LabMed

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Validation of AIA-360 for Determination of Presepsin: A Useful Tool for the Diagnosis of Sepsis at the Emergency Department

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Abstract: Sepsis is a life-threatening condition, and clinicians should diagnose it as soon as possible to enable rapid intervention. The study aims to validate the AIA-360 Presepsin (PSEP) test for its use in determination in the diagnosis of septic patients after admission to emergency departments (ED). A total of 97 blood samples were collected from patients at the ED and from blood donors of Tor Vergata Hospital. Here, 15 samples were obtained from patients with a confirmed diagnosis of sepsis, and 44 samples with non-septic inflammatory condition. A control group of 38 samples from healthy subjects was also included. The non-septic inflammatory condition group and the confirmed sepsis group had a median of 874.40 pg/mL and 1467.10 pg/mL, respectively, while the control group showed a PSEP median value of 473.90 pg/mL, thus showing a significant statistical difference among all groups. The ROC curves highlighted a good sensitivity (93.33%) and specificity (76.19%) for PSEP values, suggesting the best cut-off point of 890 pg/mL. (p-value < 0.001; Mann–Whitney test). The PSEP test can improve and speed up the diagnosis of sepsis after admission to the ED with respect to other biomarkers, mainly due to its early kinetics.

Keywords: emergency department; biomarkers; presepsin; sepsis; diagnosis

Academic Editors: Emmanouil Magiorkinis and Weiyong Liu

Received: 10 September 2024
Revised: 18 October 2024
Accepted: 29 November 2024
Published: 25 December 2024

Citation: Giovannelli, A.; Pieri, M.; Nicolai, E.; Pelagalli, M.; Calabrese, C.; Tomassetti, F.; Legramante, J.M.; Terrinoni, A.; Bernardini, S.; Minieri, M. Validation of AIA-360 for Determination of Presepsin: A Useful Tool for the Diagnosis of Sepsis at the Emergency Department. *LabMed* **2025**, 2, 1. https://doi.org/10.3390/labmed2010001

1. Introduction

Sepsis is a systemic syndrome, originally conceptualized as 'the decomposition of animal or vegetable matter in the presence of bacteria' [1]. Currently, sepsis is defined as 'a life-threatening condition that arises when the body's response to an infection injures its tissues and organs' [2]. Undoubtedly, this syndrome is a complex and multifaceted disorder that develops as a dysregulated host response to an infection associated with acute organ dysfunction, which poses a substantial risk of mortality. Sepsis occurs when the uncontrolled response of the body to an infection provokes an organ dysregulation that could evolve into a multiorgan dysfunction and septic shock. The financial burden of the sepsis syndrome is represented by a significant increase in healthcare costs, given

its frequent requirement of prolonged diagnosis and intensive care treatment [3]. Sepsis is one of the main causes of mortality in both emergency departments (ED) and intensive care units (ICU) [4], due to the main difficulty of the early recognition and appropriate identification of the etiology [5]. A meta-analysis estimated that about 31.5 million sepsis cases and 19.4 million severe sepsis cases occur each year, contributing 5.3 million deaths worldwide [6]. A wide range of pathogens can cause sepsis, such as bacteria, viruses, and fungi; however, bacterial infections represent most cases of sepsis. Currently, blood culture is considered the gold standard for the diagnosis of sepsis, although recent molecular tests performed directly on blood samples have promised faster diagnostics, with response times of a few hours [7]. Unfortunately, their implementation in clinical routine has still been hampered by critical technical and procedural problems, as well as higher costs in execution. However, blood culture is time-consuming and frequently yields almost 1/3 false negative results, and microbial contamination in the preanalytical phase can greatly affect its diagnostic value. Furthermore, up to 42% of cases of sepsis are culture-negative, suggesting a nonbacterial etiology [8]. The diagnosis of viral sepsis remains very rare, and the prevalence of viral sepsis is not known, because there is not enough information for an accurate estimation, although recent studies have described the relationship of sepsis and COVID-19 [9]. Any clinical sign or symptom could be directly related to this syndrome, making the diagnosis of sepsis particularly complex. However, for suspected cases of sepsis, a wide spectrum of laboratory tests is available. The early recognition of sepsis and adoption of the right therapeutic protocol are crucial to improving disease outcomes. It is a time-dependent disease and one of the main challenges for the ED, where the first medical contact with patients occurs [10]. The first clinical method to recognize organ damage caused by sepsis is the SOFA score (Sequential Organ Failure Assessment), and its surrogate, the quick SOFA (qSOFA), is used when the SOFA score is difficult to calculate [11]. The qSOFA evaluates altered levels of consciousness, systolic blood pressure ($\leq$100 mmHg), and respiratory rate ($\geq$22/min). Physicians can suspect sepsis when at least two of these parameters are altered together with other signs of infection. Even in the absence of the qSOFA criteria, any organ damage, not otherwise justifiable, should lead to suspicion of sepsis [12]. Clinical signs should be supported by laboratory tests that are useful in defining the severity of the disease and directing the most appropriate treatment.

