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Clinical Usefulness

PCR testing for Paediatric Acute Respiratory Tract Infections

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EDUCATIONAL AIMS:

The reader will be able to:

- Summarise the main difficulties of diagnosing respiratory infections on clinical grounds.
- Know the background for the clinical use of different diagnostic tests.
- Discuss the indications for PCR diagnostic testing.

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SUMMARY

Acute respiratory tract infection (ARI) is a frequently occurring disease in children. It is a clinical diagnosis for which no internationally accepted diagnostic test is available. The majority of ARI is viral in origin, though diagnostic tests for viruses were rarely performed in the past. In the past 2 decades, new molecular techniques have been introduced in many hospitals. They are capable of generating a high yield of viral and bacterial diagnoses, but their impact upon clinical practices is still questionable.

In this paper, we discuss the difficulties of diagnosing ARI in children, the indications for conventional and new diagnostics and their implications.

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INTRODUCTION

In developed countries, acute respiratory tract infection (ARI) is the most common cause for hospitalization of young children [1]. A rapid and accurate diagnosis is required to start adequate therapy, minimize hospital admissions and length of hospital stay and reduce unnecessary antibiotic use.

Clinical decision making in ARI is based on interpretation of clinical signs and symptoms, often supported with results of laboratory parameters such as C-reactive protein and white blood count combined with conventional viral and bacterial tests. In addition, new molecular tests have been introduced in many hospitals. However the role (and impact) of these molecular tests in clinical decision making is not known. In this paper, we discuss the role of molecular diagnostics in relation to patient care in paediatric ARI.

CLINICAL DIAGNOSIS OF PAEDIATRIC ARI

Difficulties in defining the clinical diagnosis of paediatric ARI

The anatomic approach to ARI creates 2 categories: upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI). URTI is confined to the ear, nose and throat region and is considered to be a rather harmless disease for which medical attention is generally restricted to family care. LRTI on the contrary, which involves the bronchi, bronchiole and the lung tissue, is considered a more severe disease that should be seen by a

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paediatrician. Pneumonia is thought to be more likely caused by bacteria for which prompt initiation of antibiotics is required.

However, in paediatric patients this clear distinction between URTI and LRTI is difficult to make and appears not useful for clinical decision-making. In infants, classical LRTI-symptoms like tachypnea or hypoxia are also common in paediatric URTI due to nasal obstruction and mucus plugging of the anatomically small upper airways. General symptoms such as poor feeding, lowered alertness and low transcutaneous oxygen seem to influence the clinical course more strongly than the designated anatomical site of infection [2].

Based on pathophysiologic mechanisms, LRTI can be subdivided in pneumonia and bronchiolitis. Pneumonia reflects inflammation of lung tissue (bronchi, bronchioles and alveolar tissue) in response to infection by bacterial, viral or other pathogens, while bronchiolitis primarily affects the small airways (bronchioles) and is usually of viral origin only. The clinical presentation of pneumonia overlaps substantially with that of bronchiolitis and vice versa. Pneumonia and bronchiolitis are clinical diagnoses without an internationally standardized test or definition [3,4]. Though chest radiographs are often used in clinical settings, they cannot distinguish between these conditions [5].

There have been attempts to distinguish viral from bacterial ARI because of its therapeutic consequences. Most paediatric ARI are of viral origin and the risk of concurrent (or subsequent) bacterial infection has been reported to be low in children over three months of age [6]. It is assumed that antibiotics are prescribed too often for these children [7]. This is in part explained by a high level of similarity in clinical symptoms between viral and bacterial LRTI. Respiratory Syncytial Virus (RSV) pneumonia and serologically detected pneumococcal pneumonia did have considerably overlapping clinical signs and symptoms, whereas laboratory and chest radiography findings significantly differed [8].

