Abstract
As a result of rapid developments in many fields of medicine in recent years, most pediatric emergency diagnoses can be made while the patient is still in the emergency department. Often, an accurate medical history and a physical examination are all the physician needs to make a diagnosis, but in some situations it is very difficult to identify children with dangerous conditions. Fever is one of these. We demonstrated that measuring levels of either C-reactive protein (CRP) or procalcitonin (PCT) was superior to a clinical evaluation in predicting serious bacterial infection in children aged 1 month to 3 years old. In another study, we showed that levels of proadrenomedullin, but not copeptin, may help to predict complications in children with community-acquired pneumonia and who therefore require a more aggressive therapy. As a computerized tomography scan (CT scan) is potentially harmful, we found that measuring S100B protein was helpful in decreasing the number of imaging studies needed in the management of children suffering from a mild traumatic brain injury.

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## Abstract


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Abstract

As a result of rapid developments in many fields of medicine in recent years, most pediatric emergency diagnoses can be made while the patient is still in the emergency department. Certain diagnostic investigations may take many days to provide a result, however, or they may be expensive, not readily available, or even harmful. For all these reasons, physicians would like to have rapid, safe, inexpensive tests to help them categorize or pre-select children who really need further investigations and treatment. Tests for biomarkers can meet all these requirements.

The ideal, perfectly accurate biomarker does not exist. However, because of their usefulness in helping physicians in their decision-making processes, there is a need for more research on the accuracy of biomarkers in varied pediatric emergency populations and settings. Often, an accurate medical history and a physical examination are all the physician needs to make a diagnosis, but in some situations it is very difficult to identify children with dangerous conditions. Fever is one of these. We demonstrated that measuring levels of either C-reactive protein (CRP) or procalcitonin (PCT) was superior to a clinical evaluation in predicting serious bacterial infection in children aged 1 month to 3 years old. In another study, we showed that levels of proadrenomedullin, but not copeptin, may help to predict complications in children with community-acquired pneumonia and who therefore require a more aggressive therapy. As a computerized tomography scan (CT scan) is potentially harmful, we found that measuring S100B protein was helpful in decreasing the number of imaging studies needed in the management of children suffering from a mild traumatic brain injury.

The development of biomarker use in pediatric emergency medicine will need further research to find new biomarkers or new indications for known molecules, design scores incorporating multiple biomarkers, integrate their diagnostic performance accuracy in electronic devices, and finally remove the barriers to their use in clinical practice.
1. Introduction

1.1. Pediatric emergency medicine

Pediatric emergency medicine in industrialized countries (1) has developed tremendously in the last 20 years, particularly in Switzerland.

A pediatric emergency department (ED) was formerly the place where physicians had to decide whether a child should be sent home with a simple treatment or admitted for further diagnostic investigations—because these could take many days—and/or treatment.

Pediatric EDs were segmented by specialty. Children with a suspected surgical disease or trauma were seen by a surgeon; those with medical problems, by a pediatrician. Two teams would work in parallel instead of together, leading to disparities in terms of waiting times depending on the season (more traumas in summer, more infections in winter) and the availability of the specialists.

The pediatric ED was also the place where nobody wanted to be assigned: it was a stressful environment where very sick children could arrive at any time, sometimes hidden between nearly healthy patients, and there were no specialized senior physicians.

In recent years, the number of emergency consultations at Geneva’s Children Hospital (HUG) has grown dramatically. Visits have nearly doubled in the last 10 years, reaching 27,000 per year, or a mean of 74 per day (Figure 1).
All of these issues, together with developments in other specialties, have lead to profound changes in the HUG’s pediatric ED. Firstly, the department had to grow to be able to deal with all these children. There is now a new children’s hospital building, more nursing and medical staff, and the pediatric unit has become a full department in its own right, with a chair in pediatric emergency medicine (Prof. Alain Gervaix). The ED segmentation between surgical and medical teams has now disappeared. Each child is seen by the same team of physicians, whatever the complaint, and waiting time is related solely to the estimated degree of emergency at triage. Pediatric emergency physicians work together with all the other specialties in order to take multidisciplinary decisions for the wellbeing of the children.

All of the changes occurring in Geneva also occurred in most industrialized countries, and growing worldwide interest in the field of emergency pediatrics led to specific teaching,
research, and finally a new specialty. It took five years for Switzerland to establish an *FMH Schwerpunkt*—a Swiss Medical Association post-graduate training course—to become a specialist in pediatric emergency medicine, but since 2014 it has been considered as a pediatric or pediatric surgery subspecialty.

What happens in the pediatric ED has also changed dramatically. Investigations used to take many days, during which time children often had to be hospitalized. Nowadays, thanks to advances in science, our greater experience, and the development of radiology and laboratory medicine, most diagnoses in the pediatric ED can be made within minutes or hours. Today, that speed of diagnosis has come to be expected.

### 1.2. Diagnosis in a pediatric ED

Beyond making a diagnosis, a pediatric emergency physician has to stratify patients into four categories: children with life-threatening conditions needing emergency treatment, children needing a specific treatment, children with self-limited diseases (such as viral infections, contusions, or migraine) needing only symptomatic medication, and children needing more investigations before they can be categorized.

An accurate medical history and thorough physical examination will very often provide the physician with the diagnosis. This oft-repeated idea is true for the majority of pediatric ED patients and nearly all the children seen in private pediatric practices. Thus pediatric emergency physicians do a medical history and physically examine them thoroughly, because even if these do not supply an obvious diagnosis, they may exclude dangerous conditions and help to stratify the patient into one of the categories mentioned above. Sometimes, however, this is not enough.
In most diseases, signs and symptoms do not all appear at the same time, but rather progressively. When non-specific signs are the first to develop, diagnosis is much more difficult in a disease’s early stages. In viral or bacterial infections, for example, fever may precede focal symptoms or signs, such as a cough in pneumonia, by many days.

As medicine has developed and access to it has become ever easier, parents tend to consult earlier and earlier. Whereas children in some countries are kept at home until they are nearly unconscious, at the HUG children are sometimes consulted 30 minutes after the first signs of fever. A specific history of the case and clinical signs are therefore often scarce.

It may thus be difficult, even for experienced physicians, to differentiate between a viral infection and occult bacteremia in a child aged under 3 years old who has a fever without source, between concussion and an intracranial hemorrhage, or between mesenteric adenitis and appendicitis before overt local peritonitis.

All such cases will need further investigations, but blood or urine cultures, for example, may take many days to produce a result, making any rapid decision impossible. Other tests can be expensive or not readily available, such as magnetic resonance imaging (MRI). Indeed, the latter may even require general anesthesia for younger children. Finally, some diagnostic tools may be harmful, as is the case for CT imaging; its ionizing radiation can lead to lethal malignancies, with rates as high as 1 in 1,000 following head CT scans, and younger children are even more susceptible (2).

For all these reasons, physicians and patients need rapid, safe and inexpensive tests to help categorize or pre-select children who need further investigations and treatment. Testing for biomarkers has the potential to meet all those requirements.
1.3. Biomarkers in pediatric emergency medicine

A biomarker is a biological marker. A consensus panel brought together by the US National Institutes of Health defined it as a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (3).

Some authors distinguish biomarkers from physiomarkers. Although very similar to biomarkers, the latter can be objectively or subjectively measured physiological indicators of a disease process and are also useful in diagnosing and monitoring diseases (4). One of the most popular physiomarkers of infection is an elevated body temperature (fever). There are many other physiological indicators of disease processes, such as heart rate, blood pressure, capillary refill time, or respiratory rate. When put together, physiomarkers can be used to form a clinical score, such as a dehydration score (5).

A marker may be useful before, during, and after the diagnosis (6). Before diagnosis, it can be useful in disease prevention, such as the serum cholesterol value for identifying potential cardiovascular disease (7), or in disease screening, such as the Prostate Specific Antigen level, which is higher in prostate cancer (8).

During diagnosis, a marker may simply indicate the presence of a disease (troponins in myocardial infarction [(9)]). The severity of this disease and its prognosis may also be assessed with markers, helping clinicians to choose the right therapeutic option. For example, iv antibiotics and hospitalization may be the right treatment for a child with pneumonia if the serum proadrenomedullin level is elevated as this marker is associated with bacteremia and pleural effusion in children (10). A marker may stratify a disease and help grade/stage it, such as in cancer diagnosis (11) or in intensive care (12). It may also help identify children who need no further evaluation, in order to prevent unnecessary and potentially harmful investigations, as in fever without source (13) or traumatic brain injury (14).
Finally, even after diagnosis, a biomarker may be useful for assessing treatment efficacy and monitoring therapy: measuring serum glucose is a direct indicator of insulin treatment efficacy (15) and PCT is an indirect indicator of the success of antibiotic treatment and a good guide for terminating that treatment (16, 17).

1.4. The ideal biomarker

The ideal biomarker would be safe and easy to measure. Because a venipuncture is painful, a urine marker is considered superior to a serum marker in children. The biomarker should be measureable shortly after its analysis is requested. A point of care test (POCT) is a medical test that can take place at or near the site of patient care, and is thus preferable as no time is wasted for transport and the result is available in a few seconds or minutes.

There must be a good correlation between the different measurement methods for the same biomarker. For example, we demonstrated that there was only a moderate agreement between PCT results measured semi-quantitatively (PCT-Q®) and quantitatively (PCT Kryptor®)(18).

Cost is also an important factor. This is especially true for screening markers, such as the metabolic screening of newborns, as the whole population of children is tested.

The ideal biomarker would also be consistent between genders and across a range of ethnic groups and, particularly in pediatric medicine, ages. This is not the case for PCT in newborns. Levels of PCT increase physiologically in the first 24 hours of life—sometimes reaching the very high concentrations found in sepsis (≥ 5 μg/L)—and then decrease after 36 hours (19).

Furthermore, an ideal biomarker would be perfectly accurate. A positive test would always indicate the presence of the disease and a negative test would always rule it out. Unfortunately, however, there is no ideal biomarker.
1.5. Research method in biomarkers

Although there are no ideal biomarkers, they can nevertheless help physicians in their decision-making processes, and thus the research to evaluate the real-world accuracy of those markers, as applied to the population at hand, should be performed in pediatric emergency settings (5, 10, 14, 18, 20-25).

To assess a biomarker’s validity in providing the correct answer in a particular population, we first need to define a gold standard test (i.e., the current preferred method of diagnosis) (15). This can be straightforward, such as a bacterial growth in cerebral spinal fluid for the diagnosis of meningitis. But often, even the gold standard does not deliver 100% sensitivity and specificity. Blood culture is usually the gold standard for sepsis, but it can produce false negatives or false positives (contamination). There are situations where it is impossible to make an unqualified diagnosis. Indeed, for an unqualified diagnosis of bacterial pneumonia, physicians should have access to a bacterial isolation from an alveolar aspirate, which is not feasible. Studies having bacterial pneumonia as their endpoint usually define the diagnosis via a consolidation on a chest X-ray, with or without clinical and laboratory signs (26). However, as viral and bacterial infections may exhibit the same X-ray image, it is always difficult to ensure the validity of markers in these studies. Another example of an outcome is upper urinary tract infection in young children. Official international clinical guidelines (27) recommend that a diagnosis of pyelonephritis can be made using a positive urine culture from a febrile child. Indeed, many studies have used these guidelines as the gold standard (24, 28, 29). However, renal technicium 99-m dimercaptosuccinic acid (DMSA) scintigraphy is a more precise—although more invasive—tool for the diagnosis of upper urinary tract infection. Studies that used this test for outcome showed that as many as 30% of febrile upper urinary tract infections were cystitis and not pyelonephritis (30), which is a significant gap between marker evaluation and clinical practice.
Due to the development of new diagnostic methods, gold standards for the same diagnosis may change over time. Thus, the accuracy of a biomarker may be different depending on how old the studies are. *Kingella kingae* rarely grows in standard cultures. Recently, a very sensitive and specific real-time polymerase chain reaction (PCR) assay was developed that allows a more accurate diagnosis (31). This tool was not available to Kocher *et al.* when they built their criteria (based on clinical signs and biomarkers) for detecting septic arthritis (32). However, it helped Ceroni *et al.* to differentiate osteoarticular infections caused by *Kingella kingae* from those due to typical pathogens with different marker values (33).

Biomarker accuracy is evaluated using specific tests known as test performance characteristics. The most frequently used are sensitivity and specificity. Sensitivity is defined as the proportion of subjects with the disease in whom the test gives a positive result. In other words, it refers to how good a test is at correctly identifying people who have the disease. Specificity is the proportion of subjects without the disease in whom the test is negative or how good the test is at correctly identifying people who are well (34). A test with very good sensitivity will be used to rule out a condition (such as in screening markers), and a test with very high specificity will be used to rule it in. These tests are independent of the prevalence of a disease and therefore do not vary if either the setting or the population change.