Although blood culture remains the gold standard, different serum biomarkers have been tested for the diagnosis and prognosis of sepsis, such as procalcitonin (PCT), one of the most requested biomarkers for sepsis diagnosis by clinicians. It was observed that PCT notably increases during bacterial infection [13,14]; it is also used to evaluate the efficiency of the pharmacological therapy adopted. As is known, PCT is elevated in various conditions, such as in autoimmune diseases, tumors, severe trauma, invasive surgical procedures, and critical burn injuries [15–17].

Another marker used for the diagnosis of sepsis is the C-reactive protein (CRP), although generic and nonspecific. It is frequently elevated in both infectious and non-infectious systemic inflammatory response syndrome (SIRS), such as sepsis, but also inflammation, cardiovascular disease, trauma, and malignancy [18]. However, Ryoo et al. evaluated the utility of the combination of CRP and PCT to predict the prognosis in patients with septic shock, reinforcing the idea that evaluating multiple biomarkers at one time could help the diagnosis and treatment of patients with septic shock [19].

The mid-regional pro-adrenomedullin (MR-proADM) is the precursor molecule of adrenomedullin (ADM). Unlike ADM, which is unstable and characterized by a short half-life, MR-proADM is more stable and appears as a biomarker of microcirculatory and endothelial damage. It also plays a decisive role in both the induction of hyperdynamic circulation during the early phase of sepsis and the progression towards septic shock.

Plasma levels of MR-proADM are elevated in patients with different pathologies such as heart failure, cancer, and viral infection, so it could be non-specific [20–22].

Among these new emerging biomarkers of sepsis, PSEP appears to be one of the most promising for the diagnosis and evaluation of sepsis, as it is involved in activating the innate immune system [23]. Presepsin is a small 13 kDa protein, which is a soluble N-terminal fragment of CD14, a glycoprotein receptor expressed on the surface of various immune cells, such as monocytes, macrophages, and neutrophils. In response to infection, when monocytes are activated, after binding to lipopolysaccharides (LPS) and LPS-binding protein (LPB), CD14 releases a soluble fragment (sCD14) into the bloodstream [24]. Consequently, PSEP, derived from sCD14 cleavage by plasma proteases (lysosomal enzymes, cathepsin D), can be detected in blood flow and activate the inflammatory cascade, inducing a systemic inflammatory response, which could result in septic shock [25,26]. Some recent studies have affirmed that higher levels of PSEP were associated with the rate of positive blood culture [27,28], and that PSEP has a great prognostic value in the determination of sepsis and patient treatment [29,30]. However, little is known about PSEP and its determinations. To explore the role of PSEP in sepsis determination, our study aims to validate a new automated method, the AIA-360 immunological analyzer (Tosoh Bioscience Corporation, Tokyo, Japan), for the determination of PSEP and its use in the early diagnosis of sepsis upon admission to the ED. Furthermore, this work has focused on determining the best cut-off value of PSEP in ED patients, which could be useful for rapidly discriminating patients with confirmed sepsis from patients with non-confirmed sepsis.

2. Materials and Methods

2.1. Study Design

The study was carried out on 97 patients, of which 59 (group 1 and 2) (33 males and 26 females) were admitted to the ED of Tor Vergata University Hospital from June to November 2022 with suspected infection. Samples from 38 healthy blood donors were collected for the control group (group 0).

Inclusion criteria were age $\geq$ 18 years, patients admitted to the ED with clinical signs of sepsis suspicion, and healthy blood donors for the control group. Individuals aged < 18 or >90 years old were excluded. Furthermore, blood samples with hemolysis, icterus, and lipemia (HIL) were excluded to avoid interferences in PSEP test results.

The clinical diagnosis of suspected sepsis was performed using qSOFA score by physicians at ED admission and the presence of infectious pathogens was then confirmed by blood culture tests. Furthermore, samples from patients positive for COVID-19, processed and screened by nasopharyngeal swab upon hospital admission, were excluded from the study. The retrospective study design was evaluated and approved by the local Ethics Committee of Tor Vergata University Hospital (approval number 87/20) and carried out according to the revised Declaration of Helsinki.

The total study sample was stratified into three groups, as follows:

Group 0—Here, 38 samples (from 30 males and 8 females) were collected from healthy blood donors admitted to the Transfusion department of Tor Vergata University Hospital. All patients had a normal blood count, negative PCT and CRP levels, negative erythrocyte sedimentation rate (ESR) (less than 60 mm/h), and a negative molecular swab for the diagnosis of SARS-CoV-2. This group was used as the healthy control.