Assessment of disease severity and risk factors

The majority of paediatric patients with ARI are young and previously healthy children with no known risk factors for acquiring respiratory infection. Assessment of disease severity in these children is based on patient's history, parental concern about the health of their child and objective findings by physical examination. Different clinical scoring systems are in use, adapted to local circumstances and patient groups. Examples are: The Respiratory Distress Assessment Instrument, based on wheezing and retractions [9], the (Bedside) Paediatric Early Warning Sign Scores [10–12], the Preschool Respiratory Assessment Measure, [13] the clinical scoring system described by Kristjansson [14], the Silverman-Anderson Respiratory Scale [15] and the disease severity score by Gern [16]. However, none of the above scoring systems has been validated specifically for prediction of clinical outcome in paediatric ARI. This diversity in scoring systems limits comparison of study data in the literature.

Assessment of respiratory illness in children with underlying conditions is often more difficult. Known risk factors for severe bronchiolitis are best recognized for RSV. They include young age, prematurity, low birth weight, Down syndrome, congenital heart disease, bronchopulmonary disease and immunodeficiency [17,18]. Other underlying conditions that lead to higher hospitalisation rates are cerebral palsy, chronic lung diseases like cystic fibrosis, asthma and recurrent respiratory tract infections. Use of viral diagnostics in these patient disease categories lies outside of the scope of this article.

As stated above, diagnosing paediatric ARI and assessment of disease severity is primarily based on clinical grounds. For borderline cases, laboratory results are often used in clinical decision making. Since real time polymerase chain reaction (RT-PCR) assays have been widely introduced in many hospitals, we will discuss its impact and role in clinical decision making for paediatric ARI.

VIRAL DIAGNOSTICS IN PAEDIATRIC ARI

International and national guidelines regarding use of viral diagnostics in paediatric ARI

The Pediatric Infectious Disease Society (PIDS) and the Infectious Diseases Society of America (IDSA) recommend in their guideline 'Community-Acquired Pneumonia (CAP) in Infants and Children' the use of sensitive and specific tests for the rapid diagnosis of influenza virus and other respiratory viruses in the evaluation of children older than three months of age with CAP [19]. In the case of a positive test for influenza, they strongly recommend that no antibiotic therapy be employed in the absence of clinical, laboratory or radiographic findings suggestive for bacterial co-infection. No specific recommendation is given on antibiotic use when other viruses are detected. Currently, there are no (P)IDSA guidelines for paediatric ARI in patients younger than three months.

The American Association of Pediatrics (AAP) recommends clinicians diagnose bronchiolitis and assess disease severity on the basis of history and physical examination without routinely ordering laboratory tests or radiography to make the diagnosis (evidence level B) [20]. Clinicians should assess risk factors for severe disease (age less than 12 weeks, prematurity, underlying cardiopulmonary disease or immunodeficiency) (evidence level B). Repeated observations over a short period of time will improve overall assessment and patient care. In a state of the art review in 2010, this AAP recommendation is supported by evidence of a low rate of bacterial co-infection in children younger than three months of age presenting with bronchiolitis [21]. However, rates of RSV testing did not fall after publication of the 2006 guidelines [22]. The authors suggests that hospitals continue to test for RSV in order to cohort patients after admission.

The guidelines of the Royal College of Paediatrics and Child Health (RCPCH) and the European Society of Paediatric Infectious Diseases (ESPID) recognize that rapid, sensitive and specific immunofluorescence viral tests are available and that RT-PCR is increasingly replacing immunofluorescence and serology, but they do not give recommendations when to use it and what the consequences are of the results when they become available [23].

Impact of conventional viral diagnostics in paediatric ARI

Until the 1980's, viral testing was not routinely available in paediatric clinical practice. Viral cultures were the gold standard, but they require specialized laboratory facilities and are time-consuming. Therefore, results are usually available too late to influence patient management. Serology is more easy to implement, but requires two separate blood samples over time to show (preferably) a four-fold increase in antibody response. Blood sampling is not a child-friendly procedure and the results are also not readily available. The use of serology seems restricted to epidemiologic studies in order to maximize etiologic diagnosis [24]. Rapid antigen tests like direct immunofluorescent antibody tests (DFA) provide more rapidly results, but are less sensitive than viral cultures [25,26]. They are available for RSV and influenza virus, but sensitivity ranges from 66.2% to 94.1% [27–29]. According to earlier studies, rapid viral tests contribute to reduction in hospital stay and antibiotic use [7,30–32].

Introduction of viral PCR techniques in practice

Since the 1990's PCR techniques have become more widely available. Conventional endpoint single-target PCRs were able to

report positive and negative results of RNA and DNA viruses. Later on, computerized quantitative RT-PCRs were able to correlate the amount of PCR product to a viral load. Testing a panel of respiratory viruses within a time span of 6 to 24 hours has become routine practice in many hospitals. In paediatric populations, the sensitivity has shown to be higher than for viral culture [25]. Nowadays, qualitative and even quantitative multiplex PCRs are able to detect multiple respiratory viruses in one single sample simultaneously, however sensitivity in a multiplex PCR is generally lower than in single target RT-PCR [33]. A disadvantage of commercially available multiplex PCRs is that the tests require external controls, which are difficult to perform since not all available platforms publish their targets [34]. Despite the availability of commercially quantitative multiplex PCRs, in-house quantitative single target RT-PCRs are used more frequently in current laboratory practice.

In a recent review by Jartti et al on new molecular virus detection methods, all nasopharyngeal sampling tests, including nasopharyngeal aspirates, washes, swabs or brush appear suitable for PCR analysis. Viruses in the upper airways do reflect infections of the lower airways [33]. Sputum induction methods, trying to generate sputum samples from the lower respiratory tract are not recommended for routine use as the diagnostic yield will not significantly improve.

Impact of RT-PCR in paediatric ARI

Introducing highly sensitive viral diagnostics has yielded a broad spectrum of viral diagnoses, but has not automatically lead to changes in patient management. In an adult population, implementation of RT-PCR in LRTI increased the diagnostic yield, but did not reduce antibiotic use or costs [35]. In a multicentre paediatric study, interviewing medical doctors on fictitious ARI cases, RT-PCR decreased antibiotic use. However, in real life, the same physicians did not alter their antibiotic prescriptions based on the results of RT-PCR [36]. In a Cochrane review (4 RCT's) that evaluated the impact of rapid viral tests in children with ARI in an emergency department (ED), rapid viral testing by immunofluorescence or PCR did not lead to changes in antibiotic use, length of ED visits, blood or urine testing, but did lower the rates of chest radiographs. The authors stated that routine viral testing in the ED is promising as a means to reduce antibiotic use, but there is yet insufficient evidence to support this. Large trials are needed with a focus on patient management with respect to the results [6]. In a recent retrospective study evaluating clinical differences between RSV and non-RSV patients, virological testing (enzyme linked immunoassay and / or PCR) did not help in management decisions and seemed insufficient to predict outcome at an individual level [37]. In another recent retrospective study of 177 children with ARI in a general hospital, antibiotic management was not influenced after detecting a viral respiratory pathogen, although the authors state that routine testing of common respiratory pathogens could lead to a better understanding of their role in disease in children with respiratory symptoms [38]. In a 6-year prospective study of children with community acquired pneumonia (CAP), designed to describe the frequency of respiratory viruses, antibiotics were prescribed less frequently in viral positive versus negative children, but only when they were $>$ 18 months old [39]. Our own data from a multicentre prospective controlled clinical trial of 582 previously healthy children with respiratory symptoms showed a high diagnostic yield of RT-PCR, however the rapid communication (within 24 hours) of results to the paediatrician did not change patient care versus those who received results later [40]. These findings underlie the statement of the AAP, not to recommend routine viral testing for standard ARI cases, unless the physician is willing to change his patient management based on the results [20].

