NASAL CYTOLOGY AND RESPIRATORY TRACT INFECTIONS: AN OBSERVATIONAL STUDY ON 1004 CHILDREN

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ABSTRACT

Background. Health balances, which are conducted by Pediatricians on children from 1 month up to 14 years of life, have been introduced in Italy to promote the patients' health, guaranteeing adequate interventions of health education and prophylaxis of infectious diseases. Although acute respiratory tract infections (RTIs) cause a high burden of disease among children, there are no precise indications regarding the evaluation of the health of the upper airways during health balances.

Methods. We recruited 1004 children, aged between 1 month and 13 years, who underwent health balances by Free Choice Pediatricians (PLS). We evaluated the association between a history of recurrent respiratory tract infections (rRTIs) and nasal cytology findings.

Results. 340 patients (33.9%) showed normal findings at nasal cytology, while 664 showed altered findings, such as the presence of bacteria (250 cases, 24.9%), inflammatory infiltrate (277 cases, 27.6%) or both (137 cases, 13.6%). Pearson Chi-Square Test did not showed a statistically significant association between the rRTIs and immune phlogosis (p= 0.684).

Conclusions. Although no statistically significant association was found between rRTIs and detection of bacteria or immuno phlogosis on nasal cytology, nasal cytology could be routinely included in health balances to non-invasively identify children with immune phlogosis and healthy carriers of pathogens.

INTRODUCTION

Health balances have been introduced in Italy, on an experimental basis, in 1996 according to the National Collective Agreement and, than, officially confirmed in 2000 and organically inserted in the "Health-Childhood" project. This project consists of a basic plan that provides at least six health balances, conducted by Pediatricians on children from 1 month up to 14 years of life, planned to guarantee adequate interventions of health education and prophylaxis of infectious diseases, in order to promote the patients' health, defined not only as the absence of disease, but rather as the achievement of a state of physical, mental and social well-being. These clinical examinations provide for the evaluation of anthropometric data, motor skills, posture, hearing and sight. However, there are no precise indications regarding the evaluation of the health of the upper airways.

Since acute respiratory tract infections (RTIs) are considered among the most common reasons for visiting healthcare practitioners and prescribing antibiotics to children, also causing absenteeism from school and work, effective prevention and treatment of RTIs remain a high priority worldwide[1,2]. In this context, the understanding of the factors increasing susceptibility to RTIs remains challenging. The upper respiratory tract microbiota could explain differential susceptibility of RTIS and modulate the severity of the infections[3]. While microbiota-based diagnostics typically require nasopharyngeal aspirates and real-time PCR, nasal cytology has proven to be a valuable non-invasive and cheap tool in the assessment of nasal pathologies in children, evaluating both the nasal inflammatory infiltrate and the presence of bacteria and fungi in the nasal mucosa[4].

Based on this background, the aim of our study was to evaluate the relationship between nasal cytology findings and recurrent RTIs, with a view to including nasal cytology in health balances.

MATERIALS AND METHODS

We recruited 1004 consecutive patients, including 579 males (57.67%), who underwent health balances by Free Choice Pediatricians (PLS) in the territory of Bari Local Healthcare Service. Inclusion criteria were arranged as follows: age range: 1 month-13 years; genders: both; pathology: children in apparent good health. Specific exclusion criteria were ongoing acute RTIs and anatomic sinonasal disorders. During the health balance, we carefully examined patients' clinical history, paying greater attention to recurrent respiratory tract infections (rRTIs), defined as ≥6 episodes of RTIs per year or ≥1 upper RTIs per month from September to April or ≥3 lower RTIs per year[5]. Moreover, we performed nasal cytology under anterior rhinoscopy. Cytological

samples were collected by Nasal Scaping® (EP Medica, Italy), from the middle part of the inferior turbinate, and immediately placed on a glass side, fixed by air drying and stained with May-Grunwald-Giemsa (MGG). Then, after staining, samples were read at optical microscopy, with a 1000x magnification. A minimum of fifty fields was considered to identify a sufficient number of cells[6]. The presence of bacteria and of nasal inflammatory infiltrate at nasal cytology samples were evaluated.

Informed written consent was obtained from the parents/guardians of all participants. The study was approved by the local Ethics Committees.

The association between nasal immune phlogosis and rRTIs was evaluated using Pearson Chi-Square Test. A p value of ≤ 0.05 was considered statistically significant.

RESULTS

Among the recruited patients, 579 patients (57.67%) were males (mean age 5.4 years). 425 patients (42.33%) were females (mean age 5.2 years). ***Table 1*** shows the distribution of patients by age. 340 patients (33.9%) showed normal findings at nasal cytology, while 664 showed altered findings, such as the presence of bacteria (250 cases, 24.9%), inflammatory infiltrate (277 cases, 27.6%) or both (137 cases, 13.6%) (***Table 2***). ***Table 3*** shows nasal cytology findings subdivided by age groups. The history of recurrent URTIs was investigated (***Table 4***). ***Table 5*** show the distribution of patients with low, mild and severe immune phlogosis and the relationship with the history of rRTIs. Pearson Chi-Square Test did not show a statistically significant association between the rRTIs and immune phlogosis (p= 0.684).

DISCUSSION

The introduction of health balances in the clinical practice of Italian Pediatricians has allowed to monitor the children's health over time as well as to prevent and/or possibly diagnose several diseases early, also paving the way for studies aimed at understanding the etiopathogenesis and predisposing factors of the different pathologies. In this context, we wondered if nasal cytology could be useful within health balances for identifying children most likely to develop rRTIs. As a matter of fact, nasal cytology is a non-invasive and reproducible diagnostic tool, which can be easily carried out on children to determine the characteristics of their nasal mucosa, particularly regarding the presence of infiltrating inflammatory cells and pathogens[7]. Interestingly, as shown in the results, only 340 (33.9%) of the children in apparent good health showed normal nasal cytology findings. On the contrary, most of them (n= 664, 66.1%) showed altered findings, such as the presence of bacteria (n= 250, 24.9%), immune phlogosis (n= 277, 27.6%) or both (n= 137, 13.6%), therefore deserving topical therapies[8]. Notably, the percentage of children with immune phlogosis on nasal cytology was close to the incidence of allergic diseases, which affect about 20% of the worldwide population, especially children[9]. Rhinocytograms of allergic patients are characterized by "minimal persistent Inflammation", which consist in the persistent infiltration of neutrophils and in minimal part of eosinophils, in case of perennial rhinitis, and by eosinophilic-mast cell degranulation in case of season rhinitis, if the patient is examined during the pollen season[10]. Hence the importance of investigating children with nasal immune phlogosis from an allergologic point of view, in order to identify and treat

allergic patients, avoiding the progression of the allergic march[11].

As far as the presence of bacteria is concerned, it should be emphasized that patients subjected to the health balances were in apparent good health from a clinical point of view. Thus, the presence of bacteria should not be considered as an ongoing acute infection but rather the demonstration that in the so-called "healthy carriers" the nose and nasopharynx can be colonized by bacteria, such as S. aureus[12]. The latter is a common asymptomatic constituent of the natural bacterial flora, which predominantly colonizes the mucous membranes of the upper respiratory tract, such as throat and nose. Approximately 20% of humans are persistent S. aureus nasal carriers, whereas 30% are intermittent carriers. Children are more commonly persistent carriers than adults and more than 70% of newborn babies' nasal samples are positive for S. aureus at least once[13]. Nasal colonization favors the maintenance of the microorganism in a population, whereas asymptomatic carriage, especially chronic, serves as a major reservoir for the strain assisting its spread in environment[14]. In pediatric carriers, hypervirulent S. aureus strains could cause subsequent infections, since an immature host immune system plays a vital role in thwarting the invasive process and eliminating the pathogens[15]. Interestingly, the finding of bacteria on nasal cytology was not associated with an increased risk of rRTIs. Indeed, the percentage of children with a history of rRTIs was quite homogeneous in the different groups, regardless of nasal cytology findings and degree of immune phlogosis (***Tab 4-5***). This percentage, approximately 80%, was higher than data in the literature, according to which RRTIs affect up to 25% of children aged <1 year and 18% of children aged 1 to 4 years, probably because parents of children suffering from rRTIs tend to be more adherent to health balances[16]. However, our results were unexpected precisely since we expected that the colonization of the nasal mucosa by bacteria, possibly associated with cellular inflammatory infiltrate, could predispose to acute or recurrent RTIs[17]. The absence of a statistically significant association between rRTIs and nasal cytological altered findings has highlighted the greater influence of systemic risk factors compared to local ones. As a matter of fact, it is not only the environmental exposure to infectious agents but rather a virgin immune system that predisposes to rRTIs, together with other risk factors including atopy, allergy, pollution and exposure to secondhand smoke[16]. Indeed, although the majority of patients with rRTIs do not have any recognizable immunodeficiencies or other pathologies, some of them may have mild and non-specific deviations in selected immune parameters that are an expression of immature immunity, or transient immune function decline[18]. Therefore, we believe that these results had brought to light the important social problem represented by precocious socialization: rRTIs are essentially the consequence of an increased exposure to infectious agents during the first years of life, when immune functions are still largely immature[19]. As Table 3 shows, the greatest percentage of patients with bacteria and immune phlogosis was found precisely in the age group between 1-6 years, when the immune system is still considered mature and the transmission of pathogens takes place from child to child typically in kindergartens. Indeed, children attending day-care centers have a higher risk of develop rRTIs[20]. As proof of this, as the Covid-19 pandemic which has required social distancing and the closure of schools,