Group 1—Here, 44 samples from patients (23 males and 21 females) with a heterogeneous pool of inflammatory pathologies (2 cardiovascular, 1 traumatic, 14 respiratory, 3 gastrointestinal, 4 neoplastic, 12 metabolic, 2 neurological, and 6 infectious diseases patients) were analyzed. Most patients included in this group were positive according to CRP test, and negative according to PCT test. All patients were negative according to

microbiological blood culture and molecular swabbing for the diagnosis of SARS-CoV-2. No sepsis condition was developed within 30 days.

Group 2—Here, 15 samples from patients (10 males and 5 females) admitted to the ED with a clinical diagnosis of sepsis assessed by qSOFA score, then confirmed by positive blood cultures, were analyzed.

Peripheral blood samples from patients with suspected infection were collected from residual volumes after routine tests requested by clinicians at ED triage. The results of hematological examinations (white blood cell count, neutrophil blood count, and ESR) and biochemical tests (PCT and CRP) were retrieved from a laboratory information system (LIS). The samples used in this study as groups 1 and 2 were taken within 24 h after admission to the ED.

Upon arrival at the laboratory, blood samples after hematological tests were recovered and centrifuged within 60 min of venipuncture at 2000× g for 10 min at 4 °C to obtain plasma samples for the PSEP assay. Samples with a high grade of hemolysis, icterus, and lipemia (HIL) were excluded from the study to avoid interferences and the weak accuracy of the PSEP test. The plasma HIL index was obtained by Alinity c-series instruments (Abbott, Chicago, IL, USA). After centrifuging, the plasma chosen for the PSEP assay was stored at −20 °C until the assay, following the manufacturer's instructions. At the time of analysis, the samples were kept at room temperature for approximately 30 min, the time necessary for their thawing [29]. The sample volume required for PSEP analysis in the AIA-360 immunological analyzer (Tosoh Bioscience Corporation) was 20 µL.

Other parameters, such as PCT and CRP, were processed using the Alinity c series instrument (Abbott, Chicago IL, USA) on a serum matrix. Red and white blood cell counts were performed by the hemocytometer BC-6800 plus (Mindray, Shenzhen, China).

2.2. Instrument Characteristics

The instrument used for the PSEP assay was the AIA-360 (Tosoh Bioscience, Japan), an analyzer designed for immunometric assays. The ST AIA-PACK Presepsin is a two-site enzyme immunoassay that is carried out entirely in the specific kit of reaction cups for in vitro diagnostic use. In the test sample, PSEP is bound to a monoclonal antibody immobilized on a magnetic solid phase and to an enzyme-labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled antibodies and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the concentration of PSEP in the test sample. Twenty-five samples can be analyzed at one time, and the volume required for each analysis is 20 µL. The reaction time is 15 min, making it a rapid procedure. The concentration of PSEP is calculated automatically from the generated fluorescence and expressed in pg/mL. At the first use, a calibration curve was built, and before each analytical session, quality controls were analyzed and validated.

2.3. Precision Study

The precision of the PSEP test was evaluated using commercial AIA-PACK Presepsin L1 and L2 quality control materials (QCI) recommended by the manufacturer. Precision estimation was performed by evaluating triplicate measurements of QCI aliquots for each level (L1 and L2), performed for five consecutive days. Precision was determined following National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP05-A3 9Clinical and Laboratory Standards Institute (CLSI). Statistical quality control for quantitative measurement procedures: principles and definition. 4th edition. Wayne, PA: Clinical and laboratory standards institute; 2016) [31].

The within-run and between-run values were expressed as the percentage coefficient of variation (CV %) calculated as the standard deviation (SD) divided by the mean value.

2.4. Linearity Assessment

Linearity was assessed using a series of mixes of three sample pools, prepared with different PSEP values, using serial dilutions (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256) with a dilution sample provided by the manufacturer. All serial dilutions were performed two times according to the CLSI EP6-A protocol (Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach; Approved Guideline 2003) [31].

2.5. Carryover

The carryover was carried out by dividing samples with low PSEP values in 11 aliquots (L) and samples with high PSEP values in 10 aliquots (H). Aliquots were loaded into the analyzer in the following order: L, L, L, H, H, L, H, H, L, L, L, L, H, H, L, H, H, L, H, H, L. The difference between the mean of the low measurements after a high measurement and the mean of the low measurements after a low measurement was used as a measure for carryover.

2.6. Repeatability

Sample repeatability was assessed by calculating the CV% of a normal and pathological sample repeated twelve times (EP15-A3 User Verification of Precision and Estimation of Bias; Approved Guideline-Third Edition).

