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DISSERTATION

Diagnostischer Nutzen von Procalcitonin (PCT) zur Frühdiagnostik der Sepsis in Notaufnahmepatient*innen mit mindestens einem qSOFA Kriterium

Diagnostic utility of Procalcitonin (PCT) for the early detection of sepsis in patients presenting to the emergency department with a qSOFA score of at least one

zur Erlangung des akademischen Grades

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AUC	Area under the Curve
CI	Confidence Interval
CRP	C - Reactive Protein
ED	Emergency Department
EUSEM	European Society of Emergency Medicine
ICU	Intensive Care Unit
IQR	Interquartile Range
LIFE-POC	Lebensbedrohliche Infektionen Früh Erkennen mit Point of Care
MEWS	Modified Early Warning Score
NEWS	National Early Warning Score
NPV	Negative Predictive Value
NRI	Net Reclassification Improvement
ORs	Odds ratios
PCT	Procalcitonin
PPV	Positive Predictive Value
qSOFA	quick Sequential Organ Failure Assessment
ROC	Receiver Operating Characteristic
SD	Standard Deviation
SDI	Socio-demographic Index
Sepsis-3	Third International Consensus Definitions for Sepsis and Septic Shock
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sepsis-related Organ Failure Assessment
SPSS	Statistical Package for the Social Sciences

SSC	Surviving Sepsis Campaign
TNF-a	Tumor necrosis factor- alpha

Abstract

Background

Early sepsis identification can be achieved with the help of effective screening tools and suitable point of care biomarkers. With this objective, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) introduced a new screening instrument in 2016 called the quick Sequential Organ Failure Assessment (qSOFA) score. Since the introduction of the qSOFA score, debate has continued over the lack of sensitivity of the score for sepsis recognition in the Emergency Department (ED). The combination of biomarkers of infection like Procalcitonin (PCT) with the qSOFA score might improve the early identification of septic patients in the ED and could be beneficial as a point-of-care biomarker.

Main objective

To investigate whether the early measurement of PCT improves the detection of septic patients in an ED population with elevated qSOFA score.

Methods

In this large multicentre cohort study, the LIFE-POC study, adult patients presenting with an elevated qSOFA score (≥1) were identified and prospectively recruited at three tertiary care hospital EDs; the Charité - Universitätsmedizin Berlin Campus Mitte and Campus Virchow as well as the University Hospital of Jena. Exclusion criteria were: trauma, acute ST elevation myocardial infarction; pregnancy; suspected stroke and therapy limitation due to short life expectancy. The current analysis included all (n=742) patients from the study sites of the Charité - Universitätsmedizin Berlin. PCT was measured in all enrolled patients upon ED admission. The primary endpoint was sepsis diagnosis within 96 hours after ED admission. The gold standard diagnosis of sepsis was adjudicated according to the sepsis-3 definition by an experts´ panel.

Results

Within the first 96 hours, 27.4% (n=202) of the total study population were diagnosed with sepsis. The area under the receiver operating characteristics curve (AUC) for PCT for sepsis prediction was 0.857 (95% CI: 0.83–0.89; $p < 0.0001$). PCT levels were significantly higher in septic patients (1.15µg/L; interquartile range (IQR): 0.25-5.07) as

compared with non-septic patients (0.10µg/L; IQR: 0.06-0.20; p<0.0001). The optimal cut-off value of PCT that achieved the highest accuracy was 0.5µg/L. PCT at this cut-off value had a sensitivity of 63.6% (95%-CI: 56.5-70.2%), a specificity of 89.4% (95%-CI: 86.5-91.9%), a positive predictive value (PPV) of 69.4% (95%-CI: 63.4-74.7%) and a negative predictive value (NPV) of 86.7% (84.4-88.7%).

Conclusions

This prospective cohort study showed that PCT, measured in an ED population with elevated qSOFA score, improved early sepsis identification. Based on these results, early measurement of PCT could thus be recommended as an additional and important component of sepsis screening in the ED.

Zusammenfassung

Hintergrund

Die Sepsisfrüherkennung in der Notaufnahme kann mit Hilfe wirksamer Screening-Instrumente und geeigneter Point-of-Care-Biomarker verbessert werden. Aus diesem Grund wurde über den „Dritten internationalen Konsens für Sepsis“ im Jahr 2016 ein neues Screening-Instrument eingeführt, der Quick Sequential Organ Failure Assessment (qSOFA)-Score. Seit der Einführung des qSOFA-Scores wird die mangelnde Sensitivität des Scores für die Sepsisfrüherkennung in der Notaufnahme (ED) kritisiert. Die Kombination des qSOFA-Scores mit Infektionsparameter, wie Procalcitonin (PCT), könnte geeignet sein, die frühe Identifikation von septischen Patient*innen in der Notaufnahme zu verbessern.

Ziel der Studie

Ziel der Studie ist die Untersuchung der diagnostischen Wertigkeit von PCT zur Früherkennung der Sepsis in einer Population von Notaufnahmepatient*innen mit erhöhtem qSOFA-Score.

Methodik

Bei der LIFE-POC-Studie handelte es sich um eine multizentrische, prospektive Kohortenstudie, die in den Notaufnahmen von drei Krankenhäusern der tertiären Versorgung durchgeführt wurde; Charité - Universitätsmedizin Berlin Campus Mitte und Campus Virchow sowie Universitätsklinikum Jena. Es wurden erwachsene Patient*innen mit nicht-traumatischen Vorstellungsgründen mit erhöhtem qSOFA-Score in der Notaufnahme eingeschlossen. Ausschlusskriterien waren: akuter ST-Hebungs-Myokardinfarkt, Schwangerschaft, Verdacht auf Schlaganfall und Therapielimitierung aufgrund einer kurzen Lebenserwartung. In die aktuelle Analyse wurden alle (n=742) Patient*innen aus den Berliner Studienzentren der Charité – Universitätsmedizin Berlin einbezogen. PCT wurde bei Aufnahme gemessen. Der primäre Endpunkt war Sepsis innerhalb von 96 Stunden nach Aufnahme. Die Goldstandard-Diagnose der Sepsis wurde gemäß der Sepsis-3-Definition von einem Expert*innengremium gestellt.

Ergebnisse

Von allen 742 Patient*innen wurde bei 27,4 % (n=202) innerhalb der ersten 96 Stunden eine Sepsis diagnostiziert. Die Fläche unter der Receiver Operating Characteristics (ROC)-Kurve zur Sepsisdiagnose betrug für PCT 0.857 (95% CI: 0.83–0.89; p < 0.0001). PCT war bei Patient*innen mit Sepsis signifikant höher (1,15 µg/L; IQR: 0,25-5,07) im Vergleich zu nicht-septischen Patient*innen (0,10 µg/L; IQR: 0,06-0,20; p<0,0001). Der optimale Cut-off-Wert für PCT lag bei 0,5 µg/L. Daraus ergab sich eine Sensitivität von 63,6% (95%-CI: 56,5-70,2%), eine Spezifität von 89,4% (95%-CI: 86,5-91,9%), ein PPV von 69,4% (95%-CI: 63,4-74,7%) und ein NPV von 86,7% (84,4-88,7%) für die untersuchte Studienpopulation.

Schlussfolgerungen

Diese prospektive Kohortenstudie zeigt einen hohen diagnostischen Nutzen von PCT zur Sepsisfrüherkennung bei Patient*innen mit erhöhtem qSOFA-Score in der Notaufnahme. Die frühe Messung von PCT kann basierend auf diesen Ergebnissen als eine zusätzliche, wichtige Komponente zum Sepsisscreening in der Notaufnahme empfohlen werden.

1 Introduction

1.1 Epidemiology of sepsis

Sepsis is a life-threatening medical emergency that requires early identification and treatment in order to improve prognosis (2). Despite being in the focus of acute medicine, sepsis is still one of the leading causes of death worldwide. According to a recent study of the Institute for Health Metrics and Evaluation (IHME) (3), sepsis incidence reaches over 48.9 million cases globally per year and is responsible for approximately 11 million deaths inside and outside of hospitals. This number represents one fifth of deaths worldwide and is considered to be two times higher than former estimations, which were based only on hospital administrative data and excluded countries with lower Socio-demographic Index (SDI).

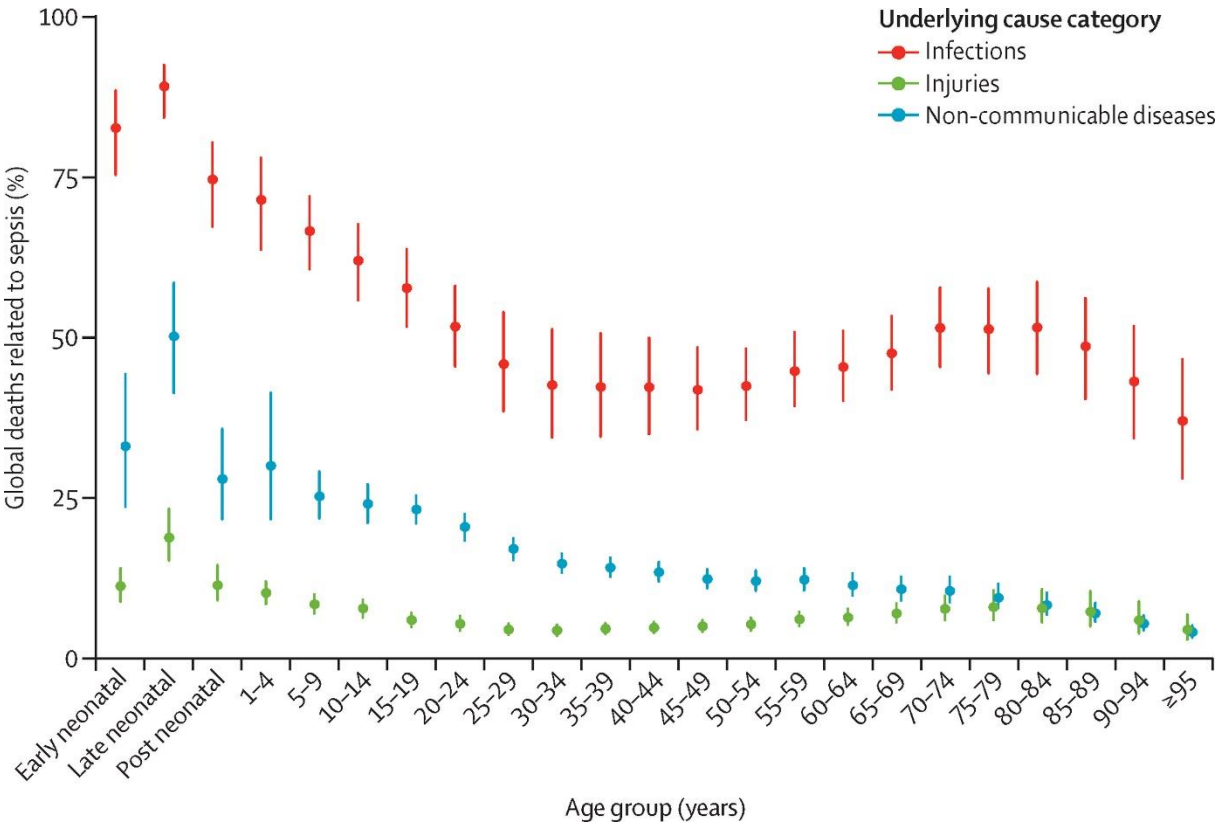

Figure 1. Percentage of all sepsis-related deaths in each underlying cause category by age group and for both sexes, in 2017, from Rudd et al.[3] , available at: https://doi.org/10.1016/S0140-6736(19)32989-7, Access Date 16.11.2021, License link: https://creativecommons.org/licenses/by/4.0/, Bars represent 95% uncertainty intervals

In Germany the estimated sepsis incidence according to the IHME study is 279,000 cases per year, also twice higher as reported in the last prospective study of Fleischmann-

Struzek et al (4). This underestimation was well known and expected, since the results of all studies before were extracted only from hospital discharge data and ICD- codes (5) .

Although mortality rates and incidence have slightly decreased within the last years, the financial burden for sepsis care all over the world remains enormous. Global and national (6) health care capacities are being exhausted. Developing sepsis is associated with prolonged hospital or ICU stay, which subsequently leads to high costs involving personnel, materials and medication. Moreover, surviving sepsis and hospital discharge is not the end of sepsis-associated costs (7). After a prolonged ICU stay, critically ill patients can require rehabilitation, cognitive therapy, physiotherapy, medication and further medical follow up in order to regain strength and mobility, while patients with permanent neurological damage will require life-time medical and nursing care (8, 9).

1.2 Definition and pathophysiology of sepsis

In 2016 the Third International Consensus for Sepsis updated the sepsis definition in order to reflect more adequately the pathophysiology of sepsis. Sepsis is now defined as a life-threatening organ dysfunction resulting from dysregulated host response to infection (10). The primary purpose of this new definition was to replace previous, confusing definitions and terminologies with simpler objective criteria and thus provide a more consistent picture of sepsis incidence and outcome. The extent to which it has achieved this aim remains a point of discussion (11).

Sepsis enhances a number of cascade reactions that begin with the insertion of a pathogen in the body of a host. Although most common pathogens are bacteria, sepsis can be a result of a viral, fungal or parasite infection as well. Pathogen components such as endotoxins, exotoxins or PAMPs (known as pathogen-derived molecular patterns) cause an immune response that triggers the activation of specific receptors (especially Toll-like receptors) and leads to the production of pro-inflammatory parameters (12). At the same time anti-inflammatory cytokines are activated. An adequate immune response, meaning a balanced pro- and anti-inflammatory pathway activation, would lead to the eradication of the infection. In sepsis both pro-inflammatory and anti-inflammatory pathways are upregulated, thus leading to inflammation and progressive tissue damage, leading to multi-organ dysfunction (13).

The task force suggested using the Sepsis-related Organ Failure Assessment (SOFA) score to assess organ dysfunction, a score that was already established in ICUs to

monitor organ function. The score is calculated based on six criteria (respiration, coagulation, liver function, cardiovascular function, Glasgow Coma Scale and kidney function). An acute change in the SOFA score of ≥ 2 plus the suspicion or confirmation of infection equals sepsis. Of course, this change in the SOFA score has to be consequent to the infection and therefore other causes have to be eliminated. In patients with unknown former values, the former SOFA score should be considered as zero.

1.3 Sepsis treatment

Since the new sepsis definition in 2016, three updates of the existing sepsis guidelines have been published by the Surviving Sepsis Campaign (SSC) (14-16). The SSC, the leading voice in Sepsis care, is an international consortium of sepsis specialists that has systemised sepsis treatment creating bundles and algorithms that altogether build the guidelines for management of sepsis and septic shock in the last years. The guidelines include many different sections and stages of sepsis therapy such as initial resuscitation, specific haemodynamic management, screening, ventilation, source control etc. in order to address all possible challenges in sepsis treatment. Evidence-based adjustments are recommended in regular intervals when needed.

Cornerstone of sepsis management and thus consistent feature of the international adult sepsis guidelines in the last years is early initiation of therapy. Fluid administration, draw of blood cultures, antibiotic therapy, lactate measurement and application of vasopressors (when indicated) belong to the first resuscitation measures that should be initiated as soon as possible after sepsis diagnosis. Today all these clinical interventions form the 1- hour sepsis bundle, which has not been without critics: The European Society of Emergency Medicine (EUSEM) raised its concerns regarding the enormous pressure that the completion of such bundles put on the EDs and emphasized the negative role that unrealistic goals can play (17). According to the EUSEM instead of extreme challenging recommendations, advanced strategies for early identification of patients at risk of sepsis are urgently needed.

1.4 State of current research

1.4.1 Screening tools up to date

To promote early treatment the SSC suggested the implementation of sepsis programs in all hospitals with the primary goal of achieving effective and adequate screening. Until 2016 several screening tools for sepsis identification were available depending on the screening location (e.g. ED, normal ward). EDs predominantly used the systemic inflammatory response syndrome (SIRS) criteria, on which the previous sepsis definition was based. SIRS provided high sensitivity on the costs of specificity, since almost every patient with infection but not necessarily sepsis met the criteria (18). Additional screening scores such as National Early Warning Score (NEWS), or Modified Early Warning Score (MEWS) (19) or SOFA score were also used in the EDs but enhanced more parameters and thus were more complex to assess. The early warning scores performed also only moderate in sepsis recognition (20, 21).

Since none of the already existing screening tools were considered ideal for sepsis identification the task force of the Third international consensus of sepsis introduced the qSOFA score along with the new definition for sepsis (10). The advantage of the score was rooted in its simplicity, since it could be assessed using only three clinical parameters: blood pressure under 100 mmHg, respiratory rate over 22 breaths per minute and altered mentation. The score originated from the work of Seymour et al. (22), who investigated death and prolonged ICU stay as potential surrogates for sepsis in more than 800,000 patients with suspected infection. In this study the qSOFA score proved its prognostic superiority to the SIRS criteria for sepsis mortality in non-ICU patients with suspected infection. Although the intention of the score was to find the ideal combination of clinical parameters to identify patients likely to develop sepsis, the Sepsis-3 Task Force concluded that a positive qSOFA score (qSOFA≥2) is ideal for flagging septic-patients likely to have a bad outcome, but the performance of the score for sepsis prediction was yet to be investigated outside the original study.

Shortly after the Sepsis-3 recommendation, the evaluation of the qSOFA score became the focus of sepsis-associated research. The results extracted from these studies were conflicting, since most of the study populations were not comparable: the study settings were different, study design could be either retrospective or prospective, the inclusion criteria varied. A systematic review and meta-analysis of Serafim et al. in 2018 (23)

revealed a low sensitivity of the qSOFA score in comparison to the old SIRS criteria for sepsis diagnosis.

The unsuitability of the qSOFA score as a sepsis screening tool was initially attributed to the absence of a parameter more specific for an infection. Subsequently several studies searched for suitable biomarkers to combine with the score, in order to achieve higher sensitivity.

1.4.2. The role of biomarkers in sepsis diagnosis and prognosis

Literature emphasizes the role of biomarkers in sepsis diagnosis and prognosis. To date, over 258 different biomarkers (24) and their relation to sepsis have been evaluated including pro-inflammatory cytokines and chemokines, inflammation responsive proteins, markers of neutrophil and monocyte activation, markers of the immune-suppressive phase of sepsis, as well as markers of organ dysfunction and coagulation.

Despite the plethora of biomarkers only two have established their role in the sepsis guidelines (SSC 2021): lactate and procalcitonin. Lactate plays a significant part in sepsis management and its measurement is recommended directly upon hospital admission for every patient presenting with sepsis suspicion in order to guide fluid management. Persisting high lactate levels along with persisting low blood pressure despite fluid resuscitation indicate the existence of septic shock. Though ineffective as a predictor of sepsis, lactate correlates to the severity of the sepsis and is therefore a useful severity/prognostic marker.

Procalcitonin on the other hand is a biomarker currently recommended as a supplement along with clinical evaluation to help physicians determine the duration of the antibiotic therapy in septic patients. PCT started as a promising infectious biomarker in the 1970s (25). Early studies showed that PCT levels rise rapidly as a response to a bacterial infection and thus can be valuable in many ways: to differentiate viral from bacterial infections (26), to guide antibiotic therapy in patients with infection (27) and to determine the severity of the infection (28). Due to these characteristics, PCT was very soon tested as a potential biomarker for sepsis with contradicting results regarding its diagnostic performance. However, two large systematic reviews (29, 30) that included studies with comparable study populations both yielded positive results for PCTs diagnostic value for sepsis identification.

1.5 Clinical need and current lack of research

Early treatment requires early identification. Since over 40% of the hospitalised septic patients initially present via the emergency department (31), an effective screening instrument for early sepsis recognition could be valuable for the emergency physician in order to implement early management as recommended by the current guidelines (32) and thus improve patients' outcome.

The assessment of the qSOFA score alone performed poorly in sepsis screening as outlined above. Studies up to date showed a benefit of combining the qSOFA score with sepsis-associated biomarkers in a non-ICU setting. Specifically, the sensitivity of the score when combined with PCT increased significantly in predicting sepsis mortality (33, 34). Furthermore, two studies that tested not only the prognostic but also the diagnostic accuracy of this combination in an ED population yielded promising results (35, 36). However, both studies had a retrospective cohort design and included only patients with suspected infection whose qSOFA score was available upon admission and thus excluding many patients due to missing data. None of the studies used the qSOFA criteria as inclusion criteria for the selection of the study population although it was suggested by the Sepsis-3 task force. This constitutes a significant lack of sepsis screening targeted research.

1.6 Study purpose

The central and primary objective of this work based on the recent publication of Bolanaki et al. (1) was to assess the diagnostic value of PCT in detecting sepsis within 96 hours after admission in patients presenting with an elevated qSOFA score at the ED. Our secondary endpoint was to evaluate the prognostic performance of PCT for the prediction of 28-day all-cause mortality. Furthermore, we explored relevant clinical outcomes in the septic subgroup of patients including septic shock, ICU admission, dialysis, surgical intervention and administration of vasopressors. Additionally, the performance of PCT to distinguish gram-negative from gram-positive and fungal sepsis was evaluated. Finally, we here provide new additional important insights on the characteristics of the subgroup of patients with elevated qSOFA score that did not develop sepsis.

