Review article

Childhood community-acquired pneumonia: Complexities and challenges of aetiological diagnosis

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Introduction

Childhood community-acquired pneumonia (CAP) is a leading cause of death in children in several parts of the world. The death rate is interconnected with poverty-related factors such as malnutrition, exposure to household pollution, overcrowding and weak immunisation programmes[1]. Southeast Asia and Africa are the worst affected regions[1]. A clear understanding of the aetiology of pneumonia is helpful in many ways. From the public health viewpoint, it helps to tailor the antibiotic policies, prioritising allocation of finances and strengthening the immunisation programmes. At an individual level, it influences the decision to start antibiotics, choice of antibiotics and treatment duration, thus minimising the immediate risks related to complications and mortality. Furthermore, appropriate therapy would reduce the long-term complications such as asthma, non-cystic fibrosis bronchiectasis and compromised lung function that is thought to be linked to severe episodes of pneumonia in otherwise healthy children[2].

The aetiological diagnosis of CAP is inherently challenging due to complexities in pathogenesis, extensive and early antibiotic use in case management, non-specific clinical manifestations and absence of gold standard laboratory investigations. The aim of this review is to describe the complexities in clinical, radiological and laboratory diagnosis and challenges of determining the aetiology.

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Determining the aetiological diagnosis

This section clarifies the microbial aetiology based on clinical, radiological, and laboratory evidence, emphasising the complexities of assigning causation.

Clinical features

Clinical manifestations of bacterial, atypical bacterial and viral pneumonia frequently overlap and therefore may not reliably distinguish between the various aetiologies. An ill child with high fever, marked tachypnoea, myalgia, and localised auscultatory findings commonly indicates a bacterial cause[3,4]. In contrast, a child with low-grade fever, runny nose, wheezing and bilateral, diffuse lung signs point more towards a viral cause[4]. Wheeze is common with viral or atypical bacterial pneumonia (*Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*). In Mycoplasma infection, commonly the symptoms appear gradually with initial pharyngitis. Subsequent hoarseness and intractable cough indicates extension of the infection into the lower airways. Symptoms are milder than with other bacterial pneumonias and it is thus called "walking pneumonia". Although the cough is initially dry, it later becomes productive[5]. Children are more susceptible to get mycoplasma pneumonia. It has been reported in 10 to 40% of CAP among children, more common in older than younger children[6]. Coryza is unusual. However, in younger children less than five years, coryzal symptoms may be present[5]. On auscultation of the chest, scattered or localised rales and expiratory wheezes may be found. Consolidation is uncommon. Extra-pulmonary manifestations such as arthritis, lymphadenitis, hepatosplenomegaly and haemolytic anaemia may also suggest mycoplasma infection[5]. However, these clinical features are not specific and can be found with *Chlamydophila pneumoniae* or viral infections as well[7]. Also, absence of these signs does not exclude mycoplasma infection.

Chest x-ray

Chest x-ray (CXR) may provide a clue to the aetiology. However, routine CXRs are not warranted for patients with suspected CAP.

Inconclusive clinical symptoms, children younger than two years with high fever and respiratory distress, poor response to antibiotics, signs of complications and rapidly worsening symptoms are some of the indications for chest radiography. Lobar or segmental consolidations are mainly seen with bacterial CAP, whereas interstitial infiltrates and peri-bronchial thickening are common with viral or mycoplasma pneumonia[8]. However, there is conflicting evidence. Robles A, *et al*[8] reported unilateral and multifocal consolidations without pleural effusions in viral CAP. The clinical features of mycoplasma pneumonia are often poorly correlated with the radiographic findings. Presence of pneumatocoeles, round opacifications, pleural effusions and empyema indicate a bacterial aetiology[9]. *Staphylococcus aureus* is known to cause necrotising pneumonia[10]. Hilar or subcarinal lymphadenopathy is associated with mycobacterium tuberculosis. Persistent opacities with inadequate clinical improvement despite antibiotics would also be an indication to investigate for tuberculosis. In adolescents, effusions and infiltrates with cavity formation in the upper zones or primary disease with segmental lung collapse are common[11].