2.7. Statistical Analysis

Statistical analysis was performed with MedCalc Software Ver. 18.2.18 (MedCalc Soft-ware Ltd., Ostend, Belgium). The Shapiro–Wilk test was used to verify the normal distribution of the data. Differences between groups 0, 1 and 2 have been represented by the median and the 1st and 3rd interquartiles (Interquartile Range, IQR); the variables were compared through the Kruskal–Wallis test. The Mann–Whitney test was used, as a nonparametric alternative test to the independent sample t-test, to evaluate the difference between non-septic inflammatory condition samples and confirmed sepsis samples. The statistical significance level established for all tests was represented by a p-value < 0.05.

Receiver operating characteristic (ROC) curves were calculated, and the pathogenicity cut-off was extrapolated. The ROC curve is an analytical method that graphically represents the performance of a binary diagnostic classification method, interpreting the data in a dichotomous form to assess the presence or absence of a specific condition [32]. For each generated ROC curve, reported as area under the curve (AUC), a confidence interval (CI) was assumed at 95%. The cut-off was determined by calculating the Youden index, a summary measure of the effectiveness of the ROC curve, while the negative predictive values (NPV) were calculated by applying the Bayes theorem.

3. Results

3.1. Discrimination Among Study Groups

Samples obtained from ED were divided into two groups (1 and 2) and analyzed with the AIA-360 instrument. As previously described, the control group (0) of healthy blood donors was enrolled in the Transfusion department. In group 1, negative PCT levels (<0.5 ng/mL) were detected in 77% of patients, while 23% of patients showed positive PCT. Positive CRP levels (>5.0 ng/mL) were detected in 93% of patients, and 7% of them had negative CRP levels. In group 2, all patients had PCT and CRP levels above the normal

cut-off (0.5 ng/mL and 5 ng/mL, respectively). The medians and IQRs for groups 1 and 2 are reported in Table 1.

Table 1. Characteristics of study population.

	GROUP 0	GROUP 1	GROUP 2
SAMPLES	38	44	15
BIOLOGICAL SEX	MALE: 30 FEMALE: 8	MALE: 23 FEMALE: 21	MALE: 10 FEMALE: 5
HOSPITAL UNIT	TRANSFUSION DEPT	EMERGENCY DEPT	EMERGENCY DEPT
CLINICAL SIGNS	NONE, GOOD HEALTH	FEVER	FEVER, TACHYPNEA, ALTERATION OF CONSCIOUSNESS STATE, HYPOTENSION
PROCALCITONIN	<0.01	77% NEG; 23% POS 0.36 (IQR 0.16 to 0.72)	100% POS 6.0 (IQR 1.31 to 14.32)
C-REACTIVE PROTEIN	<1.00	93% POS; 7% NEG 61.6 (IQR: 39.75 to 103.92)	100% POS 140.8 (IQR: 81.75 to 221.37)
SARS-CoV-2 RT-PCR SWAB	NEG	NEG	NEG
BLOOD CULTURE	N/A	NEG	POS
SEPSIS DIAGNOSIS	N/A	NEG	POS

IQR: Interquartile range.

3.2. Validation of AIA-360 Instrument

Precision was evaluated using the AIA-PACK Presepsin internal quality control (IQC) levels 1 and 2. Precision estimations were obtained by evaluating triplicate measurements of aliquots of IQC, performed for a total of 5 consecutive days. The precision results obtained were compared to those claimed by the manufacturer using the procedure recommended by the same protocol.

The percentage of coefficients of variation (CV %), calculated as repetitive measurements of AIA-PACK Presepsin control levels 1 and 2, in comparison with the CV declared by the manufacturer, are reported in Table 2.

Table 2. Precision results of PSEP assays.

	Within-Run		Between-Run		Total Precision	
	CV% Manufacturer	**CV% Laboratory**	**CV% Manufacturer**	**CV% Laboratory**	**CV% Manufacturer**	**CV% Laboratory**
ICQ Level 1	/	3.5	3.8	3.8	4.5	4.8
ICQ Level 2	/	2.7	4.5	1.8	4.5	2.8

CV%: coefficient variation.

For IQC level 1, the total precision showed a 4.8% laboratory CV, and, for IQC level 2, a laboratory 2.8% CV was seen, in contrast to the 4.5% value declared by the manufacturer. The between-run CV of 3.8% was also derived for the laboratory and manufacturer IQC level 1. However, IQC level 2 showed a laboratory CV of 1.8%, in contrast to the manufacturer's declared CV of 4.5%. The within-run CVs of the laboratory for the IQC level 1 and level 2 were 3.5% and 2.7%, respectively, while the CV percentages were not declared in the datasheet of the manufacturer.

Linearity was evaluated and the results are presented in Figure 1.

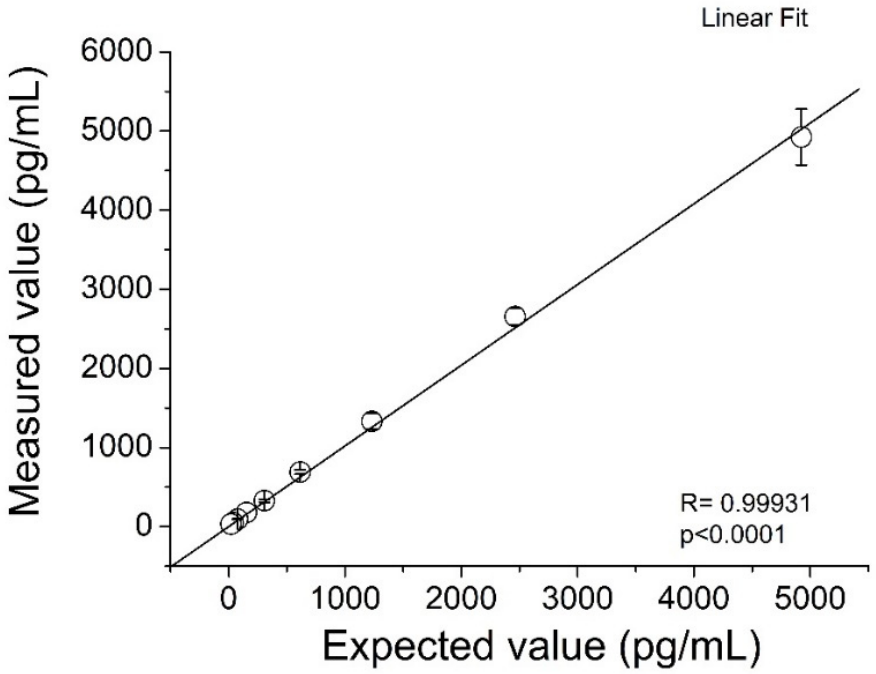

Figure 1. The results of the PSEP assay for linearity. Averages of duplicate measurements are shown.

The linearity tests indicate a p-value < 0.0001 and a correlation coefficient equivalent to 0.99931. The linearity for ST AIA-PACK Presepsin is between 20 and 20,000 pg/mL. The cut-off values recommended by the Company for PSEP are shown in Table 3.

Table 3. Criteria for assessing the risk of PSEP levels.

Presepsin pg/mL	Diagnosis
<200 pg/mL	Sepsis excluded (Negative Predictive Value, NPV = 98%)
<300 pg/mL	Systemic infection is not probable
<600 pg/mL	Systemic infection (sepsis) is possible
<1000 pg/mL	Significant risk of progression of systemic infection (sepsis), increased risk of unfavorable outcome
>1000 pg/mL	High risk of progression of systemic infection (sepsis, septic shock); high risk of 30-day mortality comparable to SOFA score ≥ 8

In the carryover analysis, the results obtained did not show a significant carryover effect (p = no significance, n.s.), as illustrated in Table 4.

Table 4. Carryover analysis.

CARRYOVER	pg/mL
HIGH–LOW Media	172.784
Minimum Concentration	167.545
Maximum Concentration	4935.995
LOW–LOW media	174.25
Carryover effect	−1.4662
Error Limit	15.40710327

Repeatability was evaluated and the results are reported in Table 5.

Table 5. Repeatability analysis.

Samples	Media (pg/mL)	SD	CV%
Normal	386.44	14.84	4
Pathological	2679.64	80.27	3

SD: standard deviation; CV%: coefficient variation.

The repeatability test indicates a 4% CV with a mean of 386.44 pg/mL (±14.84 pg/mL) for a normal sample, while a 3% CV with a mean 2679.64 pg/mL (±80.27 pg/mL) is seen for the pathological sample.

The median concentrations of PSEP in plasma samples were 473.90 pg/mL (IQR 345.35–598.82 pg/mL) in the control group (n = 38), 874.40 pg/mL (IQR 633.95–1171.55 pg/mL) in the non-septic inflammatory condition group (n = 44) and 1467.10 pg/mL (IQR 1164.20–2782.10 pg/mL) in patients with confirmed sepsis (n = 15).

The medians of the three groups differ significantly from each other (Kruskal–Wallis test, $p < 0.001$). Statistical results are reported in Table 6.

Table 6. Median values of PSEP; * the asterisk represents significance with the other two groups (Kruskal–Wallis test, $p < 0.001$).

Presepsin (pg/mL)	n	Minimum	25th Percentile	Median (pg/mL)	75th Percentile	Maximum
Control group (0)	38	84.30	345.35	473.90 *	598.82	1066.70
Non-septic inflammatory condition group (1)	44	283.60	633.95	874.40 *	1171.55	5112.20
Confirmed sepsis group (2)	15	314.70	1164.20	1467.10 *	2782.10	16,272.20

Therefore, the level of PSEP in confirmed sepsis patients (group 2) was almost twofold elevated with respect to group 1 patients.

3.3. ROC Analysis of Presepsin: Diagnostic Role as Sepsis Biomarker

The receiver operating characteristic (ROC) curves were designed to demonstrate the difference between non-septic inflammatory condition samples and confirmed sepsis samples. This analysis revealed an optimized cut-off value of 890 pg/mL for PSEP, showing a high discriminatory power (AUC = 0.876; 95% CI: 0.705 to 0.934; $p < 0.001$). These results are shown in Figure 2.

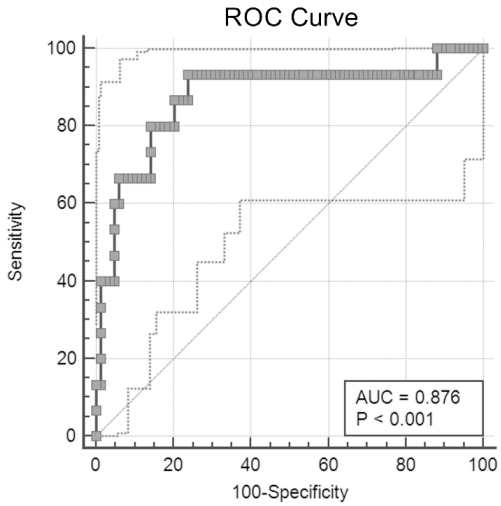

Figure 2. ROC analysis of PSEP for discrimination between the non-septic inflammatory condition group and the confirmed sepsis group.

The best diagnostic PSEP cut-off of 890 pg/mL corresponds to 93.33% sensitivity (95% CI: 68.1 to 99.8) and 76.19% specificity (95% CI: 65.7 to 84.8) (Table 7).

Table 7. Performance of PSEP, PCT and CRP for the diagnosis of sepsis.

Biomarker	AUC	Cut-Off Level	Sensitivity (%) (CI 95%)	Specificity (%) (CI 95%)	LR (+) (CI 95%)	LR (−) (CI 95%)
Presepsin (pg/mL)	0.876 (CI: 0.705 to 0.934)	890 a	93.33 (68.1−99.8)	76.19 (65.7−84.8)	3.92 (2.6−5.9)	0.088 (0.01−0.6)
Procalcitonin (ng/mL)	0.926 (CI: 0.813 to 0.982)	0.5	100 (78.2−100)	69.70 (51.3−84.4)	3.30 (2−5.5)	0
C-Reactive Protein (mg/L)	0.718 (CI: 0.579 to 0.832)	97.7	73.33 (44.9−92.2)	74.36 (57.9−87)	2.86 (1.5−5.3)	0.36 (0.2−0.8)

CI, confidence interval; LR (+), positive likelihood ratio; LR (−), negative likelihood ratio; a, according to the Youden index.

The ROC curve for PCT, PSEP and CRP is shown in Figure 3.

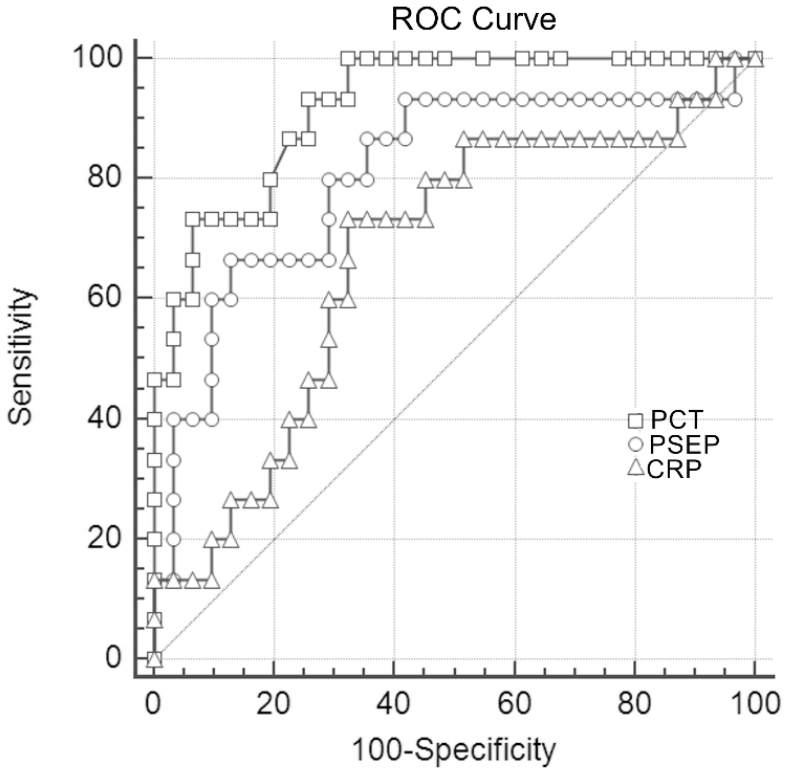

Figure 3. ROC curve for PCT, PSEP and CRP for discrimination between the non-septic inflammatory condition group and the confirmed sepsis group.

The ROC AUC for PCT was 0.926 (95% CI: 0.813 to 0.982; $p < 0.001$). This value is higher than that of the CRP ROC AUC (0.718; 95% CI: 0.579 to 0.832; $p < 0.001$) and PSEP (0.876; CI: 0.705 to 0.934; $p < 0.001$). Selected cut-off levels for CRP, PCT and PSEP and their diagnostic accuracy for sepsis are shown in Table 7.

Samples of groups 1 and 0 have been combined and used as the control because they belong to a non-septic inflammatory condition. The median values for a non-septic inflammatory condition (groups 1 and 0) and confirmed sepsis samples (group 2) are reported in Figure 4.

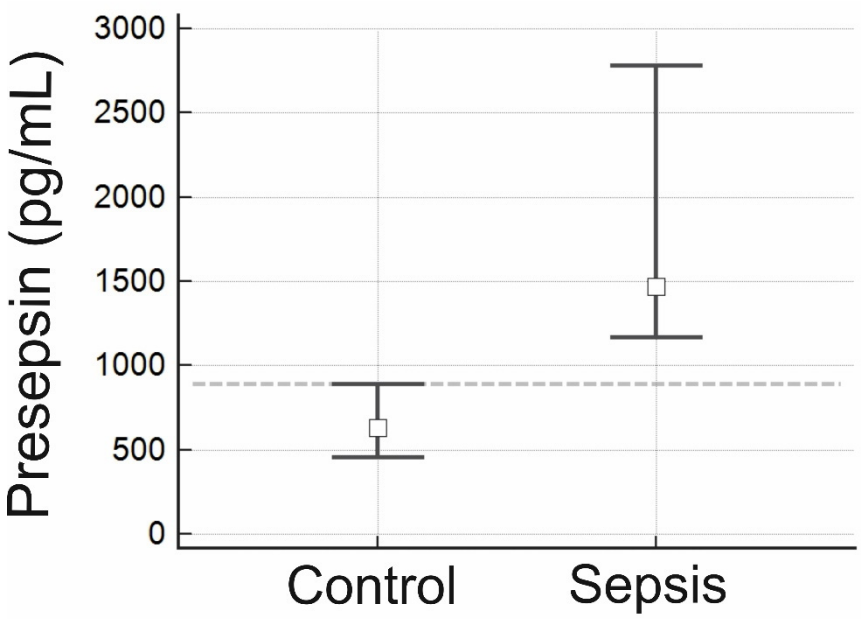

Figure 4. The median values of PSEP (boxplots) for non-septic inflammatory condition samples in comparison with confirmed sepsis samples. The dotted lines represent the cut-off of 890 pg/mL.

The results obtained, including the best cut-off value, as calculated by the PSEP ROC curve (Figure 2) and represented by dotted lines, are shown in Figure 4. The control group is below the cut-off value.

Statistical analysis showed a significant difference between the two groups, with a p-value < 0.001, given by the Mann–Whitney test.

4. Discussion

Sepsis, a global health problem, is a life-threatening multiorgan dysfunction related to a dysregulated systemic response of the host to infection [2,10]. It is one of the leading causes of death in hospitals, and its diagnosis must take place quickly to ensure the right therapeutic protocol, is adopted especially for patients admitted to the ED [33]. Nowadays, many single or combined biomarkers are used to make an early diagnosis, assessment, and prediction of mortality in patients with sepsis, such as PCT, CRP, interleukin, MR-proADM, etc. [34,35]. Unfortunately, most of them do not play a crucial role as an early biomarker [36]. Therefore, it is imperative to identify a useful biomarker for early diagnosis, with high effectiveness and efficiency, as well as good sensitivity and specificity. In addition, our results demonstrate the great performance of the automated instrument, AIA-360, showing good precision, repeatability, great linearity, and no carryover effect. From the results presented, PSEP appears to be a promising biomarker for the evaluation of sepsis in discerning the control groups from the confirmed sepsis group (p < 0.001) at the ED, and in assisting physicians in the initial evaluation of patients. The population of our study was divided into three groups: a control group, a group with non-septic inflammatory condition, and a group with confirmed sepsis. The first two groups were considered a unique group (control) due to the similar clinical and features of their conditions. The data thus obtained show that PSEP appears to be one of the more promising sepsis markers, with a diagnostic cut-off of 890 pg/mL, corresponding to high sensitivity (93.33%) and good specificity (76.19%). This cut-off is between the values of 600 and 1000, as shown in Table 3, provided by the manufacturer. Moreover, for a cut-off of 1000 pg/mL, we calculated sensitivity and specificity values of 86.67% and 80.25%, respectively.

