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Short communication

Immunofluorescence versus xTAG multiplex PCR for the detection of respiratory picornavirus infections in children

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ABSTRACT

Background: Polymerase chain reaction (PCR) is a sensitive tool for detection of respiratory picornaviruses. However, the clinical relevance of picornavirus detection by PCR is unclear. Immunofluorescence (IF), widely used to detect other respiratory viruses, has recently been introduced as a promising detection method for respiratory picornaviruses.
Objectives: To compare the clinical manifestations of respiratory picornavirus infections detected by IF with those of respiratory picornavirus infections detected by xTAG multiplex PCR in hospitalized children.
Study design: During a 1-year period, nasopharyngeal aspirates (NPA) from all children hospitalized due to an acute respiratory infection were prospectively analyzed by IF. All respiratory picornavirus positive IF samples and 100 IF negative samples were further tested with xTAG multiplex PCR. After exclusion of children with co-morbidities and viral co-infections, monoinfections with respiratory picornaviruses were detected in 108 NPA of 108 otherwise healthy children by IF and/or PCR. We compared group 1 children (IF and PCR positive, $n = 84$) with group 2 children (IF negative and PCR positive, $n = 24$) with regard to clinical manifestations of the infection.
Results: Wheezy bronchitis was diagnosed more often in group 1 than in group 2 (71% vs. 46%, $p = 0.028$). In contrast, group 2 patients were diagnosed more frequently with pneumonia (17% vs. 6%, $p = 0.014$) accompanied by higher levels of C-reactive protein (46 mg/l vs. 11 mg/l, $p = 0.009$).
Conclusions: Picornavirus detection by IF in children with acute respiratory infection is associated with the clinical presentation of wheezy bronchitis. The finding of a more frequent diagnosis of pneumonia in picornavirus PCR positive but IF negative children warrants further investigation.
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1. Background

Respiratory picornaviruses (enteroviruses and rhinoviruses) are common etiologic agents of upper and lower respiratory tract infections in pediatric patients.[1] Picornaviruses are found in 25–30% of children with acute respiratory symptoms.[2–6] Almost 80% of all children have experienced rhinovirus infections by the age of 2 years.[7] Moreover, rhinoviruses are associated with up to five hospitalizations per 1000 children <5 years of age indicating a substantial morbidity in this age group.[2]

Rapid detection of respiratory picornaviruses is important to avoid nosocomial transmission to other children, reduce unnecessary antibiotic use, decrease hospital stays, and consequently enhance the cost-effectiveness of medical management.[4,8,9] The current method used to detect respiratory picornaviruses is nucleic acid amplification, e.g., polymerase chain reaction (PCR).[10] However, this method is expensive and time-consuming and does not allow for rapid diagnosis. Recently, we have shown that detection of respiratory picornaviruses by immunofluorescence (IF) in children is feasible as a routine diagnostic procedure and may have some advantages over molecular methods.[11] Although PCR is more sensitive in detecting respiratory picornaviruses compared to IF, it remains unknown whether the additionally detected respiratory picornaviruses are always clinically relevant.[11]

2. Objectives

To address this question, we retrospectively analyzed clinical data of children hospitalized with picornavirus infections to com-

Abbreviations: PCR, Polymerase chain reaction; IF, Immunofluorescence; NPA, Nasopharyngeal aspirate; ADV, Adenovirus; RSV, Respiratory syncytial virus; PIV, Parainfluenza virus; IFA, Influenzavirus A; INB, Influenzavirus B; HMPV, Human metapneumovirus.

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Table 1
Comparison of clinical features of group 1 (IF and PCR positive) and group 2 (IF negative and PCR positive) children with respiratory picornavirus monoinfection.