However, if physicians want to use sensitivity and specificity for the accuracy analysis of a biomarker, then this requires dichotomous results and outcomes. The disease status has to be positive or negative, just as the test has to be. If a biomarker’s value is a continuous variable, such as CRP, then a cut-off must be defined at which the test is considered positive. Sensitivity and specificity will be different for each cut-off value, with lower specificity when sensitivity is higher.

This latter characteristic is best described using the receiver operating characteristic (ROC) curve which plots the true positive rate against the false positive rate for every cut-off point.
(Figure 2). The closer the curve is to the left and top sides of the plot, the more accurate the marker is in differentiating children with the disease from those without it. The diagonal line represents a test that detects an equal number of true and false positives. To summarize this ability to differentiate across the full range of cut-off values, we use the area under the curve (AUC) which lies between 1 (a perfect test) and 0.5 (a useless test) (35). The ROC and the AUC are very useful for evaluating and comparing accuracy between two tests, but also for selecting the optimal cut-off point.

Figure 2: ROC curve. Published in: Manzano et al. ADC 2011 May;96(5):440-6 (24)

Although clinicians are familiar with the concept of sensitivity and specificity, what they really want to know is the probability that their patient with the positive biomarker has the disease in question. Here lies the utility of predictive values.
Positive predictive value (PPV) reflects the probability that a patient with a positive diagnostic test result has the disease (e.g., the probability of actually having a urinary tract infection if a urinary nitrite test is positive), and negative predictive value (NPV) reflects the probability that a patient with a negative diagnostic test result does not have the disease (the probability of not having a urinary tract infection if urinary nitrite test is negative) (15). However, these tests are affected by the prevalence of the disease and are not transposable to other settings or populations as the prevalence is often different.

The likelihood ratio is a more powerful and useful statistical test for helping physicians in their diagnostic decision-making using biomarker results. It takes sensitivity and specificity into account simultaneously and is not dependent on prevalence (15). The likelihood ratio indicates how many times more likely patients with the disease are to have a positive biomarker result than patients without the disease (a positive likelihood ratio, or LR+). A negative likelihood ratio (LR-) expresses the odds that a negative test result will be observed in patients with the disease versus the odds that a negative result will be observed in patients without the disease. The higher the LR+ or the lower the LR-, the more useful the test.

1.6. Clinical use of biomarkers

Sensitivity and specificity describe how pathology predicts a biomarker result. PPV and NPV give the probability of disease if testing is positive (or negative), but they are dependent on the prevalence of the disease in the study population and can be transposed only rarely to other populations. Clinicians can use the likelihood ratios in Fagan’s nomogram to calculate the probability of a disease depending on its pre-test probability (36) (Figure 3). In children with fever without source, a CRP level < 10 mg/l has an LR- of 0.1 (24). If we consider the pre-test probability of having a serious bacterial infection (SBI) is 16% (the prevalence of SBI
in this population), the post-test probability will fall to 2% with a CRP level < 10 mg/l (Figure 3), thus nearly ruling out this diagnosis.

![Figure 3: Fagan’s nomogram. Personal data](image)

However, reducing a continuous biomarker value to a dichotomous result is very simplistic and idealistic. Indeed, pediatric emergency physicians are rarely diagnosing such clear cut conditions as fractures. Most of their diagnoses are based on probabilities. In an ideal clinical situation, a simple test result would tell physicians definitively whether or not their patients need a particular treatment, such as antibiotics for children with fever without source. But, as presented previously, biomarkers do not provide such simple answers. This could be one of the easiest errors to make when using biomarkers. A diagnostic marker should be interpreted depending on the value obtained for an individual patient: in many cases, the more significant the result, the higher the probability of having the outcome (24),
and even if a diagnostic marker’s performance is not strong enough at a particular cut-off, it might be very useful for a particular patient whose biomarker value is much higher. This point is illustrated by multilevel likelihood ratios (24, 37, 38). A positive PCT value with a 0.2 ng/ml cut-off in children with fever without source is not a very useful value for the attending physician as LR+ is only 2.8. However, if the child has a PCT value higher than 2 ng/ml and we take this value as a cut-off, LR+ is 7.1 and the probability of a serious bacterial infection is nearly 60%, making antibiotic treatment mandatory (24) (Figure 3).

Another mistake which could be made when using biomarkers in a clinical situation, would be to think that pre-test probability is the disease prevalence. Indeed, this is what most studies have chosen in order to evaluate broader populations. However, the patient’s medical history and the physical examination carried out by the physician should also be considered as markers (physiomarkers) that can increase or lower pre-test probability. This can be thought of as multi-step thinking (Figure 4). The probability that a patient will have a urinary tract infection confirmed by a positive dipstick test for either nitrites or leucocyte esterase is much higher for a 14-year-old girl with dysuria, frequency, and urgency than for a boy with signs of balanitis (39). Even if the LR for every sign or symptom is unknown, years of experience and knowledge will give the physician a sense of that LR and enable an interpretation of the biomarker result depending on this.
Figure 4: Example of multi-step thinking using clinical signs and different biomarkers post-test probabilities in a study comparing typical lobar pneumonia and complicated pneumonia.

*From* : Alcoba et al. 2016, submitted (40)
2. Specific biomarkers in pediatric emergency medicine

As stated above, the majority of pediatric emergency patients do not need any laboratory examinations in order to ensure a correct diagnosis; a thorough medical history and a physical examination are all that a good physician requires to make one. However, in some situations, it is very difficult to identify children with dangerous conditions.

2.1. Diagnostic biomarkers

Probably the most frequent of these pediatric emergency situations is a fever. The majority of the children with fever who attend a pediatric ED have nothing more than a benign viral infection needing symptomatic treatment and some reassurance for the family. However, some of these patients may have a serious bacterial infection, such as upper urinary tract infection, occult bacteremia, or even bacterial meningitis. Indeed, these may be totally asymptomatic (except for the fever) and the child may even be in a good general condition at the beginning of the disease. Many biomarkers have been described previously, such as white blood cell (WBC) count, bands, CRP, and PCT, each with conflicting but interesting results. The methodological and statistical tools mentioned above, particularly multilevel likelihood ratios (24), were therefore used to evaluate each marker’s diagnostic accuracy and its added value for the clinical examination of a Canadian population of children with fever without source. The following chapter describes a diagnostic biomarker study:

2.1.1 Markers for bacterial infection in children with fever without source (24)
Markers for bacterial infection in children with fever without source

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ABSTRACT

Objectives To compare the diagnostic properties of procalcitonin (PCT), C reactive protein (CRP), total white blood cells count (WBC), absolute neutrophil count (ANC) and clinical evaluation to detect serious bacterial infection (SBI) in children with fever without source.

Method Prospective cohort study.

Participants Children aged 1–36 months with fever and no identified source of infection.

Intervention Complete blood count, blood culture, urine analysis and culture, PCT and CRP were also measured and SBI probability evaluated clinically with a visual analogue scale before disclosing tests results.

Outcome measure Area under the curves (AUC) of the receiver operating characteristic curves.

Results Among the 328 children included in the study, 54 (16%) were diagnosed with an SBI: 48 urinary tract infections, 4 pneumonias, 1 meningitis and 1 bacteraemia. The AUC were similar for PCT (0.82; 95% CI 0.77 to 0.86), CRP (0.88; 95% CI 0.84 to 0.91), WBC (0.81; 95% CI 0.76 to 0.85) and ANC (0.80; 95% CI 0.75 to 0.84). The only statistically significant difference was between CRP and ANC (Δ AUC 0.08; 95% CI 0.01 to 0.16). It is important to note that all the surrogate markers were statistically superior to the clinical evaluation that had an AUC of only 0.59 (95% CI 0.54 to 0.65).

Conclusion The study data demonstrate that CRP, PCT, WBC and ANC had almost similar diagnostic properties and were superior to clinical evaluation in predicting SBI in children aged 1 month to 3 years.

INTRODUCTION

The introduction of the pneumococcal vaccine has significantly reduced the prevalence of serious bacterial infection (SBI) and, in particular, of occult bacteraemia in children under 3 years of age.1,2 Despite this, children less than 3 years of age with fever without source remain a clinical challenge as urinary tract infection (UTI), pneumonia or occult bacteraemia cannot be excluded in a well-appearing child.3 Thus, many decisions made by the clinician depend either on patient assessment or on interpretation of complementary laboratory tests.

As total white blood cells count (WBC) and absolute neutrophil count (ANC) have disappointing diagnostic properties,4–8 other surrogate markers of SBI have been evaluated and used in recent years. C reactive protein (CRP) and procalcitonin (PCT) were shown to be better predictors of SBI than WBC.4–7,9–11 However, the utility of these surrogate markers is not well established in the post-pneumococcal vaccination era since their diagnostic properties depend on the prevalence of the disease. In addition, it is not known whether a clinical evaluation by a physician is good enough to rule out SBI when most of the SBI are now likely to be UTIs.

The objective of the present study is to compare the diagnostic properties of PCT, CRP, WBC, ANC and clinical evaluation to detect SBI in children aged 1–36 months presenting to a pediatric emergency department with fever without source, now that most of these children have been vaccinated against Streptococcus pneumoniae.

METHOD

Study design

This prospective cohort study was part of a randomised controlled trial (RCT) assessing the impact of a rapid semiquantitative PCT test on the management of children aged 1–36 months presenting to a paediatric emergency department with fever without source.12 The institutional review board approved the study and written informed consent was obtained from a parent.
Settings and selection of participants
Patient enrolment took place in the emergency department of a tertiary care urban paediatric centre with 60 000 visits annually. To be included, the patient had to be a child between the ages of 1 and 36 months with a history of a rectal temperature over 38°C (100.4°F) with no identified source of infection after careful history taking and physical examination. All patients with known acquired or congenital immunodeficiency, as well as children already treated with antibiotics, were excluded. It is estimated that among eligible children, over 90% had received at least three doses of the PCV7 vaccine against *S pneumoniae* and over 97% at least two doses.\(^13\)\(^14\)

Study protocol
Attending paediatric emergency physicians approached the parents of children meeting the inclusion criteria to participate in the study. After consent was obtained, a blood test for complete blood count (CBC), semiquantitative PCT (for the RCT), CRP, blood culture and a bladder catheterisation or suprapubic aspiration for urine analysis and culture were performed. The attending physicians could perform any other investigations (such as lumbar puncture or chest radiography) as required and the decision to treat with antibiotics or to hospitalise was left to their discretion. A single venipuncture was performed. If this site was lost, or an insufficient amount of blood was drawn, no other attempt was made, as long as a CBC and blood culture were obtained.

The attending physicians, all paediatric emergency physicians, were asked to evaluate the SBI probability with a visual analogue scale (VAS; 0–100%) after the history had been taken and a physical examination had been carried out, but before tests results were available. This comprised the subjective clinical evaluation. Laboratory technicians were blinded to the patients’ final diagnosis.

Outcome measures
The primary outcome was to compare PCT, CRP, WBC, ANC and clinical evaluation (using the VAS) to detect an SBI in children aged 1–36 months with fever without source using the receiver operating characteristic (ROC) and the area under the ROC curve (AUC).

The secondary outcomes were to define, for the group studied, (1) the best cut-off values for the selected surrogate markers, and (2) their clinical utility when the urine analysis was normal using sensitivity, specificity, positive and negative likelihood ratios, as well as positive and negative predictive values. We also aimed to evaluate the multilevel likelihood ratios of the surrogate markers and to calculate post-test probabilities of disease using the Fagan nomogram on the basis of the pretest probability of disease.\(^15\) This enabled us to overcome the limit of a single cut-off value.\(^16\)

PCT and CRP measurement
One millilitre of blood was collected by venipuncture in a heparin/lithium Vacutainer (Becton-Dickinson, Franklin Lakes, New Jersey, USA) and centrifuged. Plasma was then frozen at −40°C. At the end of the RCT study, PCT was measured quantitatively with the ultra-sensitive immunoassay using TRACE (time resolved amplified cryptate emission) technology (Kryptor; Brahms, Hennigsdorf, Germany) in Geneva, Switzerland for the purpose of this cohort study. CRP was also measured for the same purpose using a rapid immunometric method (Nycocard CRP; Axis- Shield, Oslo, Norway) according to the instructions of the manufacturer.

Definitions
- Fever without source: Rectal temperature >38°C (100.4°F) without any signs or symptoms identifying an infectious disease
- SBI: Presence of bacteraemia, UTI, pneumonia, bacterial meningitis, osteomyelitis or septic arthritis
- Bacteraemia: Positive blood culture with bacteria not considered a skin contaminant
- UTI: Any bacterial growth on urine obtained by suprapubic aspiration or ≥10⁴ colony forming units/ml of a single pathogen on urine obtained by bladder catheterisation
- Pneumonia: Lobar consolidation diagnosed on chest radiography confirmed by a paediatric radiologist
- Bacterial meningitis: Cerebrospinal fluid leucocytes >5 cell/μl and positive bacterial culture
- Osteomyelitis: Positive bone scintigraphy
- Septic arthritis: Positive bacterial culture of synovial fluid
- Normal urine analysis: <5 white blood cells with high magnification and nitrite negative.