Additionally, further studies could demonstrate its further role in sepsis in the intensive care setting, where patients are more prone to multiple organ failure conditions [37].

Indeed, the early increase in PSEP levels during the septic cascade and other bacterial infections has made it an attractive indicator for laboratory tests [38].

Another study highlighted the important role of PSEP as an early sepsis biomarker, identifying it as a better disease predictor with respect to IL-6, CRP, and PCT in evaluating the risk of death within 30 days after the onset of sepsis [4]. Furthermore, the PSEP test with the AIA-360 instrument could have some advantages in the management of septic patients admitted to an emergency department, due to the rapid and precise results obtained.

The study by de Guadiana et al. also investigated PSEP as a biomarker in the detection of infection and sepsis with another instrument [38]. Their work supported the use of this early biomarker with a cut-off of 849 ng/L. This result agrees with our findings, although in our study, the values of sensitivity and specificity obtained were higher than in their results, suggesting the better performance of the AIA-360 instrumentation. Other possible causes of this difference should be imputed to the different populations studied.

In Figure 3, the comparison of the ROC curves highlights that the diagnostic value of PSEP (AUC: 0.876) is comparable to that of PCT (AUC: 0.926), unlike CRP (AUC: 0.718), which is a nonspecific marker of infection.

Our experience suggests that PSEP can be used in the ED to quickly identify patients who could develop sepsis. The optimal sensitivity and specificity obtained to diagnose sepsis reveal PSEP as an early-stage biomarker of sepsis, as confirmed by other investigations [39]. Rapid diagnosis and treatment play a vital role in improving prognosis in patients with sepsis. It takes only 15 min to measure PSEP and can be tested at the bedside, which means it is fast, convenient, and has financial accessibility. These features make the AIA-360 a very useful instrument in critical areas, especially at ED, where rapid quantitative results are required. For this purpose, PSEP could be a useful biomarker in reducing the workload of ED, thus favoring the hospital rule-in or rule-out of patients already at the triage stage.

These preliminary findings allow us to further support previous studies, and reinforce the idea that PSEP would be a valuable marker that could be used to distinguish between non-infection and sepsis with high accuracy. However, limitations due to sample size should be considered, and further studies are needed on PSEP clinical values [40]. Although a strong body of literature favors the validity of PSEP as a biomarker for the diagnosis and stratification of the risk of sepsis, its measurement is not yet ubiquitously available as a routine laboratory test, and it should be analyzed with other laboratory biomarkers, such as PCT, CRP, and MR-proADM.

Likewise, the complete blood count (CBC), red blood cell, white blood cell, and platelet count should always be investigated via laboratory and clinical analysis, as an instrumental method for the early identification of patients at high risk of developing sepsis [41].

5. Conclusions

Presepsin could have some value in assessing the severity of sepsis and, as test results are available within the '1 h bundle' (as indicated in the last Sepsis Surviving Campaign 2018) [42], widespread clinical use is feasible, although it is not clear at this time whether it would significantly affect clinical practice. As sepsis syndrome remains an entity with high mortality rates and increased socio-economic implications, the 'ideal' biomarker, with high specificity, sensitivity, rapid and broad-based detection and minimal invasiveness, as well as requiring clinical specimens with low sample volumes to monitor the progress of sepsis, has not yet been identified. More research is warranted to evaluate the role of PSEP alone or in combination with other biomarkers in the assessment of sepsis. Over the past few years, machine learning predictive models for the recognition of sepsis have gained enormous attention by combining PSEP with routine laboratory parameters, seeking a more accurate

model for the diagnosis of sepsis. However, no model can yet be widely adopted in the real world, due to the lack of a unified validation standard and procedure.

Author Contributions: Conceptualization, S.B. and M.M.; methodology, M.P. (Massimo Pieri); validation, A.G., C.C., M.P. (Martina Pelagalli) and F.T.; formal analysis, M.P. (Massimo Pieri); investigation, E.N.; data curation, A.T.; writing—original draft preparation, A.G. and M.M.; writing—review and editing, A.G., M.M., J.M.L. and M.P. (Massimo Pieri); visualization, A.T.; supervision, M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Tor Vergata University Hospital (approval number 87/20, approval date is 26 May 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available on specific requests.

Conflicts of Interest: The authors declare no conflicts of interest.

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