Ultrasound scan of lung (UOL)

Ultrasonography of lung is used as the primary diagnostic tool to detect pleural effusions and empyema. It may also aid in differentiation between viral and bacterial pneumonia in combination with other parameters (viz. clinical and laboratory data)[12]. Bacterial pneumonia is characterised by larger consolidations with poorly defined edges, loss of pleural line echogenicity and sonographic air bronchograms[13]. Discrete or confluent echogenic vertical lines (B-lines) and small consolidations (<1cm) without air bronchograms are common in viral pneumonia[14]. In viral bronchiolitis, sonographic pattern may be similar to viral pneumonia. Biagi C, *et al*[15] described good accuracy of UOL in diagnosing bacterial pneumonia in children who were clinically diagnosed and treated as bronchiolitis. In that study, UOL had a higher specificity to diagnose bacterial pneumonia than CXR (98.4% *vs* 87.1%) when the cut-off for the size of consolidation was taken as one centimetre. UOL in CAP has many advantages like low cost, no ionising radiation exposure, good patient cooperation, being fast, easy to learn and ability to be used as a point of care method. It has good diagnostic accuracy even when non-experts perform it. Compared to CXR, it has lesser inter-observer variability[13].

Biomarkers

The most frequently used biomarkers in clinical practice are the white blood cell (WBC) count and C-reactive protein (CRP). Procalcitonin (PCT) is the latest biomarker. In patient management, none of these biomarkers are recommended to be interpreted in isolation. Also, they should not be a substitute for one's clinical judgment. There are other biomarkers such as haptoglobin, tissue inhibitor of metalloproteinases-1, interleukin 19 and TNF receptor, mostly used in research. Procalcitonin is a calcitonin receptor peptide, detected within 2-3 hours and peaks at 6 hours. On the other hand, CRP response to infection is more delayed than PCT (secretion begins in 4–6 hours and peaks at 36–50 hours). Studies investigating the importance and the cut-off levels of CRP and PCT in the differentiation of aetiology in paediatric CAP have produced conflicting results. Thomas J, *et al*[16], in a recent systematic review, has described varying detection methods, the chosen specificity and sensitivity and the presence of co-infection with multiple pathogens as the reasons for inconsistent results. Overall, the specificity of PCT to identify viral aetiology was higher compared to CRP[16]. However, false positives of PCT could be seen in cirrhosis and burns. CRP also rises in other conditions (e.g. trauma, surgery, burns) other than infections. WBC count has the lowest positive predictive value compared to PCT and CRP. Total WBC count and neutrophil percentage tend to overlap, making it difficult to differentiate viral and bacterial CAP[17].

Microbiological and serological testing

Sputum microscopy and culture

In adults, sputum Gram stain and culture are often helpful to describe the aetiological diagnosis[18]. However, obtaining a good quality sample is often difficult in younger children. The quality of the sample is decided by the number of epithelial cells (<10/LPF) and polymorphonuclear leucocytes (>25/LPF)[19]. Sputum induction provides a good quality sputum sample collected either through expectoration or by aspiration through the nostrils. Even though the yield of organisms in the induced samples is better, routine use of induction cannot be recommended due to the unpleasantness of the procedure and experience of side effects[20]. Overall, sputum culture obtained with the above methods has a low specificity due to contamination from asymptomatic upper airway carriage[21]. If tuberculosis (TB) is suspected, three sputum samples are collected to examine for acid-fast bacilli. However, in younger children, the yield is low due to the pauci-bacillary nature of the disease. Sputum culture for *Mycobacterium tuberculosis* is a more sensitive and specific than direct smear microscopy and is recommended for children suspected to have TB.

Blood and pleural fluid cultures

Routine performance of blood cultures in children with CAP is not indicated[21]. Blood cultures have a low yield (1-10%). It does not appear to be helpful in uncomplicated CAP due to low bacteraemia[22,23]. Prior antibiotic therapy and quantity of blood taken for culture influence the blood culture results[24]. Pleural fluid cultures for bacterial pneumonia give false-negative results with previous antibiotic therapy, unsatisfactory transport and storage[25]. In patients with pneumococcal pneumonia, pleural fluid culture in conjunction with pneumococcal PCR increases detection rate significantly[26]. In addition to cytology and culture, Light's criteria, based on the ratio of serum and pleural fluid protein and lactate dehydrogenase level, are used to determine the exudative nature of the TB pleural effusion[11]. Adenosine deaminase is not very useful; it gives a higher rate of false positives in partially treated bacterial pneumonia, particularly in children[11].