Data analysis
The primary investigator, who recorded all important information regarding final diagnosis and laboratory tests results, reviewed the medical chart of each enrolled patient and all collection forms. He also contacted all discharged patients for a 1-week telephone follow-up. All data were entered in an Excel database (Microsoft, Richmond, Washington, USA) and analysed using SPSS (v 15.0; Chicago, Illinois, USA) and MedCalc (v 9.6.0.0; Mariakerke, Belgium).

Normally distributed data were reported as mean±SD. Non-normally distributed data were expressed as median and IQR. Categorical variables were reported as percentages. The diagnostic performance of PCT, CRP, WBC, ANC and clinical evaluation (using the VAS) was first evaluated by ROC analysis. The AUC were calculated for each surrogate marker and clinical evaluation. The difference between the AUC of the ROC with standard error was evaluated using MedCalc. Sensitivity, specificity, positive and negative likelihood ratios and positive and negative predictive values were reported with their 95% CI. We calculated post-test probabilities of disease on the basis of the pretest probability of disease (ie, the prevalence) and multilevel likelihood ratios. These multilevel likelihood ratios were based on the optimal cut-off obtained from our ROC analysis and other intuitive cut-offs (including previously published cut-offs).\(^4\)\(^6\)\(^7\)\(^\text{9-11}^\)\(^17\)\(^-19\) The level of significance was set a priori at p<0.05.

RESULTS
Of the 457 infants and children presenting with fever without source who met the inclusion criteria between November 2006 and November 2007, and who were approached by an attending paediatric emergency physician, 328 remained in the study. Reasons for withdrawal are summarised in the study flow chart (figure 1). Table 1 summarises the clinical characteristics of the included patients. During the study, 54 children (16%) were diagnosed with SBI of whom 48 (92%) had UTIs including two with positive blood culture, four (7%) had pneumonia, one (2%) had *Neisseria meningitidis* serogroup b meningitis and one (2%) had...
had an occult *S. pneumoniae* serotype 33 bacteraemia. Among the excluded patients, only eight had an SBI (8/112, 7.1%), all of which were UTIs.

ROC curves for PCT, CRP, WBC, ANC and clinical evaluation are shown in figure 2. The best cut-off values to detect an SBI were determined to be 0.20 ng/ml for PCT, 17.7 mg/l for CRP, 14 100×10⁶/l for WBC, 5200×10⁶/l for ANC and 14.8% for the VAS.

The AUC for the surrogate markers and clinical evaluation are listed in increasing order in table 2. PCT was better than clinical evaluation for detecting SBI: difference in AUC 0.22 (95% CI 0.12 to 0.33). There was no difference between PCT and CRP, WBC or ANC. CRP was better than ANC and clinical evaluation for detecting SBI: difference in AUC 0.08 (95% CI 0.01 to 0.16) and 0.28 (95% CI 0.18 to 0.38), respectively. There was no difference between CRP and WBC. WBC was better than clinical evaluation for detecting SBI: difference in AUC 0.21 (95% CI 0.11 to 0.32). There was no difference between WBC and ANC. ANC was better than clinical evaluation for detecting SBI: difference in AUC 0.20 (95% CI 0.10 to 0.31).

The SBI diagnostic accuracy of the various surrogate markers and of the clinical evaluation is presented in table 3. In the case of a normal urine analysis in the emergency department, a situation the clinician often faces, the diagnostic accuracy of the surrogate markers and of the clinical evaluation is likely to change because the relative SBI aetiologies would be different (table 4). Because an SBI was found later in 8/262 (3%) children (four pneumonias, two UTIs, one meningitis and one occult bacteraemia) with normal urine analysis in the emergency department, and confirmed by the telephone follow-up carried out 1 week after the initial visit to the emergency department, the surrogate markers had better negative predictive values.

The multilevel likelihood ratios and post-test SBI probability, assuming a pretest SBI probability of 16% (the SBI prevalence in our population), are presented in table 5. However, when the urine analysis was normal in the emergency department, the multilevel likelihood ratios and post-test SBI probability changed. In that situation, the pretest SBI probability was 3% in our study; the resulting multilevel likelihood ratios and post-test SBI probability are presented in table 6.

### DISCUSSION

We report, based on similar AUCs of the different ROC curves, that in a population of children 1 month to 3 years of age presenting to a paediatric emergency department with fever without source, CRP, PCT, WBC and ANC have similar diagnostic properties for detecting an SBI and are superior to clinical evaluation based on VAS. Knowing that all physicians were experienced paediatric emergency specialists in a large tertiary hospital, it is unlikely that this is due to a lack of competence. Actually, the fact that clinical evaluation had such poor accuracy is not surprising as most of the identified SBI in our study were UTIs. Many clinical scores have been developed to detect patients at high or low risk of SBI, but generally these have been disappointing.

Ancillary testing has always been recommended to help the physician detect SBI in a population such as that studied in the present report. Our study shows that in the postpneumococcal vaccination era, ancillary testing is even more important as the prevalence of SBI is lower than in the prevaccination period and that clinical evaluation to ascertain UTI is limited in the 1–36-month-old age group.

In our study, the ROC curves AUC are similar to those previously published. Isaacman et al. and Pratt et al. found no statistical difference in AUC between CRP, WBC and ANC. These observations were confirmed by Bilavsky et al., who demonstrated that there was no difference between CRP and WBC. However, using either AUC, sensitivity/specificity, positive/negative predictive values or likelihood ratios, other studies obtained better diagnostic properties for PCT and CRP than WBC and ANC.

---

**Table 1** Clinical characteristics of the 328 patients included in the cohort study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>165 (50)</td>
</tr>
<tr>
<td>Median age in months (IQR)</td>
<td>11 (6–17)</td>
</tr>
<tr>
<td>Children aged 1–6 months (%)</td>
<td>95 (29%)</td>
</tr>
<tr>
<td>CTAS* triage level (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>49 (15)</td>
</tr>
<tr>
<td>3</td>
<td>131 (40)</td>
</tr>
<tr>
<td>4</td>
<td>146 (44)</td>
</tr>
<tr>
<td>5</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Mean temperature duration in hours (SD)</td>
<td>62 (48)</td>
</tr>
<tr>
<td>Mean maximal temperature in °C (SD)</td>
<td>39.6 (0.7)</td>
</tr>
</tbody>
</table>

*CTAS, Canadian Triage Acuity Scale.*
may be explained by different inclusion criteria. We included every child with fever over 38°C without source presenting to the paediatric emergency department who required a standard work-up because of either their ill appearance or the duration of fever. Andreola et al.4 and Galetto-Lacour et al.7 included only children who had a temperature of over 39.5°C or who were ill-appearing, while Thayyil et al.11 included those with a temperature over 39°C. Fernández López et al.6 included children under 36 months of age with fever who were required to undergo blood analysis and found an SBI in 43% of their patients. Galetto-Lacour et al.7 found an SBI in 29% of children aged under 36 months with fever (>38°C) without source, which is higher than usually described.11 18 19 23 25

We observe optimal cut-offs derived from ROC curves that are lower for all markers than previously published. This is probably because most of our SBI were UTIs. In our study, the optimal cut-off value for CRP is 17.7 mg/l compared to 40 or 70 mg/l for prior studies.4 7 10 19 Similarly, we report an optimal cut-off value of 0.2 ng/ml for PCT, whereas in studies with a similar population, the reported cut-off for PCT ranged from 0.5 ng/ml6 7 11 to 0.9 ng/ml4 10 and was as high as 20 ng/ml in critically ill children.26 The fact that the PCT cut-off apparently depends on the type of population could limit the use of the available semiquantitative test with a 0.5 ng/ml detection limit.

The WBC optimal cut-off of 14 100×10^6/l is similar to the traditional and preferred 15 000×10^6/l limit described by others and official guidelines.4 7 10 11 19 Finally, the ANC cut-off found in our study (5200×10^6/l) was also lower than the 10 000–10 600×10^6/l usually described.4 18 19 23 These results are probably related again to our inclusion criteria compared with those of other studies as we previously noted.4 6 7 10 11 18 19

These differences in cut-offs highlight what is in our opinion the main error in using markers. A surrogate marker has to be interpreted depending on the value obtained in each patient: the higher the result, the higher the probability of

Figure 2  Receiver operating characteristic for procalcitonin (PCT), C reactive protein (CRP), white blood cells count (WBC), absolute neutrophil count (ANC) and clinical evaluation on a visual analogue scale (VAS) to detect a serious bacterial infection in children aged 1–36 months presenting to a paediatric emergency department with fever without source.

Table 2  Area under the curves (AUC) of the receiver operating characteristic for PCT, CRP, WBC, ANC and clinical evaluation on a VAS to detect an SBI in children aged 1–36 months presenting to a paediatric emergency department with fever without source

<table>
<thead>
<tr>
<th>Marker</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical evaluation (VAS)</td>
<td>0.59 (0.54 to 0.65)</td>
</tr>
<tr>
<td>ANC</td>
<td>0.80 (0.75 to 0.84)</td>
</tr>
<tr>
<td>WBC</td>
<td>0.81 (0.76 to 0.85)</td>
</tr>
<tr>
<td>PCT</td>
<td>0.82 (0.77 to 0.86)</td>
</tr>
<tr>
<td>CRP</td>
<td>0.88 (0.84 to 0.91)</td>
</tr>
</tbody>
</table>

See text for statistical differences.

ANC, absolute neutrophil count; CRP, C reactive protein; PCT, procalcitonin; SBI, serious bacterial infection; VAS, visual analogue scale; WBC, white blood cells count.
Table 3  Diagnostic accuracy of PCT, CRP, WBC, ANC and clinical evaluation on a VAS to detect an SBI in children aged 1–36 months presenting to a paediatric emergency department with fever without source

<table>
<thead>
<tr>
<th>Variable best cut-off</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Positive predictive value (95% CI)</th>
<th>Negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT &gt;0.20 ng/ml</td>
<td>85.2 (74.4 to 92.1)</td>
<td>69.7 (67.6 to 71.1)</td>
<td>35.7 (31.2 to 38.6)</td>
<td>96.0 (93.1 to 97.9)</td>
</tr>
<tr>
<td>CRP &gt;17.7 mg/l</td>
<td>94.4 (85.6 to 98.1)</td>
<td>68.6 (66.9 to 69.3)</td>
<td>37.2 (33.7 to 38.7)</td>
<td>98.4 (95.9 to 99.5)</td>
</tr>
<tr>
<td>WBC &gt;14 100×10⁶/l</td>
<td>81.5 (70.3 to 89.3)</td>
<td>70.8 (66.6 to 72.4)</td>
<td>35.5 (30.6 to 38.9)</td>
<td>95.1 (92.1 to 97.2)</td>
</tr>
<tr>
<td>ANC &gt;5200×10⁶/l</td>
<td>87.0 (76.5 to 93.5)</td>
<td>59.9 (57.8 to 61.1)</td>
<td>29.9 (26.3 to 32.1)</td>
<td>95.9 (92.6 to 97.9)</td>
</tr>
<tr>
<td>VAS &gt;14.8%</td>
<td>68.5 (56.5 to 78.8)</td>
<td>38.7 (36.3 to 40.7)</td>
<td>18.0 (14.9 to 20.7)</td>
<td>86.2 (80.9 to 90.7)</td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count; CRP, C reactive protein; PCT, procalcitonin; SBI, serious bacterial infection; VAS, visual analogue scale; WBC, white blood cells count.

Table 4  Diagnostic accuracy of PCT, CRP, WBC, ANC and clinical evaluation on a VAS to detect an SBI if urine analysis was normal in the emergency department

<table>
<thead>
<tr>
<th>Variable best cut-off</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Positive predictive value (95% CI)</th>
<th>Negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT &gt;0.20 ng/ml</td>
<td>87.5 (53.6 to 97.8)</td>
<td>70.5 (69.4 to 70.8)</td>
<td>8.5 (5.2 to 9.5)</td>
<td>99.4 (97.9 to 99.9)</td>
</tr>
<tr>
<td>CRP &gt;17.7 mg/l</td>
<td>87.5 (53.6 to 97.8)</td>
<td>68.7 (66.8 to 70.0)</td>
<td>6.3 (5.1 to 9.3)</td>
<td>99.4 (97.9 to 99.9)</td>
</tr>
<tr>
<td>WBC &gt;14 100×10⁶/l</td>
<td>75.0 (41.5 to 92.8)</td>
<td>71.7 (70.6 to 72.2)</td>
<td>7.7 (4.3 to 9.5)</td>
<td>98.9 (97.5 to 99.7)</td>
</tr>
<tr>
<td>ANC &gt;5200×10⁶/l</td>
<td>75.0 (41.4 to 92.8)</td>
<td>59.8 (41.5 to 92.8)</td>
<td>5.6 (3.1 to 6.9)</td>
<td>98.7 (97.0 to 99.6)</td>
</tr>
<tr>
<td>VAS &gt;14.8%</td>
<td>75.0 (41.4 to 92.8)</td>
<td>39.4 (38.3 to 39.9)</td>
<td>3.8 (2.1 to 4.6)</td>
<td>98.0 (95.4 to 99.4)</td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count; CRP, C reactive protein; PCT, procalcitonin; SBI, serious bacterial infection; VAS, visual analogue scale; WBC, white blood cells count.