Interpretation of single test results

At present, a panel of approximately 15 respiratory RT-PCR assays is available in many hospitals. Data suggest that RSV has the greatest disease burden both in hospitalized children and in outpatients, especially in children under five years of age [41]. Furthermore, Human Rhinovirus (HRV) is generally associated with the common cold, can cause severe ARI as well and is the most common pathogen in ARI in young children [42,43]. Re-infections with HRV are commonly observed, and are usually caused by different virus strains. [44] As reviewed by Kim, recent studies suggest that HRV subtype C may be more virulent than other HRV [45], but not all RT-PCRs are capable of distinguishing between the different HRV strains. Influenza virus (IV) is well known for its seasonal outbreaks of ARI during a few weeks in winter, and also for a sepsis like syndrome without respiratory symptoms. Human adenovirus (HAdV) is common in respiratory disease with a severe disease course in children with underlying immunodeficiency [46]. Para-influenza viruses (PIV) subtypes 1 and 2 are known to cause croup and subtype 3 is known to cause bronchiolitis and pneumomia [47]. PIV subtype 4 is has been reported to be a much less frequent cause of ARI [48]. The incidence of poly-omaviruses WU [49] and KI [50] is low and their clinical relevance remain unclear and require further evaluation [51].

The role of newly discovered viruses is of interest in many studies. Some of these 'new' viruses are Human Metapneumovirus (HMPV), Human Bocavirus (HBoV) and Human Coronavirus (HCoV). HMPV was first detected in 2001 [52], and its clinical symptoms overlap with those of other respiratory viruses [53]. Disease severity seems less than for RSV [54]. Another 'new' virus is HBoV, discovered in 2005 [55]. In a review by Brodzinsky, the incidence is found to be low (1.5-4.5%) and rates of co-infections are high (14-72%) [56]. The spectrum of disease is similar to RSV and hMPV. The group of HCoV's are heterogeneous. HCoV 229E [57], OC43, [58,59] NL63 [60,61] and HKU1 [62] are recognized frequently in young age and are correlated with less severe respiratory disease [54,63]. SARS coronavirus and the novel MERS coronavirus [64] are not routinely incorporated in respiratory RT-PCR test panels.

A recent Swedish study compared viral PCR findings from children with ARI versus asymptomatic matched controls. RSV, hMPV and PIVs were highly overrepresented in symptomatic patients, suggesting that they are responsible for illness. Asymptomatic controls showed high detection rates of HBoV, HRV, HAdV, HCoV and enterovirus, suggesting that prolonged virus-shedding may occur and that PCR-results need to be interpreted with caution [65].

Interpretation of multiple test results

The incidence of mixed viral infections is reported as high as 14 – 44%, depending on different populations and test panels [66]. In a multicenter study, involving 2207 children less than 2 years of age, the incidence of mixed infection in children hospitalized for bronchiolitis was 30% [67]. Several circumstances can generate positive RT-PCR results. RT-PCR assays are very sensitive and can detect small amounts of viral nucleic acids, which are still present during a convalescence period. For each individual virus it is not known how long DNA/RNA shedding may continue during this convalescence period [68,69]. Furthermore, children with a normal immune system can asymptomatically harbor viruses in their respiratory tract. Usually, a primary viral infection leads to an adaptive immune response with induction of memory T-cells, so that a second hit with the same virus usually results in less serious or even absent symptoms [70].

The relevance of finding only a single pathogen is also of interest. RT-PCR assays are sensitive tests, but detect only

pathogens that are looked for. For each micro-organism a specific primer is used, therefore non-viral pathogens like *Bordetella pertussis*, *Mycoplasma pneumonia* and *Chlamydophyla pneumonia* are not detected unless they are looked for. They can mimic viral disease as well, especially in young children [71].

One could hypothesize that mixed infections may lead to a more severe disease. Papers on this subject show somewhat contradictory results. There are a few reports suggest that there is no relation between mixed viral ARI and disease severity [72–75], while others indicate a higher disease severity in children with a mixed respiratory infection [76,77]. Further research as to how one should interpret multiple, positive RT-PCR results in one single sample is needed. Quantitative studies with interpretation of viral load will possibly help to answer this question.