Serologic testing

Routine serologic testing is not very useful for immediate patient management. Mycoplasma infection is no exception. Single serum sample for IgG or IgM antibodies during acute infection lacks specificity and sensitivity. Specificity is low because high IgG and IgM antibodies are found in some healthy, older children[26]. Sensitivity is low due to low serum antibody levels in early stages of infection[27]. The gold standard of mycoplasma pneumonia diagnosis is a fourfold rise in paired samples (acute and convalescent). In serological diagnosis of pneumococcal pneumonia, evidence of ≥2-fold increase is adequate for most pneumococcal proteins[28]. However, it is considered too complex for routine use.

Antigen testing

Antigen testing is a useful adjunct to diagnosis. Rapid antigen test for respiratory syncytial virus (RSV) is still in use due to fast results and low cost compared to molecular detection. It is widely used particularly during the flu season. In the current pandemic of COVID-19, rapid antigen testing is being used widely. Results can be obtained within a few hours, thus enabling early intervention to mitigate the spread. Urine for pneumococcal antigen is useful in research and clinical practice. In a meta-analysis, sensitivity and specificity of pooled data of urine antigen was 74% and 97.2% respectively[29]. However, cross-reactions may be seen with other closely-related streptococci. Also, previous infection within three months may lead to false-positive results[30]. Children with pneumococcal carriage excrete the antigen in the urine. Therefore, the specificity is low in populations with high pneumococcal carriage[17,24]. Evidence is limited on the effect of pre-treatment with antibiotics on the test results. Many guidelines recommend using pneumococcal antigen testing because it is a relatively low-cost and a less time consuming test[31]. As it favours early diagnosis, rational antibiotic prescribing and narrowing down the treatment could be undertaken as early as possible.

Molecular diagnostic assays

Real-time multiplex polymerase chain reaction (RT-PCR) has instituted revolutionary changes in detection of micro-organisms. It is a more sensitive test than cell culture, shell vial culture, and immunofluorescence testing[32]. PCR can detect multiple organisms simultaneously in a single real-time PCR reaction. It replicates a small amount of DNA or RNA, does not require living organisms and is not affected by the prior use of antibiotics. However, higher sensitivity is considered a disadvantage in certain situations. For example, it turns out to be positive in prolonged viral shedding following symptomatic infections[33]. Therefore, in research of CAP, to increase the accuracy of interpretation, recruitment of age-matched controls and quantification of viral load in the upper airway have been adopted. Greater viral load (low coy/cycle threshold, i.e., C_T value) in nasopharyngeal samples is associated with increased probability of lower respiratory tract infection[34].

The colonisation rate of pneumococci in the upper respiratory tract is high, particularly in resource-poor countries with overcrowding and air pollution[35]. Therefore, positive PCR of nasopharyngeal secretions does translate into a diagnosis of pneumococcal pneumonia. Some researchers have utilised PCR of whole blood to improve the identification of true cases of pneumococcal pneumonia. However, in the well-known Pneumonia Etiology Research for Child Health (PERCH) study, blood PCR demonstrated a poor specificity due to similar positivity among both cases and controls[24]. Alcoba G, *et al*[36] has presented a three-step model with high CRP, clinical signs and positive blood PCR, to increase the likelihood of detection of severe pneumococcal pneumonia. This model reduced the influence of nasopharyngeal colonisation on the true pneumococcal pneumonia cases.

RT-PCR can be performed on broncho-alveolar lavage fluid in children with severe pneumonia. Still, the bronchoscope itself might be contaminated by oropharyngeal flora while being passed through the upper airways[37]. To overcome contamination, ideal samples would be lung aspirates or biopsy; however, practical and ethical issues make it difficult in clinical practice and research. Research has shown multiple pathogens

in lung aspirates and biopsies in children with pneumonia, using molecular diagnostic assays[38]. Hence, interpreting the causality of each pathogen, even with the best sampling technique is challenging. The recent addition of WHO endorsed GeneXpert MTB/RIF is a cartridge-based nucleic acid amplification test for concurrent TB diagnosis and rapid antibiotic sensitivity test. It provides timely results to start treatment early. Another advantage is its high specificity for *Mycobacterium tuberculosis.*

Vaccine probe studies

Vaccine probe studies investigate the difference in disease occurrence between vaccinated and unvaccinated persons. In a randomised control trial in the Gambia, the conjugate type B Haemophilus influenza (Hib) vaccine prevented most cases of radiologically defined pneumonia in infants. It concluded that 20% of pneumonia in the cases was due to Hib[39]. The probe is always not necessarily a vaccine. A recent study had described using prophylactic monoclonal antibodies to investigate the RSV disease burden[40].