2 Methods

In order to test the diagnostic and prognostic performance of already established as well as experimental new biomarkers for sepsis and thus evaluate their suitability for point-of-care measurement upon admission, a prospective cohort study design was developed. The Life-POC study was conducted in three large tertiary care hospitals: The University hospital of Jena and two sites of Charité in Berlin. Overall 1,477 patients were enrolled in the participating EDs, of which 742 in Berlin, and blood was drawn within 12 hours within emergency admission. This work includes all 742 patients enrolled at the Charité study sites from January 1st 2017 to March 23rd 2018 and the results of the evaluation of the diagnostic and prognostic performance of PCT for sepsis. This study was approved from the Institutional Review Board of the University Hospital Jena (4892-08/16) and entered in the "German Clinical Trials Register" under the number DRKS00011188.

2.1 Study participants

The qSOFA criteria were applied as inclusion criteria for our study. Participants over 18 years old with a qSOFA score of at least one were eligible for enrolment. Aim of the study was to include patients likely to develop sepsis and at that time the assessment of the qSOFA score in non-ICU patients with suspicion of infection was recommended in the sepsis guidelines (14). The screening performance of the score for sepsis was yet unknown, therefore the Sepsis-3 task force suggested using the qSOFA score as entry criterion for clinical studies in order to further investigate its diagnostic value.

Before recruitment began a pre-screening study was conducted to ensure a satisfactory rate of approximately 10% sepsis patients within the study population. For 7 calendar days we retrospectively screened all non- trauma patients (n=1112) that presented in the ED based on their qSOFA score using as source administrative routine data. Only 85 eligible patients were identified with an elevated qSOFA score at emergency admission. 7 of them were coded with sepsis within 96 hours, whereas 7 were admitted in an external clinic and the outcome was unknown. Using the qSOFA score in the pre-screening process we estimated a rate of over 8% septic patients. Thus, no adjustment of the inclusion criteria was needed.

The study team screened for eligible patients on a daily 8-hour basis on alternating shifts including weekends. Since the study population we aimed for, were patients with a presumed organ dysfunction of unknown cause at the time of admission, we excluded

other medical emergencies with clear clinical signs such as ST- elevation myocardial infarction, suspected stroke and acute trauma. Furthermore, we excluded pregnant women and patients with a reduced life expectancy (below 1 month). Patients were allowed to participate only once in the study regardless how often they visited one of the participating EDs. An overview of the inclusion and exclusion criteria is offered below in Table 1.

Table 1. *Overview of In- and Exclusion criteria (own representation)*

Inclusion criteria	Exclusion criteria
• adult patients ≥ 18 years old • at least 1 qSOFA point: "altered mentation": GCS < 15 respiratory rate ≥ 22/min systolic BP ≤ 100mmHg	• ST-elevation myocardial infarction • acute trauma • pregnancy • suspicion of stroke • reduced life expectancy under 28 days • prior enrolment

Abbreviations: qSOFA: quick Sequential Organ Failure Assessment, GCS: Glasgow Coma Scale, BP: blood pressure

Written informed consent was obtained before enrolment from all patients or their legal representatives when assigned.

2.2 Study procedures and biomarker measurement

Blood was drawn by venepuncture directly after enrolment in the study within 12 hour of emergency admission and included maximum 18 ml of whole blood. The blood was then divided in 3 tubes, 9ml in an EDTA tube and 4.5ml in heparin and citrate tube each. In patients that already received a venous catheter by the paramedics or the triage nurse, the blood sample was drawn from the catheter.

In our two EDs the study team collected blood samples from a total of 742 patients within 128 minutes after admission (IQR:75-207) and the samples were stored at -20°C within a maximum time of 2 hours after centrifugation and apportion in aliquots using a Pasteur pipette. Aliquots were then placed in a matrix box. When 15 Matrix boxes were fully complete, the study team ordered the transportation in dry ice to the main laboratory at Biobank Jena to be frozen at -80°C until measurement.

PCT was measured in plasma concentrations with an automated immunoassay method thus using the TRACE Technology (Time resolved amplified cryptate emission) as thoroughly described in the manufacturers protocol time (Brahms PCT sensitive Kryptor). The assay has a direct measurement range from 0.02 to 50 µg/L, while after automatic dilution the range increases to 5,000 µg/L. The 95th percentile of plasma PCT concentrations in healthy persons with this assay was 0.064 µg/L. The limit of detection (LOD) has been assessed as being 0.02 µg/L. As recommended by the manufacturer and literature up to date (Clinical Utility and Measurement of Procalcitonin) following cut-offs were assessed for PCT within the scope of this work: 0.25, 0.5, 2.0, 5.0, and 10 µg/L.

2.3 Assessments and endpoints

By signing the informed consent, patients gave the study team permission to collect study data, to extract their routine medical information of former and current admissions as well as to document any further medical treatment within 28 days after study enrolment. Furthermore, patients consented to a follow- up phone call after 28 days.

For the first four calendar days a detailed study documentation (primary data) was conducted to assess the development of sepsis within 96 hours. Study documentation was at first conducted in paper form and then electronically and included clinical parameters, laboratory findings, medication, ventilation or dialysis parameters and diagnosis codes. All data were extracted from the hospital information system. In both EDs and in all the ICUs of the Charité data were electronically documented. In the case of patients that were admitted to a normal ward, the data were collected after hospital discharge from the patients file. When a patient was transferred to another hospital after ED presentation we received all data relevant to our study from there. The follow-up call was performed from the study team 28 days after the enrolment. The purpose of the call was to collect information regarding heath status, additional hospital admissions and possible open questions regarding study data.

Primary objective of this study was sepsis onset within 96 hours. Sepsis was diagnosed by an experts´ panel according to the Sepsis-3 criteria: acute increase of 2 points or more in the SOFA score consequent to the infection. The secondary outcome was the prognostic performance of PCT in predicting 28-days mortality. Exploratory endpoints were dialysis, ICU length of stay and septic shock as defined in the sepsis-3 definition: Persisting hypotension requiring vasopressors to maintain MAP ≥65 mm Hg plus high

levels of blood lactate >20 mg/dl despite adequate volume resuscitation. The sepsis adjudication panel was blinded to the results of all study biomarker measurements.

2.4 Statistical analyses

All statistical analyses were performed with SPSS version 25.0 (IBM). Quantitative variables were expressed as mean ± standard deviation (SD) in case of normal distribution and median and inter quartile ranges (IQR) in case of other distribution. Categorical variables were shown as number (n) and percentage. Differences in continuous variables between groups were analysed using the Student's t-test or the Mann-Whitney U-test depending on data distribution. Categorical variables were compared by chi-square test when appropriate. A p-value of <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was used to determine the area under the ROC-curve of the respective biomarkers and determine optimized cut-off values. The diagnostic performance at different cut-off values was calculated based on cross-tables and sensitivity, specificity, negative and positive predictive value according to the respective formulas. The diagnostic endpoint was sepsis within 96 hours after admission (primary endpoint).

The diagnostic accuracy of PCT was assessed, as mentioned above, at the following cut-offs: 0.25, 0.5, 2.0, 5.0, and 10 µg/L. Multivariate logistic regression analyses were conducted to assess the independency of PCT as a diagnostic marker for sepsis within 96 hours (dependent variable) from other diagnostic markers, confounders and risk modifiers. PCT was entered into the model as a binary variable at the same recommended cut-off values. PCT was assessed as a single predictor and further in three different models containing different sets of independent variables: adjusted for qSOFA (model 1); qSOFA, sex, and age (model 2; age entered as a numeric variable); and qSOFA, sex, age, C-reactive protein (CRP), and lactate (model 3; age and other biomarkers entered as numeric variables). The effect measures of logistic regression analyses, odds ratios (ORs), were illustrated together with 95%-confidence intervals (95%-CIs).

A classification tree analysis was conducted in order to further investigate a step-wise approach in the ED and optimized cut-off values in subgroups. Sepsis within 96 hours was again the dependent variable while PCT and qSOFA were independent variables in the analysis. PCT was entered as a numeric variable with a fixed number of four group

intervals while qSOFA was entered with all three categories. Minimum group sizes were defined for superior (n=70) and inferior nodes (n=10). The chi-square automatic interaction detection (CHAID) was set to a maximum of five. The node split and consolidation level of significance was 0.05 and was corrected according to Bonferroni. The model estimation criteria allowed for a maximum number of 100 iterations and a minimum change in the expected cell frequencies of 0.001. In order to validate the findings, a training and test data set was selected randomly from the study population. The algorithm was primarily identified in the training data set and the findings were then applied to the validation data set. The net reclassification improvement (NRI) was evaluated in sepsis identification initially using the already established risk cut-off values for qSOFA (2 points) and PCT 0.50 µg/L. Then the NRI index was calculated again applying the optimized cut-off values derived from the classification tree analysis (PCT: 0.13 µg/L and 0.50 µg/L).

3 Results

Most of the following results are reported in the primary research publication (1), but we here include a more detailed description of the patient population with additional analyses, a synopsis of the clinical course as well as an in depth-presentation of the essential new results.

3.1 Patient cohort and clinical course

A total of 41,852 non-trauma patients were screened by our study team during the recruiting hours. 3,001 met the inclusion criteria of which 742 patients did not meet the exclusion criteria and agreed to take part in our study providing written informed consent.

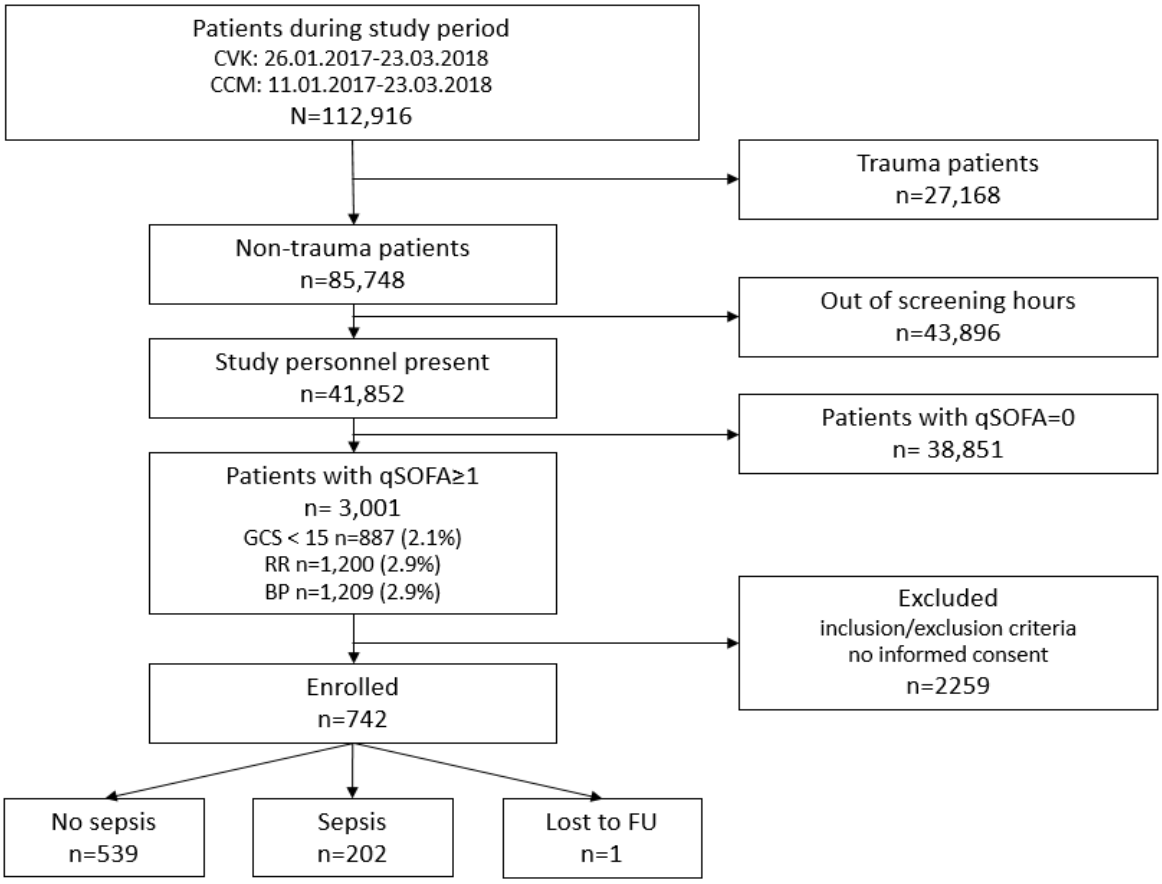

Figure 2: Consort Flow Diagram, reprinted from Bolanaki et al., 2021(1), available at: https://doi.org/10.3390/jcm10173869, access date 16.11.2022

Approximately one fourth (27%, n=202) of all patients developed sepsis in 96 hours. Only one patient was lost to follow up. 58% of the patients were female and the median age of the cohort was 68 years (IQR: 56-78). Regarding age and sex there were no significant

differences between the sepsis and the non-sepsis group. However septic patients showed a significantly higher Charlson Comorbidity Index than non-septic patients (3 vs. 2, p<0.0001). Dementia, chronic respiratory disease, metastatic solid tumor and lymphoma were significantly more frequent among septic patients. Furthermore, in the septic group the proportion of patients under immunosuppression was also significantly higher in comparison to non-septic patients (22.2% vs 6.7%, p<0.0001). A detailed description of patients' characteristics is illustrated below. Further information regarding comorbidities is provided in the supplementary section of the attached publication [(1)].

Table 2: *Demographic and clinical characteristics the Charlson Comorbidity Index and qSOFA at admission, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

	Total n=742	**Sepsis n=202***	**Non-sepsis n=539***	**p-value**
Women % (n) **	42.0 (312)	39.6 (80)	42.9 (231)	0.424
Age (median, IQR) **	68 (56-78)	70 (59-78)	67 (56-77)	0.086
Comorbidities % (n) **	85.8 (637)	91.6 (185)	83.7 (451)	0.006
Charlson Index points (median, IQR) **	2 (1-3)	3 (1-4)	2 (1-3)	<0.0001
qSOFA items **				
GCS <15 % (n) **	9.8 (73)	20.8 (42)	5.8 (31)	<0.0001
Tachypnoea (RR≥22/min) % (n) **	71.7 (532)	72.8 (147)	71.2 (384)	0.231
Systolic BP ≤100mmHg % (n) **	43.4 (322)	57.4 (116)	38.2 (206)	<0.0001
qSOFA points % (n) **				<0.0001
1	77.1 (572)	55.4 (112)	85.2 (459)	
2	20.9 (155)	38.1 (77)	14.5 (78)	
3	2.0 (15)	6.4 (13)	0.4 (2)	
GCS (median, Range) **	15 (3-15)	14 (3-15)	15 (3-15)	<0.0001
BP (mmHg) (median, IQR) **	112 (95-136)	99 (89-122)	117 (96-138)	<0.0001
RR (breaths/min) (median, IQR) **	23 (20-26)	24 (20-26)	23 (20-26)	0.028
Immunosuppression % (n)	10.9 (80)	22.2 (44)	6.7 (36)	<0.0001
WBC /nL (median, IQR) ***	10.0 (7.3-14.4)	12.7 (7.6-17.9)	9.5 (7.2-13.1)	<0.0001
CRP (mg/dl) (median, IQR) ****	34.8 (5.9-99.6)	104.4 (50.0-229.7)	18.3 (3.4-60.9)	<0.0001
Non-survivors day 28 % (n) *****	6.6% (48)	13.4% (27)	3.9 (21)	<0.0001

*Abbreviations: BP—blood pressure; GCS—Glasgow Coma Scale; IQR—Inter Quartile Range; qSOFA—quick sequential organ failure assessment; RR—respiratory rate; WBC—white blood cell count; CRP—C reactive protein * one patient was lost to follow-up ** nmiss = 0 *** nmiss = 11, **** nmiss = 13, ***** nmiss = 10.*

After stratifying the enrolled patients based on their qSOFA score, we initially created three qSOFA groups: (1) patients with qSOFA score of 1 (77.1%, n=572), (2) qSOFA score of 2 (20.9%, n=155) and (3) qSOFA score of 3 (2.0%, n=15). 29.6% of the patients

in the qSOFA 1 group developed sepsis within 96 hours (n=112), while sepsis occurred in 49.7% of the patients in group 2 (n=77) and in 86.7 % of the patients in group 3 (n=13), showing that higher qSOFA score correlates with the likelihood to develop sepsis. Correspondingly, septic patients had significantly higher qSOFA score as non-septic patients ($p < 0.0001$).

3.1.1 Clinical characteristics of the non-septic subgroup within the study population

This is the first study testing PCT in a cohort of patients with presumed organ dysfunction based on an elevated qSOFA score. Thus, a more detailed clinical description of this elevated qSOFA population and specifically of the non-septic population is illustrated here. After the initially ED presentation, 33.6 % (n= 181) of the non-septic patients were discharged from the ED on the same day, whereas 66.4% (n=358) were admitted in the hospital.

Based on the discharge diagnoses coded by the treating physician of the clinic, the most common diagnosis among non-septic patients were chronic obstructive pulmonary disease, followed by pneumonia, heart failure and atrial fibrillation. Further diagnoses are provided in Table 3.

Table 3: *Discharge diagnosis of non-septic patients (own representation)*

ICD-Codes	**Diagnosis**	Number	Percent (%)
J44	Other chronic obstructive pulmonary disease	56	10.4
J18	Pneumonia, organism unspecified	48	8.9
I50	Heart failure	30	5.6
I48	Atrial fibrillation and flutter	27	5.0
NA	Unknown	14	2.6
A09	Gastroenteritis and colitis of unspecified origin	12	2.2
J15	Bacterial pneumonia, not elsewhere classified	12	2.2
N17	Acute renal failure	12	2.2
N39	Urinary tract infection, site not specified	11	2.0

Abbreviations: ICD- International Statistical Classification of Diseases and Related Health Problems

3.1.2 Clinical course and infect foci

Of all enrolled ED patients, 75.2% were admitted in the hospital after initial treatment. 102 participants were transferred in an external hospital because of capacity reasons. For 171 patients an admission in an intensive care unit (ICU) was necessary, 96 of which

developed sepsis (47.5%). Regarding the clinical course of septic patients over 13% required treatment with vasopressors (n=27), while 47.5 % suffered acute renal failure (n=96) and approximately 8% had to undergo dialysis (n=8). According to the sepsis-3 definition 12.9% of the septic patients developed a septic shock (n=26). The 28-day mortality rate in the septic group reached 13.4 %. (Table 4)

Table 4: *Clinical course of all study participants (own representation)*

	Total n=742	Sepsis n=202	non- Sepsis n=539
ICU admission*	23.0 (171)	47.5 (96)	13.9 (75)
Vasopressors or Inotropic agents*	4.0 (30)	13.4 (27)	0.6 (3)
Acute Kidney Injury*	19.1 (142)	47.5 (96)	8.5 (46)
Dialysis*	4.8 (36)	7.9 (16)	1.5 (8)
Septic shock*	3.5 (26)	12.9 (26)	0 (0)
Mechanical ventilation*	14.3 (78)	32.7 (66)	7.4 (40)
Death % (n)*	6.4 (16)	13.4 (27)	3.9 (21)

*One patient lost to follow up

When stratifying the septic patients in groups based on the time that sepsis occurred, 20.8% (n=154) were already septic upon ED presentation. 3.9 % (n=29) developed sepsis in the first 24 hours, whereas for 2.3% (n=17) the onset of sepsis was between 24 to 96 hours. For 2 patients sepsis occurred in the first 96 hours, but we were unable to determine the exact time of onset due to lack of documentation.

Among study participants in the first 96 hours 67% had a presumed or a clinically confirmed infection (n=497). A presumed infection was assigned after consultation with the responsible treating physician based on clinical signs and laboratory findings. Clinically confirmed infection required validation through: unquestionable radiology findings, surgical intervention, positive urine test with matching symptoms for a urine infection or dermatological examination.

The focus of infection in our whole study cohort was predominately pulmonary explaining the respiratory rate as the most common qSOFA point that led to the inclusion. Following foci for infection were urogenital, abdominal and skin related. In 55 patients the infect focus remained unknown even after hospital discharge. The association of the qSOFA criteria and the infect foci on day 0 is illustrated in Table 5.

