New Microbiologica, 42, 1, 49-51, 2019, ISN 1121-7138

SHORT COMMUNICATION

Neonatal pertussis diagnosis: low procalcitonin level and high lymphocyte count are able to discriminate pertussis from bacterial and viral infections

Carlo Tascini[1], Novella Carannante[1], Giuseppe Sodano[2], Claudia Tiberio[2], Luigi Atripaldi[2], Giovanni Di Caprio[1], Emanuela Sozio[3], Francesco Sbrana[4], Andrea Ripoli[4], Camilla Menchini[5], Luisa Galli[5], Raffaele Di Sarno[1], Elena Chiappini[5], Marco Sarno[6], Paolo Siani[7]

[1]First Division of Infectious Diseases, Cotugno Hospital, Azienda Ospedaliera dei Colli, Napoli, Italy;
[2]Microbiology and Virology Unit, Cotugno Hospital, Azienda Ospedaliera dei Colli, Napoli, Italy;
[3]North-West District, Tuscany HealthCare, Spedali Riuniti Livorno, Emergency Department, Livorno, Italy;
[4]Fondazione Toscana "Gabriele Monasterio", Pisa, Italy;
[5]Division of Pediatric Infectious Disease, Anna Meyer Children's University Hospital, Department of Health Sciences, University of Florence, Florence, Italy;
[6]Department of Translational Medical Science, section of Pediatrics, University of Naples Federico II, Naples, Italy;
[7]Dipartimento di Pediatria Sistematica, Pediatria 1, AORN "Santobono-Pausilipon", Napoli, Italy

SUMMARY

Pertussis is quite frequent and severe among infants; therefore, rapid diagnosis and timely targeted therapy are essential. Although a molecular test for etiological diagnosis is now available, it may not be available everywhere, and therefore adjunctive diagnostic tests are still useful for presumptive diagnosis. We describe the use of procalcitonin (PCT) and lymphocyte count to discriminate among pertussis, bacterial and viral infections. Fourteen infants per group were studied. The decision tree, built considering all available variables, showed a major role of PCT in predicting the different groups. A PCT value equal to or greater than 0.75 ng/ml selected for bacterial infections. A PCT value lower than 0.75 ng/ml and a lymphocyte count equal to or greater than 10,400/mm^3 selected the subjects with pertussis, while a lymphocyte count lower than 10,400/mm^3 selected for viral etiology. PCT should be used in the diagnosis of infants suspected of having pertussis.

Received September 9, 2018 *Accepted* January 5, 2019

Pertussis is a vaccine-preventable disease that used to be a major cause of childhood morbidity and mortality (Nieves DJ *et al.*, 2016). It is caused by *B. pertussis,* a gram negative, pleomorphic aerobic cocco-bacillus that is exclusively a human pathogen (Nieves DJ *et al.*, 2016). Pertussis may be particularly severe in young infants, and in these individuals a very high level of leukocytosis (especially lymphocytes) is associated with a poor outcome (Cherry JD, *et al.*, 2018). In animal models, *B. pertussis* causes local damage to the respiratory tract, with invasive disease involving necrotizing bronchitis, alveolar damage and hemorrhage (Melvin JA *et al.*, 2014). These steps can lead to pulmonary hypertension, respiratory failure and even death due to aggregation of a high lymphocyte count. For a correct nasopharyngeal diagnosis, a specimen should be always obtained for culture or for a molecular test (Zlamy M. 2016). Diagnosis may be difficult if hyperlymphocytosis is not present initially, since many other respiratory pathogens might determine a similar clinical picture. However, in pertussis, fever and wheezing do not usually occur unless there is a secondary infection.

The aim of our study was to demonstrate that pertussis, bacterial and viral infections can be discriminated by combining the procalcitonin (PCT) value and the lymphocyte count. We enrolled confirmed consecutive cases of pertussis in 2016 and in the first 6 months of 2017 at Cotugno Hospital, in Naples, Italy. Patients with pertussis were compared with patients with bacterial (defined as positive microbiological culture and response to antibiotic therapy) and viral infections. The control groups were enrolled in a 1:1:1 ratio and patients were admitted for bacterial or viral infections in the same time period.

Furthermore, this analysis can support the clinician in the diagnostic roll out by suggesting a flow-chart of laboratory tests already available in the emergency department.

All patients enrolled in the study had a nasal swab for molecular analysis, and conventional cultures, blood cultures, urine cultures and procalcitonin test were performed.

Multiplex Real Time PCR analysis was employed to reveal the following pathogens: adenovirus, Influenza A and B, Coronavirus, parainfluenza, syncytial respiratory virus, metapneumovirus, human rhinovirus, Bordetella pertussis, Chamydiophila pneumoniae and Mycoplasma

Key words:
Neonatal pertussis diagnosis, Procalcitonin, Lymphocyte count, Bacterial infections, Viral infections.

Corresponding author:
Francesco Sbrana
E-mail: francesco.sbrana@ftgm.it

pneumoniae (FilmArray Respiratory Panel, Biomerieux, Mercy l'Etoile, France). Nasal swab cultures were also performed. Blood and urine cultures, biochemical tests including procalcitonin (PCT) were carried out according to standard procedures. Informed consent was waived due to the retrospective nature of the study.

Distribution of variables was assessed with the Kolmogorov-Smirnov test. Normal variables were represented as mean ± standard deviation, continuous non gaussian variables were represented as median and interquartile range, and categorical variables were represented as frequency and percentage.

Trends of variables across the three groups were studied by one-way analysis of variance, Kruskal-Wallis test or chi-square test, depending on their distribution. Analogously, post-hoc pairwise comparisons were performed with unpaired t-test, Mann-Whitney test or chi-square test with Yate's correction; in all cases, Bonferroni correction was applied.

A decision tree was built as a multivariate predictive model of pneumonia etiology. Moreover, the importance of predictors was assessed by means of multinomial ROC (Hand DJ, *et al*., 2001) and random forest (Breiman L. 2001) analysis. All analyses were performed with R statistical software. A value of $p<0.05$ was considered statistically significant.

The median age of the patients with confirmed pertussis was 3 months (Range 1-4); none of the infants and their mothers were vaccinated for pertussis. All of patients were successfully treated with macrolides (azithromicin 10 mg/kg/die e.v.) for at least 6 days, aerosol with bronchodilators (salbutamol) if necessary, and betamethasone e.v. 0.2 mg/kg every twelve hours for the first 24 hours pending molecular test results.

In infants with pertussis (*Table 1*), biochemical data confirm the presence of leukocytosis (24,230 cells/mm^3 ±6,729) with lymphocytosis (17,718 cells/ mm^3 [13,279-20,670]). In this group of patients, chest radiography was performed with no evidence of interstitial pneumonia. Blood cultures were negative in all patients with confirmed pertussis. The outcome of pertussis cases was positive in all patients.

PCT level was below the threshold of 0.5 ng/ml in all patients with pertussis (0.05±0.02).

The distribution of variables across the three etiological groups is reported in *Table 2*.

The decision tree, built considering all available variables, showed a major role for PCT in predicting the different groups. A PCT value equal to or greater than 0.75 ng/ml selected the 14 subjects with bacterial infections. For a PCT value lower than 0.75 ng/ml, a lymphocyte count equal to or greater than 10,400/mmc selected the 14 subjects with pertussis etiology, while a lymphocyte count lower than 10,400/mmc selected the 14 subjects with viral etiology (*Figure 1*).

Considering the small sample size and the instability of the decision tree, a confirmative random forest analysis was performed using 500 trees. The random forest showed a global accuracy of 92.86% and highlighted the importance of PCT, followed by lymphocytes and white cells.

The same ranking of importance of predictors was found with a multinomial roc analysis. We computed an AUC (Area Under the Curve) of 0.963 for PCT, 0.860 for lymphocytes, 0.852 for white cells, and 0.665 for age.

In Italy, pertussis vaccination coverage is high (reaching

Table 1 - *Clinical characteristics of pertussis patients.*

Patient	*Age (months)*	*Sex*	*White blood cells (mm^3)*	*Lymphocytes (mm^3)*	*Procalcitonin (ng/ml)*
1	1	M	27,360	20,793	0.06
2	2	F	25,210	18,529	0.05
3	3	F	17,240	13,257	0.08
4	1	M	25,000	18,750	0.13
5	4	F	31,580	25,264	0.04
6	1	F	24,250	20,297	0.04
7	4	F	34,000	29,308	0.04
8	4	M	15,780	11,156	0.04
9	3	F	22,930	14,468	0.05
10	2	F	36,170	25,068	0.04
11	1	F	16,730	11,878	0.10
12	4	F	15,520	12,105	0.08
13	9	F	19,060	13,342	0.06
14	3	M	21,400	16,906	0.15

Legend: F = female; M = male.

Table 2 - *Comparison of patients' clinical characteristics.*

	All patients (n = 42)	*Bacterial etiology (n = 14)*	*Pertussis etiology (n = 14)*	*Viral etiology (n = 14)*	*P*
Age (months)	3 [2-5]	4.5 [3 - 40]	3 [1 - 4]	3 [2 – 4]	0.043
Male	23 (55%)	12 (86%)*	4 (29%)	7 (50%)	0.009
Procalcitonin (ng/ml)	0.12 [0.08–2.05]	4.95 [1.78–17.75]*§	0.05 [0.04–0.09]#	0.10 [0.10–0.20]	<0.001
White blood cells (mm^3)	18,276±9,807	21,717±10,502§	24,230±6,729#	9,381±4,211	<0.001
Lymphocytes (mm^3)	6,650 [3,199-13,158]	4,965 [2,836-7,078]*	17,718 [13,279-20,670]#	3,382 [2,234-4,559]	<0.001

Legend: p-value refers to the significance of trends across the three groups. *= Bacterial etiology vs. Pertussis etiology ($p<0.05$); §= Bacterial etiology vs. Viral etiology ($p<0.05$); #= Pertussis etiology vs. Viral etiology ($p<0.05$).

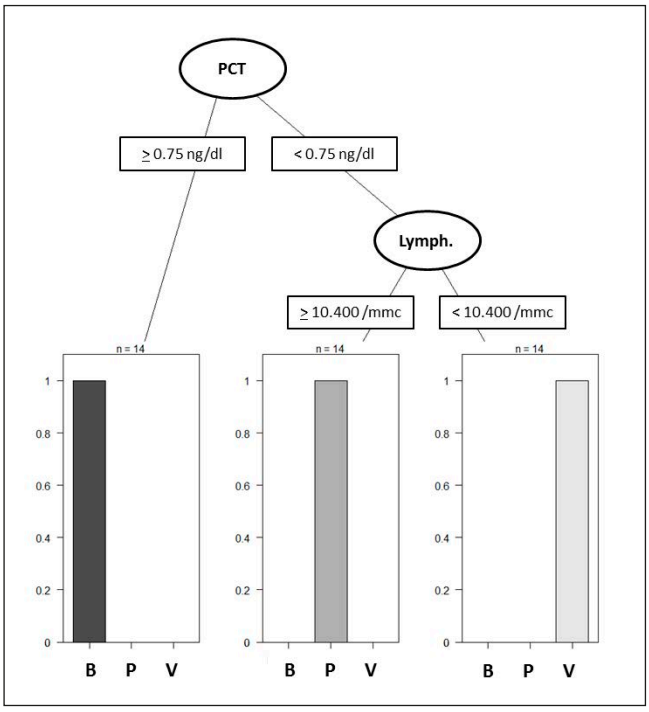

Figure 1 - *Decision tree based on the multivariate predictive pertussis model.*
Legend: PCT = procalcitonin; Lymph. = lymphocytes; B = bacterial etiology; P = pertussis etiology; V = viral etiology.

93.33% in 2015), but no data are available for pregnant women (http://ecdc.europa.eu/en/healthtopics/pertussis/Pages/Annual-epidemiological-report-2016.aspx). Therefore, this may be why pertussis is still a frequent disease among young infants.

The ability to differentiate between bacterial and viral infections affecting a child with an acute illness and, consequently, to decide whether to initiate or possibly discontinue an antibiotic therapy, still represents the most challenging situation for most clinicians (Ramilo O, *et al.*, 2016; Cherry JD. 2018).

PCT level in peripheral blood is an important diagnostic indicator of bacterial infection, especially of the respiratory tract. Furthermore, it is a sensitive indicator of distinction between bacterial pneumonia and non-bacterial pneumonia, thus being of great significance for clinical and differential diagnosis (Zhu F, *et al.*, 2015).

In a retrospective study, Lautz *et al.* analyzed the diagnostic value of PCT in unequivocally identifying bacterial infections in patients with suspected severe bacterial infection or sepsis: median PCT concentrations were lower and comparable among patients with no infection or with viral infections and showed higher values in patients with suspected and documented bacterial infections (Lautz AJDA, *et al.*, 2016).

Therefore, the presence of associated symptoms, lymphocytosis and negative PCT is potentially useful in predicting *B. pertussis* infection, especially in hospitals where PCR for diagnosis is not available.

Pertussis in infants and young children is frequently characterized by a significant rise in circulating white blood cells (Heininger *et al.*, 1997; Carbonetti NH. 2016) and especially in leukocyte count (Hodge G, *et al.,* 2003).

Furthermore, pertussis leukocytosis caused by a soluble protein toxin released during pertussis infection, is associated with poor outcome in infants hospitalized with pertussis (Winter K, *et al.*, 2015), and modern treatments, such as exchange blood transfusion, are often employed to lower the white blood cell count. Strong indications for exchange blood transfusion are leukocytes count >48,000 /mm^3 and count rise >50% in 24 hours (Cherry JD 2018).

Even though *B. pertussis* is a bacterium, and although it causes necrotizing bronchitis (an invasive infectious disease), it is unable to induce PCT production. It probably does not affect specific tissues that produce PCT. In many cases, PCT was not associated with diagnosis of bacterial community- or hospital-acquired pneumonia (Teepe J, *et al.*, 2016), but was instead associated with the duration of antibiotic therapy. Therefore, if pertussis is considered together with bacterial pneumonia, PCT might not be a reliable marker of bacterial lung infection.

In neonates, PCT should be added to routine biochemical tests at admission in order to discriminate pertussis from bacterial and viral infection, above all to avoid unnecessary antibiotic therapy.

Acknowledgements
The authors thank Dr. Laura Sabatino for helpful editing.

Conflict of Interest
C.T. has received funds for speaking at symposia organized on behalf of Pfizer, Novartis, Merck, Angelini and Astellas. All other authors: none to declare.

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