Procalcitonin and C-reactive protein-based decision tree model for distinguishing PFAPA flares from acute infections

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ABSTRACT

As no specific laboratory test has been identified, PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) remains a diagnosis of exclusion. We searched for a practical use of procalcitonin (PCT) and C-reactive protein (CRP) in distinguishing PFAPA attacks from acute bacterial and viral infections. Levels of PCT and CRP were measured in 38 patients with PFAPA and 81 children diagnosed with an acute bacterial (n=42) or viral (n=39) infection. Statistical analysis with the use of the C4.5 algorithm resulted in the following decision tree: viral infection if CRP≤19.1 mg/L; otherwise for cases with CRP>19.1 mg/L: bacterial infection if PCT>0.65ng/mL, PFAPA if PCT≤0.65 ng/mL. The model was tested using a 10-fold cross validation and in an independent test cohort (n=30), the rule's overall accuracy was 76.4% and 90% respectively. Although limited by a small sample size, the obtained decision tree might present a potential diagnostic tool for distinguishing PFAPA flares from acute infections when interpreted cautiously and with reference to the clinical context.

KEY WORDS: Periodic fever; PCT; CRP; bacterial infection; diagnosis
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INTRODUCTION

PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) is an autoinflammatory disorder characterized by recurrent attacks of high fever associated with cervical adenitis, pharyngitis and aphtous stomatitis [1]. Studies on PFAPA's pathogenesis are ongoing and no monogenic trait or other specific laboratory marker has been found [2]. Diagnosis is based on a clinical presentation and exclusion of other potential causes of fever, with infection as a primary one [1,3]. While early diagnosis provides therapeutic possibilities for the patient and comfort for the whole family, misdiagnosis of PFAPA flares as recurring infections may lead to diagnostic delay and repeated unnecessary antibiotic treatments [4]. In the search for a quick, available and cost-effective diagnostic test, the usefulness of C-reactive protein (CRP) and procalcitonin (PCT) levels have been suggested [5-9]. Both CRP and PCT levels increase in response to proinflammatory cytokines such as 1β, IL-6 and TNF-α, which are also involved in the pathomechanism of PFAPA attacks [10,11]. The ability of PCT to discriminate between bacterial infections and non-bacterial inflammatory conditions has been well documented [11-13]. Serum PCT concentrations are extremely low in healthy individuals (<0.05 ng/mL), rise moderately (up to 0.5 ng/mL) in patients with local infections and are generally greater than 1-2 ng/mL in patients with sepsis, although PCT levels greater than 0.5 ng/mL may already indicate a serious bacterial infection [14]. Studies on the usefulness of a concomitant assessment of PCT and CRP in distinguishing PFAPA febrile flares from infections are scarce [7]. Only a small study evaluating PCT values in PFAPA patients included various bacterial infections as a control group [6]. To our knowledge there have been no reports comparing CRP and PCT levels during PFAPA attacks and viral infections.

We assessed CRP and PCT values in PFAPA patients during their febrile episodes and in two control groups consisting of children with various bacterial or viral infections. We searched for a practical application of the collected data with the use of a statistical classifier.

MATERIALS AND METHODS

Thirty-eight children with PFAPA diagnosed at the Clinic of Wroclaw Medical University (Poland) between January

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2010 and August 2015 were enrolled in a population-based study. The inclusion criteria were: (1) 1-10 years of age, (2) fulfillment of the following clinical diagnostic criteria for PFAPA syndrome: (a) at least 6 (or a minimum of 3 over a 6-month period) episodes of high fever (>38.5°C) lasting no more than 7 days and recurring at regular intervals of 2-8 weeks, (b) symptoms in the absence of upper respiratory tract infection with at least one of the following clinical signs: aphthous stomatitis, cervical lymphadenitis or pharyngitis, (c) a failure of antibiotic treatment during febrile episodes, (d) exclusion of other causes for periodic fevers.

Family and patient history (including age at disease onset, a detailed description of typical febrile episodes, and comorbidities) were taken. Routine laboratory evaluation included full blood count with differential, acute-phase proteins (CRP, PCT, serum amyloid A (SAA)); serum immunoglobulins; throat swabs; blood, urine and stool cultures; and Epstein-Barr virus (EBV) serology. Additional laboratory tests and imaging were used as needed to exclude other reasons for recurrent fever. The final diagnosis was established by an experienced physician (Z.S-T., L.S.) upon physical examination and careful evaluation of all gathered information.