Table 5: *Association of qSOFA criteria and infect foci in all study participants, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

	Pulmo-nary (n=280)	Uro-genital (n=66)	Ab-dominal (n=58)	Skin or wounds (n=28)	Other (n=16)	Cardiovascular (n=6)	Central nervous system (n=2)	Unknown (n=55)
qSOFA points								
1 % (n)	76.1 (213)	59.1 (39)	75.9 (44)	78.6 (22)	81.3 (13)	50.0 (3)	0.0 (0)	72.7 (40)
2 % (n)	22.9 (64)	31.8 (21)	22.4 (13)	21.4 (6)	12.5 (2)	50.0 (3)	100.0 (2)	21.8 (12)
3 % (n)	1.1 (3)	9.1 (6)	1.7 (1)	0.0 (0)	6.3 (1)	0.0 (0)	0.0 (0)	5.5 (3)
GCS <15 % (n)	8.9 (25)	28.8 (19)	6.9 (4)	10.7 (3)	12.5 (2)	0.0 (0)	100.0 (2)	16.4 (9)
Tachypnoea (RR≥22/min) % (n)	85.0 (238)	65.2 (43)	53.4 (31)	50.0 (14)	43.8 (7)	100.0 (6)	50.0 (1)	67.3 ()37
Systolic BP ≤100mmHg % (n)	31.1 (87)	56.1 (37)	65.5 (38)	60.7 (17)	68.8 (11)	50.0 (3)	50.0 (1)	49.1 (27)

The distribution of foci in the septic group was similar: 44.6% pulmonary (n = 90), 17.3% urogenital (n = 35), 11.9% abdominal (n = 24), 6.0% skin or wounds (n = 12), 3.0% cardiovascular system (n = 6), and 1.0% central nervous system (n = 2). In 90.6 % of the septic patients, systemic microbiological samples were obtained (n=183). A relevant pathogen was identified in over 50% of the cases (n=110). The spectrum of the pathogens included gram-negative bacteria (25.6%; n = 52), gram-positive bacteria (15.9%; n = 32), multiple pathogens (7.0%; n = 14), fungal infections (4.5%; n = 9), other infections (1.0%; n = 2), and parasites (0.5%; n = 1).

3.2 Essential new results

The results of this study show that the PCT mean value in septic patients (1.17 µg/L, IQR: 0.07-0.50 µg/L) was significantly higher as in non-septic patients (0.13µg/L, IQR:0.06-0.20µg/L; $p < 0.001$)). The closer to the time of sepsis, the highest was the PCT level. When patients were already septic at the time of ED admission, the PCT mean value was

1.47 (IQR: 0.39–6.39 µg/L) (see Supplement Figure 2 and 3, Bolanaki et al. 2021). When sepsis occurred within the first 24 hours of ED admission the PCT mean value was 0.49 µg/L (0.16–2.24 µg/L), while for patients who developed sepsis 24 hours after the admission the resulted PCT value was lower (n = 17; median PCT: 0.14 µg/L; IQR: 0.10–2.50 µg/L; p < 0.001). A logarithmic illustration of PCT values depending on sepsis onset is provided in Figure 3.

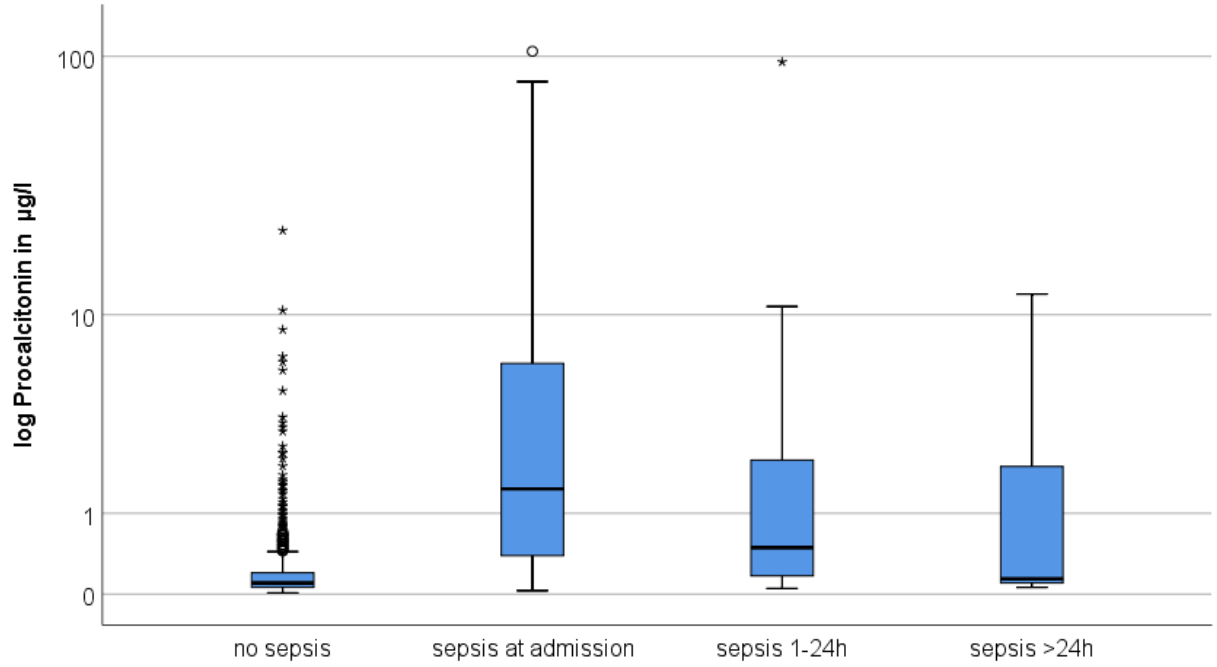

Figure 3: *Logarithmic illustration of PCT values stratified by time of sepsis, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

When dividing patients in subgroups based on the responsible pathogen detected in the microbiological tests, the median PCT value in gram-negative related infections (2.55 µg/mL, interquartile range (IQR) 1.064-6.8) was higher than that in gram-positive related infections (1.59 µg/mL, IQR 0.19-10,42), or fungal infections (1.36 µg/mL, IQR 0.31-4.98, p=0.242 for all groups). The logarithmic distribution of PCT levels depending on the responsible pathogen for sepsis is shown in Figure 4.

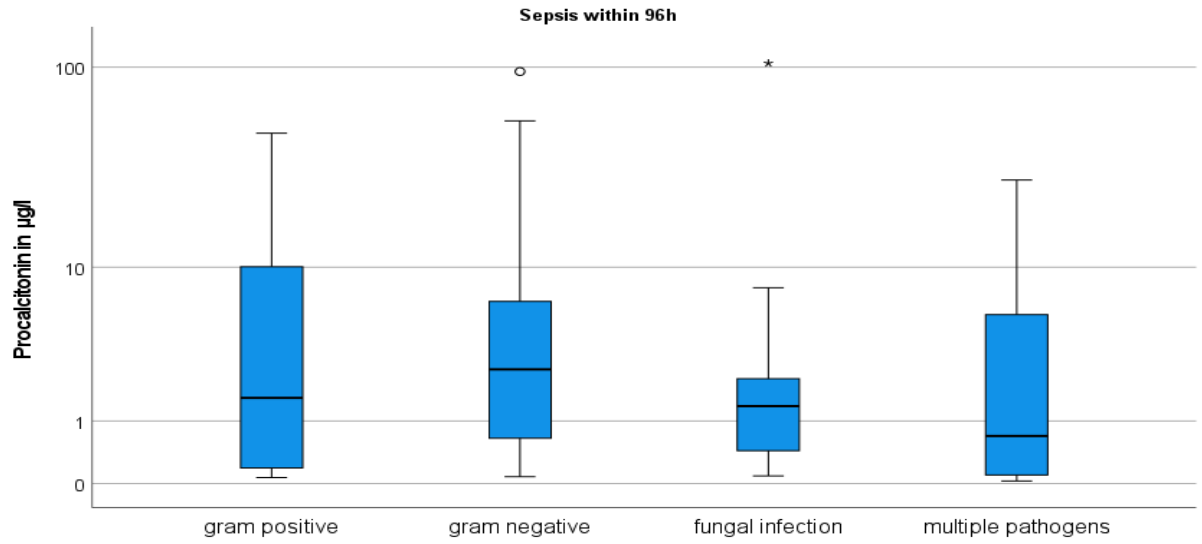

Figure 4: *PCT levels in patients with gram positive sepsis, gram negative sepsis, fungal sepsis and sepsis caused by multiple pathogens (own representation)*

The AUROC of PCT for the primary objective of sepsis development within 96 hours of ED admission was 0.86 (95% CI: 0.83–0.89; p < 0.0001), 0.82 for CRP (95% CI: 0.78–0.85; p < 0.0001) and 0.60 for lactate (95% CI: 0.55–0.64; p < 0.0001). The optimal cut-off value of PCT for sepsis diagnosis was 0.26 µg/l. Combining PCT with the qSOFA score had a negligible impact on the improvement of the AUROC (0.862, 95% CI: 0.831-0.892; p>0.001). However, the combination of qSOFA, PCT and CRP resulted in a slightly improved AUROC of 0.88 (95% CI: 0.85-0.91; p < 0.0001). (Figure 5)

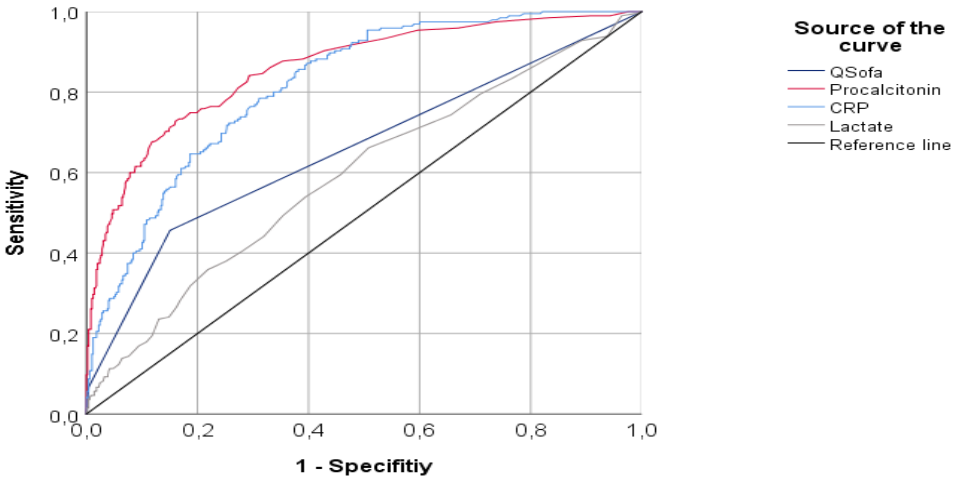

Figure 5: *ROC of qSOFA and laboratory parameters for the prediction of sepsis within 96 hours after admission., the optimized cut-off value according to ROC-analysis for PCT is 0.26 µG/L. reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022 Abbreviations CRP – C-reactive protein; PCT – procalcitonin*

3.2.1 Diagnostic performance of PCT

When applying already established PCT cut-off values the highest accuracy (82.2%) for sepsis diagnosis was achieved at a cut-off of 0.50 µg/l. The resulted sensitivity for this value was 63.4% (95% CI: 56.3–70.0), while the specificity was 89.2% (95% CI: 86.3–91.7), PPV was 68.8% (95% CI: 62.9–74.2), and NPV of 86.7% (95%CI: 84.4–88.7).

Table 6: *Diagnostic performance of PCT at different cut-off values (recommended by the manufacturer) for the diagnosis of sepsis within 96 hours after admission*, *reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

	PCT 0.20 µg/L	PCT 0.25 µg/L	PCT 0.50 µg/L	PCT 2.00 µg/L	PCT 5.00 µg/L	PCT 10.00 µg/L
True negative	400	429	481	523	533	537
False negative	44	50	74	122	151	169
False positive	139	110	58	16	6	2
True positive	158	152	128	80	51	33
Sensitivity	78.2	75.3	63.4	39.6	25.3	16.3
(95% CI)	(71.9-83.7)	(68.7-81.4)	(56.3-70.0)	(32.8- 46.7)	(19.4-31.8)	(11.5- 22.2)
Specificity	74.2	79.6	89.2	97.0	98.9	99.6
(95% CI)	(70.3-77.9)	(75.9-82.9)	(86.3-91.7)	(95.2-98.3)	(97.6-99.6)	(98.7- 100.0)
Positive Likelihood Ratio	3.0	3.7	5.9	13.3	22.7	44.0
(95% CI)	(2.6-3.6)	(3.1-4.4)	(4.5-7.7)	(8.0-22.3)	(9.9-52.0)	(10.7-181.8)
Negative Likelihood Ratio	0.3	0.3	0.4	0.6	0.8	0.8
(95% CI)	(0.2-0.4)	(0.2-0.4)	(0.3-0.5)	(0.6-0.7)	(0.7-0.8)	(0.8-0.9)
Positive Predictive Value	53.2	58.0	68.8	83.3	89.5	94.3
(95% CI)	(49.2-57.2)	(53.5-62.4)	(62.9-74.2)	(75.0-89.3)	(78.8-95.1)	(80.0- 98.6)
Negative Predictive Value	90.1	89.6	86.7	81.1	77.9	76.1
(95% CI)	(87.5-92.2)	(87.1-91.6)	(84.4-88.7)	(79.3-82.8)	(76.5-79.3)	(74.9- 77.2)
Accuracy	75.3	78.4	82.2	81.4	78.8	76.9
(95% CI)	(72.3-78.4)	(75.3-81.3)	(79.2-84.9)	(78.4-84.1)	(75.6-81.7)	(76.9-79.9)

The same cut-offs were tested for different subgroups based on qSOFA score: (1) qSOFA score of 1 and (2) qSOFA score of ≥2. The best accuracy of 84.9% was then observed in the qSOFA 1 group at a cut-off of 0.50µg/l, while the negative predictive value (NPV) was

90.8% and specificity 90.4%. In the qSOFA 2 group the best accuracy of 74.1% was observed at a cut-off of 0.25µg/L. The sensitivity at this value in this group was the highest that we observed with 78.9%.

Table 7: *Diagnostic performance of qSOFA at a cut-off value of 2 and PCT at different cut-off values within qSOFA categories for the diagnosis of sepsis within 96 hours after admission, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

	qSOFA Cut-Off Score ≥ 2	qSOFA Score = 1		qSOFA Score ≥ 2	
		PCT Cut-Off 0.25µg/L	PCT Cut-Off 0.50µg/L	PCT Cut-Off 0.25µg/L	PCT Cut-Off 0.50µg/L
True negative	459	374	415	55	66
False negative	112	31	42	19	32
False positive	80	85	44	25	14
True positive	90	81	70	71	58
Sensitivity	44.6	72.3	62.5	78.9	64.4
(95%-CI)	(37.6-51.7)	(63.1-80.4)	(52.8-71.5)	(69.0-86.8)	(53.7-74.3)
Specificity	85.2	81.5	90.4	68.8	82.5
(95%-CI)	(81.9-88.0)	(77.6-84.9)	(87.4-93.0)	(57.4-78.7)	(72.4-90.1)
Positive Likelihood Ratio	3	3.9	6.5	2.5	3.7
(95%-CI)	(2.3-3.9)	(3.1-4.9)	(4.8-8.9)	(1.8-3.6)	(2.2-6.1)
Negative Likelihood Ratio	0.7	0.3	0.4	0.3	0.4
(95%-CI)	(0.6-0.7)	(0.3-0.5)	(0.3-0.5)	(0.2-0.5)	(0.3-0.6)
Positive Predictive Value	52.9	48.8	61.4	74	80.6
(95%-CI)	(46.6-59.2)	(43.3-54.4)	(53.7-68.6)	(66.9-80.0)	(71.5-87.2)
Negative Predictive Value	80.4	92.4	90.8	74.3	67.4
(95%-CI)	(78.3-82.3)	(89.9-94.2)	(88.6-92.6)	(65.4-81.6)	(60.6-73.5)
Accuracy	74.1	79.7	84.9	74.1	72.9
(95%-CI)	(70.8-77.2)	(76.2-82.9)	(81.7-87.7)	(66.9-80.5)	65.6-79.5

Univariate and multivariate logistic regression determined that all investigated cut-off values of PCT were independent significant factors for sepsis diagnosis. For the purpose

of this analyses we created three adjusted models: for qSOFA (MODEL 1), for qSOFA, gender and age (MODEL 2) and for qSOFA, gender, age, CRP, lactate, WBC, pre-existing conditions, tumor diseases and immunosuppression.

Table 8: *Results of the logistic regression analysis of PCT at different cut-off values for the prediction of sepsis in univariate analyses (crude ORs) and adjusted for qSOFA (model 1), adjusted for qSOFA, gender and age (model 2) and adjusted for qSOFA, gender, age, C-reactive protein, lactate, WBC, pre-existing conditions and tumor diseases, immunosuppression (model 3), reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

PCT Cut-Off		OR (crude)	p-value	OR adjusted model 1	p-value	OR adjusted model 2	p-value	OR adjusted model 3	p-value
0.20µg/L	Value	10.3	<0.0001	8.7	<0.0001	8.6	<0.0001	4.3	<0.0001
	(95% CI)	(7.0-15.2)		(5.8-12.9)		(5.8-12.8)		(2.6-7.1)	
0.25µg/L	Value	11.9	<0.0001	10.1	<0.0001	10.0	<0.0001	5.4	<0.0001
	(95% CI)	(8.1-17.4)		(6.8-15.0)		(6.8-14.8)		(3.3-8.8)	
0.50µg/L	Value	14.4	<0.0001	13.1	<0.0001	13.3	<0.0001	7.7	<0.0001
	(95% CI)	(9.7-21.3)		(8.7-19.8)		(8.8-20.1)		(4.6-13.0)	
2.00µg/L	Value	21.4	<0.0001	19.4	<0.0001	21.5	<0.0001	12.7	<0.0001
	(95% CI)	(12.1-38.0)		(10.7-34.9)		(11.7-39.4)		(6.2-26.3)	
5.00µg/L	Value	30.0	<0.0001	28.5	<0.0001	29.8	<0.0001	26.4	<0.0001
	(95% CI)	(12.6-71.3)		(11.8-69.0)		(12.3-72.3)		(8.5-81.7)	
10.00µg/L	Value	52.5	<0.0001	44.7	<0.0001	47.0	<0.0001	25.0	<0.0001
	(95% CI)	(12.5-220.8)		(10.4-191.8)		(10.9-202.8)		(5.5-114.1)	

one patient was lost to follow-up

The classification and regression tree analysis showed that PCT was the best marker to identify sepsis within 96 hours after admission in the first split. Two cut-off values were identified to build three subgroups based on PCT: 0.13 µg/L, a not previously reported cut-off value, and 0.50 µg/L, which is a pre-known PCT risk cut-off. The best split value for q-SOFA in all PCT subgroups in the second step was between 1 point and 2 points which then resulted in two further risk groups (risk group 1: qSOFA 1 point, risk group 2: qSOFA 2-3 points) derived from each PCT-subgroup (6 groups in total, see Figure 6).

