions. *Microsc Res Tech.2002, 57: 3–13*.

39. 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. 2007; 35 (4):.1061–1067.*

40. 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. 2011; 55(4): 279-288.*

41. Zhao T, Su Z, Li Y, Zhang X, You, Q. Chitinase-3 like-protein-1 function and its role in diseases. *Signal transduct target ther.*2020; *5*(1): 201.

42. Yang X, Sheng G. YKL-40 levels are associated with disease severity and prognosis of viral pneumonia, but not available in bacterial pneumonia in children. *BMC Pediatrics. 2018, 18(1):381*

43. Van Houten CB, de Groot JA, Klein A, Srugo I, Chistyakov I, de Waal W, et al. A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): A double-blind, multicentre, validation study. *Lancet Infect Dis.2017;17(4):431-440.*

44. 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. 2016;193(4): 448–459.*

45. ten Oever J, Tromp M, Bleeker-Rovers CP, Joosten LA, Netea MG, Pickkers P, van de Veerdonk FL. Combination of biomarkers for the discrimination between bacterial and viral lower respiratory tract infections. *J Infect. 2012 Dec;65(6):490-5.*

46. Herrera M, Aguilar YA, Rueda ZV, Muskus C, Vélez LA. Comparison of serological methods with PCR-based methods for the diagnosis of community-acquired pneumonia caused by atypical bacteria. *J Negat Results Biomed.* 2016;*15*, 3.

47. Chang HY, Chang LY, Shao PL, Lee PI, Chen JM, Lee CY, et al Comparison of real-time polymerase chain reaction and serological tests for the confirmation of Mycoplasma pneumoniae infection in children with clinical diagnosis of atypical pneumonia. *J Microbiol Immunol Infect. 2014; 47(2):137-44.*

48. Zar H, Moore DP, Andronikou S, Argent AC, Avenant T, Cohen C, et al. Diagnosis and management of community-acquired pneumonia in children: South African Thoracic Society guidelines.' *Afr J Thorac Crit Care Med. 2020; 26(3), v26i3.104*

49. Mahajan P, Kuppermann N, Mejias A, Suarez N, Chaussabel D, Casper TC, et al. Association of RNA biosignatures with bacterial infections in febrile infants aged 60 days or younger. *JAMA. 2016; 316: 846–857.*

50. Laiakis EC, Morris GA, Fornace AJ, Howie SR. Metabolomic analysis in severe childhood pneumonia in the Gambia, West Africa: findings from a pilot study. *PLoS One 2010;5.*

51. Clish CB. Metabolomics: an emerging but powerful tool for precision medicine. *Cold Spring Harb Mol Case Stud. 2015;1(1): a000588.*

52. Florin TA, Ambroggio L. Biomarkers for community-acquired pneumonia in the emergency department*. Curr Infect Dis Rep. 2014 Dec;16(12):451.*

53. 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 Paediatrics Infectious Diseases Society and the Infectious Diseases Society of America'. *Clin Infect Dis. 2011; 53 (7): e25-76.*

54. Zar HJ, Andronikou S, Nicol MP. Advances in the diagnosis of pneumonia in children. *BMJ.2017;358: j2739.*

55. 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. *PLoS One. 2014 Aug 22;9(8): e106051.*

56. Ait-Ali L, Andreassi MG, Foffa I, Spadoni I, Vano E, Picano E. Cumulative patient effective dose and acute radiation-induced chromosomal DNA damage in children with congenital heart disease. *Heart. 2010;* 96: 269*–274.*

57. Courtoy I, Lande AE, Turner RB. Accuracy of radiographic differentiation of bacterial from nonbacterial pneumonia. *Clin Pediatr.1989;28:261–264.*

58. Wahlgren H, Mortensson W, Eriksson M, Finkel Y, Forsgren M, Leinonen M. Radiological findings in children with acute pneumonia: age more important than infectious agent. *Acta Radiol. 2005;46(4):431-436.*

59. Andronikou S, Lambert E, Halton J, Hilder L, Crumley I, Lyttle, MD, et al. Guidelines for the use of chest radiographs in community-acquired pneumonia in children and adolescents.' *Paediatr Radiol. 2017;47(11):1405-1411*.

60. Iuri D, De Candia A, Bazzocchi M. Evaluation of the lung in children with suspected pneumonia: usefulness of ultrasonography. *Radiologia Medica. 2009;* 358:321-*330.*

61. Urbankowska E, Krenke K, Drobczynski L, Korczyński P, Urbankowski T, Krawiec M, et al Lung ultrasound in the diagnosis and monitoring of community acquired pneumonia in children. *Respir Med. 2015;109 (9):1207-1212.*

62. Nazerian P, Volpicelli G, Vanni S, Gigli C, Betti L, Bartolucci M, et al. Accuracy of lung ultrasound for the diagnosis of consolidations when compared to chest computed tomography. *Am J Emerg Med. 2015;33(5):620-625.*

63. Reissig A, Kroegel C. Sonographic diagnosis and follow-up of pneumonia: a prospective study. *Respiration. 2017;*74*: 537–547.*

64. Cattarossi L. Lung ultrasound: its role in neonatology and paediatrics. *Early Hum Dev. 2013 ;*358*(suppl 1): S17-9.*

65. Reali F, Sferrazza Papa GF, Carlucci P, Fracasso P, Di Marco F, Mandelli M, et al. Can lung ultrasound replace chest radiography for the diagnosis of pneumonia in hospitalized children? *Respiration. 2014;* 88*: 112–115.*

66. Iorio G, Capasso M, De Luca G, Prisco S, Mancusi C, Laganà B, et al. Lung ultrasound in the diagnosis of pneumonia in children: proposal for a new diagnostic algorithm. *Peer J. 10;3: e1374.*

67. Pereda MA, Chavez MA, Hooper-Miele CC, Gilman RH, Steinhoff MC, Ellington LE, et al. Lung ultrasound for the diagnosis of pneumonia in children: a meta-analysis. *Pediatrics. 2015; 135(4): 714-722.*

68. Stadler JAM, Andronikou S, Zar HJ. Lung ultrasound for the diagnosis of community-acquired pneumonia in children.' *Pediatr Radiol. 2017;47(11): 1412-1419.*

69. Hirsch W, Sorge I, Krohmer S, Weber D, Meier K, Till H. MRI of the lungs in children. *Eur J Radiol. 2008; 68(2):278-288*.

70. Biederer J, Mirsadraee S, Beer M, Molinari F, Hintze C, Bauman G., et al. MRI of the lung (3/3)-current applications and future perspectives. *Insights Imaging. 2012, 3(4): 373-386.*

71. Gorkem SB, Coskun A, Yikilmaz A, Zurakowski D, Mulkern RV, Lee EY. Evaluation of paediatric thoracic disorders: comparison of unenhanced fast-imaging-sequence 1.5-T MRI and contrast-enhanced MDCT. *Am J Roentgenol. 2013;* 358*:*1352*-1357.*

72. Ozcan HN, Gormez A, Ozsurekci Y, Karakaya J, Oguz B, Unal S, et al. Magnetic resonance imaging of pulmonary infection in immunocompromised children: comparison with multidetector computed tomography. *Pediatric Radiol. 2017; 47(2):146-153.*

73. Cevey-Macherel M, Galetto-Lacour A, Gervaix A, Siegrist CA, Bille J, Bescher-Ninet B, et al. Etiology of community-acquired pneumonia in hospitalized children based on WHO clinical guidelines.' *Eur J Pediatr. 2009;168(12):1429-1436*.

74. Iroh Tam PY, Bernstein E, Ma X, Ferrieri P. Blood culture in evaluation of paediatric community-acquired pneumonia: A systematic review and meta-analysis.' *Hosp Paediatr. 2015; 5(6):324-336.*

75. Madhi SA, Petersen K, Madhi A, Khoosal M, Klugman KP. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. *Clin Infect Dis. 2000;31(1):170-176.*

76. Shah SS, Dugan MH, Bell LM. Blood cultures in the emergency department evaluation of childhood pneumonia. *Pediatr Infect Dis. 2011, 20:475-479*.

77. Driscoll AJ, Deloria Knoll M, Hammitt LL, Baggett HC, Brooks WA, Feikin DR, et al. The Effect of Antibiotic Exposure and Specimen Volume on the Detection of Bacterial Pathogens in Children with Pneumonia. *Clin Infect Dis. 2017 15;64(suppl_3): S368-S377.*

78. O'Callaghan-Gordo C, Bassat Q, Morais L, Díez-Padrisa N, Machevo S, Nhampossa, T, Nhalungo D, et al. Etiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique: a malaria endemic area with

high prevalence of human immunodeficiency virus. *Pediatr Infect Dis J. 2011 ,30(1):39-44.*

79. Pavia T. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis**.** *Clin Infect Dis. 2011, 52: S284-S289.*

80. Lee JH, Chun JK, Kim DS, Park Y, Choi JR, Kim JS. Identification of adenovirus, influenza virus, parainfluenza virus and respiratory syncytial virus by two kinds of multiplex polymerase chain reaction (PCR) and a shell vial culture in paediatric patients with viral pneumonia. *Yonsei Med J. 2010; 51: 761-767.*

81. Nichols WG, Peck Campbell AJ, Boeck M. Respiratory viruses other than influenza virus: impact and therapeutic advances. *Clin Microbiol Rev. 2008; 21: 274-290*.

82. 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. 2012;54 (Suppl 2): S190-9.*

83. El-Kholy AA, Mostafa NA, Ali AA, Soliman MMS, El-Sherbini SA, Ismail RI, et al. The use of multiplex PCR for the diagnosis of viral severe acute respiratory infection in children: a high rate of co-detection during the winter season.*' Eur J Clin Microbiol Infect Dis. 2016; 35:1607–1613.*

84. Krause JC, Panning M, Hengel H, Henneke P. The role of multiplex PCR in respiratory tract infections in children. *Deutsch Arztebl Int*. 2014; *111: 639–645.*

85. Murdoch DR, Morpeth SC, Hammitt LL, Driscoll AJ, Watson NL, Baggett HC, et al. The Diagnostic Utility of Induced Sputum Microscopy and Culture in Childhood Pneumonia. *Clin Infect Dis. 2017; 15;64(suppl_3): S280-S288*.

86. Hyams C, Williams OM, Williams P. Urinary antigen testing for pneumococcal pneumonia: is there evidence to make its use uncommon in clinical practice? *ERJ Open Res. 2020; 10;6(1):00223-2019.*

87. Pride MW, Huijts SM, Wu K, Souza V, Passador S, Tinder C, et al. Validation of an immunodiagnostic assay for detection of 13 Streptococcus pneumoniae serotype-specific polysaccharides in human urine. *Clin Vacc Immunol. 2012; 19: 1131-1141.*

88. Neuman MI, Harper MB. Evaluation of a rapid urine antigen assay for the detection of invasive pneumococcal disease in children. *Pediatrics. 2003;112(6):1279-1282.*

89. Tzeng DH, Lee YL, Lin YH, Tsai CA, Shi ZY. Diagnostic value of the Binax NOW assay for identifying a pneumococcal etiology in patients with respiratory tract infection. *J Microbiol Immunol Infect 2006; 39:39–44.*

90. Eastham KM, Freeman R, Kearns AM, Eltringham G, Clark J, Leeming J, Spencer DA. Clinical features, aetiology and outcome of empyema in children in the north east of England. *Thorax. 2004;59(6):522-525.*

91. Fletcher M, Leeming J, Cartwright K, Finn A, Southwest of England Invasive Community Acquired Infection Study Group. Childhood empyema: limited potential impact of 7-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J. 2006; 25(6):559-560.*