Diagnosis of COVID-19 infection

A rapid surge in research was observed in response to the COVID-19 outbreak, which was during the time when the manuscript was produced. Many researchers have explored clinical manifestations and diagnosis of the novel coronavirus. Most children manifested with a mild form of disease with rhino-pharyngitis (55.3%). Some were completely asymptomatic (4.4%)[41,42]. Others with moderately severe disease present with typical symptoms and signs of pneumonia without hypoxaemia or respiratory failure. Severe disease is manifested with hypoxia, respiratory distress and multi-organ involvement. Progression to acute respiratory distress syndrome or respiratory failure is linked to many complications viz. shock, encephalopathy, myocarditis, heart failure, disseminated intravascular coagulation and acute kidney disease[42]. Li Y, *et al*[43] did a retrospective study of children under five years with COVID-19 and influenza-A infection. Children with COVID-19 pneumonia had milder symptoms than children with influenza-pneumonia. Also, they had higher C-reactive protein, procalcitonin and d-dimer. Imaging results showed more ground-glass opacification of lung fields in COVID-19.

Generally, upper respiratory samples are used for microbiological diagnosis. Nasopharyngeal (NP) samples give a better yield than oropharyngeal (OP) swabs. Also, NP is more tolerable for the patient and safer to the operator[44]. Broncho-alveolar lavage can be tested when NP/OP are negative.

A real-time RT-PCR method is recommended for molecular testing. At least two molecular targets are included in the RT-PCR assay to avoid possible cross-reactions with other endemic coronaviruses[45]. Rapid antigen assays are fast and less costly. However, false negatives are not uncommon due to individual variation of the viral load and sampling variability[44]. Serology is an indirect measure of past infection, more useful for epidemiological purposes[46]. However, serology may be useful for diagnosing COVID-19, when the other antigen or molecular tests are negative in the latter part of the illness.

Challenges in establishing CAP aetiology

In clinical practice, differentiation of bacterial from viral CAP is difficult for many reasons viz. pretreatment with antibiotics, difficulty in obtaining reliable respiratory samples from small children and high cost of molecular diagnostic testing.

In aetiological research, there are additional issues. Case definition of pneumonia is controversial. The World Health Organisation has given priority to sensitivity rather than the specificity when defining CAP[47]. This definition is widely used in research and has resulted in mis-classification of cases with other respiratory disorders as CAP[24]. Most CAP studies are of cross-sectional design, and samples are collected on admission to hospital. Hence, sampling at one point will not elaborate the importance of multiple pathogens or super-infections. For example, there is considerable evidence that influenza virus and rhinovirus infection facilitate subsequent secondary bacterial pneumonia[48]. Furthermore, as most research is hospital-based, milder cases managed in the community are missed. Fatal cases of pneumonia are also under-represented in most of the aetiological studies.

Types of samples collected would influence the results. We rely more on nasopharyngeal and oropharyngeal secretions, which are not from the true site of infection. Collecting a good sputum sample not contaminated with upper respiratory flora is difficult. Although collecting lung aspirates are the best, it is difficult in clinical practice and even in research[49].

The detected organism depends on which pathogens are included in the testing panel; invariably, aetiology cannot be attributed to a pathogen not tested. On the other hand, multiple tests or highly sensitive molecular testing would give a long list of potential pathogens. It may result in assigning causal role to pathogens of less clear aetiologic significance.

Conclusion

Even with the obstacles and complexities involved, the continuous search for better testing strategies is essential. The disadvantages of current molecular diagnostic testing, particularly for *Streptococcus pneumoniae*, could be overcome using more specific gene targets. Developing point of care tests with high specificity and sensitivity will be useful in the patient management. In addition to improving tests to identify aetiology, the researchers should target examining the interplay between the host, the environment, and the aetiological agents to explain the epidemiological differences in pneumonia incidence in various parts of the world.

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