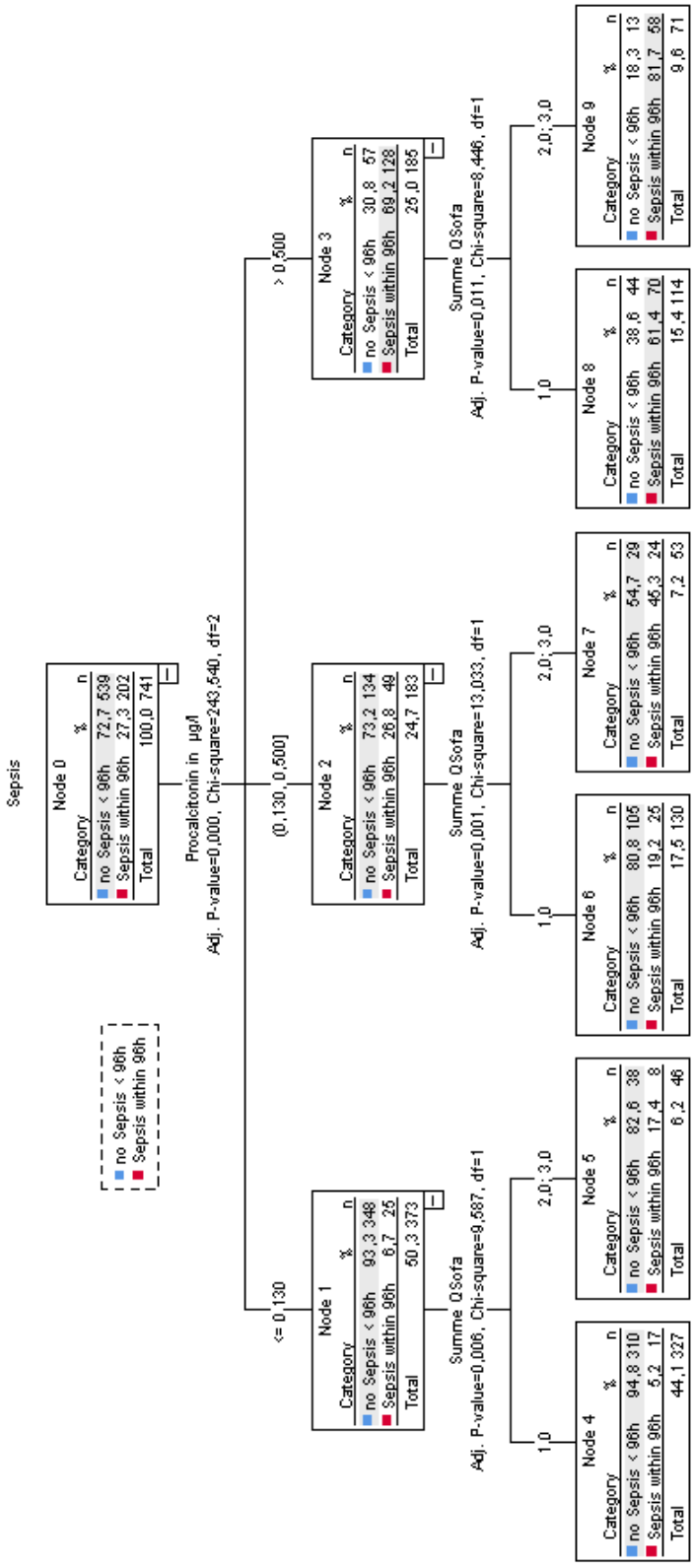

Figure 6: *Classification tree containing the independent variables PCT (numeric) and qSOFA (3 categories) as independent variables and the diagnosis of sepsis within 96 hours after admission as dependent variable, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

Since qSOFA would be the first information available in the ED, another model was conducted and qSOFA was determined to be this time the first variable in the classification tree. The same PCT cut-off values were identified in this model to further split qSOFA subgroups in the first step into further risk groups based on PCT (see Figure 7a). The validation of the findings was conducted by the split half method. When the original data set was split into a training and a validation data set, the results of the initial model could be confirmed, with a minor deviation regarding the identified cut off values. (see Figure 7b)

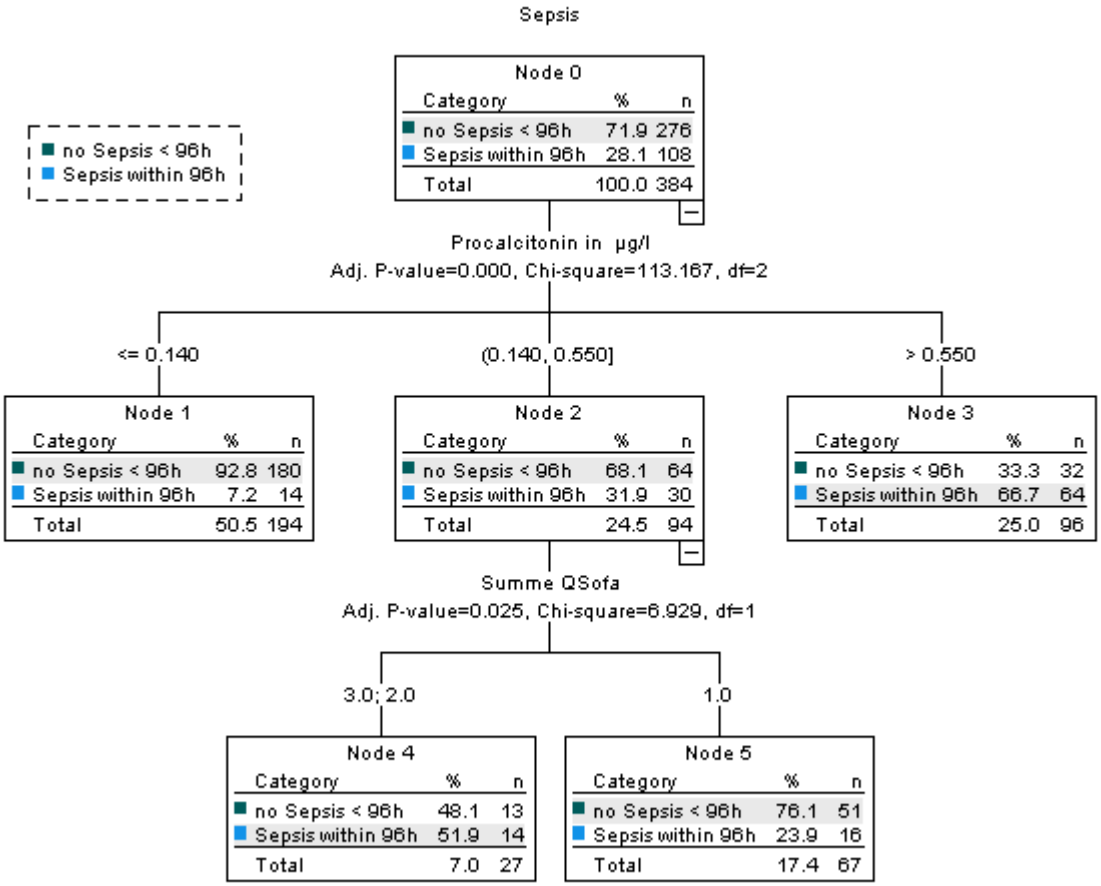

Figure 7a: Results of the classification tree analysis in the training sample, *reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

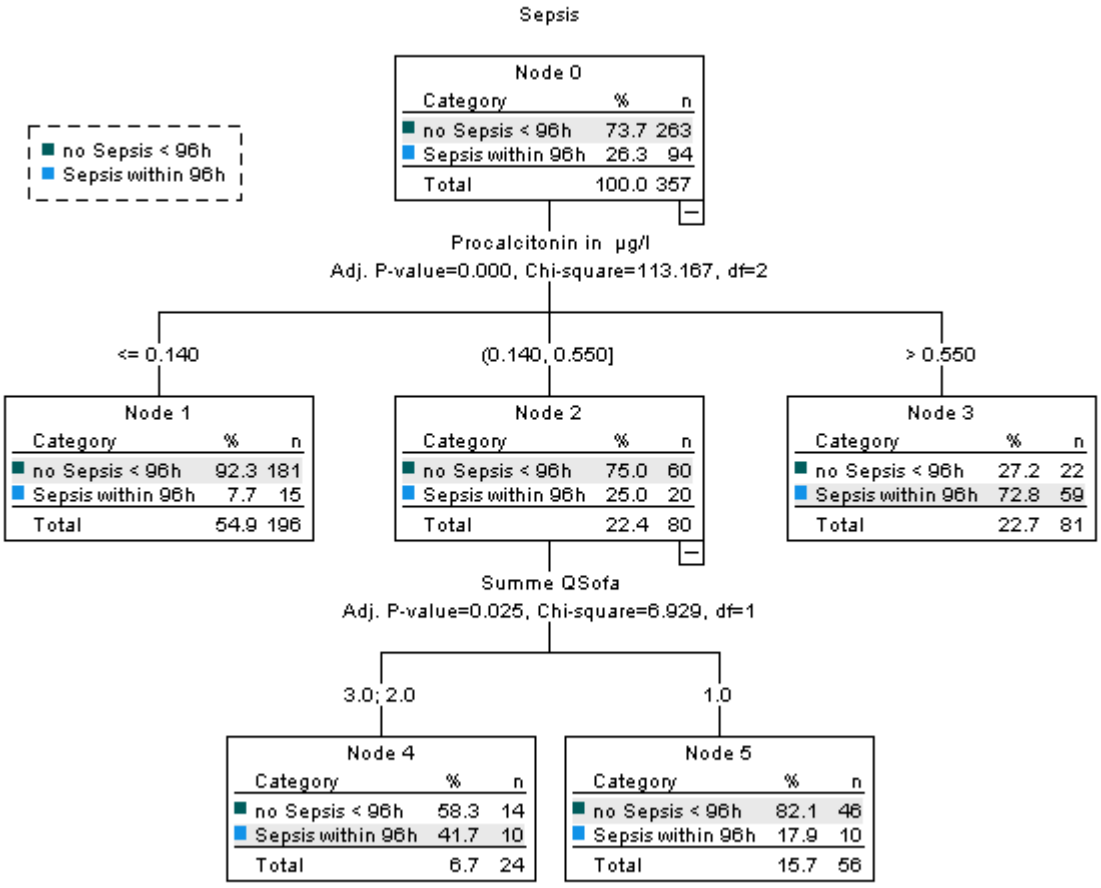

***Figure 7b**: Results of the classification tree analysis in the test sample, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

NRI analyses were conducted based on the qSOFA risk categories at 2 points. The NRI of PCT was then calculated at the established risk cut off (0.50 µg/L) and at the cut-off values identified in the classification tree analysis. The NRI was 22.9% at a cut-off value of 0.50 µg/L and increased by 39.9% at the optimised cut-off values of 0.13 µg/L and 0.50 µg/L. The results of the NRI analyses are provided in two separate tables below (Tables 8, 9)

Table 9: *Specification of change of risk categories of qSOFA by PCT applying two established risk groups and resulting Net Reclassification Improvement, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

Model without PCT	**Model with PCT at a cut-off value of 0.50 µg/L**		
	risk category 1	risk category 2	
	PCT < 0.50 µg/L	PCT ≥ 0.50 µg/L	
Patients with sepsis			**SUM**
risk category 1 (qSOFA = 1)	42	70	112
risk category 2 (qSOFA ≥ 2)	32	58	90
SUM	74	128	202
Patients without sepsis			
risk category 1 (qSOFA = 1)	415	44	459
risk category 2 (qSOFA ≥ 2)	66	14	80
SUM	481	58	539
Net reclassification improvement			**22.9%**

Table 10: *Specification of change of risk categories of qSOFA by PCT applying three risk groups and resulting Net Reclassification Improvement, reprinted from Bolanaki et al., 2021[(1)], available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

Model without PCT	**Model with PCT at a cut-off value of 0.13 and 0.50 µg/L**			
	risk category 1	risk category 2	risk category 3	
	PCT < 0.13 µg/L	PCT 0.13-0.50 µg/L	PCT ≥ 0.50 µg/L	
Patients with sepsis				**SUM**
risk category 1 (qSOFA=1)	16	26	70	112
risk category 2 (qSOFA=2)	7	21	49	77
risk category 3 (qSOFA=3)	1	3	9	13
SUM	24	50	128	202
Patients without sepsis				
risk category 1 (qSOFA=1)	294	121	44	459
risk category 2 (qSOFA=2)	35	29	14	78
risk category 3 (qSOFA=3)	1	1	0	2
SUM	330	151	58	539
Net reclassification improvement				**39.9%**

3.2.2 Prognostic performance of PCT

Secondary endpoint was mortality within 28 days. PCT levels differed significantly between survivors (94.1%, n=698) and non-survivors (6.6%, n=48) with reported mean values of 0.13 µg/l and 0.31 µg/l respectively. Kaplan-Meier curve analysis was performed to compare the cumulative survival rates between the high PCT (≥0.25µg/l) and low PCT (<0.25) groups. The optimal cut-off of PCT to predict 28-day sepsis mortality was 0.25µg/l. Cox regression analyses revealed a crude hazard ratio (HR) of 2.48 (95%-CI: 1.40-4.38, p=0.002) for PCT at this value. When adjusted for qSOFA (HR: 1.31; 95%-CI: 0.79-2.17; p=0.293), age (HR: 1.04; 95%-CI: 1.02-1.07; p=0.001), and gender (HR: 1.66; 95%-CI: 0.89-3.09; p=0.114), the HR showed a slightly decrease to 2.13 but was still found to be significant (95% CI: 1.18–3.86; p = 0.013). After adjustment for CRP (HR: 1.00; 95%-CI: 1.00-1.00; p=0.276) and lactate (HR: 1.03; 95%-CI: 1.01-1.04; p=0.003), the HR for PCT was 2.05 (95% CI: 1.03–4.06; p = 0.041).

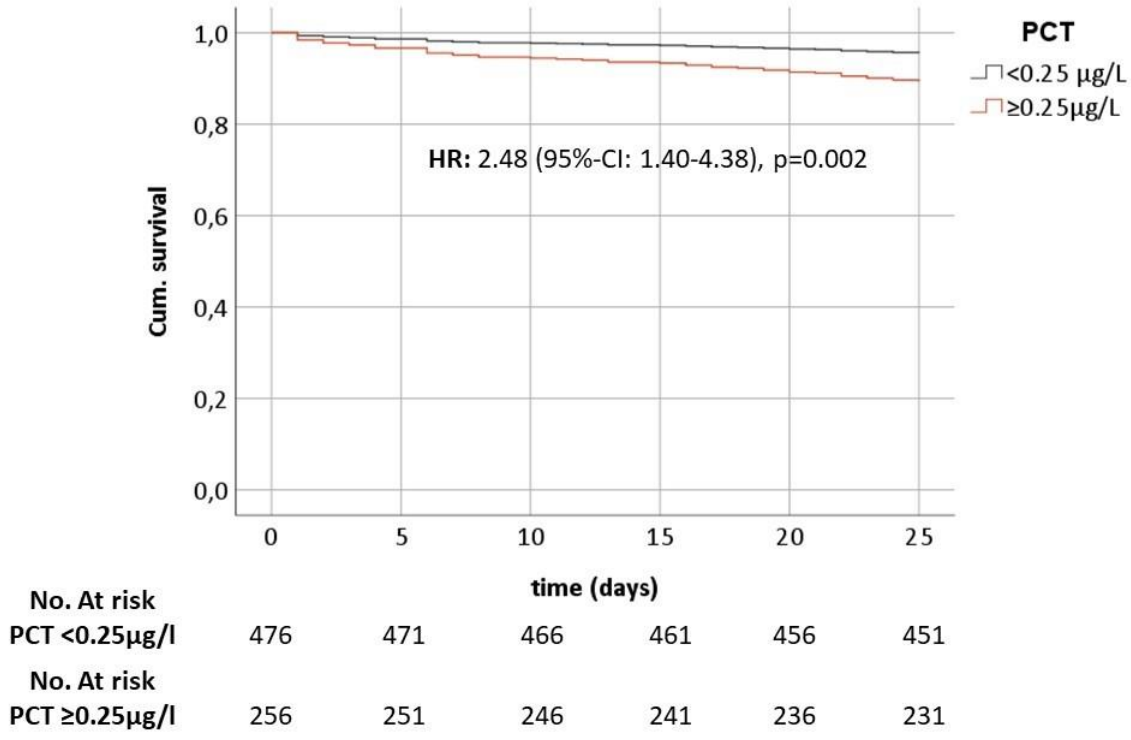

Figure 8: *Cumulative survival within 28 days after admission to the ED for patients with procalcitonin (PCT) below or at or above 0.25µg/L. Abbreviations: HR – Hazard Ratio, reprinted from Bolanaki et al., 2021(1), available at https://doi.org/10.3390/jcm10173869, access date 16.11.2022*

4 Discussion

4.1 Short summary of results

In our prospective cohort study, we found that an early single PCT measurement in patients presenting with elevated qSOFA score (≥1) at the ED performs excellent as indicator for sepsis with a resulted AUROC of 0.86. When aiming the highest diagnostic accuracy, meaning best sensitivity and specificity trade off, the optimum cut-off value of PCT was 0.5µg/l both for the whole study population as well as for the qSOFA subgroups.

The PCT cut off value of 0.5µg/l was confirmed in the decision tree analysis and additionally the cut-off value of 0.13 µg/L was proved to serve ideally for the further stratification within the lower risk group. NRI analysis underlined the diagnostic value of PCT for sepsis identification and showed an improved classification by 23.9% when a cut-off value of 0.50 µ/L was applied. When both cut-off values that derived from our tree analysis were used, NRI improved even further reaching 39.9%. The improvement of net reclassification was mostly due to the upgrading of patients with sepsis to higher risk categories, which further illustrates the great clinical benefit PCT could have for early sepsis diagnosis in the ED. We also found that PCT was an independent predictor for 28-day mortality in patients with sepsis.

4.2 Interpretation and discussion of results

4.2.1 Study population

In our study we used the qSOFA score as entry criterion, unlike all studies up to date. Our decision to prospectively recruit patients with an elevated (≥1 point) and not an already positive qSOFA score (≥2 points) was multifactorial and should be thus further elaborated: Our intention was to enroll patients who were likely to develop sepsis and not only already septic patients. This crucial study population that we aimed for, has also been formerly described as pre-septic population or as “intermediate risk” population according to the original publication of Seymour et al (22). Since the screening value of the positive qSOFA score was accused of detecting patients that had already deteriorated (11, 37), it was therefore considered unsuitable for early sepsis recognition. Furthermore, at the same time studies proved that the sensitivity of the qSOFA score in sepsis screening was higher when a cut-off value of 1 was applied, and this cut-off value even reached the high sensitivity of SIRS >2 (38). We identified 202 septic patients using the elevated qSOFA score (≥1 point) as inclusion criterion. This corresponded to 27% of the

whole study population. The majority of the septic patients derived from the qSOFA 1 group (55.4%) confirming our decision. Interestingly, sepsis prevalence in prospective studies conducted in EDs using signs of suspected infection as inclusion criterion was much lower (39, 40). This could be an effect of differences in the underlying study populations since prevalence is likely to differ between different settings but could also be interpreted as an indirect hint that the qSOFA score of ≥1 is an earlier and more sensitive screening tool for sepsis identification compared to SIRS criteria.

Sepsis is a disease that occurs most commonly in older age groups. More specifically, over 60% of the reported sepsis cases are documented in patients over 65 years of age (41). Subsequently studies on the epidemiological characteristics of sepsis usually report a significant difference of the age between patients with and without sepsis (42). However, in our study the median age of the subgroup of patients with sepsis was not significantly higher compared to the subgroup of patients without sepsis. There are two possible explanations for this deviation. First of all, a population of patients with an elevated qSOFA score is older than a population selected based on suspicion of infection, since an organ dysfunction (which is reflected by the qSOFA criteria) most likely occurs in the elderly. This led to a homogenization of older patients in both septic and non-septic groups which resulted in a similarly high median age in both groups. This hypothesis is further underlined by the fact that the most common diagnoses in the non-septic group were heart and lung diseases, diagnoses more prevalent among the elderly. Secondly, due to ethical restrictions only patients who were able to provide informed consent were eligible for study participation. Thus, selection bias regarding very old patients might have occurred since these patients are more likely to present cognitive impairment (e.g. dementia) and legal representation was very often not available. Thus, a crucial part of elderly patients who might have also been at risk to develop sepsis were excluded leading to a lower median age in the septic subgroup. This is a general problem which occurs in clinical research in the ED setting and could not be solved in our current study. This vulnerable patient population, however, needs to be further addressed in routine data analyses or in settings where a waiver of informed consent is granted by the responsible institutional review board to ensure high quality of sepsis detection also in this sub-population.

Estimated hospital mortality rates due to sepsis in Germany reach 26.7 %, while in our study only 13.4% of the septic patients died in 28 days. We identified two possible

reasons for this deviation: Firstly, we excluded patients with already poor prognosis (<28 days) due to cancer or other chronic terminal diseases. Secondly, screening for patients with elevated qSOFA score in the ED within the scope of this trial raised sepsis awareness in our EDs leading to earlier identification and thus treatment of potential septic patients. More specifically, when the study team identified a patient with elevated qSOFA score the treating physician was informed and asked regarding potential exclusion criteria in order to include the patient in the study. Subsequently the physician was alarmed for potential sepsis.

4.2.2 Diagnostic performance of qSOFA and PCT in the ED

The results of our prospective study regarding the diagnostic ability of the positive qSOFA score confirm those of previous investigations conducted strictly in EDs (40, 43, 44). The positive qSOFA score showed a poor sensitivity of 44.6% and a specificity of 85.2% for early sepsis diagnosis. Since an ideal screening tool pre-supposes a high sensitivity, the score proved to be unsuitable as a stand-alone screening instrument. However, it is important to highlight again that our analysis was performed in an already elevated qSOFA population. In a systematic review and meta-analysis of Serafim et al. (23) who compared the screening performance of the positive qSOFA score and SIRS for sepsis diagnosis the old criteria performed better. As mentioned above the low sensitivity of the qSOFA score was attributed to the lack of a parameter that is characteristic for an infection. Thus, the combination with an infectious biomarker such as procalcitonin seemed promising.

The benefits of early PCT measurement in patients with suspected infection were reported soon after Assicot et al. first observed high serum levels of the biomarker in patients with bacterial infection and sepsis (45). Numerous studies showed that PCT served excellent as early predictor of bacteremia (46-48) and sepsis in various clinical settings. Several cut-offs were examined in order to achieve the highest accuracy. Regarding sepsis prediction, PCT showed the best predictive value at cut –offs between 0.1mg/l and 0.5mg/l, depending on the time of sepsis onset and blood drawn. As already established, PCT serum levels start to increase 6 to 12 hours following initial bacterial infections and continue to increase 2 to 4 hours following the onset of sepsis. In our study we included not only pre-septic but also already septic patients with high PCT values leading to an ideal cut-off value of 0.5mg/l.