	Group 1 patients (IF+/PCR+) $n=84$ (%)	Group 2 patients (IF−/PCR+) $n=24$ (%)	*p*-Values
Median age in months (range)	18 (0–137)	17.5 (1–75)	0.994
Median hospital stay in days (range)	3 (1–8)	3.5 (2–17)	0.412
Median oxygen requirement in days (range)	1 (0–7)	1 (0–14)	0.555
Wheezing	66/84 (79)	14/24 (58)	0.046*
Retractions	50/84 (60)	9/24 (38)	0.056
Fever	21/84 (25)	11/24 (46)	0.049*
Tachypnea	66/84 (79)	21/24 (88)	0.396
Stridor	10/84 (12)	1/24 (4)	0.449
C-reactive levels (range)	10.5 mg/l (3–205)	45.9 mg/l (3–370)	0.009*
White blood cell count (range)	13.0×10^9 cells/l (4–36)	17.5×10^9 cells/l (7–187)	0.096
Antibiotic therapy	18/84 (21)	8/24 (33)	0.280

* *p*-Value is statistically significant.

pare the clinical manifestations of infections detected by both IF and xTAG multiplex PCR with those of infections detected only by xTAG multiplex PCR. We focused our study on respiratory picornavirus monoinfection in otherwise healthy children. So far, no other study has investigated this question.

3. Study design

3.1. Patients and specimens

Nasopharyngeal aspirates (NPA) were collected from all children ≤16 years of age who were admitted to the emergency room of the University Children's Hospital in Bern, Switzerland and were subsequently hospitalized due to an acute respiratory tract infection between November 1st 2006 and October 31st 2007. The specimens were prospectively tested by IF for respiratory picornaviruses (enterovirus and rhinovirus), adenovirus (ADV), respiratory syncytial virus (RSV), parainfluenza virus 1–3 (PIV 1–3), influenzavirus A (IFA), influenzavirus B (IFB), and human metapneumovirus (HMPV). For the study, all picornavirus IF positive samples and 100 IF negative NPA samples were further tested by an xTAG respiratory viral panel, a multiplex RT-PCR including respiratory picornaviruses, ADV, RSV, PIV 1–4, IFA, IFB, HMPV, and coronaviruses, as recently described,[11] provided there was frozen material left. All children with viral co-infections ($n=10$) or comorbidities ($n=133$) were excluded, resulting in a homogenous group of 108 otherwise healthy children (mean age 18 months; range 9 days–137 months; 50 female) with respiratory picornavirus monoinfection. These children were evaluated by blinded chart audits of clinical data and later classified as group 1 (IF and PCR positive) or group 2 (IF negative and PCR positive). Blood cultures were not routinely taken.

3.2. Immunofluorescence

NPA samples were processed and stained as formerly described.[11] Light Diagnostics (Chemicon International, Millipore, Temecula, CA) Respiratory Viral Screen DFA and single fluorescein-conjugated monoclonal antibody and Diagnostic Hybrid DFA Metapneumovirus Identification Kit were used to detect ADV, RSV, PIV 1–3, IFA, IFB and HMPV, respectively, according to the manufacturer's instructions. To detect respiratory picornaviruses, specimens were tested with Light Diagnostics Pan-Enterovirus Reagent "Blend".

3.3. xTAG respiratory viral panel

RNA and DNA were extracted with EasyMAG extractor (bioMérieux) using a generic 1.0.6 protocol. The xTAG respiratory viral panel assay was carried out according to the manufacturer's instructions. The detection of the amplicons was done on the Luminex IS 200 instrument. For a detailed description of the method, see Merante et al.[12] Raw data output files consisting of median fluorescence intensities for all viruses and subtypes were interpreted using the TDAS RVP-I 1.10 software (Luminex Molecular Diagnostics).

3.4. Statistical analysis

Statistical analysis was carried out using the Graph Pad Prism Software tool®.

4. Results

Eighty-four of 108 patients (78%) were both IF and xTAG multiplex PCR positive (group 1), and 24/108 patients (22%) were IF negative and xTAG multiplex PCR positive (group 2). As shown in Table 1, there was no significant difference between groups regarding age, length of hospital stay and length of oxygen requirement. Group 1 patients showed significantly more often wheezing and more often retractions when compared with group 2 patients, although the latter difference did not reach statistical significance. In contrast, group 2 patients were significantly more often febrile and showed significantly higher median CRP counts. Further, there was a trend in group 2 patients towards higher white blood cell levels and more frequent use of antibiotic therapy.

As shown in Table 2, wheezy bronchitis was more often diagnosed in group 1 compared with group 2. In contrast, group 2

Table 2
Comparison of clinical diagnosis of group 1 (IF and PCR positive) and group 2 (IF negative and PCR positive) children with respiratory picornavirus monoinfection.

	Group 1 patients (IF+/PCR+) $n=84$ (%)	Group 2 patients (IF−/PCR+) $n=24$ (%)	*p*-Values
Wheezy bronchitis	60/84 (71)	11/24 (46)	0.028*
Pneumonia	5/84 (6)	4/24 (17)	0.014*
Upper respiratory tract infection	14/84 (17)	6/24 (25)	0.378
Bronchiolitis	0/84 (0)	1/24 (4)	0.222
Other diagnosis	5/84 (6)	2/24 (8)	0.487

* *p*-Value is statistically significant.

patients were significantly more frequently diagnosed with pneumonia.

5. Discussion

Our study investigated the differences between clinical manifestations of respiratory picornavirus infections detected by both IF and xTAG multiplex PCR with those of respiratory picornavirus infections detected only by xTAG multiplex PCR in hospitalized children. Group 1 children (IF and PCR positive) developed wheezy bronchitis significantly more often when compared to group 2 children (IF negative and PCR positive). However, group 2 children were diagnosed significantly more often with pneumonia accompanied by higher body temperature and higher levels of C-reactive protein.

We have previously shown that IF is a rapid and inexpensive method for the detection of respiratory picornaviruses.[11] Here, we show that detection of respiratory picornaviruses by IF correlated well with the clinical findings of wheezing illness, the most frequent manifestation of respiratory picornavirus infections in children in a hospital setting.[2,13,14] The unexpected finding of a more frequent diagnosis of pneumonia in children with negative picornavirus detection by IF but positive detection by PCR needs to be addressed. Viral pneumonia in children has been associated with rhinoviruses.[15] However, their role in the etiology of lower tract infections is subject to debate. The presence of respiratory picornavirus RNA does not necessarily prove a cause–effect relationship and combined viral–bacterial infections are possible.[15] In our study the higher C-reactive protein levels in children who were picornavirus positive only by PCR suggest that some of their symptoms may have been caused by secondary bacterial superinfection[16–18] and that the highly sensitive xTAG multiplex PCR detected also past symptomatic or asymptomatic viral infections. It has been shown that enterovirus and rhinovirus RNA can persist for several weeks after the onset of symptomatic respiratory infection[6,19] and is detected in a substantial proportion of children without respiratory symptoms.[19–22] Thus, the relative insensitivity of IF might be a benefit for clinical practice.[11,23,24] As our clinical data were analyzed retrospectively and as we did not collect blood cultures routinely we cannot provide any data to support the hypothesis of a possible bacterial superinfection in these children, but predisposition to bacterial infection after viral infection is well-known.[15]

We evaluated the clinical course of respiratory picornavirus monoinfections in healthy children by excluding all children with co-infections and co-morbidities. We found that picornavirus infections were associated with substantial clinical and economic impacts as shown by a median hospital stay of 3 days and a median oxygen requirement of 1 day, implying the need for a fast and inexpensive detection method of these viruses.

We conclude that in our study population IF appears to be a rapid, inexpensive and reliable detection method for respiratory picornavirus that correlates well with the clinical presentation of wheezy bronchitis and therefore can be recommended for clinical application. The detection of respiratory picornavirus RNA in NPA of children during acute respiratory infection by molecular methods is expensive and may perhaps result in the discovery of a non-causative remnant of a prior symptomatic or asymptomatic infection.

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