Sepsis 'PSP test' on the abioSCOPE Device: 5 minutes to save lives

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Abstract

Sepsis, which is the second cause of mortality worldwide, is a life-threatening condition that needs immediate diagnosis and treatment to maximize the chances of survival and to reduce the risk of long-term disabilities. Due to the heterogeneous symptoms of sepsis, early recognition remains a challenge for Healthcare Professionals globally.

Current biomarkers, in particular procalcitonin (PCT) and C-reactive protein (CRP) are neither sensitive nor specific enough to aid clinicians in diagnosing sepsis nor in guiding clinical management efficiently.

At the start of 2020, Abionic received CE certification for its novel sepsis test on the rapid IVD platform abioSCOPE (also certified 2 months earlier) based on the measurement of Pancreatic Stone Protein (PSP). From a drop of fingertip blood, the test delivers results in as little as 5 minutes. Abionic exploits the special properties of fluids at the nanoscale to enhance the rate of formation of molecular interactions and thereby is able to quantify biomolecular complexes in the sub-picomolar range after a few seconds of incubation.

We demonstrate here that the PSP test on the abioSCOPE device has analytical specifications in line with clinicians' expectations. The assay reportable range spans from 0 to 699 ng/ml with an LoD of 3.21 ng/ml. The test is insensitive to interferences that may be caused by heterophilic antibodies and rheumatoid factor, as well as to clinically extreme PSP concentrations.

We also present patient case reports that support the clinical utility of quick PSP measurements at the patients' bedside for an earlier detection of sepsis, in standard practice and also in COVID19 patients in view of the current global pandemic. Sepsis is a medical emergency that must be promptly identified and correctly managed. The combination of rapid, accurate and precise diagnostic tests may contribute to reduce the burden of sepsis worldwide.

1. Introduction

1.1. The Incidence and Associated Cost of Sepsis Increases Dramatically Worldwide

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection[1]. It is a global crisis, and in January 2020 a new study published in the Lancet showed that over 48 million people are affected worldwide every year, of which more than 11 million result in casualties[2]. This is the second cause of death after cardiovascular diseases. In addition, the price tag that comes with sepsis is enormous for healthcare organizations: sepsis-related costs in U.S. hospitals surpass $24 billion annually, making it the most expensive disease to manage[3]. On May 2017, the World Health Assembly (WHA) and World Health Organization (WHO) made sepsis a global health priority and adopted a resolution urging the 194 United Nations Member States to improve the prevention, diagnosis, and management of sepsis[4]. Thus, the implementation of strategies that incorporate early recognition and timely management of sepsis are imperative to improving patient outcome[5].

1.2. Early Diagnosis of Sepsis Saves Lives

Sepsis symptoms are often unclear and unspecific and as a result sepsis is often overlooked or recognized too late. Th current international Sepsis-3 definition relies on a sequential organ failure score (SOFA). Mortality from sepsis increases by about 8% per hour of delayed appropriate administration of antibiotics[6]. Early identification and treatment of sepsis can also reduce the cost of sepsis-related care[7,8]. Therefore, early diagnosis along with timely and appropriate clinical management of sepsis, such as optimal antimicrobial use and fluid resuscitation, are crucial to increase the chances of survival and currently recommended in the latest international guidelines of the Sepsis Surviving Campaign[9].

1.3. PSP is a Novel Biomarker to Identify Sepsis Better and Earlier

Pancreatic stone protein (PSP) is a 16 kDa C-type lectin protein produced mostly by the pancreas and the intestine and whose blood level increases early in the onset of sepsis[10]. The pathophysiological mechanisms of PSP functions remain only partially understood (Figure 1). In a landmark paper comparing several emerging sepsis biomarkers in an unselected ICU and high-dependency care population, Llewelyn and colleagues found that PSP was the best marker to distinguish sepsis from non-infectious inflammation (sensitivity 90%, specificity 83%)[22]. In the past years several other independent clinical trials have evaluated the diagnostic accuracy of PSP versus reference biomarkers in critically ill patients and confirmed Llewelyn's findings (reviewed in [11]).

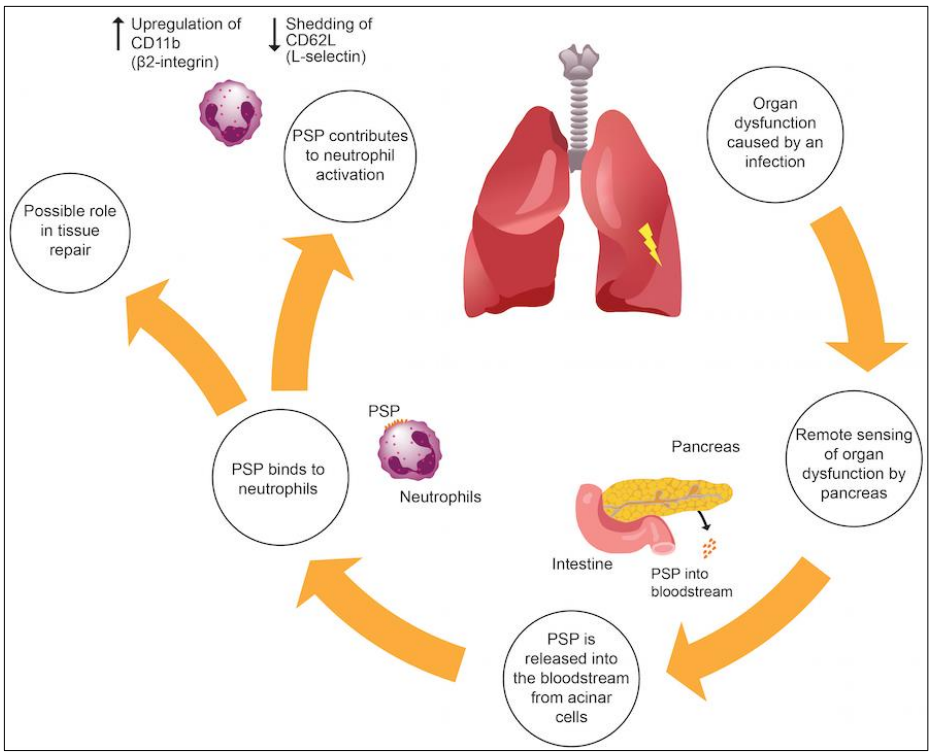


Figure 1 | Hypothetical model of the role of PSP in sepsis. In response to an infection-caused organ dysfunction (1), the pancreas remotely senses this event and responds by releasing PSP from acinar cells into the blood stream (2)[10,12]. PSP is a C-type lectin protein family member[14], one which is well known to be involved in the response to inflammatory stress and infection[15]. PSP has been shown to promote the shedding of CD62L and upregulate the expression of CD11b on the cell surface of neutrophils (3)[13]. Neutrophils with an increased CD11b expression interact with ligands on damaged endothelial cells, resulting in high affinity adhesion to the vascular endothelium, extravasation and migration to the site of tissue damage[16,17]. Proteolytic enzymes and reactive oxygen species (ROS) are subsequently released by neutrophils to kill pathogens[16]. However, in an exaggerated inflammatory response during sepsis, this may also cause unwanted tissue and microvascular damage that can ultimately lead to organ dysfunction (4). It has also been shown that PSP plays a role in tissue regeneration[4], but this remains to be demonstrated in sepsis (5).

1.4. Abionic CE marked the first PSP Sepsis Test for its abioSCOPE Platform

Nanofluidics is the study of a liquid's behavior at the nanoscale[18]. In such dimensions, it is in fact possible to take advantage of the 'forced' biomolecular interactions happening in the nanospace in order to develop immunoassays able to detect minute concentrations of analyte in complex matrices, such as blood[19]. We developed a nanofluidic PSP immunoassay that quantifies PSP from a drop of capillary whole blood in as little as 5 minutes. The test principle relies on the passage of a specimen, previously mixed for a few seconds with a solution containing the fluorescently labelled detecting antibody, through a nanometric-size channel in which anti-PSP antibodies are immobilized. These antibodies capture the PSP bound to the fluorescent detecting anti-PSP antibodies. The abioSCOPE, a tabletop size, easy-to-operate device, reads the fluorescence emission from the PSP sensor and converts the signal, employing advanced signal processing, into a concentration thanks to the assay's embedded, lot-specific calibration.

We demonstrate here that the technical performances of the PSP assay in the abioSCOPE device fulfil clinician's needs and match the analytical specifications of similar assays routinely performed in clinical laboratories on large automates. The abioSCOPE received the European certification CE March 2020

2. Methods and Analytical Performances

2.1. Nanofluidic Homogeneous Immunoassay Enables Ultra-Fast Total Turnaround Time

The principle of the PSP nanofluidic immunoassay relies on the enhanced biomolecular interactions that occur between the anti-PSP capture antibodies immobilized on the sensing area of the biosensors and the PSP molecules present in the sample (Figure 2a). For the ease of manipulation, the nanofluidic biosensors are embedded in a capsule holder onto which the sample is deposited (Figure 2b). Passive capillary forces drive the sample through the system. This single-use capsule is then introduced into the tabletop size reader (the 'abioSCOPE'), which automatically analyzes the signal. It takes no more than 5 minutes for the results to be displayed on the large touchscreen of the device.
In agreement with the nanofluidic principles published in several academic works[19, 20] and confirmed for several analytes in the abioSCOPE device[21], a near 100% analyte capture efficiency is observed in the sensors with analyte capture profiles showing a dose-dependent signal increase (Figure 3).

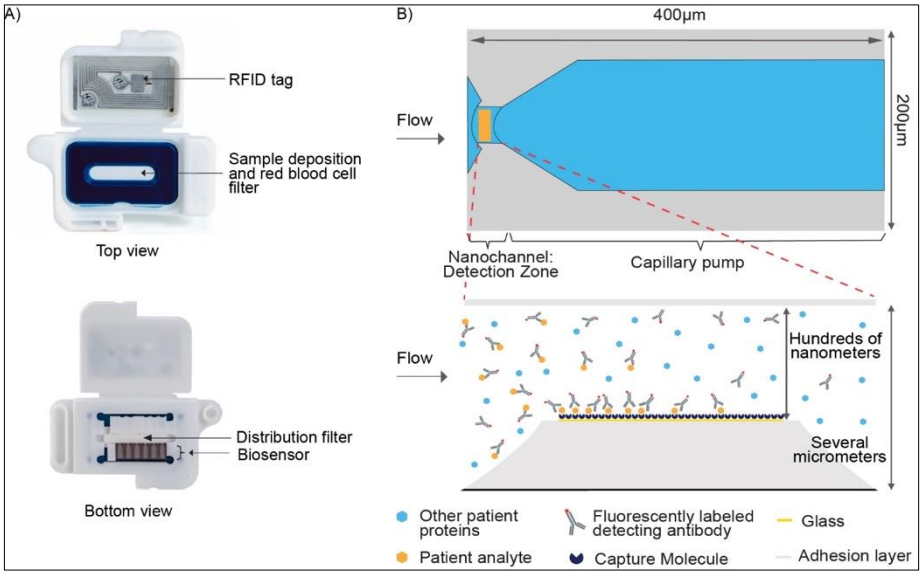


Figure 2 | Fabrication and functionalization of MEMS nanofluidic sensors embedded in a plastic injection molded capsule. (A) Picture of the top and bottom view of the open capsule holder in which the sensors are embedded. A RFID tag placed in the capsule lid to communicate with the device. The sample is deposited onto a membrane that filters the blood and drives the sample by capillary action to the entry of the biosensors. (B) Conceptual drawing of the sensors (top panel) top view showing the microfluidic entry channels (on the left side) and the connected nano-scaled channel (orange area) that is biofunctionalized to capture PSP. The sample flows from the left to the right through passive capillary action triggered by the high surface-to-volume ratio of the hydrophilic reservoir. Magnification on the functionalized nanochannel (side view, not to scale, bottom panel). The sensing surface area is specifically modified to immobilize capture molecules, while the surrounding surfaces are coated with a hydrophilic polymer to prevent non-specific adsorption of biomolecules.

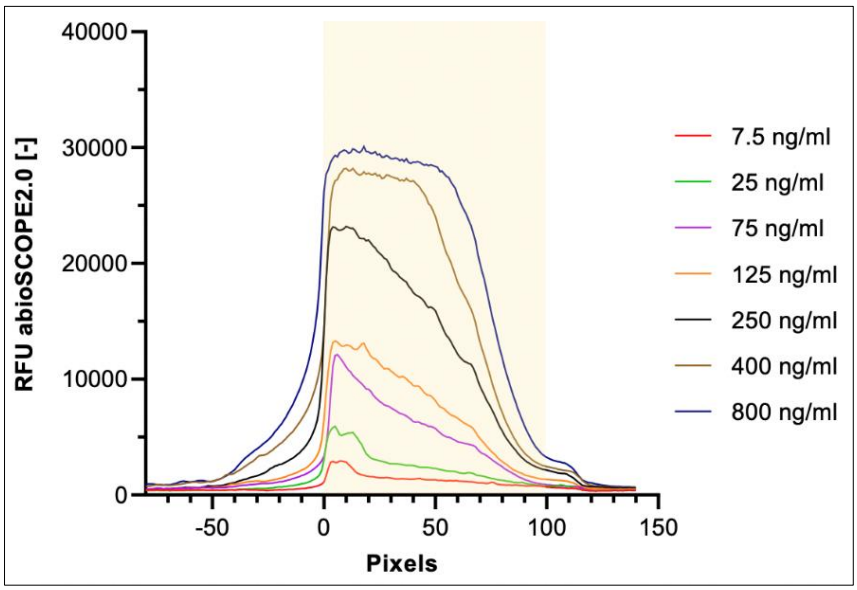


Figure 3 | Highly efficient capture of analyte thanks to nanofluidics. Average of the relative fluorescent unit (RFU) values are plotted as a function of the position in the nanochannel, expressed in pixels, for different concentrations of PSP. The yellow area delineates the boundaries of the sensing area. Data are from one sensor per dose, representative of 15 replicates.

2.2. Assay Calibration

From the signal output of the different PSP doses, a calibration curve is generated using a standard 5 parameter logistic (5-PL) fitting strategy to maximize accuracy and precision in the clinically relevant range of the assay (Figure 4). Abionic has developed internal calibrators along with a calibration strategy that allows a mean percentage recovery of 99.98% (ranging from 96.5% – 103.1%) and an average imprecision of 6.71% in the range 20 to 600 ng/ml.

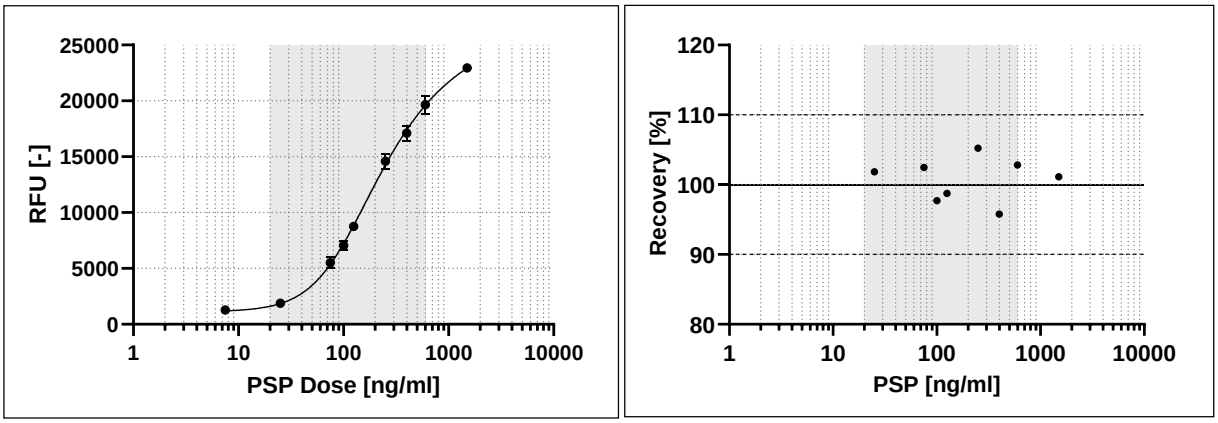
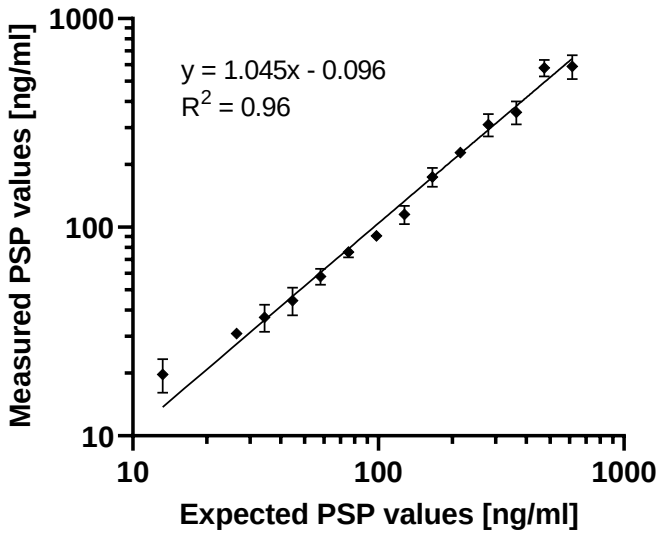


Figure 4 | Semi-log graph representing a dose-response calibration curve for PSP in the abioSCOPE device. Left panel shows the 5PL fit of 9 calibrator doses when plotted against measured RFU; right panel shows the percentage recovery back to the theoretical values (%RE) for each individual dose after back-calculation with the curve. Each calibrator dose was measured in triplicate and data points correspond to the mean of replicates. RFU: relative fluorescent unit, PSP: pancreatic stone protein.

2.3. Assay Linearity and Detection Capabilities

The linearity of the PSP test in the abioSCOPE was demonstrated through a dilution-recovery analysis from a pool of six human sera with clinically elevated PSP levels that was diluted in PSP-depleted serum. The PSP assay was found to be linear from the lower limit of 20.0 ng/ml to an upper limit of 591.0 ng/ml (Figure 5).



Order	Coef. Symbol	Coef. Value	R^2
First	b_0	-0.096	0.96
	b_1	1.045	
Second	b_0	-11.198	0.98
	b_1	1.204	
	b_3	$3 \cdot 10^{-4}$	
Third	b_0	20.521	0.98
	b_1	0.418	
	b_3	$32 \cdot 10^{-4}$	
	b_4	$-4 \cdot 10^{-6}$	

Figure 5 | Linearity of the PSP assay on the abioSCOPE device. The left graph displays the linear regression fit and equation of the average of triplicates measured per dilution. Data are shown as the PSP value measured in abioSCOPE [ng/ml] against the theoretical value [ng/ml]. Standard deviation error bars displayed per dose as well as the 95% CI shown in dotted lines. The table on the right displays the first, second and third polynomial fits of the data, showing no significant improvement of the R^2 between the first, second and third polynomials, thus supporting the claim that the assay is linear across the range of 20 - 591.0 ng/ml. PSP: Pancreatic stone protein, CI: Confidence interval.

The limit of blank (LoB) and limit of detection (LoD) were measured to determine the detection capabilities of the PSP assay. The LoB was determined to be 2.72 ng/ml and LoD was found to be 3.21 ng/ml.

2.4. Precision Testing

The precision of the PSP assay on the abioSCOPE device was estimated in a between-run study that included 10 replicates covering 3 doses of PSP (low, intermediate and high) as measured on the same device, same day, same operator and same product lot. The average imprecision of the test was 8.9% across three doses (Table 1). The device-to-device and lot-to-lot imprecision was then evaluated with a limited number of replicates. The measurements indicate a good comparability of test results across devices and lots (Table 2).

PSP Dose [ng/ml]	N [-]	Mean [ng/ml]	SD [-]	CV [%]
Low	10	49.5	2.3	7.2
Intermediate	10	110.8	10.8	8.0
High	10	176.8	25.4	11.5
Average imprecision				8.9

Table 1 | Between-run imprecision of the PSP assay on the abioSCOPE device. The average between-run imprecision, calculated as the mean coefficient of variation issued from 10 replicates obtained on a same device with a same lot, was 7.2% for a low dose sample, 8.0% for an intermediate dose sample and 11.5% for a high dose sample. PSP: Pancreatic stone protein, N: Number of replicates, SD: Standard deviation, CV: Coefficient of variation.

	Sample #1 (low)			Sample #2 (intermediate)		
	Mean [ng/ml]	SD [-]	CV (%)	Mean [ng/ml]	SD [-]	CV (%)
Device-to-device	73.8	2.5	3.5	118.8	3.9	3.3
Lot-to-lot	76	3.1	4.1	112.6	8.8	7.9
Total imprecision	75.7	9.1	12.0	113.5	17.1	20.1

Table 2 | Device-to-device and lot-to-lot imprecision of the PSP assay on the abioSCOPE device. Two PSP doses (Sample #1 and Sample #2) were measured in triplicate on four devices and two product lots, for a total of 24 datapoints. Device-to-device and lot-to-lot imprecisions were computed as the ratio, expressed in percent (coefficient of variation), of the standard deviation of the replicates by the mean value of the replicates (either by taking the devices as a variable or the lots). Total imprecision is computed as the ratio, expressed in percent (coefficient of variation), of the standard deviation of all replicates for a given dose by the average value of these replicates. CV: coefficient of variation; SD: standard deviation.

2.5. Interference Testing

The PSP assay was evaluated for endogenous substances known to potentially interfere with results of immunoassays. At a PSP concentration of 400 ng/ml, negligible biases in test results were observed when clinically elevated concentrations of Human anti-mouse antibodies or rheumatoid factors were present (Table 3). No high dose Hook effect was observed at clinically extreme PSP concentration (5000 ng/ml was the highest dose tested).

Tested substance	Highest tested doses	PSP [ng/ml]	Bias [%]
HAMA[1]	651 ng/ml	400	1.8
Rheumatoid factor	1164 ng/ml	400	4.0
High dose PSP	5000 ng/ml	5000	Reported as "> ULOD"[2]

Table 3 | Interferences caused by HAMA and RF, as well as high PSP doses. The bias is computed as the difference in the results between the control sample (without the interferent) and the test sample

(contains the interferent), expressed in percent. Bias exceeding 10% was considered as relevant interference with the test results. Triplicates were tested for HAMA, Rheumatoid factor and the high dose of PSP. [1] Human anti-mouse antibodies; [2] ULOD: upper limit of detection.

3. Clinical Utility

Recently, a pivotal study confirmed that the PSP assay provides an earlier and more accurate detection of sepsis compared to PCT and CRP in intensive care patients at the University Hospital Zurich[24].

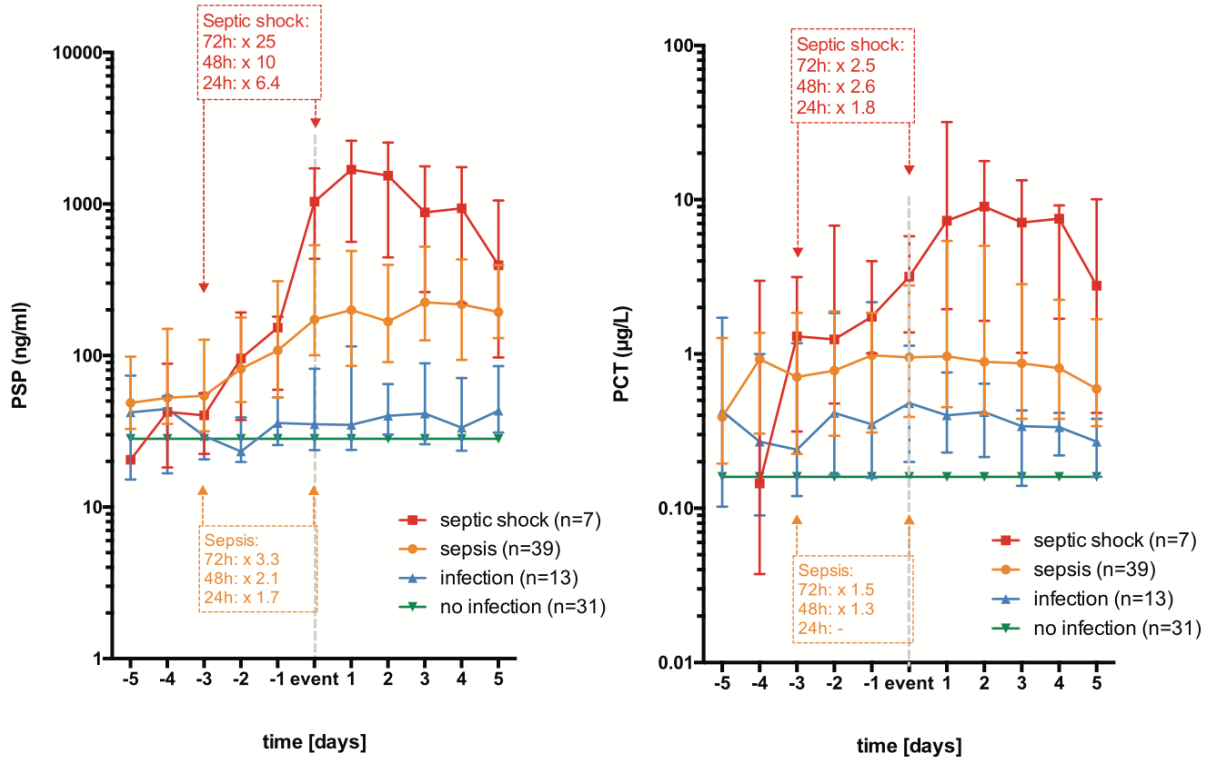
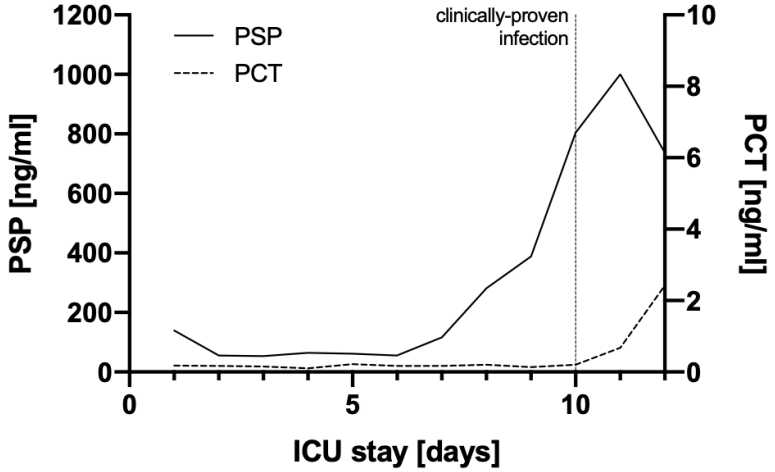


Figure 6 | