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Infectious Pathogens that Could Trigger Cough in Children Outside Winter Epidemic Season

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ABSTRACT

Background: *Children's cough is the most prevalent reason for consultation with doctor. For this age group most of the trigger factors are attributed to infectious pathogens. During epidemic seasons the causative agents are quickly identified and unified treatment guidelines are easily created and covered. Outside epidemic season however the etiological profile could be in a very broad spectrum and thus could hamper the fast appropriate management.*

Methods*: A real life observational study for 6 months (outside winter) on 74 children divided in 4 groups: 24 children with bronchial asthma (BA), 20 with chronic wet cough (CWC), 24 with bronchiolitis and bronchitis (AB) and 10 healthy children (HC) as a control. We collected serum, nasopharyngeal and deep throat swabs from specific pathogen detection (culture examination, PCR, ELISA).*

Results: *In the HC we didn't identify any pathogens in the throat samples. In 20% of the nasal swabs we cultured Staphylococcus aureus. In 33% of the patients from the AB group we found only viruses (RSV, hRV and hMPV), in 25% we found combined infection with virus and bacteria (mainly Moraxella catarrhal is and Streptococcus pneumoniae). The BA group in 25% we found only viruses (Adenovirus, hRV and RSV). In 56% of the cases Streptococcus pneumoniae was confirmed in the throat swabs vs. only 33% for isolated Moraxella catarrhal is. Only in 10% of the CWC group we found viral infection (hMPV, Adenovirus and RV), 50% had Streptococcus pneumoniae and the rest 40% - polymicrobial etiology incl. S.aureus, H.influenzae, S. pyogenes, E.aerogenes.*

Conclusion: *For immunocompetent children outside winter seasons the most common infectious cough triggers are M.catarrhalis, S.pneumoniae, hRV, RSV and Adenoviruses.*

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Keywords: *chronic wet cough, bronchial asthma, immunocompetent children*

INTRODUCTION

One of the main symptoms for which parents seek medical attention is cough of their child. The dry cough usually accompanied by expectoration could be very frightening for the parents, for in their mind (providing they are not medical specialist) it is a sign of pneumonia – which for a small child could be dreadful. Such cough however besides pneumonia is also typical for bronchitis, conchiectasis, sinusitis or congenital anomalies of the respiratory system [1].

As a general rule, in most patients the acute cough is self-limiting and is easily coped with any antitussive medications, often without the intervention of a doctor. An acute cough with infectious trigger could be first symptom of bronchitis or bronchiolitis [1]. The availability of molecular-detection techniques has made it possible

to identify a diverse group of viruses that are capable of causing bronchiolitis – RSV, hRV, parainfluenza virus, hMPV, Corona virus, Adenovirus, IV, Enterovirus, Human bocavirus (copathogen in bronchiolitis). There is no evidence has been found for a primary role of bacteria as a cause of bronchiolitis, although Bordetella pertussis, Chlamydia trachomatis, Mycoplasma pneumoniae Haemophilus influenzae and Streptococcus pneumoniae are also found in children with acute lower respiratory tract infection [2].Persisting cough is almost always a sign for an underlying disease. Though difficult to determine the exact problem, which had provoked the cough, the most common triggers are cigarette smoke exposure, postnasal drip, and asthma or gastroesophageal reflux [1].

The most common cause of chronic wet cough in preschool children is protracted bacterial bronchitis (PBB) [3-4]. PBB is caused by bacterial respiratory tract infection in otherwise healthy children. If left without treatment, the persisting end bronchial infection is predisposition for bronchiectasis formation. For example in the study of Wurzel et al. has been established that the isolation of H influenzae. (From broncho-alveolar lavage) increases over 7 times the risk for bronchiectasis (OR 7.55; 95%, CI: 1.66 – 34.28; p = 0.009) compared to children without H. influenzae[5].

There are much aetiology that may lead to an asthma exacerbation including respiratory infection (bacterial/viral), allergens, irritants and occupational exposures. From the infection triggers the most commonly reported to be related to exacerbation are human rhinovirus (hRV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza virus (IV), Mycoplasma pneumoniae and chlamydia pneumoniae [6]. It was suggested that some asthmatic subjects have a dysfunctional antiviral response, and this abnormality may make them more susceptible to the consequences of an infection [6].

During epidemic seasons the causative agents are quickly identified and unified treatment guidelines are easily created and covered. Outside epidemic season however the etiological profile could be in a very broad spectrum and thus could hamper the rapid and appropriate management and thus possibility for long-term consequences arise. Therefore, we aimed to describe the most common infectious triggers of cough in Bulgarian children outside the winter season.

Material and Methods

Patients

The study material comprised the data from 74 children (36 females and 38 males, aged 1.5 – 11.9 years) – 10 healthy controls and 64 patients. The inclusion criteria that were met by all patients was acute episode of cough, for which the parents sought advice from pediatric pulmonologist for a period of six months (April-October 2018) – outside winter epidemic season. All patients with prior antibiotic use, with known immune-deficiency, cystic fibrosis, bronchiectasis or other congenital chronic lung diseases were excluded. We excluded also all patients that had X-ray changes suggestive for pneumonia. The patients were divided in 3 groups – bronchial asthma (BA), acute bronchitis/bronchiolitis (AB) and chronic wet cough (CWC). The patients from the BA group – 24 children, had confirmed bronchial asthma by pediatric pulmonologist, and have been on controller therapy with inhaled corticosteroids. The samples were obtained during exacerbation episode of the asthma (with excluded allergen exposition or weather changes as triggers for the exacerbation).

Twenty four children with acute lower respiratory tract infection (LRTI) classified either as bronchiolitis or bronchitis was included in AB group. None of these children had any family or personal history for bronchial asthma or allergy. For most of them it was their first episode of LRTI, the patients with more than two previous episodes to LRTI were not included in the study. The last group of patients – CWC, comprised by 20 children with wet coughs lasting more than 3 months, without any personal or family history for bronchial asthma or allergy.

All children with CWC over the age of 5 had undergone spirometry with bronchodilator response test for excluding reversible bronchial hyperesponsivness. The healthy controls (HC) children were chosen to best match the gender and age distribution, as the patients, also they too had no personal or family history for asthma. All of the tests were performed after obtaining a signed informed consent of the legal guardian.

There was no personal patient information in the database. The Hospital's Ethics Committee granted study approval. The therapy prescribed was as all the clinical practice and guidelines approved, according the isolated pathogen.

Clinical, Imaging and Laboratory Data Methods

Clinical information, including demographic characteristics, immunization record, and history of illness, symptoms, the onset of the cough, medication record and onset of exacerbation was documented on case report forms.

X-Ray Confirmation

All patients had chest X-ray done in the process of evaluation for their cough, reviewed by senior specialist in imaging diagnosis and confirmed absence of any imaging signs of pneumonia. The radiologist assigned mostly reports as bronchial obstruction or no pathological findings.

Microbiology

We collected nasopharyngeal and deep throat swabs. All the samples were obtained by a pediatrician and then right away sent to the laboratory in a transport medium. The standard protocol for microbiological diagnostic follow-up and tests was followed in the laboratory. For isolation and detection of the rigorous etiologic bacterial agents we used routine nutrition media such as Blood agar base supplemented with 5% sheep blood, MacConkey agar, selective media for Bordetella, Candida chrome agar and developed and implemented by our team selective media chocolate agar with vancomyc in for Moraxellae and Haemophilae, for their identification, as well as a quantitative method for correct evaluation of the clinical significance of isolates [7].The cultivation of inoculated samples on different media was performed in aerobic and in microaerophilic atmosphere respectively for various pathogens. For identification of the isolated clinical strains we used mainly the products and systems by Crystal BD BBL (BBL; Becton, Dickinson, Germany) and RapID System Remel Thermo Fisher Scientific (Remel, Thermo Fisher Scientific Remel Products; Santa Fe, USA).

All bacterial isolates in a microbial number over the critical one for an infectious process (>100 000 CFU/mL), were considered as significant [7]. Susceptibilities to investigated pathogens were determined by Kirby-Bauer disk-diffusion method. Due to the necessity for some peculiar cases and for a representative sample strain, minimal inhibitory concentration according the criteria of CLSI were measured [8]. Isolated Streptococcus pneumoniae strains were serogrouped using the latex agglutination method (Pneumotest-Latex kit, Statens Serum Institute-SSI, Copenhagen, Denmark) as previously described [9]. Additionally nasopharyngeal swabs were obtained for Real-Time Amplification test for the qualitative detection of Mycoplasma pneumoniae and Chlamydia pneumoniae - (Kit Mycoplasma pneumoniae / Chlamydophila pneumoniae Real-TM, Sacace Biotechnologies Srl) following the manufacturer instructions.

For viral detection combined nasal and pharyngeal specimens were collected from the enrolled patients by means of commercial polyester collection swabs (Deltalab, Spain). Following collection, swabs were stored at 4 °C for up to 72 hrs. Specimens were processed immediately or stored at −80 °C prior to analysis. Viral nucleic acids were extracted automatically from 500 µl of each respiratory specimen in a final eluate volume of 75 µl using an ExiPrep Dx Viral DNA/RNA kit (BioNeer, Korea) in accordance with the manufacturer'

s instructions. Detection and typing/sub typing of influenza viruses were carried out by a real time RT-PCR method and the Superscript III Platinum ® One-Step qRT-PCR System (Nitrogen, Thermo Fisher Scientific, USA) as previously described [10]. The detection of RSV, hMPV, PIV 1/2/3, RV and AdV was performed using singleplex real time PCR assays and an AgPath-ID One Step RT-PCR kit (Applied Bio systems, Thermo Fisher Scientific, USA). The primers, probes and PCR conditions used in the study were identical to those previously described [11]

Inflammatory Indices

Complete blood count with with manuallyverified differential count as well as CRP was performed in all children. For CRP detection (positive test 6 mg/L or higher) we used serums collected by standard procedures and slide agglutination test C-Reactive protein (CRP) latex (BioSystems S.A.; Costa Brava, Barcelona, Spain).

Statistical Analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, Version 19.0. (SPSS Inc.; Chicago, IL, USA). Chi Square was used for testing relationships between categorical variables. We considered p values of ≤ 0.05 to indicate statistical significance.

Results and Discussion

The 10 healthy children were 5 females and 5 males with mean age of 5.21±1.4 years. From the 64 patients (31 females and 33 males, aged 1.5 – 11.9 years) the younger group was expectedly the children from AB group – 24 children (male: female = 10:10) – 3.83±1.6 years, followed by the CWC group – 20 children (male: female = 9:11) – 5.43±0.75 years and the ones from BA group had

highest mean age 6.05 ±0.8 years – 24 children (male: female = 14:10), the differences were not statistically significant (p=0.07).

On the X-ray images the AB and BA patients all had bronchial obstruction, while the patients from CWC group were all without pathological findings. From ethical point of view we didn't perform X-ray to the healthy controls.

The inflammatory indices in all patients were within normal limits. None of the children had positive result for Bordetella pertussis, Mycoplasma and influenza or para influenza virus. Perhaps due to excluded cases with pneumonia and the season we obtained the samples. Although we had expected some patients with Mycoplasma pneumonia to be identified [12].

In the HC we didn't identify viral pathogens or any bacterial colonization in the throat samples. In 20% of the nasal swabs we cultured Staphylococcus aureus. All viral samples also came clean. We have expected at least half of the children to carry S.aureusand some Streptococcus pneumoniae, as it was previously published[13].We presume that our data is due to described age effect on the carriage for S.aureus which is expected around 10 years and the age of our HC group, which was selected to match the patients groups [13].

In 33% of the patients from the AB group we found only viruses – RSV, hRV and hMPV, in 25% we found combined infection with virus and bacteria. Isolated bacteria -mainly Moraxella catarrhalis and S.pneumoniae had equal prevalence of 33% of the children in this group. Only one child had S. aureus, found in the nose sample but not in the throat swab, as in our control group. We couldn't find a study stating the bacterial etiology in obstructive bronchitis or bronchiolitis in children, but the articles about viral infections [14-15]. The one we found states the importance of Mycoplasma, Chlamidia and Bordetella, none of which we have identified in our patients [16].

In the BA group in 25% we found virus infection only predominantly Adenovirus, followed by hRV and RSV. Which comes additionally to show the importance of viral tests in every asthma exacerbation, which could avoid unnecessary antibiotic perscriptions [17-19]? In 56% of the cases S. pneumoniae was confirmed in the throat swabs vs. only 33% for isolated M. catarrhalis. There were no other bacteria isolated in BA group. These results could be expected since the infection related asthma is more common during preschool and early childhood (as our patients group), also viral-induced exacerbations, and increases in prevalence during both fall and spring are reported [20]. We also found in 10% of the patients co-infections between hRV and bacteria. These results are concordant with previously publish data for asthma exacerbations [21-22].

Only in 10% of the CWC group we found viral infection mainly hMPV, followed by Adenovirus and hRV. There is other studies supporting the role of viruses in patients with PBB [23], but viral only infection is a rarity for this group. Half of the children had *S. pneumoniae* isolated (predominantly non-vaccine serogroups 76% vs. 24% for vaccine strains) and the rest 40% - polymicrobial etiology incl. *S.aureus, H.influenzae, S. pyogenes, E.aerogenes* was found. The most commonly described pathogen in PBB is *H.influenzae,* but in our children we detect if only in a few patients in combination with other bacteria, maybe because most of the published study relay on invasive sampling procedure as broncho-alveolar lavage [24-25].

Conclusion

Our study through expanded diagnostic investigations aimed to define the etiological profile of the pathogens that can be infectious triggers for acute cough in healthy children and in children with asthma or chronic cough. Although a significant part could be attributed to viruses, in international studies was found that up to 60% of young children with acute respiratory infection are treated with antibacterial agents [27]. Overuse of antibiotics and inappropriate prescriptions can increase the virulence of pathogens, increasing the damage to the wall of the airway and potentiated the rise of antibiotic resistance. The unnecessary antibiotic usage may be reduced through patient and physician education and rapid detection of the causative pathogen [28-29].

Nevertheless the recommendable antibiotic strategy,, wait before usage and see" for young children, is not advisable for children with CWC since only in 10% there are viruses isolated and in about 40% they have polymirobial flora found, thus antibiotics should be given for these children as soon as possible.It is essential to confirm any possible infectious pathogen and the associated conditions for the so called "hard-to-treat patients". The presence of airway inflammatory response can result in irreversible damage to the structure of the lung and even under the most restrictive conditions, if not treated.

We can conclude that outside winter epidemic seasons in Bulgarian children the pathogens we should be aware of and treat are mainly *M.*

*catarrhalis and S. pneumoniae (*non vaccine serotypes*)*from bacteria species and hRV, RSV and Adenoviruses as viral cough triggers.

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