Additionally, CRP and PCT serum levels were obtained from children aged 1-10 years, who were referred to the clinic for fever >38°C and a suspected infection, and who were considered for recruitment to one of the control groups ("bacterial" or "viral") according to the final diagnosis. The final diagnosis of bacterial or viral infection was adjudicated by two researchers (L.Sz., B.K-G.).

Patients (1) aged younger than 1 or older than 10 years old, (2) without a clear diagnosis, (3) who received more than a single dose of antibiotic within 48 hours before admission, or (4) with known immunodeficiencies were excluded from the study.

PCT and CRP levels were measured within 24 hours of admission for all study participants. At the time of sampling the mean age was 4.4±2.3, 4.5±2.6 and 4.1±2.6 years in PFAPA, a bacterial, and a viral group respectively, and the median time from the onset of fever was 2 days (range 1 – 7) in all groups. CRP levels were determined by immunoturbidimetric assay (Konelab, Thermo Fisher Scientific) and PCT levels were measured using enzyme-linked fluorescent assay (VIDAS B.R.A.H.M.S PCT, BioMérieux). Normal reference ranges for CRP and PCT values were 0-10 mg/L and <0.05 ng/mL, respectively.

The study was approved by the ethical committee of our institute and a written informed consent was obtained from the parents of examined children.

Statistical analysis

A statistical evaluation was carried out in two stages. The first stage of analysis involved data collected from children with either PFAPA, bacterial infections, or viral infections who were enrolled in the study until the end of January 2014 (training cohort). PCT and CRP values were applied to the C4.5 algorithm (statistical classifier) to construct a decision tree and a 10-fold cross validation was applied for the initial estimation of the accuracy of the model [15]. To verify how accurately the model performs in practice, it was then validated in an independent test cohort, consisting of PFAPA patients and controls enrolled after January 2014. Additionally, a comparison between the groups was performed by the Mann-Whitney U test (p values lower than 0.05 were considered significant) in both training and testing datasets.

RESULTS

In the 6-year study period (2010-2015) PCT and CRP were measured in 38 patients diagnosed with PFAPA. Demographic and clinical characteristics of all PFAPA patients are presented in Table 1. Table 2 shows the frequency of signs and symptoms associated with febrile episodes.

Altogether 81 children were recruited for two control groups: Group 1, 42 children with a bacterial infection, diagnosed upon typical clinical symptoms supported by a positive culture (sepsis-7, meningitis-5, pyelonephritis-5, gastroenteritis-4, peritonitis-2, tonsillitis-3, scarlet fever-5), histological changes (appendicitis-1), classic X-ray (pneumonia-7, sinusitis-1), ultrasound (adenitis-1) findings, or characteristic

TABLE 1. Demographic and clinical characteristics of 38 PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) patients

Total number of patients	38
Male/female	22/16
Age at disease onset (years)[a]	2.0/1.6 (0.6-7.4)
Age at diagnosis (years)[a]	3.9/3.6 (1.0-8.4)
Duration of febrile episode (days)[a]	4.1/4.0 (2-7)
Interval between episodes (weeks)[a]	5.0/5.0 (2-8)

[a]Values are given as mean/median (range)

TABLE 2. Frequency of signs and symptoms associated with febrile episodes in 38 PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) patients

Symptom	Present	Always	Often	Sometimes	Never
Pharyngitis	37 (97.4)	33 (86.8)	3 (7.9)	1 (2.6)	1 (2.6)
Aphthous stomatitis	21 (55.3)	3 (7.9)	6 (15.8)	12 (31.6)	17 (44.7)
Cervical adenitis	37 (97.4)	27 (71.1)	7 (18.4)	3 (7.9)	1 (2.6)
Malaise	38 (100)	32 (84.2)	2 (5.3)	4 (10.5)	0
Headache	15 (39.5)	7 (18.4)	3 (7.9)	5 (13.2)	23 (60.5)
Abdominal pain	22 (57.9)	8 (21)	9 (23)	5 (13.2)	16 (42.1)
Vomiting	10 (26.3)	1 (2.6)	1 (2.6)	8 (21)	28 (86.4)
Diarrhea	12 (31.6)	0	5 (13.2)	7 (18.4)	26 (68.4)
Osteoarticular pain	18 (47.3)	9 (23)	3 (7.9)	6 (15.8)	20 (52.6)
Skin rash	2 (5.3)	0	1 (2.6)	1 (2.6)	36 (94.7)
Chills	18 (47.4)	14 (36.8)	1 (2.6)	3 (7.9)	20 (52.6)