Table 5  Multilevel likelihood ratios and post-test SBI probability for each surrogate marker and clinical evaluation at different cut-off values

<table>
<thead>
<tr>
<th>Marker and cut-off</th>
<th>Pretest probability (%)</th>
<th>LR+ (95% CI)</th>
<th>Post-test probability if test positive (%)</th>
<th>LR− (95% CI)</th>
<th>Post-test probability if test negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT &gt;0.2 ng/ml</td>
<td>16</td>
<td>2.8 (2.3 to 3.2)</td>
<td>35</td>
<td>0.2 (0.1 to 0.4)</td>
<td>4</td>
</tr>
<tr>
<td>PCT &gt;0.5 ng/ml</td>
<td>16</td>
<td>3.9 (2.6 to 5.5)</td>
<td>42</td>
<td>0.5 (0.4 to 0.7)</td>
<td>9</td>
</tr>
<tr>
<td>PCT &gt;2 ng/ml</td>
<td>16</td>
<td>7.1 (3.4 to 14.9)</td>
<td>58</td>
<td>0.8 (0.7 to 0.9)</td>
<td>13</td>
</tr>
<tr>
<td>PCT &gt;10 ng/ml</td>
<td>16</td>
<td>∞ (4.0 to ∞)</td>
<td>100</td>
<td>0.9 (0.9 to 1.0)</td>
<td>15</td>
</tr>
<tr>
<td>CRP &gt;10 mg/l</td>
<td>16</td>
<td>2.1 (1.9 to 2.3)</td>
<td>29</td>
<td>0.1 (0.03 to 0.3)</td>
<td>2</td>
</tr>
<tr>
<td>CRP &gt;17.7 mg/l</td>
<td>16</td>
<td>3.0 (2.6 to 3.2)</td>
<td>36</td>
<td>0.1 (0.03 to 0.2)</td>
<td>2</td>
</tr>
<tr>
<td>CRP &gt;40 mg/l</td>
<td>16</td>
<td>4.4 (3.3 to 5.5)</td>
<td>46</td>
<td>0.3 (0.2 to 0.5)</td>
<td>4</td>
</tr>
<tr>
<td>CRP &gt;80 mg/l</td>
<td>16</td>
<td>5.9 (3.5 to 9.9)</td>
<td>53</td>
<td>0.6 (0.5 to 0.8)</td>
<td>11</td>
</tr>
<tr>
<td>WBC &gt;10 000×10⁶/l</td>
<td>16</td>
<td>1.7 (1.5 to 1.9)</td>
<td>25</td>
<td>0.2 (0.1 to 0.5)</td>
<td>4</td>
</tr>
<tr>
<td>WBC &gt;14 100×10⁶/l</td>
<td>16</td>
<td>2.8 (2.2 to 3.2)</td>
<td>35</td>
<td>0.3 (0.1 to 0.4)</td>
<td>5</td>
</tr>
<tr>
<td>WBC &gt;20 000×10⁶/l</td>
<td>16</td>
<td>5.1 (3.2 to 7.9)</td>
<td>49</td>
<td>0.6 (0.5 to 0.7)</td>
<td>10</td>
</tr>
<tr>
<td>ANC &gt;5200×10⁶/l</td>
<td>16</td>
<td>2.2 (1.8 to 2.4)</td>
<td>30</td>
<td>0.2 (0.1 to 0.4)</td>
<td>4</td>
</tr>
<tr>
<td>ANC &gt;10 000×10⁶/l</td>
<td>16</td>
<td>3.6 (2.4 to 5.2)</td>
<td>41</td>
<td>0.6 (0.4 to 0.7)</td>
<td>10</td>
</tr>
<tr>
<td>ANC &gt;15 000×10⁶/l</td>
<td>16</td>
<td>5.6 (2.5 to 12.9)</td>
<td>52</td>
<td>0.8 (0.8 to 0.9)</td>
<td>14</td>
</tr>
<tr>
<td>VAS &gt;14.8%</td>
<td>16</td>
<td>1.1 (0.9 to 1.3)</td>
<td>17</td>
<td>0.8 (0.5 to 1.2)</td>
<td>14</td>
</tr>
<tr>
<td>VAS &gt;25%</td>
<td>16</td>
<td>1.5 (1.3 to 2.0)</td>
<td>22</td>
<td>0.7 (0.6 to 1.0)</td>
<td>13</td>
</tr>
<tr>
<td>VAS &gt;50%</td>
<td>16</td>
<td>2.2 (1.1 to 4.4)</td>
<td>30</td>
<td>0.9 (0.8 to 1.0)</td>
<td>15</td>
</tr>
</tbody>
</table>

A pretest SBI probability of 16% (the SBI prevalence) was assumed. LR+, positive likelihood ratio; LR−, negative likelihood ratio.

ANC, absolute neutrophil count; CRP, C reactive protein; PCT, procalcitonin; SBI, serious bacterial infection; VAS, visual analogue scale; WBC, white blood cells count.

having an SBI. It should not only be positive or negative. This is illustrated by multilevel likelihood ratios. For example, a PCT concentration >2 ng/ml or a CRP concentration >80 mg/l raises the SBI probability from 3% to 20% if urine analysis is normal, and a PCT >10 ng/ml is diagnostic of SBI in these children (table 6). Conversely, a CRP <10 mg/l in that situation lowers SBI probability to less than 1%. This has also been reported by other authors. Thus, regardless of their respective AUC, these markers can play an important role in the decision making process when multilevel likelihood ratios are used, even when the urine analysis is normal.

In our study, UTIs were the most frequent SBI in patients, accounting for nearly 90% of all SBI. In contrast, occult bacteraemia was unusual, with only 1% (0.5%) occurrence. This is consistent with other postpneumococcal vaccine studies that showed a drop in the rate of occult bacteraemia from 2–4% to less than 1%. The utility of blood culture in children with fever without source is more and more challenged as a result. Our study confirms that blood cultures are generally not helpful. Indeed, assuming that only 5–20% of the untreated pneumococcal occult bacteraemias will result in an SBI such as pneumonia or cellulitis and less than 2% in meningitis or sepsis, this represents only 0.05–0.2% of the well-appearing children aged 1 month to 3 years with fever without source with a 1% occult bacteraemia prevalence. If the prevalence is less, as in our study, this number is even lower, reaching 0.0015–0.06%. Nevertheless, as the very young infant, not fully protected by the streptococcal vaccine, has a higher risk of bacteraemia, care must be taken in this population.
Since urine infection is the most frequent SBI in children aged 1–36 months with fever without source, a urine analysis is considered necessary in such patients. However, the risk of SBI when the urine analysis obtained by an appropriate method (catheterisation or suprapubic aspiration) is normal in the emergency department is not well known. In this situation, surrogate markers may become useful for the clinician for detecting SBI because, as we show in this study, clinical evaluation is inferior to those markers. Because of the rarity of other SBI, surrogate markers have an excellent negative predictive value (98–99.4%) but poor positive predictive value with the optimal cut-offs when the urine analysis is normal in the emergency department.

**Limitations**

We considered the usual clinical diagnosis for UTI and pneumonia (positive urine culture and lobar consolidation on chest radiography). Nevertheless, studies that assessed UTI by renal 99mTc-dimercaptosuccinic acid scintigraphy show that as many as 30% of febrile UTIs are cystitis and not pyelonephritis. Also, viral or bacterial pneumonias can be indistinguishable. This could have influenced the real diagnostic properties of the markers used in our study. The study took place in a paediatric emergency department of a large tertiary hospital. As results could be different in smaller community hospitals or other settings, it is not known if the results are generalisable. Another potential limitation is that not all markers were available in every patient as some were missing in 15% (56/384) of the children included in the RCT. Finally, although the AUC is a strong descriptor to explore the diagnostic properties of a marker, it does not weigh its clinical consequence.

**Conclusion**

In our population of children 1 month to 3 years of age with fever without source, CRP, PCT, WBC and ANC had similar diagnostic properties to detect an SBI. Clinical evaluation was inferior to all of these markers. Although we report lower best cut-off values than previously described, a marker has to be interpreted depending on the value obtained in each patient: the higher the result, the higher the probability of having an SBI. By reporting multilevel likelihood ratios, we showed that these markers can play an important role in the decision making process.

**Funding** The investigators received 200 PCT-Q kits from Brahms (Germany). Reagents for the Kryptor PCT measurements were provided by Brahms (Switzerland).

**Ethics approval** This study was conducted with the approval of CHU Sainte-Justine.

**Competing interests** None.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**REFERENCES**


When clinicians wish to apply the conclusions of biomarker studies, they should not only take into account the population studied, but also the measurement methods used, and there can be many of these. PCT, for example, can be measured using various methods: with a semiquantitative assay (PCT-Q®, Brahms, Germany); with a quantitative assay such as the luminometric immunoassay (Lumitest®, Brahms, Germany); or with the ultra-sensitive immunoassay using TRACE (Time Resolved Amplified Cryptate Emission) technology (Kryptor®, Brahms, Germany). Comparison studies should be carried out in order to be aware of the potential for different results when using different methods:

2.1.2 Comparison of procalcitonin measurement by a semi-quantitative method and an ultra-sensitive quantitative method in a pediatric emergency department (18)
Comparison of procalcitonin measurement by a semi-quantitative method and an ultra-sensitive quantitative method in a pediatric emergency department

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Available online 16 July 2009

Abstract

Objective: To compare procalcitonin measurements between semi-quantitative and quantitative assays.

Method: Procalcitonin was measured with the PCT-Q® and the Kryptor® assays in a pediatric emergency department.

Results: Among the 359 pairs of results, 103 had discordant results. The linear weighted kappa was 0.44 (95% CI 0.36, 0.51). The concordant/discordant results distribution varied depending on the laboratory technician (p=0.018).

Conclusion: Agreement between procalcitonin measured semi-quantitatively and quantitatively was moderate. This is probably due to a subjective interpretation of the assay result.

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Keywords: Procalcitonin; Inter-rater reliability; Semi-quantitative assay; Quantitative assay; Emergency department; Children

Introduction

Almost 10 years ago, Gendrel et al. [1] demonstrated the superiority of procalcitonin (PCT) over C-reactive protein (CRP) in distinguishing bacterial from viral infections in children in the emergency department. This initial observation has since been confirmed by others [2–7].

PCT can be measured by various methods: with a semi-quantitative assay (PCT-Q®, Brahms, Germany) or with a quantitative assay such as the luminometric immunoassay (Lumitest®, Brahms, Germany) or the ultra-sensitive immunoassay using TRACE (Time Resolved Amplified Cryptate Emission) technology (Kryptor®, Brahms, Germany). In the emergency department where results need to be obtained rapidly so timely clinical decisions can be made (use of antibiotic or not, and discharge or hospitalization), a simple immunochromatographic assay such as PCT-Q® is highly desirable. It is easy to use, inexpensive and does not require calibration. Nevertheless, the color intensity of the band (result) must be evaluated by a technician, and is therefore subject to individual observer variation.

The objective of our study was to compare PCT values obtained from the semi-quantitative PCT-Q® assay to those obtained from the most sensitive and precise quantitative immunoassay, Kryptor®, when the former was performed by various laboratory technicians in real-time 24/24 h, 7 days a week in 1 to 36-month-old children presenting to a pediatric emergency department (ED) with a fever without a source.

Method

The prospective cohort study was part of a randomized controlled trial (RCT) assessing the impact of PCT on the management of children aged 1 to 36 months presenting to a
pediatric ED with fever without a source [8] and took place in a tertiary pediatric hospital emergency department with an annual census of more than 60,000 visits. The CHU Sainte-Justine’s Institutional Review Board approved the study.

Inclusion criteria consisted of children between 1 and 36 months with a rectal temperature over 38.0 °C (100.4 °F) and no identified source of infection. All children with a history of acquired or congenital immunodeficiency were excluded from our study. None of the children was receiving antibiotics before the study. After consent was obtained, all children included in this study had blood drawn for complete blood count, blood culture and PCT measurement. Furthermore, a urine sample was also obtained either by bladder catheterization or suprapubic aspiration for urine analysis and culture.

Before the start of the study, a procedure for PCT measurement using PCT-Q® (Brahms, Germany) was developed for in-house use. All laboratory technicians read the written procedure and received training for this procedure by one of the study biochemists.

One milliliter of blood was collected by venipuncture in a heparin/lithium vacutainer and centrifuged. A total of 200 μL of plasma was applied to each individual PCT-Q® strip. After 30 min of incubation at room temperature (maximum 45 min), a visual reddish color band of variable intensities was visible. The colored intensity of the assay band, directly proportional to the PCT concentration of the sample, was compared to a colored block of the reference chart with the following concentrations: ≤0.5 ng/mL, ≥0.5 ng/mL, ≥2 ng/mL, and ≥10 ng/mL. PCT values were available within 1 h of the venipuncture.

At the end of the study that lasted 12 months, a quantitative measurement of procalcitonin was performed on leftover plasma, frozen at −40 °C, using Kryptor® (Brahms, Germany) in Geneva, Switzerland. The laboratory staff was blinded to the initial results obtained by the PCT-Q®. According to the manufacturer of Brahms’s Kryptor®, samples can be frozen and thawed up to three times. This has been confirmed by Meisner et al. [9].

The primary outcome measured was a comparison of inter-rater reliability between procalcitonin measured by PCT-Q® and by PCT Kryptor®. Unweighted, linear weighted and quadratic weighted kappa statistics were calculated [10]. A priori, linear weighted kappa was the primary outcome for this study. Unweighted kappa scores reflect only exact agreement, while weighted kappa take into account close agreement, near misses, and the relative values of close agreement versus marked disagreement. Quadratic weighted kappa assigns greater relative credit to closer near misses than linear weighted kappa. Kappa agreement was defined a priori as excellent (>0.8), good (between 0.6 and 0.8), moderate (between 0.4 and 0.6), fair (between 0.2 and 0.4) or poor (<0.2) [11].

The secondary outcome was the distribution of concordant and discordant PCT values for each laboratory technician that performed the PCT-Q® analyzed by Chi-square. Serious bacterial infection rate and PCT and WBC sensitivity and specificity were also analyzed [8]. All data were entered in an Excel database (Microsoft Inc., Richmond, WA) and analyzed using MedCalc version 9.6.0.0 (Mariakerke, Belgium).

Results

A pair of PCT-Q®–PCT Kryptor® results was available for 359 children among the 384 children that were initially included in the randomized controlled trial between November 25th, 2006 and November 21st, 2007. No plasma was available for the other 25 children. The median age of the children was 11 months (IQR 6, 17). During the study, 53 urinary tract infections, 4 pneumonia, 1 occult bacteremia (Streptococcus pneumoniae) and 1 bacterial meningitis (Neisseria meningitidis) were diagnosed.

The distribution of each result obtained by PCT Kryptor®, according to the results obtained by PCT-Q®, is presented in Fig. 1. A total of 29% (103/359) of the patients had discordant results. Agreement between procalcitonin measured semi-quantitatively and quantitatively was moderate (Table 1). A total of 61 different laboratory technicians performed the PCT-Q® and read the result using the semi-quantitative assay. The distribution of concordant/discordant results varied depending on the laboratory technician (p=0.018).

PCT-Q® sensitivity and specificity to detect a serious bacterial infection (bacteremia, urinary tract infection, pneumonia, meningitis, osteomyelitis or septic arthritis) with a cut-off value of ≥0.5 ng/mL were 77% (95% CI 66, 86) and 64% (95% CI 59, 69) respectively, while those of PCT Kryptor® with the same cut-off value were 56% (95% CI 45, 66) and 86% (95% CI 84, 88) respectively and those of WBC at >15,000 × 10⁹/L were 71% (95% CI 59, 81) and 75% (95% CI 70, 80) respectively.

Discussion

Our study demonstrates that, in the context described, agreement between procalcitonin results measured semi-quantitatively (PCT-Q®) and quantitatively (PCT Kryptor®) is moderate.

Few studies have examined the agreement between the semi-quantitative assay and the quantitative measurement of PCT. In 2000, Guerin [12] compared the PCT-Q® assay with the Lumitest®. He selected 75 frozen serum samples previously measured with Lumitest® to cover a wide range of concentrations. The results demonstrated a discordance of 11/75 (15%) cases. Gervaix et al. [13] measured PCT with PCT-Q® and Lumitest® in 54 children with a urinary tract infection. A discordant result was found in only 6/54 (11%). The same assays, evaluated in 445 children aged 1 to 36 months with fever in a pediatric emergency department [4], revealed 14% discordance (66/445) with an unweighted kappa of 0.80. Prat et al. [14] measured PCT using PCT-Q® and Lumitest® in 55 children aged from 1 month to 12 years presenting with fever of less than 12 h duration. Comparison showed significant correlation (p<0.0001). It appears from the methodology of these studies that only one or two technicians performed the PCT-Q® assay. Under those conditions, they all had lower rates of discordance than those observed in our study.

Two studies have shown similar results to ours. Meisner et al. [15] measured PCT in 237 patients in an undescribed population
Author's personal copy

Discordant results were found in 25% (60/237) of the patients when results obtained by PCT-Q® were compared to results from the Lumitest®. Interestingly, numerous technicians performed the PCT-Q® assay. Kordek et al. [16] in a study involving cases of suspected sepsis in 1 to 7-day-old neonates, also observed a large number of discordant results [108/151 (71%) with an unweighted kappa score of only 0.10] between PCT-Q® and Lumitest®. It was however unclear how many technicians performed the PCT-Q® assay in that study.

Thus, it appears that when PCT-Q® results are read by a limited number of technicians (which is often the case in clinical studies) they give better agreement than when the results are read by several technicians (which would be the case in real-life situations). In our opinion, this difference between studies arises from the fact that there is certain amount of subjectivity in the reading of the color band intensity with the PCT-Q®. Some authors [12,15,17] stated that the interpretation of the result was difficult, mostly for the ranges of interpretations that are of interest for pediatric infections (0.5–2 ng/mL). In our study, the significant difference in the distribution of concordant/discordant results among the 61 technicians, suggests that the results may be dependent on the technicians. Moreover, Meisner et al. [15] have pointed out that the assay has to be read 30 min but no later than 45 min after the beginning of the test. Later than that, the color band shifts from red to purple and the interpretation may be erroneous. This time restriction, not evaluated in our study, is often difficult in a busy clinical laboratory. This should be evaluated in a future study. Given this, photography of the strip may help evaluate the accuracy of the reading.

Our study is in many ways an effectiveness study. It took place in a pediatric emergency department and PCT-Q® was measured in real-time by one of the laboratory technicians on duty at that time, explaining why many different technicians took part in the analysis. Another particularity of our study is the comparison of PCT-Q® with the most sensitive assay (Kryptor®) available.

In conclusion, when PCT is measured in a clinical setting, agreement between procalcitonin measured semi-quantitatively (PCT-Q®) and quantitatively (PCT Kryptor®) is moderate as calculated with the linear weighted kappa. This is probably due to a subjective interpretation of the semi-quantitative assay.

Table 1
Agreement between procalcitonin measured semi-quantitatively (PCT-Q®) and quantitatively (PCT Kryptor®) in children presenting to a pediatric emergency department with a fever without a source.

<table>
<thead>
<tr>
<th>PCT-Q® (ng/mL)</th>
<th>PCT Kryptor® (ng/mL)</th>
<th>&lt;0.5</th>
<th>≥0.5</th>
<th>≥2</th>
<th>≥10</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5</td>
<td>220</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥0.5</td>
<td>67</td>
<td>26</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥2</td>
<td>7</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>≥10</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Kappa (95% CI)
- Unweighted: 0.35 (0.24, 0.46)
- Linear weight: 0.44 (0.36, 0.51)
- Quadratic weight: 0.52 (0.43, 0.61)

Data in bold means concordant results.

Fig. 1. Distribution of the results obtained by PCT Kryptor® according to the results obtained by PCT-Q®.
results, and should be taken into consideration whenever such point-of-care technology is introduced.

Financial disclosure

We received 200 PCT-Q® from Brahms (Germany). Reactants for Kryptor® PCT dosages were provided by Brahms (Switzerland).

References


2.2. Prognosis biomarkers

In pediatric EDs, as in other wards, it is important to diagnose the specific condition in order to prescribe the right treatment. If a clinical evaluation cannot be discriminative enough, the physicians may need help from a biomarker, as seen previously.

However, pediatric ED physicians must also know how the disease is supposed to evolve with the treatment prescribed. Again, most of the time, the physician’s clinical judgment and knowledge are all that are needed to evaluate this. In some situations, however, it is impossible to identify children with a worse outcome. Community-acquired bacterial pneumonia can usually be dealt with after a few days of oral antibiotic treatment. Despite this therapy, a few children will present a complication such as an empyema or bacteremia. If these children can be identified early, then a more aggressive treatment can be prescribed, such as intravenous and broader spectrum antibiotics, and they can be hospitalized for closer observation. Because these children cannot be clinically differentiated in the early stages of their disease, a biomarker that could help ensure that differentiation would be highly significant. We prospectively studied proadrenomedullin and copeptin as biomarkers in children with community-acquired pneumonia (10). The following chapter describes a prognosis biomarker study:

2.2.1 Proadrenomedullin and copeptin in pediatric pneumonia: a prospective diagnostic accuracy study (10)
Proadrenomedullin and copeptin in pediatric pneumonia: a prospective diagnostic accuracy study

Gabriel Alcoba 1,2†, Sergio Manzano 1†, Laurence Lacroix 1, Annick Galetto-Lacour 1 and Alain Gervaix 1

Abstract

Background: Community-acquired pneumonia is the leading cause of child mortality worldwide. Very few studies have explored the predictive value of Proadrenomedullin and Copeptin in pediatric severe pneumonia and bacteremia.

Methods: Proadrenomedullin and Copeptin were assessed as predictors for complicated community-acquired pneumonia (bacteremia, empyema) in 88 children aged 0 to 16 years presenting to the pediatric emergency department, using B.R.A.H.M.S. Kryptor Compact pro-ADM and Copeptin with the TRACE technology (time-resolved amplified cryptase emission). STARD standard reporting was used.

Results: A complicated community-acquired pneumonia was found in 11 out of 88 children (12.5%). Proadrenomedullin median values increased more than twofold, in complicated vs. uncomplicated (0.18 vs. 0.08 nmol/L, p = 0.039), and fivefold in bacteremic vs. non-bacteremic pneumonia (0.40 vs. 0.08 nmol/L, p = 0.02). Proadrenomedullin > 0.16 nmol/L showed 100% sensitivity (95% CI 39.8 – 100.0) and 70% (95% CI 58.7 – 79.7) specificity for bacteremia. Copeptin showed no added-value.

Conclusions: Proadrenomedullin seems a reliable and available predictor for complicated CAP, and could therefore help the physician with the decision to hospitalize, and choose the antibiotics administration route. Larger studies are needed.

Background

Community-acquired pneumonia (CAP) is the leading cause of child mortality worldwide [1]. The decision to treat with intravenous antibiotics and to hospitalize will depend on the severity of the disease and the risk of complications such as sepsis, empyema or abscess. Proadrenomedullin (ProADM) and copeptin (CoPEP) are peptides co-synthesized together with adrenomedullin and vasopressin in endothelial cells and pituitary gland respectively. These peptides have vaso-active, immune modulating, and metabolic properties. They are increased in sepsis, but they have a short half-life. ProADM and CoPEP are more stable and easier to measure than the active hormones [2, 3].

These novel biomarkers seem useful in predicting severity and complications in severe pneumonia in adults [4–6], but very few studies have explored the predictive value of these markers in children [7, 8]. Our objective was to assess ProADM and CoPEP’s diagnostic accuracy for predicting complications in a cohort of children with community-acquired pneumonia (CAP).

Methods

Population and setting

We performed a secondary analysis of a prospective cohort study on biomarkers of pediatric pneumonia [9], including children aged 0 to 16 years presenting to the pediatric emergency department of three tertiary hospitals (Geneva, Lausanne, and Sion) with CAP, defined as fever (>38 °C), cough, tachypnea, and a radiographic lung infiltrate. Children with chronic heart, lung, or neurological diseases were excluded. A venous blood sample was drawn from each child for white blood cell count, C-reactive
protein, blood culture (one sample), and subsequently from those with a clear-cut diagnosis of complicated or uncomplicated CAP, we also measured ProADM and CoPEP. Every child had a chest X-ray and, if a pleural effusion was present, a pleural liquid sample was drawn and sent for culture. We defined the diagnosis of “complicated CAP” as CAP with bacteremia (positive blood culture) or empyema (positive pleural culture). CAP with simple pleural effusions without empyema, was considered as “uncomplicated CAP”. The three hospitals ethics committees approved this sub-study within the main pneumonia study [9], (Geneva, Vaud, and Valais states’ Human Research Ethics Committees http://www.swissethics.ch/eks_e.html) and participants’ parents or teenage patients provided informed written consent.

Laboratory methods
Serum samples were immediately stored at −80 °C. ProADM and CoPEP values were determined using TRACE (time-resolved amplified cryptase emission) technology with the B.R.A.H.M.S. Kryptor Compact pro-ADM and Copeptin® (Brahms, Hennigsdorf, Germany). White blood cell counts (WBC) with differential (Band neutrophil percentage) and C-reactive protein (CRP) had already been performed in all children. The laboratory team who performed ProADM and CoPEP was blinded to the clinical data.

Statistics
Using STATA 11.0 (Texas, USA) we analyzed baseline demographic and clinical characteristics expressed as percentages for categorical data, and median with interquartile ranges (IQR) for continuous data due to their non-normal distribution. Statistical associations were assessed using a chi-square test or Fisher exact test for categorical data, and a student’s t-test or Wilcoxon-Mann–Whitney rank-sum test for continuous data; and multiple logistic regression using odds-ratios (adjusting for age, sex, previous medication and pneumococcal vaccination) was used to identify variables (ProADM, CoPEP, etc.) independently associated with the outcome variable, namely the cases of complicated CAP. Diagnostic performance was expressed through sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (LR+ and LR-) with 95 % Confidence Intervals (95 % CI), and receiver-operator characteristic with area under the curve (AUC). STARD, the Standard Reporting for Diagnostic studies was used, and its checklist is provided in Additional file 1.

Results
We analyzed 88 samples from children meeting the inclusion criteria for CAP. These children were included from January 2008 to September 2010. Among these 88 children with CAP, eleven (12.5 %) presented with complicated CAP, (9 empyema, 4 bacteremia, and 2 with both complications). The pathogens isolated in blood and empyema cultures were all S. pneumoniae. The comparison between the two groups (complicated vs. uncomplicated CAP) showed no significant differences for baseline characteristics: age (median 3.1 years, IQR 1.81 – 5.76), sex, daycare attendance, passive tobacco exposure, and pneumococcal vaccination (p > 0.05).

Fig. 1 Performance of proadrenomedullin, C-reactive protein, and white blood cell count, for predicting bacteremic pneumonia. ROC area: Receiver-Operator Characteristic area under the curve for sensitivity and 1-specificity to predict bacteremic pneumonia; CRP: C-reactive protein; WBC: white blood cell; ProADM: proadrenomedullin
Regarding the main objective, children with bacteremic pneumonia \((n = 4)\) or empyema \((n = 9)\) showed a general statistical association with ProADM (logistic regression \(p = 0.007\) and 0.036 respectively), but not with CoPEP \((p > 0.05)\). We found a significant difference in ProADM median values in complicated cases: complicated vs. uncomplicated \((0.18 \text{ vs. } 0.08 \text{ nmol/L, } p = 0.039)\) and bacteremia vs. no-bacteremia \((0.40 \text{ vs. } 0.08 \text{ nmol/L, } p = 0.02)\), as shown in the boxplot of Additional file 2.

This shows a twofold increase for complicated and a fivefold increase for bacteremic pneumonia. On the contrary, CoPEP did not distinguish complicated from uncomplicated CAP \((p = 0.95)\).

The diagnostic accuracy of these biomarkers in predicting complicated CAP was analyzed and compared with classical biomarkers (WBC, band neutrophils, CRP). We found an optimal cut-off at 0.16 nmol/L for ProADM with 100 \% sensitivity \((95 \% \text{ CI } 39.8\text{ – }100.0)\) and 70 \% \((95 \% \text{ CI } 58.7\text{ – }79.7)\) and 10 pmol/L for CoPEP with a specificity of 67.5 \% \((95 \% \text{ CI } 56.1\text{ – }77.6)\), but a low sensitivity 50 \% \((6.8\text{ – }93.2)\). Table 1 shows that ProADM with a 0.16 nmol/L cut-off was very efficient to rule out bacteremia (sensitivity and NPV = 100 \%, LR- 0.14 \([0.01\text{ – }2.00]\)), as accurate as CRP (>100 mg/L) and better than band neutrophils (>1.5 G/L) or leukocytosis (>15 G/L). ProADM >0.16 nmol/L, with a specificity of 70 \% for bacteremia, was as accurate as band neutrophils, or CRP and better than leukocytosis to rule it in. With an overall ROC AUC of 0.85, the accuracy of ProADM was comparable to CRP for the diagnosis of bacteremia, as shown in Fig. 1. In contrast, CoPEP did not perform as well to exclude or confirm complicated CAP.

### Discussion

This secondary analysis of 88 children with CAP supported the hypothesis that ProADM strongly predicts serious complications of pediatric community-acquired pneumonia (CAP), such as bacteremia and empyema. CoPEP on the contrary did not show such performances for predicting complicated CAP in our small population, but should be retested in larger samples.

Diagnostic performances of ProADM seem excellent, especially to rule-out bacteremia (sensitivity 100 \%), and as accurate as classical markers (WBC, Band neutrophils, and CRP) with regards to specificity. Only true complications, such as bacteremia or empyema, seem to cause a significant elevation of ProADM above 0.16 nmol/L.

**Table 1** Performance of proadrenomedullin, copeptin, C-reactive protein, white blood cell and band neutrophils (band cells) counts, for predicting "complicated" (with empyema or bacteremia) and "bacteremic"community-acquired pneumonia

| Complicated CAP \((n = 11; 12.5 \%)\) vs. non-complicated CAP \((n = 77)\) |
|---|---|---|---|---|---|
| Proadrenomedullin >0.16 nmol/L | Copeptin >10 pmol/L | CRP >100 mg/L | White blood cells >15G/L | Band cells >1.5G/L |
| Sensitivity % | 72.7 (39.0–94.0) | 45.5 (16.7–76.6) | 100.0 (71.5–100) | 72.7 (39.0–94.0) | 90.9 (58.7–99.8) |
| Specificity % | 71.4 (60.0–81.2) | 688 (57.3–78.9) | 77.0 (65.8–86.0) | 58.7 (46.7–69.9) | 75.7 (64.3–84.9) |
| LR+ | 2.55 (1.5–4.2) | 1.46 (0.70–3.0) | 4.11 (2.68–6.29) | 1.76 (1.12–2.76) | 3.55 (2.26–5.56) |
| LR- | 0.38 (0.1–1.0) | 0.79 (0.45–1.39) | 0.05 (0.00–0.82) | 0.46 (0.17–1.24) | 0.17 (0.04–0.75) |
| PPV% | 26.7 (12.3–45.9) | 172 (5.8–35.8) | 39.3 (21.5–59.4) | 20.5 (9.3–36.5) | 35.7 (18.6–55.9) |
| NPV% | 94.8 (85.6–98.9) | 89.8 (79.2–96.2) | 100.0 (93.7–100) | 93.6 (82.5–98.7) | 98.2 (90.6–100) |
| ROC area | 0.72 (0.6–0.9) | 0.57 (0.41–0.73) | 0.89 (0.84–0.93) | 0.66 (0.51–0.81) | 0.83 (0.73–0.93) |
| Odds ratios | 6.67 (1.7–25.3) | 1.84 (0.54–6.30) | 75.6 (4.2–1348) | 3.78 (1.00–14.2) | 21.4 (3.57–128) |

| Bacteremic CAP \((n = 4; 4.5 \%)\) vs. non-bacteremic CAP \((n = 84)\) |
|---|---|---|---|---|---|
| Proadrenomedullin >0.16 nmol/L | Copeptin >10 pmol/L | CRP >100 mg/L | White blood cells >15G/L | Band cells >1.5G/L |
| Sensitivity % | 100 (39.8–100.0) | 50.0 (6.8–93.2) | 100 (39.8–100) | 75.0 (19.4–99.4) | 75.0 (19.4–99.4) |
| Specificity % | 70.0 (58.7–79.7) | 67.5 (56.1–77.6) | 70.5 (59.1–80.3) | 57.0 (45.3–68.1) | 71.8 (60.5–81.4) |
| LR+ | 2.98 (1.91–4.63) | 1.54 (0.55–4.31) | 3.03 (1.93–4.73) | 1.62 (0.87–3.04) | 2.46 (1.26–4.81) |
| LR- | 0.14 (0.01–2.00) | 0.74 (0.27–2.00) | 0.14 (0.01–1.98) | 0.53 (0.14–2.04) | 0.42 (0.11–1.61) |
| PPV% | 14.3 (4.0–32.7) | 7.1 (0.9–23.5) | 14.8 (4.2–33.7) | 8.1 (1.7–21.9) | 12.0 (2.5–31.2) |
| NPV% | 100.0 (93.6–100.0) | 96.4 (87.7–99.6) | 100 (93.5–100) | 97.8 (88.5–99.9) | 98.2 (90.6–100) |
| ROC area | 0.85 (0.80–0.90) | 0.59 (0.30–0.88) | 0.85 (0.80–0.90) | 0.66 (0.41–0.91) | 0.73 (0.48–0.98) |
| Odds ratios | 20.8 (1.1–400.5) | 2.08 (0.35–12.5) | 21.3 (1.1–411) | 3.08 (0.43–21.9) | 5.86 (0.81–42.2) |

CAP Community-acquired pneumonia, CRP C-reactive protein, ROC area Receiver-Operator Characteristic area under the curve, LR+ and LR Likelihood ratio of a positive or negative result, NPV and PPV Negative and Positive Predictive Values (NPV, PPV); *All odds ratios (OR) are multivariable-adjusted-OR (age, sex, previous medication and pneumococcal vaccination)
Compared to similar studies, Renaud et al. [6] showed that ProADM in elderly patients improved the prediction of early admission to ICU for severe CAP. In their study, ProADM showed a sensitivity of 95.0 % at a cut-off value of 0.75 nmol/L, and a specificity of 81.0 % at a cut-off value of 2.0 nmol/L.

One pediatric study by Sardà Sanchez et al. [8] showed that ProADM could predict simple pneumonia versus pneumonia with complications (2.32 vs. 1.18 nmol/L, \( p = 0.014 \)) or pleural effusion (2.94 vs. 1.14, \( p < 0.001 \)), but only fifty patients were included in this study, including ten with complications, of which seven with pleural effusions. Another pediatric study by Michels et al. [7] concluded that ProADM also predicts capillary damage, leakage and risk of shock in hemorrhagic dengue and dengue shock, showing that these lesions similar to sepsis can be predicted by this marker in non-bacterial sepsis-like syndromes.

Initially a marker of severity in non-infectious diseases such as stroke, CoPEP also seemed promising as a prognostic marker in CAP. This was suggested by Katan et al.’s review on adults with ventilator-associated pneumonia [5], and in a Swiss study on lower respiratory tract infections by Müller B et al. [10]. In the latter, CoPEP levels were significantly lower in survivors. However, in our study CoPEP did not show any added value as a predictor for complicated pediatric CAP, but the sample size may limit such conclusions. This could also be due to the low prevalence of severe CAP in our population compared to that of intensive care units in these studies, and the absence of mortality, even in cases with bacteremia and empyema. This hypothesis should be tested in future studies among children with ventilator associated pneumonia or sepsis.

A few limitations need to be stated: the design of this study was a secondary analysis of a larger study focused on the etiology of childhood CAP, with selection of samples with typical characteristics rather than random selection, implying a slight risk of selection bias. Although most statistical analyses seem coherent and logical compared to other studies, the design of this study did not include a sample size calculation, so that the significance levels could be inaccurate in small groups. The laboratory team who performed ProADM and CoPEP was blinded to the clinical data, reducing the risk of bias.

**Conclusion**

In conclusion, we found that ProADM, in contrast to CoPEP, seems to be an interesting marker of complicated CAP, very similar to CRP in sensitivity and specificity. It could therefore help the physician with the decision to hospitalize and choose the antibiotics administration route. Larger studies should assess the promising performances of ProADM in pneumonia and other serious pediatric infections, as well as further comparisons with CRP and Procalcitonin.
2.3. Categorization biomarker:

Nearly every laboratory or radiological investigation has the potential to be harmful. Some have more potential for harm than others. Even a simple venipuncture or a lumbar puncture can be perceived as quite a hostile, painful act by a young child who does not understand the reason for it. Another potential harm may arise from false-positive results that lead to further unnecessary examinations, medical treatment, or even surgery. Finally, some tests may induce severe diseases such as cancers: CT imaging is one of these. Its ionizing radiation can lead to lethal malignancies and the rate can be as high as 1 in 1,000 CT scans, with younger children being more susceptible (2). As stated previously, pediatric ED physicians have to stratify patients into four categories: children with life-threatening conditions needing emergency treatment, children needing a specific treatment, children with self-limited diseases (such as viral infections, contusions, or migraine) needing only symptomatic medication, and children needing more investigations before they can be categorized. Some children with mild traumatic brain injury (Glasgow Coma Scale ≥ 13) fall into this latter category. As the signs and symptoms may not be specific enough to be able to distinguish children with an intracranial hemorrhage, initial evaluations should seek to identify children at a very low risk in order to avoid unnecessary CT scans. The S100B protein has recently been described and is associated with brain cell injury. This biomarker was studied in a population of children with mild traumatic brain injury in order to identify those needing further investigations (CT scan) (14). The following chapter describes a categorization biomarker study:

2.3.1 Diagnostic performance of S100B protein serum measurement in detecting intracranial injury in children with mild head trauma (14)
Diagnostic performance of S100B protein serum measurement in detecting intracranial injury in children with mild head trauma

Sergio Manzano,1 Iris Bachmann Holzinger,2 Christian J Kellenberger,3 Laurence Lacroix,1 Dagmar Klima-Lange,4 Martin Hersberger,5 Giorgio La Scala,6 Stefan Altermatt,7 Georg Staubli2

ABSTRACT
Objective To assess the accuracy of S100B serum level to detect intracranial injury in children with mild traumatic brain injury.

Methods A multicenter prospective cohort study was carried out in the paediatric emergency departments of three tertiary hospitals in Switzerland between January 2009 and December 2011. Participants included children aged <16 years with a mild traumatic brain injury (GCS ≥13) for whom a head CT was requested by the attending physician. Venous blood was obtained within 6 h of the trauma in all children for S100B measurement before a head CT was performed. As the S100B value was not available during the acute care period, the patient’s management was not altered. The main measures were protein S100B value and the CT result.

Results 20/73 (27.4%) included children had an intracranial injury detected on CT. S100B receiver operating characteristics area under the curve was 0.73 (95% CI 0.60 to 0.86). With a 0.14 µg/L cut-off point, S100B reached an excellent sensitivity of 95% (95% CI 77% to 100%) and 100% (95% CI 81% to 100%) in all children and in children aged >2 years, respectively. The specificity, however, was 34% (95% CI 27% to 36%) and 37% (95% CI 30% to 37%), respectively.

Conclusions S100B has an excellent sensitivity but poor specificity. It is therefore an accurate tool to help rule out an intracranial injury but cannot be used as the sole marker owing to its specificity. Used with clinical decision rules, S100B may help to reduce the number of unnecessary CT scans.

Key messages
What is already known about this subject?
▸ Studies in adults show that serum S100B is a possible adjunct to clinical decision rules to detect intracranial injury, and the American College of Emergency Physicians suggests that S100B measurement could reduce the number of unnecessary CTs.
▸ Research in children on this biomarker is limited and it shows interesting but conflicting results.

What does this study add?
▸ In this multicentre, prospective cohort study in patients aged <16 years, S100B was found to have excellent sensitivity, making it an accurate tool to rule out intracranial injuries. Its poor specificity is a limiting factor that precludes its use as the sole marker.
▸ Used with clinical decision rules, S100B may help to reduce the number of unnecessary CTs in children.

INTRODUCTION
Traumatic brain injury (TBI) is a very frequent cause of presentation to the emergency department. This condition accounts for the USA for more than 500 000 visits a year in children.1 The vast majority of children with mild TBI, defined as GCS 13–15,2 have no intracranial injury (ICI);3 a lesion is seen in 3–7% of these children undergoing CT4–6 and only 0.1–0.6% need a neurosurgical intervention.3,7 Consequences of a missed ICI may be devastating and its symptoms or signs, such as vomiting, headache or amnesia, are often misleading. CT is therefore performed in almost 50% of children with mild TBI8 as this is the only diagnostic tool to detect an ICI in the emergency setting.

However, overuse of CT scans has been called into question because the ionising radiation can lead to lethal malignancies. This rate can be as high as 1 in 1000 head CT scans, with younger children being more susceptible.7,8

Several decision rules based on clinical signs have been described to reduce the number of unnecessary CT scans.3,5,9,10 Although these guidelines have excellent sensitivity and good specificity, they are not widely used,11 and a sharp increase in the use of CT has been noted.12,13 Groups at very low risk for ICI have been identified. However, for all other children, the decision to obtain a CT scan is left to the physician and often based on his/her own experience.3

S100B is a calcium-binding protein which is located predominantly in the cytoplasm and nucleus of astrocytes and Schwann cells.14 It is released by damaged cells and enters the systemic circulation only when the blood–brain barrier is disrupted. It is then excreted in urine within 6 h from injury.3,15 S100B is also expressed to a lesser extent in adipocytes and chondrocytes, and thus long-bone fractures may increase the systemic S100B level even in the absence of brain injury.16

Many adult studies have shown that serum S100B is a possible adjunct to clinical decision rules to detect ICI.\textsuperscript{17–21} with the American College of Emergency Physicians suggesting that S100B measurement could reduce the number of unnecessary CT scans by 30%.\textsuperscript{22}

A few studies have evaluated S100B as a tool to reduce the number of CT scans in children, with interesting but conflicting results.\textsuperscript{23–27}

The objective of our study was to assess the accuracy of S100B serum level to detect ICI in children with mild TBI.

METHODS
Study design
This was a multicentre prospective cohort study. The study received institutional review board approval of each participating hospital and was conducted in accordance with Good Clinical Practice guidelines and provisions of the Declaration of Helsinki.

Settings and selection of participants
The study was conducted in the paediatric emergency departments of three tertiary hospitals in Switzerland on a consecutive sample of patients between January 2009 and December 2011. These three centres see 33 000, 24 000 and 12 000 patients a year. We included all children aged <16 years with a mild TBI (GCS \( \geq 13 \)) for whom a head CT was requested by the attending physician. We excluded those children who arrived at the hospital more than 6 h after the trauma, children with Down syndrome (since in these patients S100B is overexpressed\textsuperscript{28}) or patients with a history of convulsion in the past 7 days.

Study protocol
The attending paediatric emergency physicians obtained consent from the parents of children meeting the inclusion criteria for participation in the study. After consent was obtained, venous blood was drawn and centrifuged. The serum was then frozen (\(-20^\circ \text{C}\)) and sent in batches to the division of clinical chemistry and biochemistry of the University Children’s Hospital Zurich for S100B measurement. As the S100B value was not available during the acute care period, the patient’s management was not altered.

All head CT scans were reviewed for traumatic injuries by one paediatric radiologist (CJK), who was blinded to the patient’s clinical signs and the S100B value.

S100B measurement
All S100B (\( \alpha \) and \( \beta \) dimers) measurements were done on an Elecsys 2010 using the commercial S100 assay from Roche (Roche Diagnostics, Rotkreuz, Switzerland), with an interassay coefficient of variation <2.8% according to the manufacturer.

Outcome measures
Primary outcome was evaluation of the diagnostic value of S100B in detecting intracranial injuries in children aged <16 years with mild head trauma. Secondary outcomes were evaluation of the same parameters excluding children aged <2 years (age group where normal S100B values vary physiologically with age) and comparison of signs and symptoms between the groups with and without ICI (all children).

Definitions
Mild TBI: acute head trauma with a GCS 13–15, with confusion or loss of consciousness (<30 min) or amnesia or transient neurological abnormality.\textsuperscript{2}

ICI: any collection of blood within the cranial vault or cerebral oedema.

Data analysis and sample size
A \( \chi^2 \) test was used to describe categorical data. A t test was used to compare continuous variables as normality was observed in our data. The 95% CIs of the results are reported. A level of \( p<0.05 \) was considered significant. Statistical analyses were performed using PASW V.18 (SPSS Inc, Chicago, USA).

RESULTS
Between January 2009 and December 2011, 2595 children were admitted for a mild TBI and 342 (13.2%) had a head CT scan. A total of 80 children with mild TBI meeting the inclusion criteria were enrolled (see figure 1). Seven patients were excluded (no S100B measurement: three patients; venous puncture \( >6 \) h after trauma: one patient; no CT performed: three patients). Of the 73 included children, 20 (27.4%) had an ICI detected on CT. The lesion was an epidural haematoma in nine children, a subarachnoid haemorrhage in four, an epidural haematoma and subarachnoid haemorrhage in three. The remaining four children had respectively a subdural haematoma, an epidural and subdural haematoma, a subdural haematoma and subarachnoid haemorrhage and haemorrhagic parenchymal contusion. No surgical intervention was required. There was no statistically significant difference between the patients with or without ICI in age, gender and time between trauma and venous puncture for S100B measurement (table 1).

Clinical features that might influence a physician’s decision to obtain a CT scan are shown and compared between groups in table 1. The only statistically significant difference between groups was the S100B value.

The S100B receiver operating characteristics (ROC) curve is shown in figure 2. The area under the curve was 0.73 (95% CI 0.60 to 0.86). After excluding children aged <2 years, the area under the curve was 0.77 (95% CI 0.65 to 0.89) for the remaining 64 children.

Using the best cut-off value derived from the ROC analysis (0.14 \( \mu \)g/L), S100B reached a sensitivity of 95% (19/20) (95% CI 77% to 100%) and 100% (18/18) (95% CI 81% to 100%) in all children and in children aged >2 years, respectively. With this cut-off point, one (1.4%) child (aged 17 months) with a 1.5 cm temporoparietal epidural haematoma would have been missed. The corresponding specificity and the derived positive and negative likelihood ratio for all children and children aged >2 years are shown in table 2. This cut-off point has been chosen to take into account the best sensitivity/specificity ratio. If the aim was 100% sensitivity, specificity would fall to 4% (with a 0.07 \( \mu \)g/L cut-off value).

DISCUSSION
This study shows that S100B protein has good diagnostic properties with an area under the curve of 0.73 and, with 0.14 \( \mu \)g/L cut-off value, a very good sensitivity but poor specificity, in children aged <16 years with mild TBI (GCS 13–15).

We evaluated the same parameters after exclusion of children aged <2 years. As expected these are slightly better: the area under the curve rises to 0.77 and sensitivity reaches 100% with similar specificity. The reason for this is probably because the S100B value is age-dependent with physiologically higher levels in the youngest subjects.\textsuperscript{29–31} Reference value studies mainly use two age groups: under or over 2–3 years of age.\textsuperscript{29–31}

Taking S100B as the only identifier of ICI, 18 (24.7%) head CT scans would have been avoided. However, one (1.4%) child...
with a 1.5 cm epidural haematoma would have been missed. Thus, the decision to perform a CT scan cannot rely on the biomarker alone. In order to reduce unnecessary CT scans and radiation-induced malignancies, several decision rules based on clinical signs have been described. The strongest validated rule to date published by Kuppermann et al suggests obtaining a CT scan in all children with GCS <15 or other signs of altered mental status or signs of basilar skull fracture, but leaves the decision to the physician if the child has any history of loss of consciousness, vomiting, a severe mechanism of injury or severe headache. According to these authors, the decision should be based, among other factors, on the physician’s experience. In our opinion, this is the situation in which S100B could help to avoid unnecessary CT.

Most evaluations of the diagnostic accuracy of S100B come from reports on adults. Unden and Romner included 12 studies with 2466 patients in a systematic review and meta-analysis. They found sensitivity and specificity results similar to ours (97% and 40%, respectively). The largest prospective cohort comparing S100B values with CT results in 1559 adults showed the same sensitivity (98.6%) but worse specificity (12%); the area under the curve was 0.76.

Our results are also comparable to those of the few paediatric studies published. Babcock et al included children aged 0–18 years with TBI of all grades; the area under the curve was 0.71. However, to achieve optimal sensitivity and specificity, the cut-off value was much lower than ours (0.006 µg/L). The reason might be a different definition of ICI, comprising skull fractures. Castellani et al selected 109 children with only mild TBI (GCS 13–15). With a cut-off value comparable to ours (0.16 µg/L) and they again found similar sensitivity (100%) and specificity (42%); the area under the curve was 0.68.

In contrast, Hallen et al published much higher S100B diagnostic properties. The area under the curve was 0.93, sensitivity 100% and specificity 88%. The difference between their study and other studies was the definition of a negative outcome. Children were considered to be without ICI if CT was normal or the course after observation was satisfactory, with only 10% of patients having a CT scan. This is an interesting point and possibly a limitation of our study. We considered positive any intracranial abnormality, even, probably, lesions without consequences, as in previous studies. It is, however, difficult to recognise those with potential for deterioration. Because a S100B value is undetectable after 6 h from injury in 36% of patients with initially detectable levels, we decided to include only children within 6 h. It is not clear whether earlier blood sampling would have altered the diagnostic properties. Bouvier et al found a 100% sensitivity and 33% specificity when including children within 3 h of the TBI. However, others had the same sensitivity with better specificity when including children within 6 h.

S100B protein is expressed in astrocytes and Schwann cells, but also in chondrocytes and adipocytes. This raised the
question as to whether other lesions, such as long-bone fractures, could raise the serum S100B level, with conflicting results in the paediatric literature. Unlike Berger et al., who found no influence, Bechtel et al. showed that children with ICI and long-bone fractures had significantly higher levels of S100B than those without a fracture, possibly affecting the specificity of the biomarker. This aspect could not be evaluated in our study because only one child had long-bone fracture (with a S100B of 1.62 μg/L).

Another interesting finding is that we found no statistically significant difference between the groups in the presence of ICI-related worrying symptoms, whereas S100B levels were higher in the group with ICI. That point was also evaluated by Babcock et al., but with opposite results. Their two groups significantly differed in their responses concerning nausea or vomiting, headache and amnesia. The difference might be explained by their study population that included TBI of all severities. Also, there may be a selection bias for our patients, possibly identified as needing a CT scan based on these symptoms. Indeed, ICI was found in 27.4% of our population, compared with 17.4% in the study of Babcock et al.

