"Do we Need to Investigate Respiratory Virus in Hospitalized Infants with Suspected Pertussis?"

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Introduction

Respiratory virus (RV) and *Bordetella pertussis* (BP) are common etiological agents of respiratory infections in infants during the first year of life. Those infants younger than 6 months, still partially immunized for BP, are also the most susceptible to severe viral infections leading to hospitalization, such as in cases of bronchiolitis caused by respiratory syncytial virus [1,2]. Although some striking clinical characteristics of pertussis are common in older children and adults, in these infants the clinical picture may be less typical. Co-infection with RV and BP can also occur, due to co-circulation of these agents and the susceptibility of infants [1,3,4].

In a previous retrospective study, the authors pointed to the need to investigate RV in infants with suspected pertussis [4]. In this short communication, preliminary results of a prospective cohort study that compares the characteristics of these different etiologies are presented. The results can contribute to strategies of prevention, diagnosis and treatment of respiratory infections in infants.

Material and Methods

The prospective study included all hospitalized infants less than one year of age with suspected pertussis, between June 2014 and December 2015. The criterion of inclusion to select pertussis suspected cases was based on the orientation of the São Paulo State Health Department. According to the health department the definition of suspected case of whooping cough is: "any person without other apparent cause and regardless of immunization status with dry cough for at least two weeks, followed by one of the following manifestations: paroxysmal cough, inspiratory winch and vomiting after coughing". We reduced to ten days or more of cough to increase the sensibility for inclusion. Those children that have used macrolides or other antibiotics and viral agents prior to diagnoses were excluded. The Ethics Committee of the Hospital approved this study.

Following a standardized protocol, we collated demographic, clinical, laboratory, and outcome data. According to the service routine, all these patients had nasal swab for BP identification (culture techniques and polymerase chain reaction (PCR), standardized at the Adolfo Lutz Institute [5]) and nasopharyngeal secretions for RV identification (indirect immunofluorescence assay) collected at admission.

The patients were divided into two groups, with single agent detection, for comparison: the group with pertussis (BP) and the group with respiratory virus (RV). The clinical/laboratory characteristics and the severity of infection and treatment were compared.

Statistical analyses

A convenience sample was analyzed. For descriptive analysis, the categorical variables are presented as total numbers and percentages and continuous variables as averages and standard deviation. Comparative analysis of categorical variables using the chi-square test and the t test, considering significant $p=0.05$ was performed. The variables with $p<0.20$ in the univariate analysis were subjected to multivariate analysis model. The positive and negative predictive values for outcomes "BP infection" and "RV infection" were calculated.

Results

We reported 74 suspected cases of whooping cough between June 2014 and December 2015. During this period, 59 children were included in the study, according to the inclusion criteria. Two cases were excluded due to use of macrolide prior to diagnosis. There was a loss of 7 patients, because collecting BP test and RV test were not possible. Fifty patients were analyzed: 14 patients presented positive BP and 13 presented positive viral test (10 respiratory syncytial virus, 2 parainfluenza-3 and 1 influenza), all of them as single infections. Three samples

presented simultaneous detection of BP and RV (respiratory syncytial virus, parainfluenza virus and adenovirus); therefore co-detection with RV occurred in 17.6% cases of laboratory confirmed pertussis. In 20 patients (38%) no etiologic agent could be identified. The clinical, demographic data and comparative analysis are presented in Tables 1-3. All infants were born at term and showed no comorbidities.

Table 1: Descriptive analysis of 50 infants with suspected pertussis, *Cell count in peripheral blood; **Pulse oxymetry; BP= *Bordetella pertussis*; VR=Respiratory virus; ***All doses of vaccines recommended for age according to Brazilian Immunization Program.

CLINICAL AND DEMOGRAPHIC DATA	
	N (%)
Male	30 (60)
Cyanosis	42 (84)
Vomiting	39 (78)
Dyspnea	36 (72)
Wheezing	22 (44)
Apnea	18 (36)
Fever	17 (34)
Inspiratory whoop	16 (32)
Anorexia	10 (20)
mean (standard deviation)	
Age - days	93.9 (60.3)
Leukocytes*(n/mm^3)	19750 (21706)
Lymphocytes *(n/mm^3)	9479 (8340)
BACKGROUND	
	N (%)
Contact with smokers	18 (36)
Updated Immunization ***	25 (50)
Maternal BP immunization	02 (04)
Nursery	02 (04)
Breast-feeding	35 (70)
mean (standard deviation)	
Number of people at home	4.3 (+/- 1.5)
Number of brothers	1.5 (+/- 1.4)
THERAPEUTIC AND OUTCOME	
	N (%)
Intensive care unit	14 (28)
Oxygen therapy	42 (84)
Bronchodilator	11 (22)
Corticosteroid	05 (10)

Antibiotics	49 (98)
mean (standard deviation)	
Length of oxygen therapy (days)	4.6 (+/- 6.2)
Length of stay (days)	6.8 (+/- 7.3)
Oximetry (O$_2$ %)**	92.2(+/- 6.1)
ETIOLOGY	
	N (%)
BP	14 (28)
RV	13 (26)
Co-detection (BP + RV)	03 (06)
Undetermined	20 (40)

Table 2: Comparative analysis between the groups with infection by BP or VR, as single etiologic agent, * Cell count in peripheral blood; ** Pulse oxymetry; BP= *Bordetella pertussis*; VR= Respiratory virus; *** All doses of vaccines recommended for age according to Brazilian Immunization Program.

	BP (n=14)	RV (n=13)	Univariate analysis
	N (%)		p
Male	09 (64)	06 (46)	
Masculino	09 (64)	06(46)	0.34
Feminino	05 (36)	07 (54)	
Cyanosis	14 (100)	09 (69)	0.03
Vomiting	12 (86)	09(69)	0.30
Dispneia	09 (64)	11 (85)	0.23
Wheezing	02 (14)	10 (77)	<0.001
Apnea	05 (36)	05 (38)	0.88
Fever	02 (14)	07 (54)	0.03
Inspiratory whoop	04 (29)	03 (23)	0.74
Anorexia	04 (29)	03 (23)	0.84
Age (days)	68.1 (31.3)	95.2 (34.8)	0.04
Leukocytes*	27674 (33250)	16260 (12499)	0.26
Lymphocytes*	10810 (6920)	8026 (6444)	0.30
	N (%)		
Contact with smokers	04 (29)	05 (39)	0.48
Updated Immunization***	04 (29)	07 (58)	0.13
Maternal BP immunization	00 (00)	01 (7.7)	0.29
Nursery	00 (00)	00 (00)	nsa
Breast-feeding	12 (86)	09 (69)	0.49
Number of people at home	4.4 (1.0)	4.8 (2.5)	0.52

Number of brothers	1.2 (1.1)	2.3 (1.9)	0.94
	N (%)		
Intensive care unit	04 (29)	05 (38)	0.59
Oxygen therapy	13 (93)	12 (92)	0.96
Bronchodilator	01 (7.1)	04 (31)	0.09
Corticosteroid	00 (00)	02 (17)	0.11
Antibiotics	14 (100)	12 (92)	0.29
Length of oxygen therapy (days)	5.4 (5.9)	6.5 (8.3)	0.67
Length of stay (days)	7.9 (7.9)	8.5 (9,0)	0.85
Oximetry (O2 %)**	93.5 (6.8)	90.4 (6.8)	0.26

Table 3: Multivariate analysis of clinical signs and symptoms possibly associated with the etiology, adjusted for age, *OR=odds ratio; **R^2=coefficient of regression

	OR*	p	R^2 **
Outcome: wheezing whooping cough age	0.04 0.96	0.01 0.05	0.68
Outcome: fever whooping cough age	0.23 0.97	0.16 0.04	0.43

In the univariate analysis, cyanosis and younger age were significantly associated with BP and wheezing and fever were associated with RV. In multivariate analysis, the presence of wheezing remained associated with the respiratory viruses infections and underage associated with infection by BP (p=0.01 and p=0.05, respectively) this model explains 68% of our results (R^2=0.68). The presence of wheezing had positive predictive value (PPV) for viral infection of 83% and negative predictive value (NPV) of 86%. The higher incidence of fever in viral infections has not remained significant in the multivariate analysis adjusted for age (p=0.16; R^2=0.43) (Table 3)

The leukocytes count in peripheral blood above 20.000/mm^3 showed PPV for BP 36% and NPV of 66%. The lymphocytes count above 15.000/mm^3 had a PPV of 56% and NPV of 71%.

Discussion

The preliminary results of this prospective cohort study highlight the importance of RV test on infants with suspected pertussis, since infections RV were frequent, there have been cases of co-detection with BP and RV and the classical signs of pertussis were not sensitive enough to identify the etiology of the infection.

Several previous studies have shown the importance of BP investigation in patients with viral bronchiolitis [1,6,7]. This study differs from the other because it drives up the need to expand the etiological diagnoses test and point the viral etiology

in infants with a clinical diagnosis of pertussis, especially those partially immunized for BP.

These preliminary results show similar prevalence of BP and RV. It is possible that such proportions can be modified until the end of the inclusion period. In a previous retrospective study, conducted at the same institution, the authors showed prevalence of 44% of BP and 26% of RV [4]. We already can highlight the importance of viral etiology in such preliminary results. In fact, pertussis in partially immunized infants may be associated with less typical clinical picture, which makes the suspicion raised even in the absence of such characteristic signals [8-11]. Such behaviour increases the sensitivity of screening for pertussis however decreases its specificity. In this study, age was significantly lower in infants with whooping cough, which can be linked to their immunization status [8]. The average age of the infants with pertussis was about 2 months, the same age of the first dose of vaccine BP. Previous studies show that, in fact, the frames of severe pertussis requiring hospitalization occur preferentially in under two months. The immune response improves after the subsequent doses of vaccine that contribute to the reduction of the incidence of pertussis [8-10,12,13].

Respiratory virus infections more often presented with wheezing, so that wheezing had positive and negative predictive values above 80%. Fever was more common in viral infections, but this association was not maintained independently when adjusted for age. The presence of post cough cyanosis was more frequent in pertussis and was present in all cases. Despite the significant difference, it is important to emphasize that about 70% of cases of viral infections also presented with cyanosis. Moreover, pertussis typical signs, as inspiratory whoop, where not significantly different between the two etiologies. In our retrospective study conducted previously, inspiratory whoop has only been described in patients with BP (p<0.001) and post cough apnoea was reported in 23% of positive patients and 8% of BP with RV (p=0.22) [4].

Laboratory tests also showed limited value for the pertussis diagnosis. The leukocytes count in the peripheral blood above 20.000/mm^3 had low positive and negative predictive values for BP, as well as the lymphocytes count above 15.000/mm^3. The absence of leukocytosis and lymphocytosis were better makers than other features to exclude BP, but with low NPV.

Some previous studies have called attention to the simultaneous detection of BP and RV [3,4,14]. Kruijssen and colleagues found co-detection of BP and other agents as RV, *Mycoplasma pneumoniae* and *Chlamydia pneumonia* in 37% of suspected cases of BP [15]. Two other previous studies analyzed the presence of respiratory viruses in children with confirmed BP infection. Moshal and colleagues showed that among 55 children positive for BP, 25 had test for VR and 5 were positive (20% - 2 RSV and 3 influenza A virus). All co-detection occurred in children under 6 months unimmunized or partially immunized for BP [16]. In a study conducted at the Federal University of Health Sciences of Porto Alegre, among 161 cases of confirmed BP infection, 21.4% had co-detection with respiratory viruses, the most common virus were: adenovirus (40.9%), RSV (22.7%), parainfluenza virus type 3 (22.7%), influenza B,

Metapneumovirus, parainfluenza virus type 1 and Bocavirus [13]. The aforementioned study consistent with the results presented here, but with higher rates of co-detection with RV, which may be due to the age, and sensitivity of the laboratory tests used [17]. It is possible that the use of molecular methods for RV resulted in greater co-detection index in our study.

The small number of cases did not allow analyzing the severity of co-infection. Although it is not possible to correlate co-infection with poor clinical outcome, it is noted that two in three patients who had co-infection (one with adenovirus and one with RSV) required hospitalization in an intensive care unit and ventilatory support, taking time total hospitalization of 18 and 22 days. In the retrospective study previously conducted by the authors there was one death among the 3 patients with co-detection [4]. In addition, the study of Moshal and collaborators, one patient with both infections at the same time, were admitted to the ICU and required mechanical ventilation [16].

This short communication presents preliminary results that need to be confirmed by the end of the cohort study.

Conclusion

The preliminary results of this prospective cohort suggest that RV detection as single agents was common in infants with suspected pertussis cases and also show BP and RV co-detection. BP infection occurred more often in younger infants and wheezing was more common in viral infections. The typical clinical signs and symptoms of pertussis as well as laboratory tests were not suitable to differentiate etiologies. These results support the contribution of RV tests in infants hospitalized with suspected pertussis, particularly for those not fully immunized.

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