The diagnostic performance of PCT for sepsis in our elevated qSOFA population was similarly high as reported in critical ill patients according to the systematic review of Walker et al (30). A more recent systematic review of Tan et al (49) also reported a similar AUC of 0.85 for sepsis diagnosis in adults. When compared with other biomarkers of inflammation, PCTs diagnostic capability was proven superior due to its higher correlation to infections of bacterial origin and the severity of the infection. Accordingly, in our study PCTs performance was superior to that of CRP. The majority of studies that examined the clinical value of PCT in sepsis diagnosis and prognosis selected their study populations based on the SIRS criteria, positive blood cultures or clinical signs of infection.

After the introduction of the qSOFA score several studies evaluated the diagnostic and prognostic accuracy of PCT in combination with the score (33-36, 50-51). All studies yielded positive results, despite the heterogeneity of the study populations and the study design. None of these studies used the qSOFA criteria to select their study population as already mentioned above, but rather calculated the qSOFA score retrospectively in patients diagnosed with sepsis or patients selected based on the SIRS criteria, positive blood cultures or clinical signs of infection. The results of the prospective study of Spoto et al (35), revealed a posttest probability of 0.99 for sepsis diagnosis when using the combination of PCT 0.5µg/l with the positive qSOFA score, suggesting a diagnostic algorithm similar to ours. However, the study was conducted in a normal ward of a hospital and included patients who were already admitted with sepsis.

4.3 Strengths and limitations

The new sepsis definition focuses on the dysregulated immune response that leads to organ dysfunction rather than the cause of infection. Screening for patients with a screening tool oriented on presumed organ dysfunction and then stratifying them with the help of a biomarker of infection consists the novelty of this study. Previous studies included patients who were suspected of having an infection and retrospectively calculated the existing screening scores. Patients with missing values were therefore excluded from these studies.

To the best of our knowledge, the LIFE-POC study is the first study that investigated the diagnostic utility of PCT for early sepsis recognition in patients prospectively recruited based on their qSOFA score at ED presentation. The septic population was large enough

to provide safe conclusions, whereas the non- septic group served as the control group. We conducted the study in accordance with the recommendations of the Sepsis-3 task force, not only regarding the sepsis -3 definition but also using the qSOFA criteria as entry criteria to our study population. We examined both, diagnostic and prognostic parameters.

Due to ethical reasons, patients with altered mentation (GCS<15) and no legal representative were excluded from this study. Although in the screening process the proportion of patients that presented with an altered mentation was similarly high to the other two qSOFA points, the GCS was the smallest attributor to the qSOFA groups. This consists the most important limitation of our study.

4.4 Implications for practice or future research

Shortly after the publication of our research article based on the data of the LIFE-POC study, the new sepsis guidelines were published. Regarding sepsis screening, the qSOFA score is no longer recommended as a stand-alone screening instrument (strong recommendation based on moderate evidence). Although the positive qSOFA score should alert the physician to sepsis, the assessment of the score alone showed a low sensitivity (40, 43, 44) in identifying septic patients as mentioned above, thus confirming the results of our study and further pointing out the necessity to combine qSOFA with further diagnostic parameters.

Until today there is still no clear recommendation by the SSC which sepsis screening tool should be used in the ED since all screening tools proved to have limitations as single instruments. In view of this deficiency in the sepsis guidelines, the need of biomarkers to support early sepsis diagnosis rises. However, finding the right combination of objective criteria and point of care biomarkers can be challenging.

The qSOFA score is easily assessed and focusses on presumed organ dysfunction thus remaining more oriented to the new sepsis definition. In our study we confirmed that the qSOFA score is fast obtainable- within minutes after ED presentation- and that a single early PCT measurement in ED patients presenting with a qSOFA score of at least 1 could facilitate the timely identification of septic patients.

5 Conclusions

Sepsis early recognition is a top priority and at the same time a big challenge for EDs around the world. Our present data show that PCT, measured in an ED population with elevated qSOFA score, improved early sepsis identification. Thus, the combination of qSOFA with an early PCT measurement could be recommended for sepsis screening in the ED.

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Statutory Declaration

I, Myrto Bolanaki, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "Diagnostic utility of Procalcitonin (PCT) for the early detection of sepsis in patients presenting to the emergency department with a qSOFA score of at least one/ Diagnostischer Nutzen von Procalcitonin (PCT) zur Frühdiagnostik der Sepsis in Notaufnahmepatient*innen mit mindestens einem qSOFA Kriterium", independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.

Date Signature

Declaration of my contribution to the publication

My contribution for the conduction of the LIFE-POC study and the successful publication of the article “Diagnostic Performance of Procalcitonin for the Early Identification of Sepsis in Patients with Elevated qSOFA Score at Emergency Admission” can be summarised as followed.

As I was assigned the role of primary investigator of the LIFE-POC study in the Charité, the study design and protocol were developed by the Board of the clinical directors. The institutional review board of Jena had already approved the study and the Federal Ministry of Education and Research of Germany (Bildungsministerium für Bildung und Forschung – BMBF) provided the funds. I contributed in evaluating the paper CRF, suggesting changes or excluding unnecessary data, organising the study team and training the ED personnel.

After consulting my doctor father to be, Professor Möckel, and my second supervisor, Professor Slagman, we generated the main hypothesis of my dissertation. I recruited 777 patients working on a daily 8 –hour basis and during my shifts in the emergency department. Two to three students contributed in the recruitment. The study nurses were mostly assigned the paper documentation which was conducted under my supervision. Blood samples collection, centrifugation and transport were performed strictly by our study team. At the same time, I was in touch with the study site at Jena University Hospital to ensure standardized study conduction and data assessment. Furthermore, I was in charge of reporting the current status of the study to the other study site and the BMBF. After completing the enrolment phase of the project, we proceeded with the intensive documentation of medical data. Due to prolonged ICU stays and complex course of patients I reviewed every complex case. As the study team of the University hospital in Jena completed the recruitment a panel of specialists proved and confirmed the sepsis diagnosis. I prepared and supported the sepsis adjudication at all study sites.

After the exportation of the data in SPSS by Prof. Slagman, I conducted a plausibility check for all data. Statistical analysis and interpretation of the data were performed under the guidance and statistical advice from Prof. Slagman. I created all figures and tables of the publication except from figures 6,7a+7b and tables 9+10. Tree analysis and NRI analysis were performed by Professor Slagman, who also contributed in writing the first

draft of the manuscript with me. After taking into consideration all comments of the co - authors I composed the final draft. I was corresponding author and thus responsible for manuscript submission and communication with the editor and reviewers. Following the reviewing process, I replied to all the reviewer's comments under the valuable guidance of my supervisors as well as adjusted the manuscript accordingly. The manuscript was accepted after one single extensive revision. All extra analyses performed for this dissertation were conducted by me.

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Signature, date and stamp of first supervising university professor

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Signature of doctoral candidate

Excerpt from Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2020** Selected Editions: SCIE,SSCI
Selected Categories: **"MEDICINE, GENERAL and INTERNAL"**
Selected Category Scheme: WoS
Gesamtanzahl: 168 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NEW ENGLAND JOURNAL OF MEDICINE	464,351	91.245	0.631180
2	LANCET	369,601	79.321	0.445240
3	JAMA-JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION	224,147	56.272	0.279750
4	Nature Reviews Disease Primers	14,221	52.329	0.043550
5	BMJ-British Medical Journal	158,747	39.890	0.150630
6	ANNALS OF INTERNAL MEDICINE	72,588	25.391	0.082030
7	Lancet Digital Health	1,260	24.519	0.003000
8	JAMA Internal Medicine	25,002	21.873	0.077060
9	Journal of Cachexia Sarcopenia and Muscle	5,908	12.910	0.009440
10	PLOS MEDICINE	42,445	11.069	0.061920
11	Cochrane Database of Systematic Reviews	81,212	9.266	0.121830
12	JOURNAL OF INTERNAL MEDICINE	13,801	8.989	0.011960
13	BMC Medicine	20,511	8.775	0.040120
14	JOURNAL OF TRAVEL MEDICINE	5,260	8.490	0.004900
15	JAMA Network Open	12,653	8.483	0.039940
16	CANADIAN MEDICAL ASSOCIATION JOURNAL	19,683	8.262	0.014960

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
17	MEDICAL JOURNAL OF AUSTRALIA	14,127	7.738	0.011150
18	MAYO CLINIC PROCEEDINGS	20,504	7.616	0.025820
19	AMYLOID-JOURNAL OF PROTEIN FOLDING DISORDERS	2,202	7.141	0.003280
20	Translational Research	5,766	7.012	0.007980
21	Deutsches Arzteblatt International	6,542	5.594	0.007550
22	MEDICAL CLINICS OF NORTH AMERICA	4,487	5.456	0.005110
23	BRITISH JOURNAL OF GENERAL PRACTICE	8,303	5.386	0.009300
24	JOURNAL OF THE ROYAL SOCIETY OF MEDICINE	4,981	5.344	0.002310
25	PANMINERVA MEDICA	1,003	5.197	0.000930
26	ANNALS OF FAMILY MEDICINE	6,770	5.166	0.009280
27	JOURNAL OF GENERAL INTERNAL MEDICINE	26,727	5.128	0.028950
28	Frontiers in Medicine	6,143	5.091	0.013050
29	AMERICAN JOURNAL OF PREVENTIVE MEDICINE	28,400	5.043	0.037310
30	AMERICAN JOURNAL OF MEDICINE	29,186	4.965	0.021220
31	Journal of Personalized Medicine	1,071	4.945	0.002290
32	MINERVA MEDICA	1,338	4.806	0.001280
33	PALLIATIVE MEDICINE	7,332	4.762	0.009100
34	ANNALS OF MEDICINE	5,619	4.709	0.004060

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
35	EUROPEAN JOURNAL OF CLINICAL INVESTIGATION	8,197	4.686	0.005980
36	AMERICAN JOURNAL OF CHINESE MEDICINE	4,572	4.667	0.003020
37	European Journal of Internal Medicine	7,083	4.487	0.009330
38	BRITISH MEDICAL BULLETIN	5,494	4.291	0.003290
39	Journal of Clinical Medicine	21,502	4.241	0.031360
40	PREVENTIVE MEDICINE	20,705	4.018	0.028980
41	POSTGRADUATE MEDICINE	3,169	3.840	0.003850
42	DM DISEASE-A-MONTH	951	3.800	0.000600
43	PAIN MEDICINE	10,086	3.750	0.012300
44	International Journal of Medical Sciences	5,677	3.738	0.005680
45	Diagnostics	2,557	3.706	0.003180
46	JOURNAL OF URBAN HEALTH-BULLETIN OF THE NEW YORK ACADEMY OF MEDICINE	5,637	3.671	0.005810
47	JOURNAL OF PAIN AND SYMPTOM MANAGEMENT	15,063	3.612	0.015920
48	Journal of Translational Internal Medicine	457	3.451	0.000990
49	Internal and Emergency Medicine	3,446	3.397	0.004890
50	Military Medical Research	2,279	3.329	0.001380
51	Archives of Medical Science	4,209	3.318	0.005000

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Diagnostic Performance of Procalcitonin for the Early Identification of Sepsis in Patients with Elevated qSOFA Score at Emergency Admission

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Abstract: Infectious biomarkers such as procalcitonin (PCT) can help overcome the lack of sensitivity of the quick Sequential Organ Failure Assessment (qSOFA) score for early identification of sepsis in emergency departments (EDs) and thus might be beneficial as point-of-care biomarkers in EDs. Our primary aim was to investigate the diagnostic performance of PCT for the early identification of septic patients and patients likely to develop sepsis within 96 h of admission to an ED among a prospectively selected patient population with elevated qSOFA score. In a large multi-centre prospective cohort study, we included all adult patients (n = 742) with a qSOFA score of at least 1 who presented to the ED. PCT levels were measured upon admission. Of the study population 27.3% (n = 202) were diagnosed with sepsis within the first 96 h. The area under the curve for PCT for the identification of septic patients in EDs was 0.86 (95% confidence interval (CI): 0.83–0.89). The resultant sensitivity for PCT at a cut-off of 0.5 µg/L was 63.4% (95% CI: 56.3–70.0). Furthermore, specificity was 89.2% (95% CI: 86.3–91.7), the positive predictive value was 68.8% (95% CI: 62.9–74.2), and the negative predictive value was 86.7% (95% CI: 84.4–88.7). The early measurement of PCT in a patient population with elevated qSOFA score served as an effective tool for the early identification of sepsis in ED patients.

Keywords: qSOFA; SOFA; sepsis; procalcitonin

1. Introduction

1.1. Background

In 2016 the Third International Consensus Definitions for Sepsis and Septic Shock redefined sepsis as a life-threatening organ dysfunction resulting from a dysregulated host response to an infection [1]. Organ dysfunction was defined as an acute increase of at least 2 points in the patient's Sequential Organ Failure Assessment (SOFA) score, a well-known measure first introduced in 1994 [2] as a tracking tool for organ failure in intensive care units (ICUs). The worldwide incidence of sepsis is estimated to be approximately 48.9 million cases per year [3,4], with persistently high morbidity and mortality rates [5]. This high incidence of sepsis coupled with poor clinical outcome places a heavy burden on health care systems and consumes a high proportion of already scarce hospital resources (e.g., personnel, hospital beds, intensive care capacity). According to a recent study by Buchman

et al. conducted in the USA, the human and economic costs of sepsis care continue to grow [6–8].

To improve sepsis outcomes and avoid excessive resource consumption due to extended but preventable in-hospital treatment and intensive care stays, early identification of septic patients and patients likely to develop sepsis is a top clinical priority to enable early strategy implementation. According to the latest update to the surviving sepsis campaign guidelines, early management should involve early fluid resuscitation, lactate measurement, obtaining of blood cultures, early administration of broad-spectrum antibiotics, and, where necessary, the application of vasopressors within one hour of diagnosis [9].

In order to achieve early sepsis recognition, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) searched for the most suitable criteria for the development of an effective sepsis-screening tool. Consequently, the quick SOFA (qSOFA) score was introduced. This score consists of three easy-to-assess parameters: SBP $\leq$ 100 mmHg, respiratory rate $\geq$ 22 breaths per minute, and altered cognitive state. To define altered cognitive state the use of the Glasgow Coma Scale (GCS) was recommended. This may have limited the detection of patients at risk, since the GCS was intended to describe the extent of impaired consciousness and not altered mentation [10]. Seymour et al. [11] proved the qSOFA score's prognostic superiority to the previously used systemic inflammatory response syndrome (SIRS) criteria for non-ICU patients with suspected infection and underlined its ideal use outside ICUs and in emergency departments. According to the 2018 Surviving Sepsis Campaign (SSC) guidelines for the management of sepsis and septic shock, a qSOFA score of 2 or higher (i.e., a positive qSOFA score) should prompt physicians to look for evidence of organ dysfunction and to search for sepsis in order to improve the patient's outcome. However, the absence of a positive qSOFA score should not be misinterpreted as the absence of sepsis. The Sepsis-3 task force suggested that further research should be conducted to evaluate the prognostic and diagnostic performance of the score and proposed using the qSOFA criteria as entry criteria in clinical trials in the future.

After the introduction of the qSOFA score, numerous studies tested the prognostic performance of the score in variant study populations; the results were conflicting. Specifically, studies involving ED patients with suspected or confirmed infection exhibited relatively low sensitivity for the qSOFA score in predicting in-hospital mortality [12–14]. Similarly disappointing results were published regarding the sensitivity of the score for sepsis diagnosis [15,16]; however, sensitivity increased slightly when a qSOFA score cut-off of 1 was applied [16].

Because of these results, improving the prognostic performance of the qSOFA score became the primary goal of research. Subsequent studies [17–19] revealed that the addition of multiple infectious disease biomarkers—including procalcitonin (PCT), mid-regional proadrenomedullin, and multiple interleukins—could counterbalance the absence of a parameter that reflects infection in the qSOFA score and therefore improve its prognostic accuracy. Procalcitonin, a member of the calcitonin family, is a biomarker that rises rapidly during an infection and has already been established to monitor response to and guide antimicrobial therapy in septic patients [20,21]. Furthermore, PCT has been proved to be an important biomarker for sepsis diagnosis and prognosis as summarised in several systematic reviews [22]. Additionally, several studies [23,24] also showed that procalcitonin could be used to differentiate infectious and non-infectious inflammatory diseases, making it the ideal additional biomarker for a qSOFA-elevated population.

To date, only two studies have evaluated the diagnostic performance of PCT combined with qSOFA [25,26]. The previously mentioned studies provided encouraging results; however, they were not conducted in ED settings and did not apply the qSOFA criteria as inclusion criteria to select their study populations. They rather analysed patients who had been identified as having suspected infection or had been retrospectively diagnosed with sepsis.

1.2. Research Aim

The aim of the current study was to determine the diagnostic performance of PCT for early sepsis recognition in a population of ED patients with a qSOFA score of at least 1 upon admission. PCT was additionally investigated as a potential predictor of 30-day mortality in patients with elevated qSOFA scores in the ED.

2. Materials and Methods

2.1. Study Design

The LIFE-POC study was a large multi-centre prospective cohort study conducted in three EDs at three tertiary care hospitals: Charité University Hospital Berlin (Campus Virchow Klinikum and Campus Charité Mitte) and Jena University Hospital. The analysis included all adult patients (n = 742) admitted between 1 January 2017 and 23 March 2018 at the Berlin study sites with a qSOFA score of at least 1.

This study was approved by the institutional review boards of the respective universities. Written informed consent was obtained from all patients or their legal representatives where appropriate.

2.2. Patient Selection

All patients aged $\geq$ 18 years who had a qSOFA score of at least 1 were included in the study. Patient recruitment was conducted for seven days a week on a daily 8-h basis in alternating day and night shifts. The qSOFA score of each patient who presented at the ED during the study period was assessed, regardless of suspicion of infection, and confirmed by the study team at enrolment. The inclusion of patients with one qSOFA point was chosen to also detect patients likely to develop sepsis in the ED.

Patients suffering from acute trauma, an acute ST-elevation myocardial infarction, or suspected stroke or had been admitted for palliative care with a life expectancy of less than 1 month were excluded from the study. Further exclusion criteria involved pregnancy and referrals from other hospitals following prior in-hospital treatment. Additionally, patients were only included once in the study. All the study patients were treated according to the standard best practices of the department in question and current clinical guidelines.

2.3. Blood Sampling and Biomarker Measurement

Blood samples were collected within 12 h of presentation at the ED following written informed consent. For each patient, a sample of 18 mL of whole blood was taken after venipuncture under aseptic conditions, divided into three sampling tubes (ethylenediaminetetraacetic acid (EDTA), lithium heparin, and serum separator tubes) and centrifuged at room temperature. Plasma was immediately aliquoted, and the aliquots of all materials were stored at −20 °C within four hours. Blood samples were shipped on dry ice to the research laboratory at Charité University Hospital Berlin on the same day and stored at −80 °C within 72 h. Once a week, all the aliquots were shipped to the central biobank at the Jena University Hospital, where they were stored at −80 °C until measurement.

PCT was measured in all the study patients with an automated immunofluorescent assay using a Brahms PCT sensitive Kryptor. The direct measurement range of the assay was 0.02 to 50 µg/L, and the measurement range with automatic dilution was 0.02 to 5000 µg/L. The 95th percentile of serum or plasma PCT concentrations in healthy persons with this assay was 0.064 µg/L. The detection limit, calculated using the imprecision profile, was 0.02 µg/L. The following cut-offs for PCT [27,28] were investigated in the current analysis: 0.25, 0.5, 2.0, 5.0, and 10 µg/L.

2.4. Data Collection

Primary data were obtained in the EDs, starting with screening at triage. Clinical routine data were then extracted from the clinical patient records system. All data were then entered into a study-specific electronic case report form by members of the study personnel. The clinical study data included the patient's medical history, admission data,

process data, clinical in-hospital courses (including medications), vital signs, laboratory findings, and diagnostic procedures. In addition, 28-day follow-up calls were arranged to assess further clinical courses and 28-day mortality. The study database was coordinated by the clinical study centre of the University of Jena. Data were monitored and checked for plausibility by data managers on a regular basis.

2.5. Endpoints

The patients' electronic or paper medical files were consistently reviewed for 4 calendar days or until discharge from the hospital. The primary endpoint of this study was the diagnosis of sepsis within 96 h. Our aim was to include all patients with community-acquired sepsis, since these are the most common ones [29,30] and usually present to EDs in need of early sepsis management [31]. Furthermore, the board of the clinical trial aimed to include late sepsis development so that early biomarker diagnostic performance could be assessed. Direct referrals from other clinics were excluded.

In accordance with the Sepsis-3 criteria, sepsis was diagnosed as an acute change in a patient's total SOFA score of $\geq$2 points due to infection. Therefore, each patient's SOFA score was calculated daily for the first 4 days. Sepsis diagnoses were adjudicated by an expert panel. PCT values, if measured in clinical routine, were available for the expert panel to examine. However, the PCT values measured as part of the study were not available. Non-septic patients served as the control group for this study. The secondary study endpoint was 28-day mortality, which was defined as all-cause mortality within 28 days of initial enrolment.

2.6. Statistical Analysis

The study data were analysed in SPSS version 25 (IBM Deutschland GmbH, Ehningen, Germany). The intended sample size was 750 patients, as determined based on feasibility considerations. Relative and absolute frequencies were reported, and the chi-squared test was conducted for statistical comparisons among two or more groups in terms of categorical variables. The distribution of numeric variables was investigated graphically using histograms, normal distribution approximation curves, and the Kolmogorov–Smirnov test. Owing to skewed distributions of numeric variables, median values and interquartile ranges (IQRs) were reported, and non-parametric statistical tests (Mann–Whitney U Test) were conducted. A p-value below 0.05 was considered statistically significant. The diagnostic utility of biomarkers was primarily assessed and graphically illustrated by the area under the receiver operating characteristic curve (AUROC). Diagnostic performance was quantified based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy and was calculated from crosstabs for the diagnosis of sepsis within the first 96 h (primary endpoint). Logistic regression analyses were performed for the primary endpoint as the dependent variable, with PCT as a binary variable at the aforementioned cut-off points. Odds ratios (ORs) and in addition, 95% confidence intervals (CIs) were reported. Logistic regression was conducted for PCT as a single predictor (crude OR), adjusted for qSOFA (model 1); qSOFA, sex, and age (model 2; age entered as a numeric variable); and qSOFA, sex, age, C-reactive protein (CRP), and lactate (model 3; age and other biomarkers entered as numeric variables).

In the classification tree analysis, sepsis within 96 h was entered as the dependent variable, and the independent variables were (1) PCT with a fixed number of four group intervals and (2) the qSOFA score for all three categories. The minimum group sizes were set as 70 for the superior nodes and 10 for the inferior nodes. The chi-square automatic interaction detection (CHAID) method was used, allowing for a maximum of five steps, and the significance level for both nodes was split, and node consolidation was 0.05. Significance correction was conducted using the Bonferroni method. The model estimation criteria allowed for a maximum number of 100 iterations and a minimum change in the expected cell frequencies of 0.001. For validation purposes, a training and test data set was

randomly sampled from the study population, and the algorithm derived from the training data set was then applied in the test (validation) data set.

The net reclassification improvement (NRI) was calculated using established risk cut-off values for qSOFA (2 points) and PCT 0.50 µg/L and again applying optimised cut-off values, which were identified in classification tree analysis (PCT: 0.13 µg/L and 0.50 µg/L).

3. Results

3.1. Patient Population

All patients who presented at one of the two EDs in Berlin during the study period were screened, and 742 patients who met the inclusion criteria and agreed to participate in our study were included (Supplementary Figure S1, CONSORT flow diagram). Of these 742 patients, 42.0% (n = 312) were women, and the median age was 68 (IQR: 56–78) years. Regarding qSOFA scores the highest proportion of patients had a score of 1 (77.1%; n = 572), while 20.9% (n = 155) had a score of 2, and 2.0% (n = 15) had a score of 3. The median time between presentation at the ED and study blood drawing was 2 h (IQR: 1–3 h).

Further details on clinical characteristics are provided in Table 1 and further information on comorbidities is available in Supplementary Table S1.

Table 1. Demographic and clinical characteristics the Charlson Comorbidity Index and qSOFA at admission.

	Total n **= 742**	**Sepsis** n **= 202 ***	**Non-Sepsis** n **= 539 ***	p**-Value**
Women % (n) **	42.0 (312)	39.6 (80)	42.9 (231)	0.424
Age (median, IQR) **	68 (56–78)	70 (59–78)	67 (56–77)	0.086
Comorbidities % (n) **	85.8 (637)	91.6 (185)	3.7 (451)	0.006
Charlson Index points (median, IQR) **	2 (1–3)	3 (1–4)	2 (1–3)	<0.0001
qSOFA items **				
GCS < 15% (n) **	9.8 (73)	20.8 (42)	5.8 (31)	<0.0001
Tachypnoea (RR ≥ 22/min) % (n) **	71.7 (532)	72.8 (147)	71.2 (384)	0.231
Systolic BP ≤ 100 mmHg % (n) **	43.4 (322)	57.4 (116)	38.2 (206)	<0.0001
qSOFA points % (n) **				<0.0001
1	77.1 (572)	55.4 (112)	85.2 (459)	
2	20.9 (155)	38.1 (77)	14.5 (78)	
3	2.0 (15)	6.4 (13)	0.4 (2)	
GCS (median, Range) **	15 (3–15)	15 (3–15)	15 (3–15)	<0.0001
BP (mmHg) (median, IQR) **	112 (95–136)	99 (89–122)	117 (96–138)	<0.0001
RR (breaths/min) (median, IQR) **	23 (20–26)	24 (20–26)	23 (20–26)	0.028
Immunosuppresion % (n)	10.9 (80)	22.2 (44)	6.7 (36)	<0.0001
WBC/nL (median, IQR) ***	10.0 (7.3–14.4)	12.7 (7.6–17.9)	9.5 (7.2–13.1)	<0.0001
CRP (mg/dL) (median, IQR) ****	34.8 (5.9–99.6)	104.4 (50.0–229.7)	18.3 (3.4–60.9)	<0.0001
Non-survivors day 28% (n) *****	6.6% (48)	13.4% (27)	3.9 (21)	<0.0001

Abbreviations: BP—blood pressure; GCS—Glasgow Coma Scale; IQR—Inter Quartile Range; qSOFA—quick sequential organ failure assessment; RR—respiratory rate; WBC—white blood cell count; CRP—C reactive protein * one patient was lost to follow-up ** nmiss = 0 *** nmiss = 11, **** nmiss = 13, ***** nmiss = 10.

3.2. Further Clinical Course and Clinical Endpoints

After initial treatment in an ED, 24.8% (n = 184) of the study patients were discharged home, 44.2% (n = 328) were admitted to a general ward, 17.5% (n = 130) were admitted to an ICU, and 13.7% (n = 102) were transferred to another hospital.

The diagnosis of sepsis based on the Sepsis-3 definition within the first 96 h was assigned to 27.3% (n = 202) of the study patients. The onset of sepsis was prevalent upon admission in 20.8% (n = 154), occurred within the first 24 h after admission in 3.9% (n = 29), and occurred between 24 and 96 h after admission in 2.3% (n = 17) (the time of onset was unknown in two septic patients). After stratification based on qSOFA scores, it was determined that sepsis had occurred in 19.6% (n = 112) of all the patients with a qSOFA score of 1, 49.7% (n = 77) of all those with a qSOFA score of 2, and 86.7% (n = 13) of all those with a qSOFA score of 3.

The suspected focus of infection on day 0 was pulmonary in 38.0% (n = 280) of the study patients, urogenital in 8.9% (n = 66), and abdominal in 7.8% (n = 58). Less frequent infection foci were skin or wounds (3.8%; n = 28), other (2.2%; n = 16), the cardiovascular system (0.8%; n = 6), and the central nervous system (0.3%; n = 2). The infection focus was unknown in 7.4% (n = 55) of the patients, and no focus of infection was suspected in 31.3% (n = 231). The distribution of qSOFA points within infect foci is shown in Supplementary Table S2.

The distribution of infectious foci was similar in patients with sepsis within the first 96 h after admission, with 44.6% pulmonary (n = 90), 17.3% urogenital (n = 35), 11.9% abdominal (n = 24), 6.0% skin or wounds (n = 12), 3.0% cardiovascular system (n = 6), and 1.0% central nervous system (n = 2).

Microbiological tests were performed on 53.1% (n = 394) of the study patients within 96 h of admission. Regarding the patients who were transferred to other hospitals (n = 102), the study team was informed of only the microbiological tests with positive results as opposed to all such tests.

In the septic subgroup, microbiological samples were obtained from 90.6% (n = 183) of the study patients, and a relevant pathogen was identified in 54.7% (n = 110). The most common pathogens detected in the first microbiological examination within the first 96 h were Gram-negative bacteria (25.6%; n = 52), Gram-positive bacteria (15.9%; n = 32), multiple pathogens (7.0%; n = 14), fungal infections (4.5%; n = 9), other infections (1.0%; n = 2), and parasites (0.5%; n = 1).

Overall, a clinically confirmed infection was diagnosed in 28.7% (n = 213) of the study patients. A clinically confirmed infection was defined as an infection verified by (1) imaging features or after surgical intervention, (2) a positive urine dip test with symptoms of urinary tract infection, or (3) in cases of skin infections, typical appearance and symptoms according to a dermatologist. Suspected infection, defined as suspicion of infection according to the treating physician based on clinical signs and laboratory findings, was diagnosed in 38.4% (n = 285) of the study patients. The remaining patients (32.9%, n = 244) did not have a clinically confirmed infection or suspicion of an infection.

In the sepsis subgroup, a clinically confirmed infection was diagnosed in 54.4% (n = 110) of the study patients, and a suspected infection was present in 45.6% (n = 92). In the septic subgroup, surgical intervention was performed in 11.4% (n = 23) of the patients, while 32.7% (n = 66) needed mechanical ventilation, 13.4% (n = 27) were treated with vasopressors or inotropic agents, and 7.9% (n = 16) underwent dialysis. Of the patients with sepsis, septic shock occurred in 12.9% (n = 26), and acute renal failure occurred in 47.5% (n = 96). Mortality after 28 days was observed in 13.4% (n = 27) of the patients with sepsis and 3.9% (n = 21) of the patients with other diagnoses. Of the septic patients, 0.5% (n = 1) died on day 0, 3.0% (n = 6) died on day 1, and 0.5% (n = 1) died on day 2.

3.3. Diagnostic Performance of Biomarkers

Of all PCT values, 25.1% (n = 186) were above the reference value of 0.05 μg/L, whereas this was true for 76.7% (n = 559) of the CRP values (cut-off: 5 mg/L) and 31.7% (n = 223) of the lactate values (cut-off: 20 mg/dl).

The median PCT value was 0.13 μg/L (IQR: 0.07–0.50 μg/L) and PCT was significantly higher in the patients with sepsis (1.17 μg/L; IQR: 0.25–5.10 μg/L) than in those without sepsis (0.10 μg/L; IQR: 0.06–0.20 μg/L; $p < 0.0001$) (Supplementary Figure S2). The median PCT value in patients who presented with prevalent sepsis at the ED (n = 154) was 1.47 μg/L (IQR: 0.39–6.39 μg/L). In patients who became septic within 24 h of admission (n = 29), the median PCT value was 0.49 μg/L (0.16–2.24 μg/L), while PCT was lower in patients who developed sepsis more than 24 h after admission to the ED (n = 17; median PCT: 0.14 μg/L; IQR: 0.10–2.50 μg/L; $p < 0.001$) (Supplementary Figure S3).

The AUROC for the PCT value at admission for the diagnosis of sepsis within 96 h was 0.86 (95% CI: 0.83–0.89; $p < 0.0001$). The AUROC for sepsis diagnosis within the first 24 h was 0.87 (95% CI: 0.84–0.90), which was slightly higher than that for the diagnosis of sepsis

on day 1 (0.82; 95% CI: 0.78–0.86) and higher still than that for diagnosis on day 2 (0.82; 0.77–0.86 ($p < 0.0001$ for all)). Compared with the other biomarkers, PCT showed higher AUROC values than CRP (AUROC: 0.82; 95% CI: 0.78–0.85; $p < 0.0001$) and lactate (AUROC: 0.60; 95% CI: 0.55–0.64; $p < 0.0001$) (Figure 1). The combination of PCT and qSOFA resulted in an AUROC of 0.86 (95% CI: 0.83–0.89; $p < 0.00001$), while the combination of qSOFA, PCT, and CRP showed an AUROC of 0.88 (95% CI: 0.85–0.01; $p < 0.00001$).

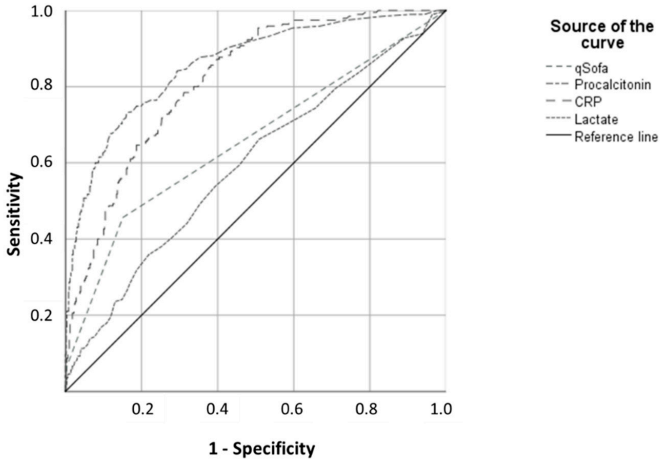

Figure 1. ROC of qSOFA and laboratory parameters for the prediction of sepsis within 96 h of admission. the optimized cut-off value according to ROC-analysis for PCT is 0.26 μG/L. Abbreviations: CRP—C-reactive protein; PCT—procalcitonin. qSOFA—the quick Sequential Organ Failure Assessment.

3.4. Diagnostic Performance of PCT

The diagnostic performance measures of PCT at various cut-off values for the diagnosis of sepsis are detailed in Table 2. The highest accuracy level of 82.2% correctly classified patients was observed at a cut-off value at 0.50 μg/L. This value resulted in sensitivity of 63.4% (95% CI: 56.3–70.0), specificity of 89.2% (95% CI: 86.3–91.7), a PPV of 68.8% (95% CI: 62.9–74.2), and an NPV of 86.7% (95%Cl: 84.4–88.7).

Table 2. Diagnostic performance of PCT at different cut-off values (recommended by the manufacturer) for the diagnosis of sepsis within 96 h of admission.

	PCT 0.20 μg/L	**PCT 0.25 μg/L**	**PCT 0.50 μg/L**	**PCT 2.00 μg/L**	**PCT 5.00 μg/L**	**PCT 10.00 μg/L**
True negative	400	429	481	523	533	537
False negative	44	50	74	122	151	169
False positive	139	110	58	16	6	2
True positive	158	152	128	80	51	33
Sensitivity (95% CI)	78.2 (71.9–83.7)	75.3 (68.7–81.4)	63.4 (56.3–70.0)	39.6 (32.8–46.7)	25.3 (19.4–31.8)	16.3 (11.5–22.2)
Specificity (95% CI)	74.2 (70.3–77.9)	79.6 (75.9–82.9)	89.2 (86.3–91.7)	97.0 (95.2–98.3)	98.9 (97.6–99.6)	99.6 (98.7–100.0)
Positive Likelihood Ratio (95% CI)	3.0 (2.6–3.6)	3.7 (3.1–4.4)	5.9 (4.5–7.7)	13.3 (8.0–22.3)	22.7 (9.9–52.0)	44.0 (10.7–181.8)
Negative Likelihood Ratio (95% CI)	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.4 (0.3–0.5)	0.6 (0.6–0.7)	0.8 (0.7–0.8)	0.8 (0.8–0.9)
Positive Predictive Value (95% CI)	53.2 (49.2–57.2)	58.0 (53.5–62.4)	68.8 (62.9–74.2)	83.3 (75.0–89.3)	89.5 (78.8–95.1)	94.3 (80.0- 98.6)
Negative Predictive Value (95% CI)	90.1 (87.5–92.2)	89.6 (87.1–91.6)	86.7 (84.4–88.7)	81.1 (79.3–82.8)	77.9 (76.5–79.3)	76.1 (74.9–77.2)
Accuracy (95% CI)	75.3 (72.3–78.4)	78.4 (75.3–81.3)	82.2 (79.2–84.9)	81.4 (78.4–84.1)	78.8 (75.6–81.7)	76.9 (76.9–79.9)

One patient was lost to follow-up. CI—confidence interval.

Further details regarding the diagnostic performance of PCT in the qSOFA subgroups are provided in Table 3.

Table 3. Diagnostic performance of qSOFA at a cut-off value of 2 and PCT at different cut-off values within qSOFA categories for the diagnosis of sepsis within 96 h of admission. 95%-CI, 95% confidence intervals.

	qSOFA Cut-Off Score ≥ 2	qSOFA Score = 1		qSOFA Score ≥ 2	
		PCT Cut-Off 0.25 µg/L	PCT Cut-Off 0.50 µg/L	PCT Cut-Off 0.25 µg/L	PCT Cut-Off 0.50 µg/L
True negative	459	374	415	55	66
False negative	112	31	42	19	32
False positive	80	85	44	25	14
True positive	90	81	70	71	58
Sensitivity	44.6	72.3	62.5	78.9	64.4
(95% CI)	(37.6–51.7)	(63.1–80.4)	(52.8–71.5)	(69.0–86.8)	(53.7–74.3)
Specificity	85.2	81.5	90.4	68.8	82.5
(95% CI)	(81.9–88.0)	(77.6–84.9)	(87.4–93.0)	(57.4–78.7)	(72.4–90.1)
Positive Likelihood Ratio	3	3.9	6.5	2.5	3.7
(95% CI)	(2.3–3.9)	(3.1–4.9)	(4.8–8.9)	(1.8–3.6)	(2.2–6.1)
Negative Likelihood Ratio	0.7	0.3	0.4	0.3	0.4
(95% CI)	(0.6–0.7)	(0.3–0.5)	(0.3–0.5)	(0.2–0.5)	(0.3–0.6)
Positive Predictive Value	52.9	48.8	61.4	74	80.6
(95% CI)	(46.6–59.2)	(43.3–54.4)	(53.7–68.6)	(66.9–80.0)	(71.5–87.2)
Negative Predictive Value	80.4	92.4	90.8	74.3	67.4
(95% CI)	(78.3–82.3)	(89.9–94.2)	(88.6–92.6)	(65.4–81.6)	(60.6–73.5)
Accuracy	74.1	79.7	84.9	74.1	72.9
(95% CI)	(70.8–77.2)	(76.2–82.9)	(81.7–87.7)	(66.9–80.5)	65.6–79.5

One patient was lost to follow-up. CI—confidence interval.

3.5. Logistic Regression Analysis

The logistic regression analyses revealed that at all investigated cut-off values, PCT was a significant predictor of sepsis within 96 h of admission in univariate and all adjusted logistic regression models (Table 4).

Table 4. Results of the logistic regression analysis of PCT at different cut-off values for the prediction of sepsis in univariate analyses (crude ORs) and adjusted for qSOFA (model 1), adjusted for qSOFA, gender and age (model 2), and adjusted for qSOFA, gender, age, C-reactive protein, lactate, WBC, pre-existing conditions and tumour diseases, IMMUNOSUPRESSION (model 3). One patient was lost to follow-UP.

PCT Cut-Off		OR (Crude)	*p*-Value	OR Adjusted Model 1	*p*-Value	OR Adjusted Model 2	*p*-Value	OR Adjusted Model 3	*p*-Value
0.20 µg/L	Value (95% CI)	10.3 (7.0–15.2)	<0.0001	8.7 (5.8–12.9)	<0.0001	8.6 (5.8–12.8)	<0.0001	4.3 (2.6–7.1)	<0.0001
0.25 µg/L	Value (95% CI)	11.9 (8.1–17.4)	<0.0001	10.1 (6.8–15.0)	<0.0001	10.0 (6.8–14.8)	<0.0001	5.4 (3.3–8.8)	<0.0001
0.50 µg/L	Value (95% CI)	14.4 (9.7–21.3)	<0.0001	13.1 (8.7–19.8)	<0.0001	13.3 (8.8–20.1)	<0.0001	7.7 (4.6–13.0)	<0.0001
2.00 µg/L	Value (95% CI)	21.4 (12.1–38.0)	<0.0001	19.4 (10.7–34.9)	<0.0001	21.5 (11.7–39.4)	<0.0001	12.7 (6.2–26.3)	<0.0001
5.00 µg/L	Value (95% CI)	30.0 (12.6–71.3)	<0.0001	28.5 (11.8–69.0)	<0.0001	29.8 (12.3–72.3)	<0.0001	26.4 (8.5–81.7)	<0.0001
10.00 µg/L	Value (95% CI)	52.5 (12.5–220.8)	<0.0001	44.7 (10.4–191.8)	<0.0001	47.0 (10.9–202.8)	<0.0001	25.0 (5.5–114.1)	<0.0001

OR: odds ratio, CI—confidence interval.

3.6. Classification Tree Analysis

In the classification tree analysis, PCT and qSOFA were investigated as independent variables to predict sepsis within the first 96 h of admission. The most accurate discrimination was achieved by PCT in the first place at cut-off values of 0.13 and 0.50 µg/L. The most accurate split value for qSOFA in all subgroups was between 1 (first category) and 2 (second category: qSOFA 2 and 3). When qSOFA was forced to be the first variable in the model, the same PCT cut-off values were identified to further discriminate among patients in the qSOFA 1 and qSOFA 2 and 3 subgroups (Supplementary Figure S4). Supplementary Figure S5A,B shows the validation of the decision tree analysis by the split half method.

3.7. Net Reclassification Improvement

The NRI of qSOFA by PCT was 22.9% when the established risk categories were applied (qSOFA: 2 points; PCT: 0.50 µg/L; Supplementary Table S3). The NRI increased to 39.9% when the optimised cut-off from classification tree analysis was applied (PCT: 0.13 µg/L and 0.50 µg/L; Supplementary Table S4).

3.8. PCT and Mortality

Regarding patients who died within 28 days of admission (6.5%; $n = 48$), the median PCT value was 0.31 µg/L (IQR: 0.12–0.95 µg/L), which was significantly higher than that for those who survived the first 28 days after ED presentation (median: 0.13; IQR: 0.07–0.47; $p = 0.001$). Mortality was significantly higher in patients with a PCT value of at least 0.25 µg/L (10.5%, $n = 27$) than in those with a PCT value below 0.25 µg/L (4.4%, $n = 21$; $p = 0.001$). In the Cox regression analysis, the crude hazard ratio (HR) for PCT at a cut-off value of 0.25 µg/L was 2.48 (95% CI: 1.40–4.38; $p = 0.002$; Figure 2). After adjustment for qSOFA (HR: 1.31; 95% CI: 0.79–2.17; $p = 0.293$), age (HR: 1.04; 95% CI: 1.02–1.07; $p = 0.001$), and gender (HR: 1.66; 95% CI: 0.89–3.09; $p = 0.114$), the HR decreased slightly to 2.13 but was still significant (95% CI: 1.18–3.86; $p = 0.013$). When adjusted for CRP (HR: 1.00; 95% CI: 1.00–1.00; $p = 0.276$) and lactate (HR: 1.03; 95% CI: 1.01–1.04; $p = 0.003$), the HR for PCT was 2.05 (95% CI: 1.03–4.06; $p = 0.041$).

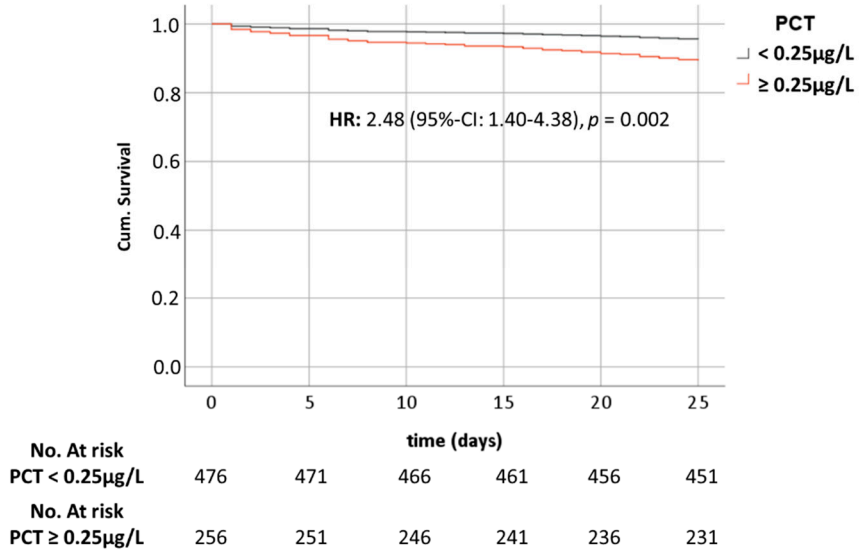

Figure 2. Cumulative survival within 28 days of admission to the ED for patients with procalcitonin (PCT) below, at, or above 0.25 µg/L. Abbreviations: HR—Hazard Ratio. CI- confidence intervalX; Cum: cumulative.

4. Discussion

4.1. Summary of Findings

To the best of our knowledge, this study is the first prospective study to investigate the diagnostic performance of PCT for sepsis and to employ the qSOFA as entry to the study criteria to focus on a patient population with presumed organ dysfunction rather than infection suspicion alone, as in similar studies to date. This study performed analyses for all qSOFA-elevated subgroups and included the qSOFA 1 population for two reasons. Initially we were seeking patients likely to develop sepsis in addition to already septic patients. Based on our clinical observations, a significant number of patients with qSOFA 1 will deteriorate to qSOFA 2 if not treated promptly. Therefore, qSOFA scores require re-evaluation in EDs. Our results justified our decision, since 19.6% (n = 112) of the qSOFA 1 population developed sepsis within 96 h.

Secondly, in the validation cohort of the original study of Seymour et al., among non-ICU encounters the subgroup of qSOFA 1 reported as intermediate-risk encounters was the largest and presented a high mortality rate. In our study, the qSOFA 1 population was also the largest; thus, it was a crucial ED population to include in the analysis.

PCT alone provided an exceptional AUROC of 0.86 (95% CI: 0.83–0.89; $p < 0.0001$), with a slight improvement when combined with a qSOFA score of $\geq$2 for sepsis prediction. Discriminatory analyses highlighted the diagnostic abilities of PCT on top of qSOFA, proving that PCT acts independently of other established risk markers like CRP and lactate, as revealed in the logistic regression analyses. Furthermore, PCT was an independent predictor of 28-day mortality and was therefore identified as a risk predictor marker.

4.2. Clinical Endpoints

In all 742 patients analysed in this study, 202 were diagnosed with sepsis. This high prevalence of sepsis was a result of our selection criteria and accurate assessment of qSOFA score. There were no missing data regarding qSOFA, and the parameters were repeatedly re-evaluated.

In the present study, most of the septic patients (44.6%) were diagnosed with a respiratory infection. Urogenital infection was the second most common source of sepsis in our study population, exceeding abdominal infection; this result contradicted previous studies, including the Impress Study [32], which demonstrated the abdomen to be the second most frequent source of sepsis in Western Europe. A simple explanation for this deviation is that our hospital is the only hospital with a urology clinic in central Berlin and the only one with a kidney transplant unit within a 190-km area.

Furthermore, in opposition of previous studies [33] our results revealed that the age factor showed no statistical significance between the sepsis and non-sepsis groups. The reason for this discrepancy is that we studied a prospectively identified population with elevated qSOFA, namely, patients with presumed organ dysfunction. This led to a homogenisation of critically ill patients of older age with a high proportion of comorbidities in the selected study population, and thus, age did not show a significant association with the occurrence of sepsis in our study. In Germany, the in-hospital mortality of patients diagnosed with sepsis, according to the former definition, was estimated in 2013 to be approximately 41.7% in ICU-treated patients [34]. More recent evidence on mortality regarding community-acquired sepsis using the Sepsis-3 definition is not available. In our study, the 28-day mortality rate was low at only 6.6% since we excluded patients with an already low survival rate (<28 days) attributed to cancer or other conditions, as well as patients with a Glasgow Coma Scale score of below 13 who had no legal representation.

4.3. Diagnostic Performance of Biomarkers and qSOFA

Our results regarding qSOFA were consistent with those of previous studies; however, we must acknowledge that the present patient population was an already evaluated qSOFA population. Since the introduction of the qSOFA, numerous studies have attempted to test the performance of the assessment in the screening, diagnosis, and prognosis of sepsis,

yielding a range of results from a variety of heterogeneous study populations and leading to many subsequent systematic reviews. In a meta-analysis of 23 studies of patients with infections outside the ICU, Song et al. [35] revealed that a positive qSOFA score had high specificity in predicting in-hospital mortality and sepsis severity but somewhat low sensitivity, making the qSOFA unsuitable as a screening tool. In our study, we confirmed a low sensitivity of 44.6% in predicting sepsis but a high specificity of 85.2%, as expected. However, these results are hardly comparable since the patients in the present study were selected based on their qSOFA scores at admission to the ED. Furthermore, most of the patients in the present study (77.1%) still presented with a qSOFA score of 1 at admission, and further diagnostic measures, including such biomarkers as PCT, would be required to identify the still considerably high proportion of sepsis patients (approximately 20% in the current study) in this intermediate-risk group.

The role of PCT in sepsis, not only in the ICU but also in the ED, has been widely explored in previous studies, revealing contradictory results. In a population with elevated qSOFA scores, our data revealed the notably high diagnostic performance of PCT. Moreover, the AUROC in the present study was similar to the AUROC of the systematic review conducted by Miechun Tan et al. [36], who reviewed nine studies evaluating the diagnostic accuracy of PCT.

Although the low sensitivity of the qSOFA and the high diagnostic performance of PCT were similar to the results of several previous studies, most of those studies were retrospective and investigated the qSOFA score and PCT for sepsis mortality prediction and prognosis [18,19,25]. The results of the present study were consistent, indicating an undeniable benefit from the combination of PCT and qSOFA compared with qSOFA alone for the prediction of sepsis severity and mortality.

In the current study, PCT was investigated prospectively in EDs by a single early measurement promptly after admission, and the main focus was early sepsis diagnosis. PCT exhibited high and independent diagnostic performance at several cut-off values derived from the literature. To achieve the highest possible level of accuracy and thus the optimal trade-off between sensitivity and specificity, a cut-off value of 0.50 µg/L proved to be the optimal value in both the general study population and the qSOFA subgroups. This finding was confirmed through decision tree analysis when PCT was added as a numeric value, and decision tree analysis also revealed another optimised cut-off value of 0.13 µg/L, which could aid in the further identification of low-risk groups. NRI analysis confirmed that PCT was indeed of incremental diagnostic value and improved classification by 23.9% at a cut-off value of 0.50 µ/L. When both cut-off values were applied, NRI improved to 39.9%. The improvement of NRI was mainly triggered by the upgrading of patients with sepsis to higher risk categories, which further illustrates the great clinical benefit PCT can have for early sepsis diagnosis in the ED. Given that the qSOFA score is easily obtainable at admission to an ED, PCT measurement could be recommended for ED patients with a qSOFA score of at least 1 upon admission for further risk stratification. Therefore, the availability of point-of-care PCT measurement in EDs can facilitate the early identification of already septic patients and patients likely to develop sepsis.

4.4. Strengths and Limitations

In the present prospective study, no patients had to be excluded for missing values; therefore, we had an advantage over previous studies. On a daily 8-h basis, we screened all patients (approximately 34,000) that presented in the study EDs based on their qSOFA scores.

For the diagnosis of sepsis, the Sepsis-3 definition was used and adjudicated by a panel of experts. For ethical reasons, patients with dementia or a severely altered cognitive state, who had no legally authorised representation, were excluded from the study. This exclusion could be considered as a limitation of our study and may have contributed to the fact that in the qSOFA assessments mental status changes were the smallest contributor to the qSOFA score. In addition, this may have caused a minor reduction in the number

of patients with a qSOFA score of 3. The prediction of mortality through PCT should be investigated in further studies since mortality in the present study was low and the subgroup analyses of patients with and without sepsis, as well as full adjustment for all possible other predictors and confounders, was not possible.

5. Conclusions

In a cohort of ED patients selected based on current guideline-recommended clinical criteria, PCT exhibited excellent diagnostic performance. PCT can improve early sepsis identification in EDs by 40% (NRI), especially for the majority of patients presenting with qSOFA scores of at least 1 upon admission. Thus, PCT can support clinicians in the early application of targeted measures to improve clinical courses and outcomes. PCT can thus serve as an ideal biomarker for point-of-care measurement in EDs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10173869/s1, Figure S1: Consort Flow Diagram, Figure S2: Logarithmic illustration of the distribution of PCT in patients with and without a diagnosis of sepsis within the first 96 h after admission. Figure S3: Procalcitonin values stratified by time of sepsis (logarithmic scale). Figure S4: Classification tree containing the independent variables PCT (numeric) and qSOFA (3 categories) as independent variables and the diagnosis of sepsis within 96 h after admission as dependent variable. Figure S5A: Results of the classification tree analysis in the training sample. Figure S5B: Results of the classification tree analysis in the test sample. Table S1: Comorbidities, Table S2: Association of qSOFA criteria and infect foci in all study participants, Table S3. Specification of change of risk categories of qSOFA by PCT applying two established risk groups and resulting Net Reclassification Improvement. Table S4. Specification of change of risk categories of qSOFA by PCT applying three risk groups and resulting Net Reclassification Improvement.

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Institutional Review Board Statement: This study was performed in accordance with the ethical guidelines of the Helsinki Declaration (WMA Declaration of Helsinki), approved by the Institutional Review Board of the University Hospital Jena (4892-08/16) and registered in the German registry for clinical trials DRKS00011188. Written informed consent was obtained from all patients or their legal representatives where appropriate.

Informed Consent Statement: No individual participant data are reported in this article that would require consent from the participant. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data sets used and/or analysed during the present study are available from the corresponding author upon reasonable request.

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Table S1. Comorbidities.

	Total ***n*** **= 742**	**Sepsis** ***n*** **= 202 ***	**Non-sepsis** ***n*** **= 539 ***	***p*-value**
Previous myocardial infarction % (*n*)	15.9 (99)	13.4 (23)	16.9 (76)	0.279
Heart failure % (*n*)	37.9 (232)	32.9 (53)	39.3 (179)	0.148
Peripheral arterial disease % (*n*)	12.9 (79)	13.0 (20)	12.9 (59)	0.988
Cerebrovascular dieses % (*n*)	12.8 (92)	15.8 (31)	11.7 (61)	0.140
Dementia % (*n*)	5.4 (39)	4.2 (22)	8.5 (17)	0.020
Chronic respiratory disease % (*n*)	36.5 (257)	29.2 (56)	39.3 (201)	0.013
Collagenosis % (*n*)	2.8 (20)	2.5 (5)	2.8 (15)	0.806
Ulcera % (*n*)	6.2 (39)	6.0 (10)	6.3 (29)	0.891
Mild liver disease % (*n*)	2.8 (20)	6.2 (12)	1.6 (8)	0.001
Severe liver disease % (*n*)	2.1 (15)	3.5 (7)	1.5 (8)	0.088
Hemiplegia % (*n*)	2.6 (19)	4.0 (8)	2.0 (11)	0.143
Diabetes mellitus without end organ damage % (*n*)	20.2 (147)	25.0 (50)	18.4 (97)	0.048
Diabetes mellitus with end organ damage	5.4 (38)	5.7 (11)	5.2 (27)	0.790
Medium to severe renal disease % (*n*)	6.4 (47)	8.5 (17)	5.6 (30)	0.154
Tumor % (*n*)	10.9 (75)	13.2 (25)	10.1 (50)	0.248
Metastatic solid tumor % (*n*)	7.2 (50)	13.6 (26)	4.5 (23)	<0.0001
Leukemia % (*n*)	1.4 (10)	2.5 (5)	0.9 (5)	0.099
Lymphoma % (*n*)	3.2 (23)	6.0 (12)	2.1 (11)	0.007
AIDS % (*n*)	0.5 (4)	1.5 (3)	0.2 (1)	0.032

*one patient was lost to follow up.

Table S2: Association of qSOFA criteria and infect foci in all study participants**.**

	Pulmonary (*n* = 280)	**Urogenital (*n* = 66)**	**Abdominal (*n* = 58)**	**Skin or wounds (*n* = 28)**	**Other (*n* = 16)**	**Cardiovascular (*n* = 6)**	**Central nervous system (*n* = 2)**	**Unknown (*n* = 55)**
qSOFA points								
1 % (*n*)	76.1 (213)	59.1 (39)	75.9 (44)	78.6 (22)	81.3 (13)	50.0 (3)	0.0 (0)	72.7 (40)
2 % (*n*)	22.9 (64)	31.8 (21)	22.4 (13)	21.4 (6)	12.5 (2)	50.0 (3)	100.0 (2)	21.8 (12)
3 % (*n*)	1.1 (3)	9.1 (6)	1.7 (1)	0.0 (0)	6.3 (1)	0.0 (0)	0.0 (0)	5.5 (3)
GCS <15 % (*n*)	8.9 (25)	28.8 (19)	6.9 (4)	10.7 (3)	12.5 (2)	0.0 (0)	100.0 (2)	16.4 (9)
Tachypnoea (RR ≥ 22/min) % (*n*)	85.0 (238)	65.2 (43)	53.4 (31)	50.0 (14)	43.8 (7)	100.0 (6)	50.0 (1)	67.3 ()37
Systolic BP ≤100mmHg % (*n*)	31.1 (87)	56.1 (37)	65.5 (38)	60.7 (17)	68.8 (11)	50.0 (3)	50.0 (1)	49.1 (27)

Qsofa: the quick sequential organ failure assessment; GCS: Glasgow Coma Scale

Table S3. Specification of change of risk categories of qSOFA by PCT applying two established risk groups and resulting Net Reclassification Improvement.

Model without PCT	**Model with PCT at a cut-off value of 0.50 μg/L**		
	risk category 1	risk category 2	
	PCT < 0.50 μg/L	PCT ≥ 0.50 μg/L	
Patients with sepsis			**SUM**
risk category 1 (qSOFA = 1)	42	70	112
risk category 2 (qSOFA ≥ 2)	32	58	90
SUM	74	128	202
Patients without sepsis			
risk category 1 (qSOFA = 1)	415	44	459
risk category 2 (qSOFA ≥ 2)	66	14	80
SUM	481	58	539
Net reclassification improvement			**22.9%**

Table S3 shows the calculation table for net reclassification improvement when applying two risk categories for both parameters: qSOFA (cut-off value of 2 points), PCT (cut-off value of 0.50 μg/L). PCT: procalcitonin

Table S4. Specification of change of risk categories of qSOFA by PCT applying three risk groups and resulting Net Reclassification Improvement.

Model without PCT	**Model with PCT at a cut-off value of 0.13 and 0.50 μg/L**			
	risk category 1	risk category 2	risk category 3	
	PCT < 0.13 μg/L	PCT 0.13-0.50 μg/L	PCT ≥ 0.50 μg/L	
Patients with sepsis				**SUM**
risk category 1 (qSOFA = 1)	16	26	70	112
risk category 2 (qSOFA = 2)	7	21	49	77
risk category 3 (qSOFA = 3)	1	3	9	13
SUM	24	50	128	202
Patients without sepsis				
risk category 1 (qSOFA = 1)	294	121	44	459
risk category 2 (qSOFA = 2)	35	29	14	78
risk category 3 (qSOFA = 3)	1	1	0	2
SUM	330	151	58	539
Net reclassification improvement				**39.9%**

Table S4 shows the calculation table for net reclassification improvement when applying three risk categories for both parameters: qSOFA points, and the PCT cut-off values of 0.13 μg/L and 0.50 μg/L which were derived from classification tree analysis.

Figure S1. Consort Flow Diagram.

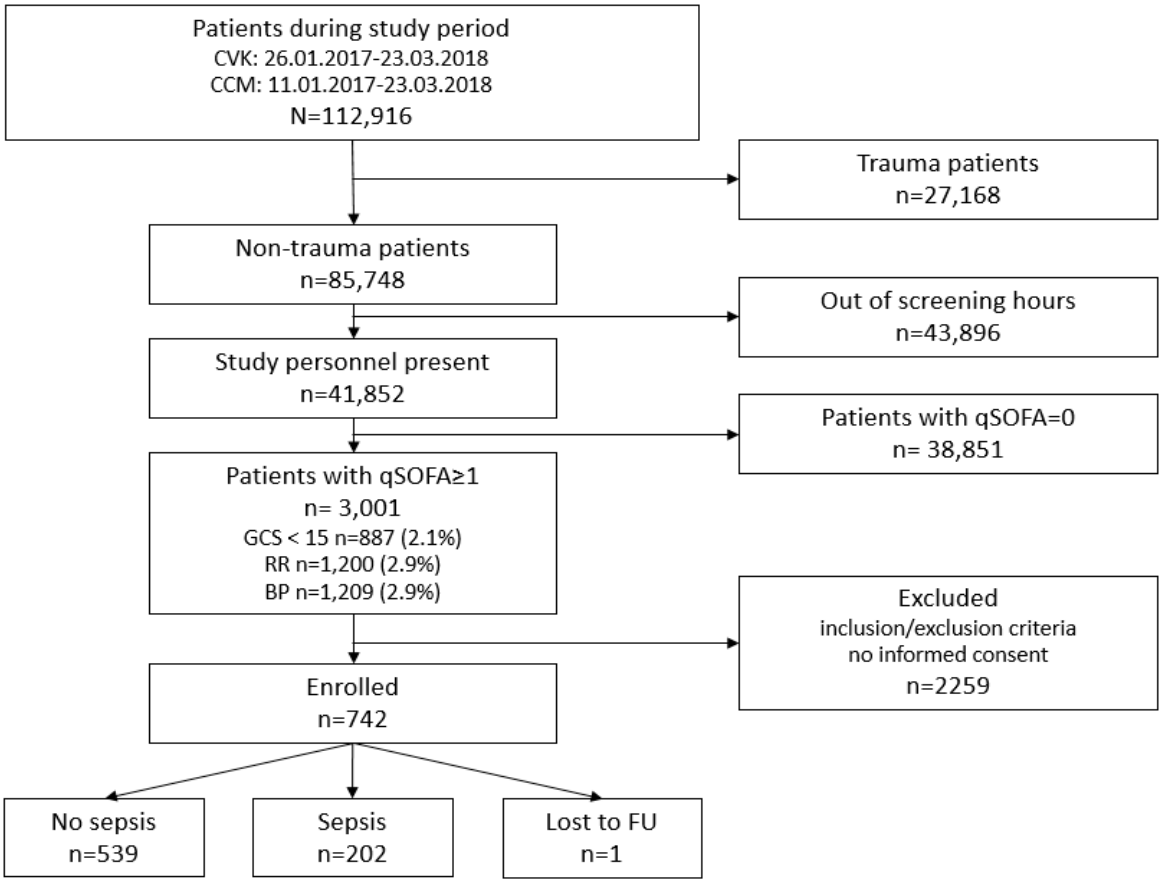


Figure S2. Logarithmic illustration of the distribution of PCT in patients with and without a diagnosis of sepsis within the first 96 hours after admission.

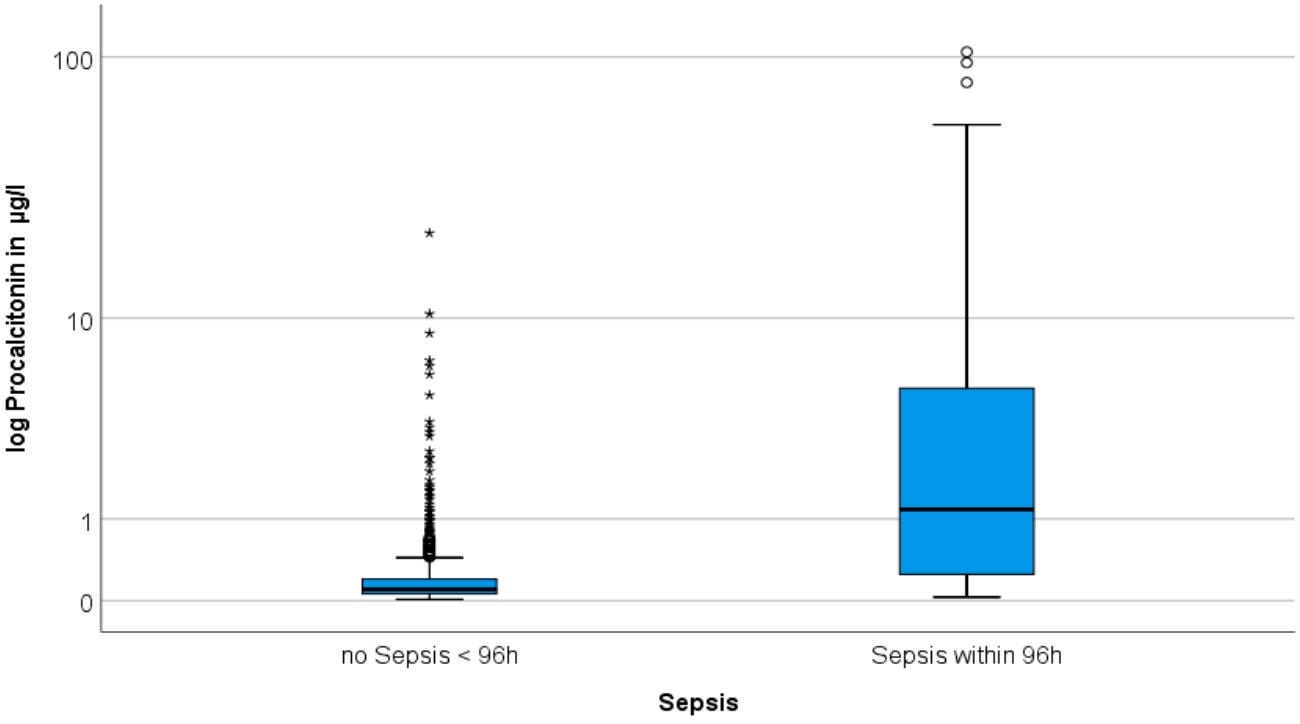


Figure S3. Procalcitonin values stratified by time of sepsis (logarithmic scale).

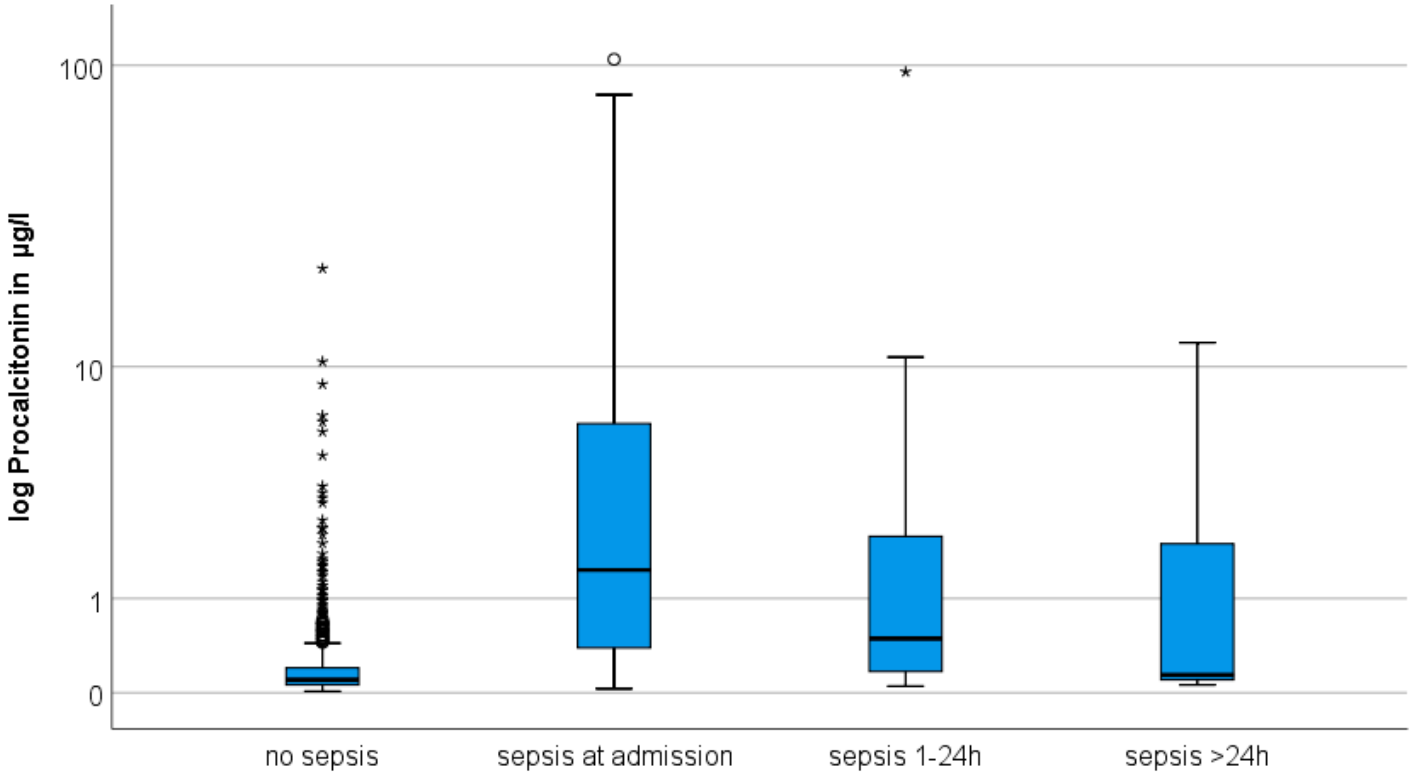


Figure S4. Classification tree containing the independent variables PCT (numeric) and qSOFA (3 categories) as independent variables and the diagnosis of sepsis within 96 hours after admission as dependent variable.

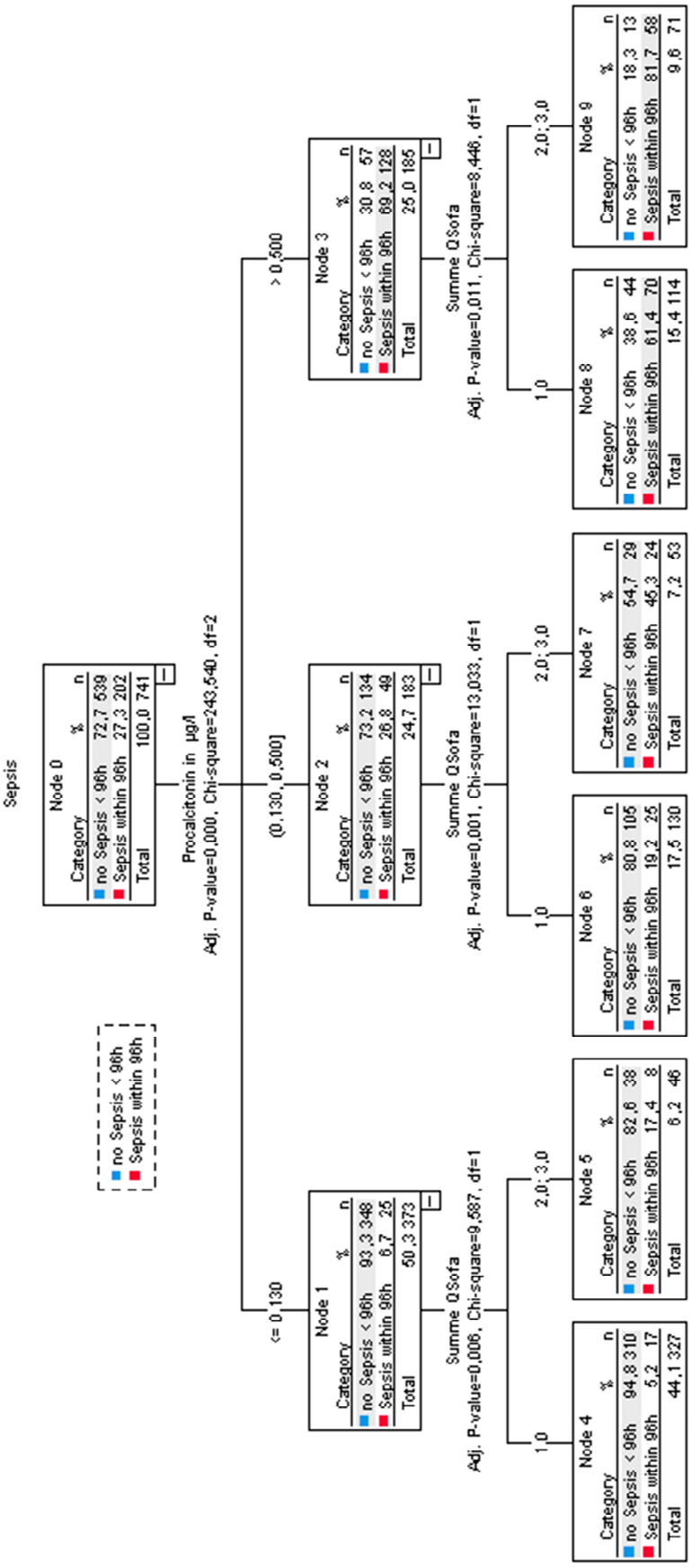


Figure S5. Validation of the classification tree analysis by the split half method. Figure S5A shows the results in the training sample and Supplement Figure 5B in the validation sample of the original data set.

Figure S5A: Results of the classification tree analysis in the training sample.

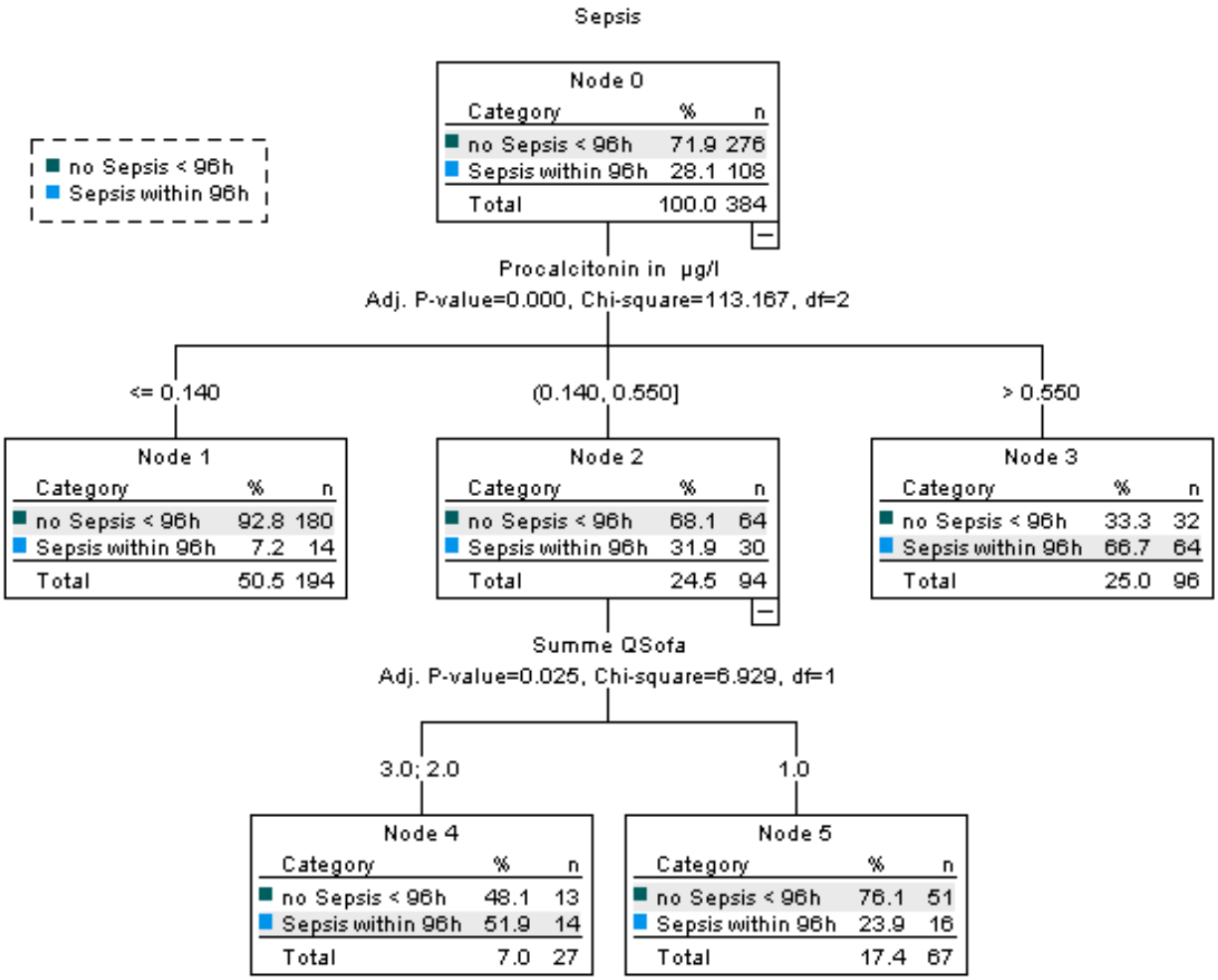
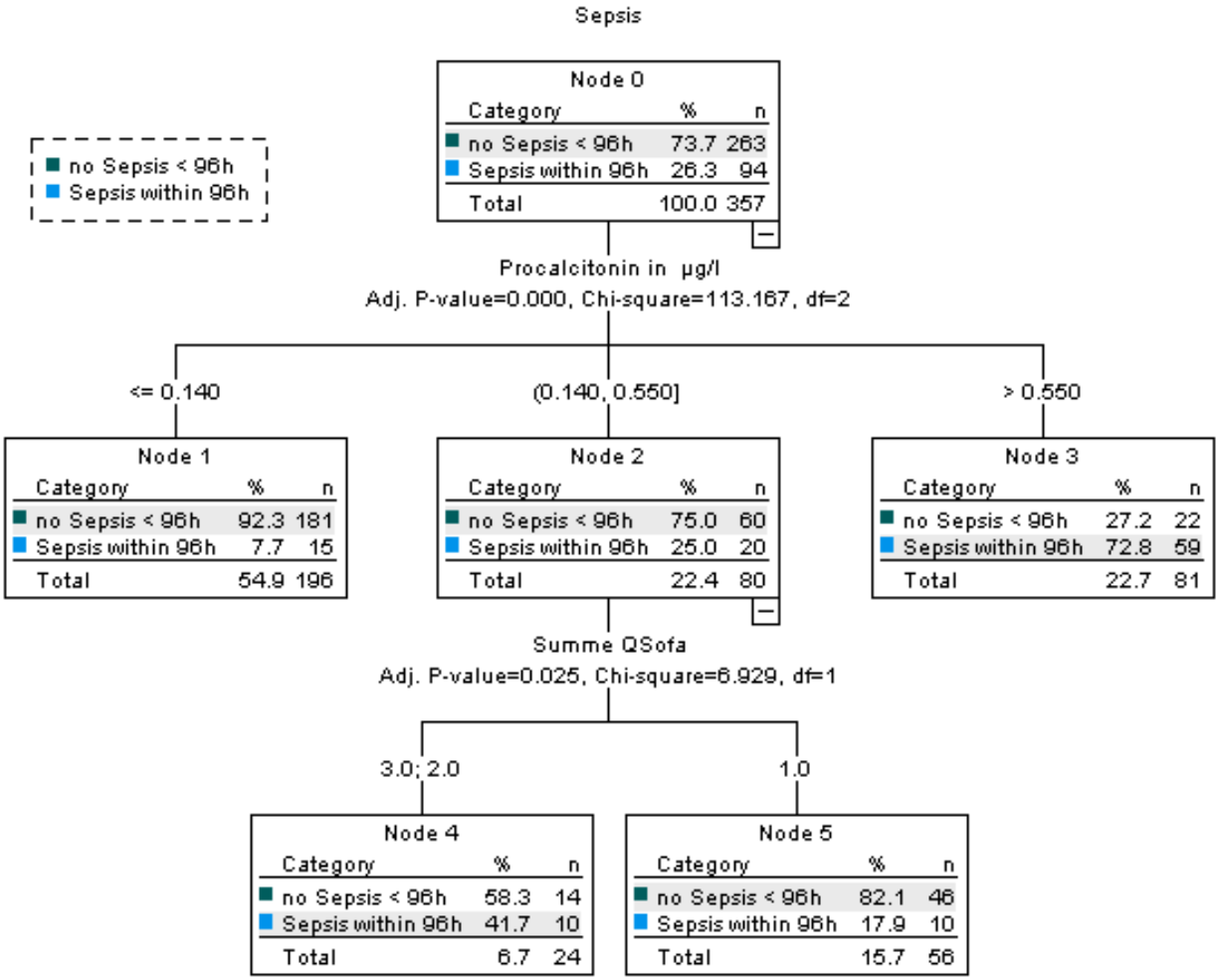


Figure S5B: Results of the classification tree analysis in the test sample.



Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

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Publication list

Myrto Bolanaki contributed the following to the below listed publications:

Publication 1: Bolanaki M, Möckel M, Winning J, Bauer M, Reinhart K, Stacke A, Hajdu P, Slagman A., Diagnostic Performance of Procalcitonin for the Early Identification of Sepsis in Patients with Elevated qSOFA Score at Emergency Admission, J Clin Med. 2021 Aug 28;10(17):3869. doi: 10.3390/jcm10173869. PMID: 34501324; PMCID: PMC8432218 (Impact Factor: 4,241)

Publication 2: Napierala H, Kopka M, Altendorf MB, Bolanaki M, Schmidt K, Piper SK, Heintze C, Möckel M, Balzer F, Slagman A, Schmieding ML. Examining the impact of a symptom assessment application on patient-physician interaction among self-referred walk-in patients in the emergency department (AKUSYM): study protocol for a multi-center, randomized controlled, parallel-group superiority trial. Trials. 2022 Sep 20;23(1):791. doi: 10.1186/s13063-022-06688-w. PMID: 36127742; PMCID: PMC9490986. (Impact Factor 2,728)

Publication 3: Möckel M, Bolanaki M, Hofmann J, Stein A, Hitzek J, Holert F, Fischer-Rosinský A, Slagman A. SARS-CoV-2 screening in patients in need of urgent inpatient treatment in the Emergency Department (ED) by digitally integrated point-of-care PCR: a clinical cohort study. Diagn Microbiol Infect Dis. 2022 Apr;102(4):115637. doi: 10.1016/j.diagmicrobio.2022.115637. Epub 2022 Jan 16. PMID: 35123377; PMCID: PMC8761116. (Impact Factor: 2,803)

Publication 4: Slagman A, Greiner F, Searle J, Harriss L, Thompson F, Frick J, Bolanaki M, Lindner T, Möckel M. Suitability of the German version of the Manchester Triage System to redirect emergency department patients to general practitioner care: a prospective cohort study. BMJ Open. 2019 May 6;9(5):e024896. doi: 10.1136/bmjopen-2018-024896. PMID: 31064804; PMCID: PMC6527986. (Impact Factor: 2,496)

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