preventing children from coming into contact with other people and visiting places that expose them most to infectious risk has been shown to greatly improve children's health, particularly regarding the upper airway, even resulting in modifications in medical and surgical therapeutic indication[21]. Thus, the exposure of children to pathogens at school, possibly transmitted by healthy carrier, determines a greater risk of developing rRTIs regardless of the findings in nasal cytology, whether normal or altered.

CONCLUSIONS

Although no statistically significant association was found between rRTIs and detection of bacteria or immuno phlogosis on nasal cytology, we believe that nasal cytology could be routinely included in health balances to non-invasively identify children with immune phlogosis. Specifically, children with nasal immune phlogosis should undergo an allergologic evaluation, both to identify allergic and non-allergic rhinitis, such as non-allergic rhinitis with eosinophils (NARES) and non-allergic rhinitis with eosinophils and mast-cells (NARESMA), which are still poorly diagnosed but which require suitable treatments[22]. On the other hand, the discovery of bacteria colonizing the nasal mucosa of clinically healthy children should indicate the condition of healthy carriers, responsible for the maintenance of the bacteria in the population, and therefore deserving of therapies aimed at nasal decontamination[23]. In this context, school environments represent the primary source of transmission of pathogens for the presence of several carriers, especially among children under the age of 6, who have an immature immune system.

TABLES

TABLE 1.

Age, years	*Frequency, n*	*Percentage, %*
1	65	6,6
2	94	9,5
3	146	14,8
4	157	15,9
5	143	14,5
6	85	8,6
7	82	8,3
8	51	5,2
9	46	4,7
10	43	4,4
11	32	3,2
12	21	2,1
13	21	2,1
Tot	986	100,0
Missing values	18	
	1004	

TABLE 1. Distribution of patients by age.

TABLE 2. Nasal cytology findings.

Nasal cytology findings	*Frequency, n*	*Percentage, %*
Normal	340	33,9
Bacteria	250	24,9
Immunophlogosis	277	27,6
Immunophlogosis + Bacteria	137	13,6
Tot	1004	100,0

TABLE 2. Nasal cytology findings.

TABLE 3. Nasal cytology findings subdivided by age groups.

	Age groups, n (%)						***Tot***
	< 1	1-3	4-6	7-9	10-12	13-15	
Normal	7 (2.1)	94 (27.6)	133 (39.1)	53 (15.6)	45 (13.2)	8 (2-4)	340 (100)
Bacteria	9 (3.6)	71 (40.0)	100 (40.0)	52 (20.8)	10 (4.0)	8 (3.2)	250 (100)
Immunophlogosis	15 (5.4)	67 (24.2)	106 (38.3)	49 (17.7)	26 (9.4)	14 (5.1)	277 (100)
Immunophlogosis + Bacteria	3 (2.2)	42 (30.7)	46 (33.6)	25 (18.2)	15 (10.9)	6 (4.4)	137 (100)
Tot	34	274	385	179	96	36	1004

TABLE 3. Nasal cytology findings subdivided by age groups.

TABLE 4.

	rRTIs		***Tot***
	Yes, %	***No, %***	
Normal	77,4	22,6	100,0
Bacteria	80,0	20,0	100,0
Immunophlogosis	78,7	21,3	100,0
Immunophlogosis + Bacteria	83,2	16,8	100,0
Tot	79,2	20,8	100,0

TABLE 4. Relationship between history of rRTIs and nasal cytology findings.

TABLE 5.

Grade of immunophlogosis	*rRTIs*		*Tot*
	Yes, %	*No, %*	
Low	77,8	22,2	100,0
Mild	80,9	19,1	100,0
Severe	82,1	17,9	100,0
Tot	80,1	19,9	100,0

TABLE 5. Relationship between grade of immunophlogosis and history of rRTIs.

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