Special indications for RT-PCR

Epidemiologic surveillance programs benefit from viral RT-PCRs. Examples include the worldwide search for SARS and MERS coronaviruses, well known for their febrile and atypical pneumonia and the dramatic course in some affected people [64,78–81]. Other national and WHO-supported surveillance programs include seasonal influenza viruses. Currently, the 'bird flu' influenza A subtype H7N9 is of special interest for its infection of humans and possible easy transmission from wild birds to humans [82].

Prevention of nosocomial infections should be of high priority in any hospital and isolation or cohorting is a well-established policy. RT-PCR has shown to be a useful tool for epidemiologic studies. However, the epidemiology of many nosocomial respiratory infections is not well known. For RSV, the incidence of nosocomial spread seems low. Although information about management of outbreaks is sparse and not well studied, normal prevention measurements appear to be rational [83]. For cohorting, rapid testing for RSV has shown to be a safe, cost-effective and efficient way to improve bed management [84]. Although we do not doubt that isolation as strategy helps to prevent nosocomial infections, we still have concerns when cohorting is based on RT-PCR results of only a limited number of viruses. Mixed infections occur in a substantial proportion of children and whether these children spread other non-detected viruses during cohorting is not yet clear. Rapid antigen tests like DFA are limited useful for cohorting issues, since they may have a high proportion of false negatives. RT-PCR obviously is much more sensitive and specific, but the turnaround time is not sufficient to act in time for infection control measurements at admission. Theoretically, one would expect that RT-PCR would be beneficial for infection control, however no large study has demonstrated a positive effect to date. The risk of cross-infection in children sharing a room was studied recently in a prospective observational cohort study of 48 children with bronchiolitis. Room sharing between RSV-positive and RSV-negative children on the first day of admission did not influence the risk of co-infection [85].

Children with respiratory failure induced by viral infection and admitted to paediatric intensive care units for mechanical ventilation frequently have concomitant bacterial infections [86]. Use of an extended RT-PCR panel of respiratory pathogens seems to be justified in those conditions to determine the causing micro-organisms. However, it is unclear what the exact relevance of the RT-PCR results is, except for those viruses for which treatment is available (like influenza and adenovirus)

Children with underlying immunodeficiencies are susceptible to severe complications of viral infections. Indications for RT-PCR diagnostics in children with hematologic malignancies, hematopoietic stem cell transplantation, solid organ transplantation, premature infants and children with cystic fibrosis lies outside the scope of this article and was well reviewed by Vallieres and Renaud recently [34].

Viral load

An objective laboratory parameter, reflecting disease severity, would be helpful to estimate disease course in an early stage. Viral load was thought to serve as such a parameter. Viral load is the quantity or copy number of viral RNA or DNA detected per milliliter body fluid. In RT-PCR, the cycle threshold (CT) value is defined as the number of RT-PCR cycles required for a positive fluorescent amplification signal to cross the threshold. This is inversely correlated with the viral load. Some authors indeed found a significant correlation between viral load and disease severity [87–89], thereby justifying broad use of RT-PCR. However viral load does not always correlate well [90–94]. Some viruses, like RSV, are short lived and others like HBoV are long-lived, which is a complicating factor in demonstrating this relationship. The question how well disease severity is correlated with viral load is not answered yet.

CONCLUSIONS

Paediatric ARI is a clinical diagnosis and most infections are caused by viruses. Use of clinical scoring systems helps to assess disease severity for the individual patient and could be used for clinical management. Thus far, an extended panel of RT-PCR assays contributes little to clinical decision making for the majority of children with ARI; in fact RT-PCR for detecting respiratory infection is not routinely available in many hospitals. In today's paediatric practice, RT-PCR is used in patients with high-risk of complications or with an unexpected disease course. The panel of micro-organisms should at least include pathogens for which a specific treatment is available, e.g. influenza virus and *Bordetella pertussis*.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

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PRACTICE POINTS

- RT-PCR will help to explore the role of certain viruses in ARI with mixed viral aetiology, to discover new viruses in future, and possibly to assess disease severity by repeated viral load quantification.
- RT-PCR is a powerful tool in public health surveillance.
- The role of RT-PCR in hospital hygiene is currently limited.

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