92. Mathew JL. Etiology of Childhood Pneumonia: What We Know, and What We Need to Know: Based on 5th Dr. IC Verma Excellence Oration Award. *Indian J Pediatr. 2018;85(1):25-34.*

93. Vuori-Holopainen E, Peltola H. Reappraisal of lung tap: review of an old method for better etiologic diagnosis of childhood pneumonia*. Clin Infect Dis. 2001; 1;32(5):715-726.*

94. Carrol ED, Mankhambo LA, Guiver M, Banda DL, IPD Study Group, Denis B, et al. PCR improves diagnostic yield from lung aspiration in Malawian children with radiologically confirmed pneumonia'. *PLoS One. 2011;6 (6): e21042.*

95. Tsai CM, Wong KS, Lee WJ, Hsieh KS, Hung PL, Niu CK, et al. Diagnostic value of bronchoalveolar lavage in children with nonresponding community-acquired pneumonia. *Pediatr Neonatol. 2017 ;58(5): 430-436.*

96. De Schutter I, De Wachter E, Crokaert F, Verhaegen J, Soetens O, Piérard D, et al. Microbiology of bronchoalveolar lavage fluid in children with acute nonresponding or recurrent community-acquired pneumonia: identification of nontypeable Haemophilus influenzae as a major pathogen. *Clin Infect Dis. 2011; 52(12):1437–1444.*

97. Shapiro MF, Greenfield S. The complete blood count and leukocyte differential count. An approach to their rational application. *Ann Intern Med. 1987;106(1):65-74.*

98. 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. 2015;19(22):4300–4303.*

99. Segel GB, Halterman JS. Neutropenia in pediatric practice. *Pediatr Rev. 2008; 29(1): 12–23.*

100. Celkan T, Koç BŞ. Approach to the patient with neutropenia in childhood. *Turkish Archives of Pediatrics. 2015; 50(3):136–144.*

Table 1: Summary of the diagnostic strengths of current investigatory modalities for childhood pneumonia

Investigatory modality	Diagnosis of childhood pneumonia	Diagnosis of bacterial vs. viral childhood pneumonia
Clinical features		
Clinical features?		
Serum investigations		
White blood count		
C-reactive protein		
Procalcitonin		
Serum Lipocalin -2	Sensitivity of 77% and specificity of 95% [33].	
Serum Myxoma Resistance Protein		96.4% sensitivity and 66.7% specificity at threshold of 200ng/ml [36].
Soluble triggering receptor expressed on myeloid cells	AUC of 0.50 with a sensitivity of 31.8% and specificity of 73.7%.	
Serum mid regional pro-atrial natriuretic peptide (MR-proANP)		AUC of 0.52 with sensitivity of 76.1% [29].
Serum mid regional pro-adrenomedullin (MR-proADM)		AUC of 0.58 with sensitivity of 78% [29].
Combination ImmunoXpert (CRP, TRAIL, interferon gamma protein 10)		Sensitivity of 86.7% and specificity of 91% [43].
Combination biomarkers (haptoglobin, TIMPS, IL-19)	Sensitivity 96% and specificity 86% [44].	
Micro array-based genome expression (RNA biosignatures)		Sensitivity of 94% and specificity of 95% in differentiating bacterial and non-bacterial infections [49].
Plasma metabolomics	Uric acid, hypoxanthine and glutamic acid levels were higher in children with pneumonia compared to controls [50].	
Radiological investigations		

Lung Ultrasonography	Pooled sensitivity of 96% and specificity of 93% when compared to CXR, or CXR with clinical/laboratory parameters [67].	
MRI	Sensitivity of 97% using CT as gold standard in diagnosing pneumonic changes [71].	
Microbiological investigations		
Urinary pneumococcal antigen	Sensitivity of 96-100% and specificity of 62-92% in detecting childhood pneumococcal pneumonia when compared to blood cultures [88,89].	