Values are number of patients (%)

otoscopic signs (otitis media-1); Group 2, 20 children with a specified viral infection, diagnosed upon positive antigen detection or serology (Epstein-Barr virus-6, adenovirus-1, rotavirus-1, cytomegalovirus-1), or pathognomonic symptoms (varicella-7, herpes simplex dermatitis-1, roseola-1, erythema infectiosum-1, hand, foot and mouth disease-1); and 19 children with an unspecified viral infection diagnosed upon typical symptoms, negative cultures, and spontaneous recovery without antibiotic treatment (viral meningitis-7, upper respiratory tract infection-6, rash-5, diarrhea-1). Ten patients with a bacterial infection, 2 patients with a viral infection and none of the PFAPA patients were treated with a single dose of antibiotic before blood collection.

Training cohort

The training cohort included 26 PFAPA patients (16 males) and 62 controls – children diagnosed with acute bacterial (n=32, 13 males) or viral (n=30, 18 males) infections. Table 3 presents PCT and CRP values, as well as a statistical comparison between groups. The results of PCT and CRP analyses are presented in Figure 1. Data analysis with the C4.5 algorithm resulted in a decision tree presented in Figure 2.

As demonstrated with a 10-fold cross validation, the rule was effective in 76.4% of the cases. Febrile episodes during PFAPA flares, bacterial infections, and viral infections were classified with a sensitivity of 74.1%, 78.1% and 76.7% and a specificity of 79.0%, 87.7% and 98.3% respectively.

Test cohort

The test cohort consisted of 11 PFAPA patients (5 males), 10 children with an acute bacterial (4 males) infection and 9 children with an acute viral disease (3 males). At the time of sampling the mean age was 3.3±1.8, 4.1±2.6 and 4.4±1.8 years in PFAPA, bacterial and viral groups, respectively, and the

TABLE 3. Levels of procalcitonin (PCT) and C-reactive protein (CRP) during febrile episodes in children diagnosed with PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) and in control groups (children with an acute bacterial or viral infection)

	Bacterial infection[1]	Viral infection[1]	PFAPA[1]	*p*-value PFAPA vs. bacterial infection	*p*-value PFAPA vs. viral infection
Training cohort					
CRP (mg/L)	160.26±80.73 155.85 (20.20-377.60)	13.36±14.91 5.30 (1.20-64.90)	101.53±59.13 94.50 (26-216.70)	p=0.005	$p<10^{-9}$
PCT (ng/mL)	6.85±7.35 4.96 (0.24-29.5)	0.21±0.30 0.08 (0.00-1.13)	0.67±0.92 0.33 (0.00-4.42)	$p<10^{-8}$	p=0.002
Test cohort					
CRP (mg/L)	134.09±77.72 115.60 (43.60.-288.50)	4.53±0.91 4.20 (3.90-6.90)	113.± 54.59 94.8 (59.60-216.70)	p=0.704	$p<10^{-4}$
PCT (ng/mL)	9.67±11.19 3.07 (0.38-31.51)	0.19±0.28 0.08 (0.00-0.91)	0.39±0.35 0.35 (0.00-1.28)	$p<10^{-4}$	p=0.05

Results are given as follows: Mean value, median (range)

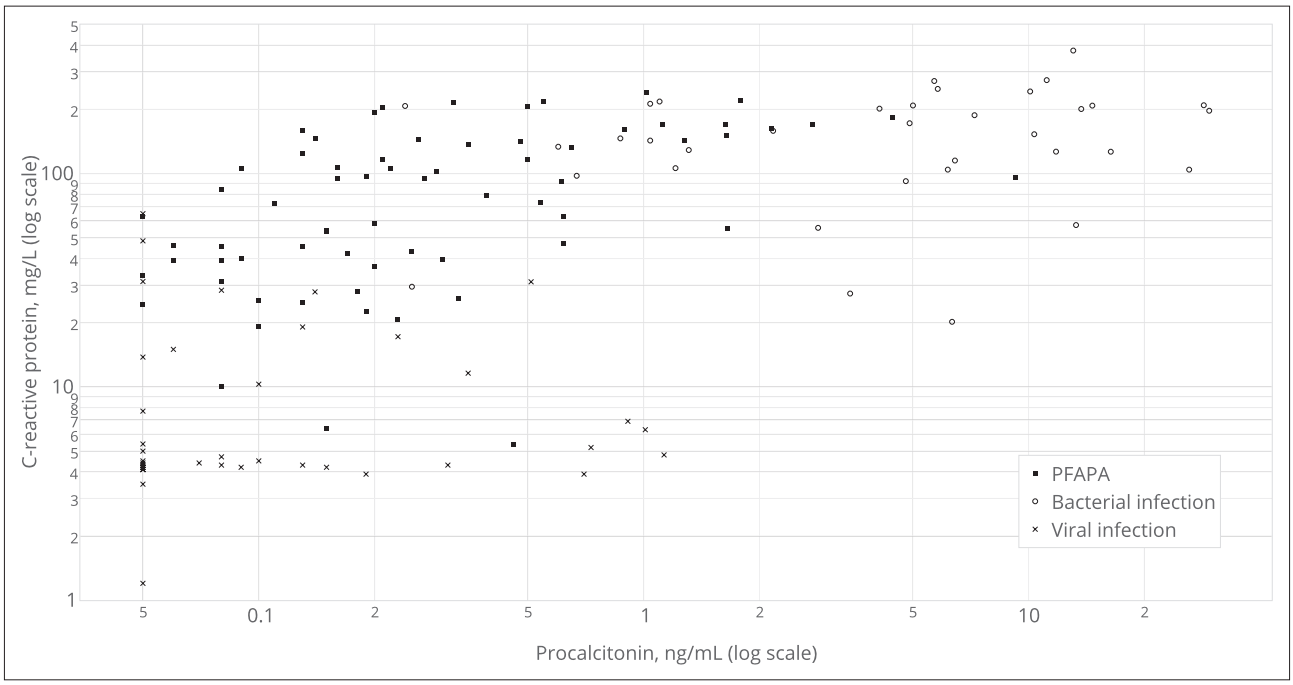

FIGURE 1. Levels of C-reactive protein (CRP) and procalcitonin (PCT) in children with PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) syndrome and in control groups (children diagnosed with an acute bacterial or a viral infection).

TABLE 4. Confusion matrixes for a decision tree model based on procalcitonin and C-reactive protein levels in children with diagnosis of PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis) and in control groups (children with an acute bacterial or viral infection)

Studied group	Number of patients classified as					
	10-fold cross validation			Validation in the test cohort		
	PFAPA	Bacterial infection	Viral infection	PFAPA	Bacterial infection	Viral infection
PFAPA	20	7	0	9	2	0
Bacterial infection	6	25	1	1	9	0
Viral infection	7	0	23	0	0	9

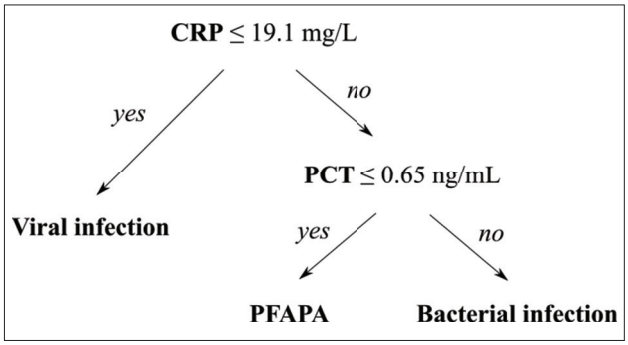

FIGURE 2. Procalcitonin (PCT) and C-reactive protein (CRP) - based decision tree model for distinguishing PFAPA flares from acute infections

median time from the onset of fever was 2 days (range 2 – 7) in all groups. Table 3 shows the laboratory values and a statistical comparison between groups. The overall accuracy of the obtained decision tree was 90% after validation in the testing cohort. Febrile episodes during PFAPA flares, bacterial infections, and viral infections were classified with a sensitivity of 81.8%, 90% and 100% and a specificity of 94.7%, 90% and 100% respectively (the confusion matrix is given in Table 4).

DISCUSSION

Since the first description of PFAPA syndrome almost 30 years ago, our understanding of its clinical manifestations has advanced greatly, but reliable tests to aid in the diagnosis are still needed. Nonspecific findings such as elevated markers of inflammation (CRP, white blood cells (WBCs), sedimentation rate, serum amyloid A), an increase of neutrophils and monocytes, and a decrease of lymphocytes and eosinophils during PFAPA attacks have been described before [3,5,9,16-19]. Recently, normal or moderately elevated PCT concentrations in association with the increase of other inflammatory markers have been indicated as distinctive of PFAPA febrile episodes [6-9].

We found CRP levels to be substantially increased during febrile episodes in patients with PFAPA, which is consistent with other studies [5,7,9]. In the obtained decision tree, CRP is used as the initial split variable to distinguish viral infections from bacterial infections and PFAPA flares. As shown in the 10-fold cross validation, CRP >19.1 mg/L should be expected in all PFAPA patients and almost all patients with a bacterial infection (see Figure 1 and Table 3). This was confirmed by validation in the test cohort. Statistical significance between the PFAPA group and viral controls was also reached for PCT, indicating its potential use in distinguishing between the two conditions. In line with previous studies, we found CRP inferior to PCT in distinguishing PFAPA attacks from bacterial infections [7,9].

Mean PCT value in children with PFAPA was higher than in the previous reports [6,7,9]. Yoshira et al. found undetectable levels of PCT during 13 PFAPA attacks [6]. In a study by Yazgan et al. PCT levels stayed within normal limits for 78 PFAPA flares [7]. In two other cohorts a slight to moderate elevation of PCT concentrations during some of the PFAPA inflammatory attacks was observed [8,9]. As indicated by the median value (0.3 ng/mL), most of our PFAPA patients had PCT concentrations similar to those reported by other authors [7-9]. Nevertheless, in contrast to previous reports, we noted quite high PCT levels in some of the PFAPA patients (see Figure 1). Several children had PCT >0.65 ng/mL and were consequently misclassified by the constructed decision tree as having a bacterial infection. Those patients were sampled during their PFAPA febrile flares, which in the parents' opinion were no different from the previous ones and could not be explained otherwise after a thorough clinical and laboratory evaluation. One possible explanation could be the time of sampling (mean time from fever onset: 3.3 days ±1.8 in subjects with PCT>0.65 ng/mL vs 2.2 days±1 in the entire PFAPA group), however the PCT kinetics during PFAPA flares are unknown and definite conclusions cannot be drawn. Besides, in previously published works normal PCT values were observed in patients sampled on the third through fifth day of fever, while elevated PCT levels were reported in PFAPA patients sampled within the first 24 hours of a PFAPA febrile episode [6,8].

Most children with a serious bacterial infection were correctly identified by the decision tree: 2 patients with local infections (streptococcal tonsillitis-1, adenitis-1), 1 child with scarlet fever and 1 with bacterial enteritis were misclassified as PFAPA; 1 case of pyelonephritis was misclassified as a viral infection. None of those patients was treated with antibiotics before blood collection and except for the child with adenitis, who was admitted on the fifth day of fever, they were all sampled on day 2±1 of fever.

This study has several limitations and our results must be interpreted with caution. We realize that our sample size is not ideal for this kind of statistical modeling, however PFAPA is a rare disease and to our knowledge, studies on PCT and CRP in bigger PFAPA cohorts have not been published. We assume that the model's accuracy validated in the test cohort was probably inflated by a small sample size and will likely drop once more patients are recruited. Until then, results of the 10-cross validation appear more plausible. In our study the time of sampling differed depending on the time of admission. Blood was taken 2 days after the fever onset in most patients, although all samples ideally should be collected on the same day of fever in all patients. Since many, if not most, of our patients with serious bacterial infections are referred from other facilities, we decided not to exclude those controls who had received a single dose of antibiotic, which might have lowered the levels of inflammatory markers in the bacterial group. However, it is unlikely that this would affect the construction or accuracy of our model, as most of the patients treated before admission had serious bacterial disease and PCT levels higher than the cut-off level.

In line with other studies, we found significantly lower procalcitonin levels in patients with PFAPA compared to the bacterial control group, indicating the potential usefulness of PCT for discriminating between PFAPA flares and bacterial infections. However, our results do not support the hypothesis of normal PCT values being typical of PFAPA febrile episodes. Additionally, we demonstrated that CRP and (to a lesser degree) PCT might be helpful in distinguishing PFAPA flares from viral infections. When interpreted cautiously and with reference to the clinical context, the presented decision tree model might facilitate the diagnostic process in children with PFAPA. To obtain a more reliable prediction rule, data from larger groups of PFAPA patients should be collected for analysis, possibly as part of an extensive international project.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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