Limitations
Our study has some limitations. First, is the small sample size. Because of this, we could not draw conclusions in the subgroup aged <2 years and we had no surgical intervention. It would have been interesting to compare these children with those who received conservative treatment. However, despite our small sample size, we obtained results similar to those of much larger adult and paediatric studies. Also, the study was conducted in three tertiary hospitals, but the inclusion period and contribution were not equal, with most of the study patients coming from the two biggest centres. As stated before, our study population is limited to children undergoing head CT, with the risk of considering positive any intracranial abnormality, even, possibly, lesions without consequences. However, the benefit is to have objective outcomes, as it is difficult to identify patients at risk of deterioration.

In conclusion, the biomarker S100B is a valuable tool to help the physician decide whether head CT is indicated for children aged <16 years with mild head trauma. Its excellent sensitivity indicates that it could be an accurate tool to ‘rule out’ an ICI. However, its poor specificity is a limiting factor that precludes

| Table 2 Diagnostic characteristics of S100B in children aged <2 years, children aged 2–16 years and all children (<16 years) |
|---|---|---|---|
| | S100B | Children <2 years | Children 2–16 years | All children <16 years |
| n total (n with ICI) | 9 (2) | 64 (18) | 73 (20) |
| Area under the curve | 0.57 (0.00–1.00) | 0.77 (0.65–0.89) | 0.73 (0.60–0.86) |
| Sensitivity (95% CI)* | 50.0% (5% to 97%) | 100.0% (81% to 100%) | 95.0% (77% to 100%) |
| Specificity (95% CI)* | 14.3% (1% to 28%) | 37.0% (30% to 37%) | 34.0% (27% to 36%) |
| LR+ (95% CI)* | 5.08 (0.05 to 1.35) | 1.59 (1.16 to 1.59) | 1.44 (1.05 to 1.55) |
| LR− (95% CI)* | 3.50 (0.10 to 74.19) | 0.00 (0.00 to 0.63) | 0.15 (0.01 to 0.84) |
| S100B value, mean(SD) | 0.40 (0.35) | 0.54 (0.86) | 0.52 (0.81) |
| Time trauma to venous puncture (min), mean (SD) | 182.1 (91.3) | 168.2 (91.3) | 169.9 (90.8) |

*Cut-off value 0.14 μg/L.
ICI, intracranial injury; LR, likelihood ratio.
its use as a sole marker. Used with clinical decision rules, S100B may help to reduce the number of unnecessary CT scans.

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Contributors IBH, GS and SM conceived the study, designed the trial and obtained research funding. IBH and GS supervised the conduct of the trial and data collection. LL, DK-L, GLS and SA undertook recruitment of participating centres and patients and managed the data, including quality control. CJK interpreted all study CT scans. MH supervised the S100B laboratory analysis and its data. SM and LL provided statistical advice on study design and analysed the data. SM drafted the manuscript and all authors contributed substantially to its revision.

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Roche Switzerland supplied the S100 reagents without charge.

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Provenance and peer review Not commissioned; externally peer reviewed.

Correction notice Since this paper was updated so that it displays two different ROC curves.

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3. Conclusion and perspectives

As a medical history and physical examination may not be sufficient to diagnose a disease or to identify children whose condition is at risk of deterioration, biomarkers can play an important role in the decision-making processes of physicians in pediatric emergency medicine. As the ideal biomarker does not exist, we have to evaluate each one’s real weight (accuracy) for every particular condition. We have demonstrated that, if at all possible, a biomarker should not be reduced to a dichotomous value. Multilevel likelihood ratios can show a biomarker’s real accuracy at different levels, allowing physicians to interpret every biomarker result in association with the patient’s clinical condition.

In certain situations, CRP or PCT may help physicians to identify those children with fever without source who are most at risk of a serious bacterial infection. Proadrenomedullin levels, but not copeptin levels, may detect children with community-acquired pneumonia who need a more aggressive therapy, such as intravenous and broader-spectrum antibiotics. Finally, when used with clinical decision rules, measuring S100B protein may help to reduce the number of unnecessary CT scans in children with mild traumatic brain injury. This will lower the risks of ionizing exposition for this particularly vulnerable population.

The perspectives for biomarker research cover many fields. First, we will have to discover the many new biomarkers that are candidates for revealing particular conditions. There are two main approaches used for identifying these molecules: knowledge-based approaches and unbiased approaches (15).

The knowledge-based approach is the more traditional method. The investigator must have a very strong knowledge of the disease’s pathophysiology in order to identify the possible candidates a priori. The investigator will then have to design a hypothesis-based study to prove a possible correlation between the biomarker and the pathological condition.
molecules may be new discoveries, but they may also well-known ones: very recently, Tekin et al. found a correlation between the well-known mean platelet volume (MPV) and upper urinary tract infection in children (41). The advantage of the traditional method is that it is focused and follows the usual hypothesis-driven scientific design. Its disadvantage is that it depends on current knowledge of the disease’s pathophysiology.

The unbiased approach falls within the field of clinical proteomics, where quantitative measurement of proteins is compared between two states, usually disease versus no disease. This method allows researchers to find many candidate biomarkers without any a priori assumptions. It is therefore particularly appropriate for the first step in biomarker discovery, but it is also a costly and complex analytical challenge. Moreover, finding a new molecule using mass spectrometry is still a long way away from proving its clinical usability. A good example of this is the urine alpha-2-glycoprotein found to correlate with acute appendicitis using a proteomics approach. Its validation study showed that the commercial ELISA was not suitable for clinical use due to an immunoassay interference effect (42).

Advances in other fields of molecular biology will also benefit biomarker development. Polymerase chain reaction (PCR) assays are becoming increasingly fast and easy to perform. One day, they will be fast and easy enough to be useful in emergency settings. This will allow physicians to diagnose, for example, a Kingella kingae osteoarticular infection using a throat swab without doing any other test. Indeed, we demonstrated that a Kingella kingae positive throat swab has 100% sensitivity and 90% specificity for osteoarticular infection in children with compatible clinical features (20).

One important development in the field is a combination of multiple biomarkers. This is already well-known with physiomarkers: some signs or symptoms show poor individual predictive performance but may have strong diagnostic accuracy when put together, such as dry mucous membranes and sunken eyes in dehydration scores (5). In Geneva, Galetto-
Lacour et al. developed a score combining CRP and PCT levels with a urine dipstick test (positive leucocytes esterase or nitrite test result), known as the Lab-score (43). This score has a higher diagnostic accuracy than each test taken individually: the Lab-score’s area under the ROC curve was 0.91 versus 0.84 for PCT and 0.86 for CRP alone.

Another multi-biomarker combination (TNF-related apoptosis-inducing ligand [TRAIL], interferon gamma-induced protein-10, and CRP) is currently being evaluated in children consulted in the HUG’s pediatric ED(44). This score has the particularity of being able to combine viral (TRAIL and interferon gamma-induced protein-10) and bacterial (CRP) biomarkers, and the result is given as a probability of viral or bacterial infection (Figure 5). This is very innovative as the vast majority of the markers for fever are intended to detect bacterial infections only.

Figure 5: In-vitro diagnostic test that uses a computer algorithm to combine the immunoassay measurements of three host immune-response proteins present in human serum, in order to help distinguish between bacterial and viral etiologies. Source: ImmunoXpert™, MeMed Company (Israël), www.me-med.com
However, most of the scores, such as the Lab-score, use dichotomized biomarkers very early on in the process of analysis, and this simplicity has a cost. Dichotomized biomarkers may lead to a decreased power and then to reduced applicability across different settings (45). It takes a much more complex statistical analysis to refine the scores and improve their robustness. We are currently refining the Lab-score by maintaining biomarkers as continuous variables for as long as possible and dichotomizing them only if necessary, based on observed data distributions (that is, instead of dichotomizing them right away). Furthermore, we are also demonstrating that the model provides a more statistically robust test and better diagnostic accuracy than PCT, CRP, WBC count, and the original Lab-score for all threshold probabilities(46).

Once again, the way for the physician to produce the best results in clinical practice would be to have a probability of the patient having a particular disease considering his or her past medical history, a physical examination, and the value or levels of one or more biomarkers as a continuous variable. Thus, the next step towards giving attending physicians the answers they need will be the ability to integrate refined scores and continuous biomarker likelihood ratios with post-test probabilities (Figure 6). As this calculation is very complex, it should be carried out in and communicated by an electronic device or computer.
However, before the field of biomarker research can properly be developed, there are also some barriers to remove.

Despite numerous high-quality studies on biomarkers, it is still difficult to implement the results of their measurement into daily practice, as is the case for official guidelines (23, 25, 47).

We conducted a prospective, randomized, controlled impact study on the utility of Lab-score (based on the combined determination of PCT and CRP levels and urinary dipstick results) to safely decrease antibiotic prescriptions in children with fever without source. We found that if the Lab-score had been strictly followed, a safe reduction of antibiotic prescription rates would have been observed. However, there was no statistically significant difference in the
initial antibiotic treatment rate between our two groups (with or without Lab-score), because there was such a low adherence rate to Lab-score’s recommendations (23). In another biomarker impact study conducted in Canada (25), we used a visual analog scale to evaluate the influence of PCT and WBC count on the attending physician’s perception of serious bacterial infection. We found that an abnormal PCT level with a normal WBC count, or abnormal WBC count with a normal PCT level, appeared to have had no influence on the physician’s perception of serious bacterial infection compared to when both were normal. However, an abnormal PCT level and WBC count resulted in a higher perception of SBI than a normal PCT level and WBC count. Despite this, these abnormal results appear to have had no impact on patient management (antibiotic use or hospitalization). There are several reasons for this:

Pediatric emergency medicine requires science, common sense, and experience. Common sense is probably innate. The science is learned at medical school and is (or at least should be) updated continuously by reading the latest medical literature and attending scientific meetings. And experience is progressively acquired via the physician’s exposure to clinical cases. That experience is a significant factor, one that has to be considered in diagnostic reasoning and patient management. Biomarkers and guidelines are important safeguards for relatively inexperienced physicians and, indeed, some recommendations take experience into account, such as the PECARN traumatic brain injury rule, where physicians may choose between a head CT scan or patient observation, depending on their experience (48). In this situation, a biomarker (S100B) could even be used to overrule a physician’s decision, taken due to his relative lack of experience, as demonstrated in the author’s multicenter study (14).

With experience, however, physicians can also acquire a degree of self-confidence that may make them less prone to change their practices. Developing on their previous personal experiences, their diagnostic reasoning can shift progressively from mainly analytical
reasoning (Bayesian) to non-analytical reasoning (intuitive) (49-51). Analytical reasoning refers to an explicit, controlled, rational, burdensome, and relatively slow process. Considering the performance of biomarkers or other diagnostic tests in clinical decision-making is a part of this. It is also called Bayesian reasoning, based on Bayes’ theorem, in which the probability of a hypothesis is modified by further data (39). A non-analytical cognitive process is implicit, based on automatic and effortless thought processes; it is rapid, associative, and intuitive (51). As they become increasingly experienced, physicians tend to use non-analytical reasoning more and more, but they are able to switch to an analytical approach when the non-analytical approach proves insufficient to explain the patient’s situation. Some authors think that there is a third analytical track called “gut feeling”. This is an intuitive sense of alarm or sense of reassurance that has more of an affect on the expected course of child’s condition or its prognosis than the diagnosis itself (51). When pediatricians sense that “alarm bells are ringing”, they automatically slow down their diagnostic reasoning process and switch to a more analytical reasoning process.

Another barrier in using biomarkers is that even experienced physicians sometimes fail to understand the implications of Bayesian reasoning (52). If a disease has a prevalence of one in a thousand, and a test is 100% accurate for people who have the disease and 95% accurate for those who do not (meaning that 5% of the people who do not have the disease will be wrongly diagnosed as having it), then a person selected at random who tests positive has a less than 2% probability of having the disease. This is known as the “Harvard question.” When questioned, however, most physicians think that the probability of having the disease is much higher, because the test is very accurate (52): they forget to consider the prevalence. This is another reason why physicians may distrust biomarkers: they see the many false positives when biomarkers are used for rare conditions and project their performance onto other indications.
In conclusion, if physicians wish to see ever more effective pediatric emergency medicine then they will need biomarkers. There is a significant potential for development in this field, but it will require lots of further research. New biomarkers must be discovered, and new indications for known molecules must be found. Scores involving multiple biomarkers must be developed and their diagnostic performance accuracy must be integrated with that of other physiomarkers into electronic devices that can give the disease probability for a particular patient. Finally, physicians need to develop new ways of thinking about diagnosing diseases in pediatric emergency medicine, so that they can embrace biomarkers, technology, and statistics as parts of the science that accompanies their common sense and experience.
4. References:


