C-reactive Protein, Procalcitonin and the Lab-Score for Detecting Serious Bacterial Infections in Febrile Children at the Emergency Department

A Prospective Observational Study

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Background: C-reactive protein (CRP) and procalcitonin (PCT) are useful diagnostic tools to estimate the risk of serious bacterial infection (SBI) in febrile children at the emergency department (ED). The Lab-score combines these 2 biomarkers with urinalysis in an easy to use validated model. Kinetics of inflammatory markers suggests a differentiating role of duration of disease.

Aim: Appraisal of the diagnostic role of CRP and PCT in febrile children at risk of SBI, determining the differentiating value of duration of fever, and validating and updating the Lab-score.

Methods: In this prospective observational study previously healthy children with fever, 1 month to 16 years of age, attending the EDs of a university hospital and a teaching hospital (Rotterdam, the Netherlands) between 2009 and 2012 were included. Standardized information on clinical signs and symptoms, CRP, PCT and urinalysis were collected prospectively. Logistic multivariable regression analysis was used to assess diagnostic performance. The original Lab-score included CRP, PCT and urinalysis and the total score ranged 0–9 points.

Results: One thousand eighty-four children were included, median age was 1.6 years (interquartile range: 0.8–3.5), 170 children (16%) had SBI. CRP [receiver operating characteristic (ROC)-area 0.77 (95% confidence interval [CI]: 0.69–0.85)] and PCT [ROC-area 0.75 (95% CI: 0.67–0.83)] were both strong predictors of SBI. Duration of fever had no added diagnostic value to CRP and PCT. The Lab-score performed well [ROC area 0.79 (95% CI: 0.72–0.87)], but threshold values performed similar to often used cutoffs of single biomarkers. An updated Lab-score improved only moderately [ROC area 0.83 (95% CI: 0.76–0.90)]. PCT did not alter post-test probabilities for SBI substantially in patients with low (<20 mg/L) or elevated CRP (≥100 mg/L) levels (67% of population).

Conclusion: CRP and PCT were both strong predictors of SBI. The original and updated Lab-score performed well, but thresholds values lacked diagnostic value for ruling out SBI. Depending on clinical risk thresholds, diagnostic testing can be limited to CRP or PCT, rather than both, in many febrile children.

Key Words: C-reactive protein, procalcitonin, serious bacterial infection, emergency department, children, fever

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Biomarkers, such as C-reactive protein (CRP) and procalcitonin (PCT) are useful for discriminating febrile children with a serious bacterial infection (SBI) from those with self-limiting febrile illnesses.[1,2] However, superiority of one biomarker above the other has not yet been proven convincingly, and neither CRP nor PCT has the ability to be used as a solitary predictor of SBI without other clinical features or diagnostic markers.[1–5] Recently, a promising prediction model was developed and validated, the so-called Lab-score, which combined both markers and urinalysis, and which showed remarkable discriminative ability in both derivation and validation studies.[6,7] One downside of the Lab-score is the requirement of having all three components available, possibly implying overusing diagnostic tools. On the basis of differences in protein kinetics it has been suggested that the diagnostic values of PCT and CRP to detect SBI are related to the duration of disease, and that PCT and CRP could play differentiating diagnostic roles at different stages of the disease.[8–12]

In this study, we aimed to position the clinical usefulness of CRP and PCT as diagnostic tests in febrile children at risk of SBI, we determined the differentiating value of duration of fever, and we aimed to externally validate and update the Lab-score.

METHODS

Design

In this prospective observational study, we first determined the diagnostic value of CRP and PCT in a cohort of febrile children, and studied the association of PCT and CRP with duration of fever. Next, we externally validated and updated the Lab-score, and compared the diagnostic value of the Lab-score with those of single biomarkers. Finally, we critically appraised the practical diagnostic approach of febrile children as we aimed to optimize the diagnostic management while minimizing the amount of diagnostic tests.

Patients and Setting

This prospective observational study was conducted at the Erasmus MC–Sophia children's hospital and the Maasstad hospital, both located in Rotterdam, the Netherlands. The Erasmus MC–Sophia children's hospital is a tertiary pediatric university hospital, and its pediatric emergency department (ED) is visited by 9000 children annually.[13] The Maasstad hospital is a general

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teaching hospital, and has a mixed adult and pediatric ED, visited by nearly 10,000 children yearly. Patients with fever aged 1 month to 16 years, attending the EDs between February 2009 and May 2012 in the Erasmus MC–Sophia and between May 2011 and May 2012 in the Maasstad hospital, were eligible. Fever was defined as a rectal temperature ≥38.5°C, fever as positive discriminator in the Manchester triage system,[14] fever as reason for referral to the ED or high fever noted at home in the previous 24 hours. Children with a chronic underlying disease were excluded. Also, well appearing children presenting with fever and a clear focus of an upper airway infection were excluded from the study as this group of children pose a minimal diagnostic challenge for the physician with a very low risk of SBI and diagnostic tools not contributing to clinical decision-making. Eligibility of children was determined at the point of triage after which children were enrolled in the study, clinical signs and symptoms were recorded and informed consent was sought; according to a predefined study protocol CRP and PCT were determined routinely in eligible children, sampling of CRP and PCT occurred at any stage of the ED visit. Hospitalization followed directly after ED discharge, except in a minority of cases in which patients were hospitalized after a revisit. Urinalysis was performed according to a national guideline, resembling the National Institute for Health and Care Excellence (NICE) guidelines on the diagnosis and the management of children at risk of urinary tract infections (UTIs)[15,16] A midstream or clean catch urine sample urine sample was collected routinely in all children with fever without apparent source or at an increased risk of UTI. A first urine sample tested positive for leukocyte esterase and/or nitrates had to be confirmed by a positive catheterized sample in children <12 months of age.[17] Additional diagnostic tests were executed at the discretion of the physician. When patients returned to the ED within 5 days of their initial visit, we considered only the clinical signs and symptoms and laboratory values of their first visit in our analyses.

Ethics Approval

The study protocol was approved by the institutional ethical committees of both the Erasmus MC (MEC-2007–066) and the Maasstad hospital (2010/64). Patient informed consent was required and obtained for all included children.

Data Collection

In the Erasmus MC–Sophia, clinical data and patient characteristics of eligible children were collected prospectively using a standardized data entry form which was embedded in the hospital's electronic patient file. In the Maasstad hospital, data were collected similarly, except for data on specific clinical signs and symptoms of febrile children, for which a standardized paper research form was used. Physicians and nurses were trained in patient inclusion, sampling of biomarkers and data collection using the data entry forms; feedback on compliance to the study protocol was supplied at regular intervals.

Outcome Measures

We specified pneumonia, UTIs, bacteremia, meningitis, cellulitis orbitae, erysipelas, bacterial gastro-enteritis, bacterial arthritis and bacterial osteomyelitis as SBI. Final diagnoses were coded by trained medical students that were blinded for the specific aim of this study; they were blinded for PCT values, but not for CRP values or clinical data. Final diagnoses were defined according to a reference standard, based on positive bacterial cultures from otherwise sterile sites, abnormal radiologic findings and clinical consensus diagnoses by the investigators (R.N. and R.O.).[18] Available data from all consecutive consultations were considered to determine final diagnoses. Hospital records were checked for any return visits and a standardized telephonic follow-up after 72 hours was installed to ensure no diagnoses of SBI were missed.

Variables of Interest

CRP was measured either by a validated bedside test or by a laboratory test. Laboratory CRP was determined in heparin plasma with an immunoturbidimetric CRP assay using a Modular system (Roche Diagnostics, Basel, Switzerland) in the Erasmus MC–Sophia, and with a nephelometric CRP assay using a Dimension VISTA 1500 system (Siemens, Erlangen, Germany) in the Maasstad hospital. Laboratory CRP was executed on-going, and results were available to the physician for further patient assessment. CRP bedside testing was performed using the Afinion™ AS100 (Axis-Shield PoC AS, Dundee, Scotland, distributed by Clindia Benelux BV).[19] The bedside test was evaluated extensively before implementation: the correlation coefficient of the Afinion™ with the reference method was: Afinion™ = 0.96 × CRP (Modular) + 0.38. The Afinion™ AS100 CRP bedside test requires 1.5 μL of ethylenediaminetetraacetic acid or heparin blood. Results of the test are available within 4 minutes, with values ranging from 8 to 200 mg/L (values smaller than 8 are presented as <8 mg/L, values greater than 200 are presented as >200 mg/L). The interassay and intra-assay coefficients of variation at a concentration of 12.6–17.4 mg/L were 4% and 9.3%, respectively. The CRP bedside test was performed on site at the ED by trained and certified nurses, and results were available immediately. PCT was measured using the Brahms Kryptor Assay, which has a measuring range of 0.02–5000 ng/mL (0.02–50 ng/mL directly and up to 5000 ng/mL after sample dilutions). PCT samples from both the Maasstad and the Erasmus MC–Sophia were determined in the Erasmus MC–Sophia in batches of 50–100 samples; samples were stored at −70°C until assayed; PCT results were available for study purposes only. CRP was truncated at the 97th centile at 225 mg/L and PCT at 6.0 ng/mL. Duration of fever was entered in the data entry form rounded to half days. We truncated duration of fever at the 97th centile at 6 days. Urine dipstick was considered positive if either leukocyte esterase or nitrates were positive. For children without urinalysis (703/1084, 65%), urinalysis was considered to be normal, as standardized outpatient or telephonic follow-up ruled out any missed and clinically relevant, symptomatic diagnosis of UTI.

Lab-score

The Lab-score was presented in detail previously[7]; in short, PCT, CRP and urinalysis were the only predictors contributing to a multivariable logistic regression model predicting SBI in febrile children. A simple score was constructed based on the univariate odds ratios (ORs), and points could be obtained for CRP (<40 mg/L: 0 points; 40–99 mg/L: 2 points; ≥100 mg/L: 4 points), PCT (<0.5 ng/mL: 0 points; ≥0.5–1.99 ng/mL: 2 points; ≥2.0 ng/mL: 4 points) and positive urine dipstick (1 point). The Lab-score ranged from 0 to 9 points and a cutoff of 3 points was proposed.

Statistical Analysis

First, we calculated diagnostic performance measures, including the area under the receiver operating characteristic curve (ROC-area), sensitivity, specificity, positive likelihood ratio (LR), negative LR and percent accuracies, of the individual variables CRP, PCT, duration of fever and urinalysis. We used the natural logarithm of CRP and PCT (hence referred to as LnCRP and LnPCT) as this approached optimal modeling as explored by restricted cubical splines.[5] Second, we performed multivariable logistic regression analyses to evaluate the association between

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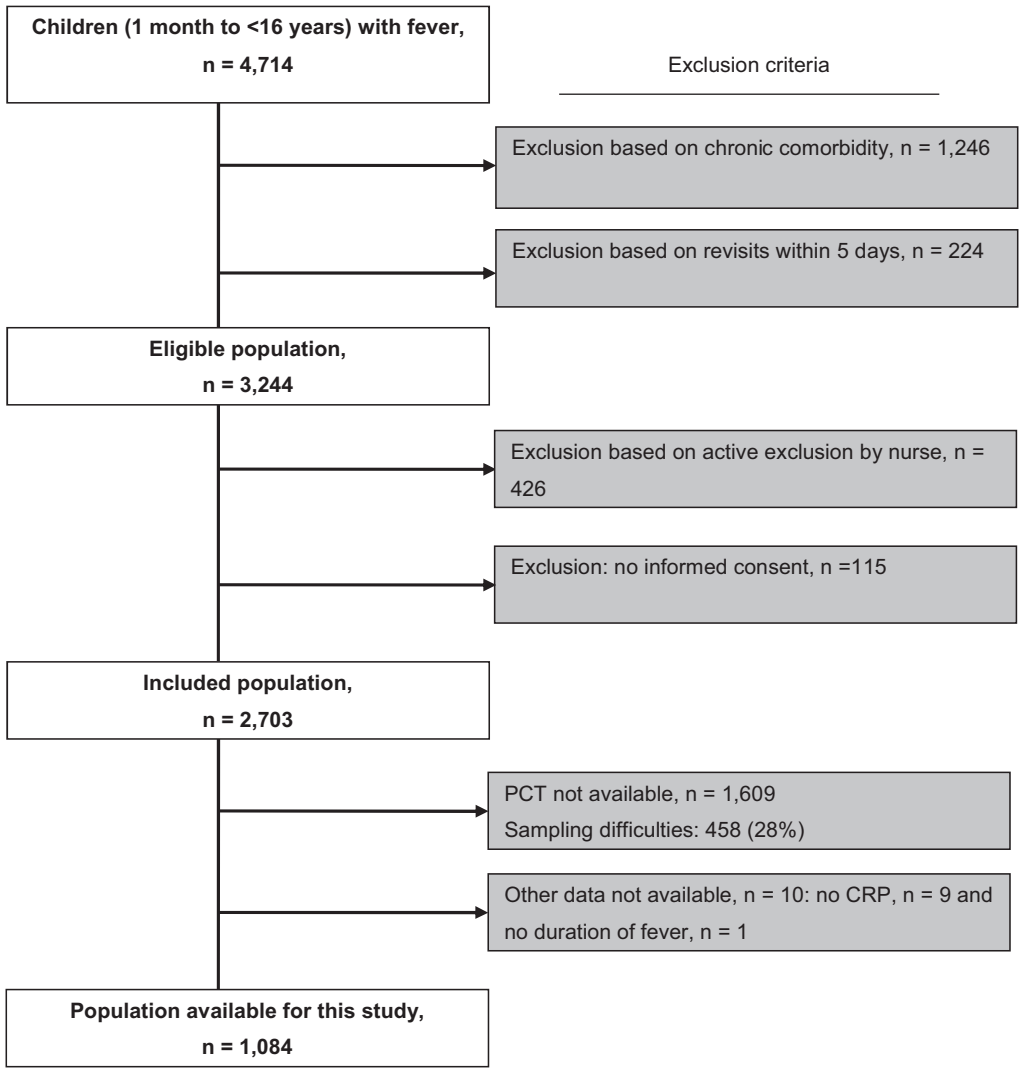

FIGURE 1. Patient selection.

CRP, PCT and duration of fever. We included interaction terms (inflammatory markers and duration of fever) and stratified for duration of fever ≤24 hours versus >24 hours. Third, we determined the discriminative ability (ie, ROC-area) of the Lab-score in our external validation cohort.[6,7] We assessed calibration by comparing predicted and observed risks. Fourth, we updated the Lab-score by using LnCRP and LnPCT. which allowed for comparison of the diagnostic performance of an optimized model with that of the existing Lab-score. Finally, we investigated the clinical usefulness, by means of alterations of pretest probabilities to post-test probabilities, of additional PCT values to often used threshold values of CRP, ie, 20, 40 and 100 mg/L. Number needed to test (NNT) to identify 1 SBI was calculated by taking the inverse of having a true positive test among all tested children: 1/[(true positive)/(true positive + true negative + false positive + false negative)]. Model performance was assessed by means of the ROC-area, R^2 and model χ^2.[20] We performed a complete case analysis for children who had PCT, CRP, duration of fever and urinalysis available. We tested for differences between the Erasmus MC–Sophia and the Maasstad cohorts by including the cohorts as statistical interaction terms with the predictor variables (interaction by cohort).[21] All analysis were performed using R statistical software.[22]

RESULTS

Population Characteristics

Of 2703 included children with fever, 1084 children, who had both CRP and PCT determined and duration of fever registered, were available for this study (Fig. 1). Median age was 1.59 years (interquartile range: 0.84–3.45) and 170 children (16%, 95% CI: 14–18%) had an SBI, of which 73 children had pneumonia (7%), 56 children had UTI (5%) and 41 had another SBI (4%) (Table 1). In 458 of 1609 (28%) children with PCT sampled, PCT could not be determined because of insufficient amount of serum.

Univariate Analyses

LnCRP and LnPCT were both significant predictors of SBI: CRP with a ROC-area of 0.77 (95% CI: 0.69–0.85) for LnCRP, versus a ROC-area of 0.75 (95% CI: 0.67–0.83) for LnPCT (Table 2). Model performance decreased substantially when PCT and CRP were dichotomized using different cutoffs. High cutoffs of 100 mg/L

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TABLE 1. General Characteristics of Included Population

Variable, Median (25th to 75th percentile)*	Study Population (n = 1084)	SBI (n = 170)	NonSBI (n = 914)	*P*-value†
Patient characteristics				
Age (years)	1.59 (0.84–3.45)	2.32 (0.87–5.50)	1.50 (0.84–3.14)	0.003
Gender (female), n (%)	478 (44%)	99 (58%)	379 (41%)	<0.001
Temperature (°C)‡	38.9 (38.2–39.5)	39.1 (38.4–39.8)	38.9 (38.1–39.5)	0.008
Lab-score variables				
Duration of fever (days)	2.00 (1.00–4.00)	2.00 (1.00–4.75)	2.00 (1.00–4.00)	0.007
CRP (mg/L)	15 (8–40)	53 (20–135)	12 (8–30)	<0.001
PCT (ng/mL)	0.19 (0.10–0.57)	0.74 (0.25–3.17)	0.16 (0.09–0.41)	<0.001
Urinalysis (positive), n (%)	96 (9%)	57 (34%)	39 (4%)	<0.001
Lab-score total	0 (0–2)	4 (1–7)	0 (0–2)	<0.001
Outcome measure				
SBI, n (%)	170 (16%)			
Pneumonia	73 (7%)			
SBI other§	97 (9%)			
No SBI	914 (84%)			

*Unless otherwise noted.
†Nonparametric Kruskall–Wallis test for continuous variables, χ^2 analysis for dichotomous variables.
‡Data available for 1069 children (98%).
§Others include UTI, sepsis/meningitis, erysipelas, cellulitis, bacterial gastro-enteritis, cellulitis orbitae, ethmoiditis, arthritis and osteomyelitiss.

for CRP (specificity 0.96, 95% CI: 0.94–0.97, positive LR 8.43, 95% CI: 5.77–12.31) or 2.0 ng/mL for PCT (specificity 0.94, 95% CI: 0.92–0.95, positive LR 5.19, 95% CI: 3.74–7.21) were useful to rule in SBI. Threshold values of CRP and PCT were not useful for ruling out SBI (Table 3). Urinalysis (odds ratio: 11.32, 95% CI: 7.20–17.78) was a strong predictor of SBI (Tables 2 and 3). Duration of fever had a small, but significant effect (odds ratio: 1.13 per day, 95% CI: 1.04–1.24, Tables 2 and 3), but could not be used to correctly classify children with or without SBI.

Multivariable Analyses

Duration of fever had no significant additional diagnostic value to a model containing LnCRP, LnPCT and urinalysis (Table 2). We observed no interaction between the variables in the multivariable model and no differences in model performance when stratifying for duration of fever (cutoff at >24 hours). We found no significant differences between our 2 cohorts.

Validating and Updating of Original Lab-score

Validation of the original Lab-score in our population resulted in a ROC-area of 0.79 (95% CI: 0.72–0.87). In children with 1 to ≤3 points, the Lab-score calibrated poorly, with much higher observed than predicted risks of SBI among both children with pneumonia, UTI or other SBI. The Lab-score calibrated well for low risk children (0 points) or children with >3 points. At a cutoff of ≥3 points, sensitivity was 0.60 (95% CI: 0.52–0.67), while specificity was reasonable (0.86, 95% CI: 0.84–0.88). Negative LR at a cutoff of ≥3 points could not be used to rule out SBI (0.46, 95% CI: 0.39–0.56); positive LR was useful to identify children with SBI (4.32, 95% CI: 3.53–5.29). This was similar to commonly used cutoffs of single biomarkers (ie, CRP: 40 mg/L and PCT: 0.5 ng/mL, Table 3. A subgroup analysis in children under the age of 3 [total n = 761 (70%), SBI n = 97 (13%)] showed similar results, namely a ROC-area of 0.81 (95% CI: 0.72–0.91), and a cutoff at 3 points had a sensitivity of 0.62 (95% CI: 0.51–0.72), specificity of 0.87 (0.84–0.90), positive LR of 4.83 (95% CI: 3.75–6.22) and a negative LR of 0.44 (95% CI: 0.34–0.56). Updating the Lab-score using LnPCT, LnCRP and urinalysis resulted in a ROC-area of 0.83 (95% CI: 0.76–0.90) (Table 2). Models with either CRP and urinalysis or PCT and urinalysis performed well, but not as well as the Lab-score.

Targeted Diagnostic Approach

PCT did not alter pretest probabilities on a clinically relevant scale for children with low CRP levels [<20 mg/L, n = 628 (58%)] or high levels of CRP [≥100 mg/L, n = 95 (9%)]. Additional PCT results altered pretest probabilities in children with CRP values between 20 and 100 mg/L in particular (Fig. 2). Duration of fever did not alter post-test probabilities sufficiently to be used as a differentiating variable. In children with low levels of CRP (<20 mg/L) and PCT (<0.5 ng/mL), urinalysis had to be performed in 69 children (ie, NNT) to assist in correctly classifying 1 SBI.

DISCUSSION

Principal Findings

CRP and PCT were both strong predictors of SBI. Duration of fever did not improve the predictive value of CRP and PCT for detecting SBI. The Lab-score, combining both these biomarkers with urinalysis, performed well in our prospective cohort of febrile children, although thresholds for the Lab-score only marginally outperformed thresholds of single biomarkers. The discriminative ability of an updated Lab-score increased moderately. In many children, there was no indication for determining both CRP and PCT: in children with high levels (≥100 mg/L) or low levels (<20 mg/L) of CRP, accounting for 67% of our population, PCT did not alter pretest probabilities substantially. Contribution of urinalysis to clinical risk assessment in children with high or low values of CRP and PCT was limited.

Comparison with Existing Literature

The diagnostic values of CRP and PCT corresponded to those previously reported.[1,2] As is a common feature of validation studies, the discriminative ability of the Lab-score was lower than those originally reported, underlying the importance of external validation studies in different settings.[5–7,23–26] However, even a newly derived and updated Lab-score did not match the diagnostic properties of the originally reported Lab-score. Especially, the diagnostic strength for ruling out SBI was considerably higher in the original studies, as reflected by higher sensitivities and negative LRs.[6,7] Higher incidences of SBI in the original studies compared with our incidence could explain these differences as they indicate differences in severity of disease between the populations, and

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TABLE 2. Diagnostic Performance of Individual Markers and Lab-score for Predicting SBI

	Odds Ratio (95% CI)	ROC-area (95% CI)	R^2	χ^2	Degrees of Freedom Used	P value
Univariate Analysis						
CRP						
Continuous (natural logarithm) mg/L*	2.71 (2.28–3.22)	0.77 (0.69–0.85)	0.22	148.52	1	<0.001
Cutoff: ≥20 mg/L	5.29 (3.63–7.70)	0.69 (0.62–0.76)	0.14	88.19	1	<0.001
Cutoff: ≥40 mg/L	5.67 (4.01–8.01)	0.69 (0.61–0.77)	0.15	97.75	1	<0.001
Cutoff: ≥100 mg/L	12.27 (7.77–19.38)	0.65 (0.58–0.72)	0.18	115.97	1	<0.001
PCT						
Continuous (natural logarithm) ng/mL†	1.94 (1.72–2.19)	0.75 (0.67–0.83)	0.18	123.09	1	<0.001
Cutoff: ≥0.5 ng/mL	5.32 (3.77–7.51)	0.69 (0.61–0.77)	0.14	92.73	1	<0.001
Cutoff: ≥2.0 ng/mL	7.25 (4.78–10.99)	0.63 (0.56–0.71)	0.13	81.50	1	<0.001
Urinalysis						
Positive	11.32 (7.20–17.78)	0.65 (0.57–0.72)	0.17	109.44	1	<0.001
Duration of fever						
Days (per day, rounded to half a day)	1.13 (1.04–1.24)	0.56 (0.47–0.66)	0.01	7.72	1	0.005
Cutoff: >24 hours	1.42 (1.00–2.00)	0.54 (0.46–0.62)	0.01	3.95	1	0.05
Multivariable analysis						
Original Lab-score						
Per point, max. 9 points (validation)‡	1.55 (1.45–1.66)	0.79 (0.72–0.87)	0.27	186.37	1	<0.001
≥3 points (validation)‡	9.30 (6.49–13.32)	0.73 (0.65–0.81)	0.23	152.25	1	<0.001
Lab-score with updated coefficients		0.83 (0.76–0.90)	0.35	248.68	3	
CRP						
Continuous (natural logarithm) mg/L	1.98 (1.59–2.47)					<0.001
PCT						
Continuous (natural logarithm) ng/mL	1.42 (1.21–1.67)					<0.001
Urinalysis						
Positive	9.95 (5.94–16.68)					<0.001
Updated Lab-score with addition of duration of fever		0.83 (0.76–0.90)	0.35	249.25	4	
CRP						
Continuous (natural logarithm) mg/L	1.96 (1.57–2.45)					<0.001
PCT						
Continuous (natural logarithm) ng/mL	1.42 (1.21–1.67)					<0.001
Urinalysis						
Positive	9.99 (5.96–16.75)					<0.001
Duration of fever						
Continuous, in days	1.04 (0.93–1.17)					NS

*CRP value of 10 mg/L: LnCRP = 2.30; CRP value of 20 mg/L: LnCRP = 3.00; CRP value of 40 mg/L: LnCRP = 3.69; CRP value 100 mg/L: LnCRP = 4.61.
†PCT value of 0.5 ng/mL: LnPCT = −0.69; PCT value of 1.0 ng/mL: LnPCT = 0; PCT value of 1.5 ng/mL: LnPCT = 0.41, PCT value of 2.0 ng/mL: LnPCT = 0.69.
‡ Validation of original Lab-score: diagnostic performance of the original Lab-score was calculated by using the score system as described in the original studies.

imply less disease heterogeneity and thus less discriminative ability in our study population. A recent retrospective validation study of the Lab-score largely corresponded with our observations,[27] but had included well appearing infants younger than 3 months of age only, and their findings might be less applicable to a general population of febrile children. Also, the Lab-score was originally developed targeting children up to 3 years old, possibly contributing to the lower diagnostic performance in our population. However, a subgroup analysis in children up to 3 years was in line with our overall findings. In addition, differences could partly have been instigated by a more stringent definition for UTI in the original studies, which required an additional positive dimercaptosuccinic acid scan in the acute phase, accompanying a positive culture and clinical signs and symptoms. To the contrary, among low-scoring SBIs in our population were not only children with UTI but also many children with pneumonia and other SBI.

In this study, we failed to replicate evidence supporting PCT being a stronger predictor of SBI in children with a short duration of fever.[8–10] Perhaps, PCT and CRP already reached their plateau concentration levels at our threshold of 24 hours, as physiologic changes in levels of PCT and CRP occur mostly within the first 24 hours.[11,12,28] Only 54 children (3 with SBI) had a duration of fever of 12 hours or less, limiting power of this analysis, but also suggesting limited value in practice.

Clinical Approach of Febrile Children

With overcrowding of EDs becoming an increasing worry,[29,30] there is need to identify those children in whom additional testing can be omitted from the diagnostic work-up. We observed similar diagnostic values for CRP and PCT. From a pragmatic, and biologically plausible, perspective, we would suggest measuring PCT in febrile children with a short onset of fever, and CRP in children with a longer duration of fever, notwithstanding the lack of statistical evidence to support this in our study. From Fig. 2, one can derive that neither in children with low levels of CRP (<20 mg/L) nor in children with high values of CRP (≥100 mg/L) additional PCT results changed the risk of having an SBI on a clinical relevant scale. A targeted approach of PCT testing in children with intermediate values of CRP thus seems to reduce redundant diagnostic testing without influencing clinical decisions. However, it should be noted that the clinical usefulness of such a targeted diagnostic approach also depends on clinical signs and symptoms, which need to be considered when interpreting these findings.[5] Even though urinalysis was useful for ruling in SBI. it lacked diagnostic ability for ruling out SBI. For practical reasons, such as to prevent a prolonged length of stay awaiting the collection of urine samples, we propose a more selective approach of urine sampling in febrile children, in particular for children with intermediate values of CRP or PCT. For example, the NNT to test

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TABLE 3. Diagnostic Performance (95% Confidence Intervals) of Lab-score and Inflammatory Markers for SBI

	N (%)	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	Positive LR (95% Confidence Interval)	Negative LR (95% Confidence Interval)
Lab-score					
≥1 point	449 (41%)	0.82 (0.75–0.87)	0.66 (0.63–0.69)	2.41 (2.15–2.70)	0.28 (0.20–0.38)
≥2 points	408 (38%)	0.73 (0.66–0.79)	0.69 (0.66–0.72)	2.35 (2.05–2.68)	0.39 (0.31–0.50)
≥3 points*	**229 (21%)**	**0.60 (0.52–0.67)**	**0.86 (0.84–0.88)**	**4.32 (3.53–5.29)**	**0.46 (0.39–0.56)**
≥4 points	215 (20%)	0.54 (0.46–0.61)	0.86 (0.84–0.89)	3.95 (3.18–4.89)	0.54 (0.46–0.63)
≥6 points	112 (10%)	0.36 (0.29–0.44)	0.95 (0.93–0.96)	6.67 (4.77–9.32)	0.67 (0.60–0.75)
≥8 points	52 (5%)	0.22 (0.16–0.29)	0.98 (0.97–0.99)	13.26 (7.45–23.62)	0.80 (0.73–0.86)
CRP					
≥20 mg/L	470 (43%)	0.76 (0.69–0.82)	0.63 (0.59–0.66)	2.03 (1.81–2.29)	0.38 (0.29–0.50)
≥40 mg/L	275 (25%)	0.58 (0.50–0.65)	0.81 (0.78–0.83)	2.98 (2.47–3.58)	0.53 (0.44–0.63)
≥100 mg/L	95 (9%)	0.34 (0.27–0.42)	0.96 (0.94–0.97)	8.43 (5.77–12.31)	0.69 (0.62–0.77)
PCT					
≥0.5 ng/mL	303 (28%)	0.60 (0.52–0.67)	0.78 (0.75–0.81)	2.73 (2.29–3.24)	0.51 (0.43–0.62)
≥2.0 ng/mL	114 (11%)	0.33 (0.26–0.41)	0.94 (0.92–0.95)	5.19 (3.74–7.21)	0.72 (0.64–0.80)
Urinalysis					
Positive	96 (9%)	0.34 (0.26–0.41)	0.96 (0.94–0.97)	7.86 (5.41–11.451)	0.69 (0.62–0.77)
Duration of fever					
>24 hours	660 (61%)	0.68 (0.60–0.75)	0.40 (0.37–0.44)	1.13 (1.01–1.28)	0.80 (0.64–1.01)

*A cutoff at 3 points was proposed in the original publications.

in children with low levels of PCT and CRP to assist in classifying 1 SBI correctly was high, namely 69. Sampling in these low risk children should therefore depend on additional clinical features to prevent unnecessary false positive test results. Likewise, in children with a high risk of SBI, for example due to high levels of CRP or PCT, urinalysis and cultures are required for focus identification rather than for altering clinical risk assessment.

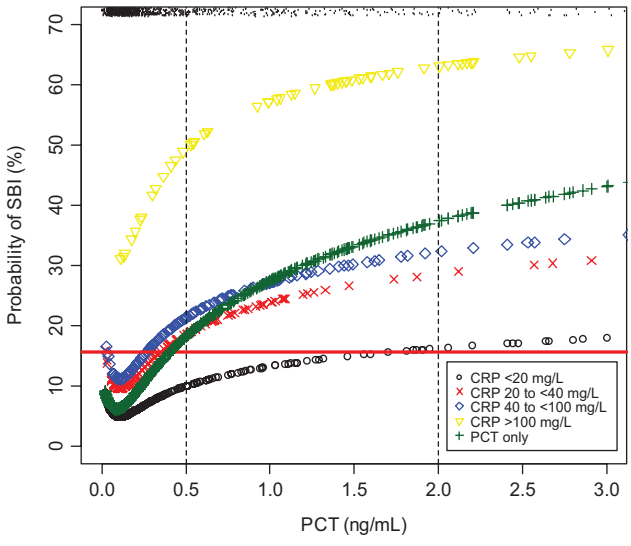

FIGURE 2. Probabilities of SBI. The risk of having an SBI (y-axis) is plotted for values of PCT (ng/mL) (green "+" signs), and is stratified for different values of CRP (mg/L). The risk of having SBI is calculated with 1/[1 + exp(–linear predictor)], where the linear predictor from logistic regression analyses varies for each threshold category of CRP and each value of PCT. The red horizontal line represents the pretest probability, or incidence of SBI. The 2 dashed vertical black lines are set at the cutoff values for PCT as used in the Lab-score. The scatter bar at the top of the graph reflects the number of observed values of PCT. PCT alters the risk substantially for children with CRP values between 20 and 100 mg/L. For children with values of CRP <20 or ≥100 mg/L additional PCT does not significantly influence the post-test probabilities of having an SBI.

Strengths and Limitations

Main strength of our study is the large, prospective cohort of febrile children with a large number of children with SBI, ensuring sufficient power for our validation study.[31] Also, whereas previous validation studies selected young infants or febrile children with an increased risk of SBI, we considered a general population of febrile children up to 16 years, reflecting the population of patients visiting the ED daily. Moreover, we combined 2 different cohorts that, rather than posing a problem of heterogeneous case-mix, supported the Lab-score's capacity of broad external validity. Although PCT was not determined in some 50% of the included children, partly because of sampling difficulties, we feel our study findings are generalizable to other emergency care settings, and can be applied to those children in whom physicians will consider additional laboratory tests in particular. Overall, the children available for this study had a slightly higher incidence of SBI, were hospitalized more often and were prescribed antibiotic therapy more frequently compared with the total included population. The diagnostic value of CRP in children who did not have PCT sampled, or had too little serum sampled, was similar to that in the study population, supporting the generalizability of our findings.

One of the main limitations is that we did not perform required reference tests to establish final diagnoses in all children. Rather, as it would be impractical and unethical to perform these tests in all children, diagnostic testing was left to the discretion of the attending physician and we ensured final diagnosis of SBI by implementing standardized follow-up of all children after ED discharge. In addition, assuming urinalysis to be negative if not performed, might have resulted in an underestimation of false-positive results detracting from the true diagnostic performance of the test. However, this effect should be limited if urine samples were taken appropriately. In addition, as we ensured not missing any clinically relevant UTI, the selective sampling of urine did not result in missed true-positives, or false-negatives.

CONCLUSION

CRP and PCT are useful diagnostic tools in the evaluation of the febrile child. Duration of fever did not influence the

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performance of CRP and PCT. The Lab-score performed well in a prospective cohort of febrile children, confirming its external validity in emergency care settings.

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Authors' contributions: All authors substantially contributed to the study and the writing (ie, drafting and/or critical revision) of the manuscript. In particular, R.G.N. was responsible for protocol development, data collection, data analysis and was the main author of the article; H.A.M. supervised protocol development, interpretation of the results and writing of the manuscript; F.J.S. was responsible for protocol development and supervised data collection and patient inclusion in the Maasstad hospital; A.G. was involved in writing and critical appraisal of the manuscript, and in interpreting results; F.W. was responsible for data collection and laboratory analyses in the Maasstad hospital; Y.V. supervised data analysis and contributed to the interpretation of the data; Y.B.d.R. contributed to the protocol development, acquisition of data and laboratory analyses; R.O. supervised protocol development, patient inclusion, data analysis and writing of the manuscript and was responsible for obtaining funding. R.O. will act as corresponding author, and confirms that she had full access to the data and had final responsibility for the decision to submit for publication. All authors read and approved the final manuscript. All authors had full access to all of the data in the study and can take full responsibility for the integrity of the data and the accuracy of the data analysis.

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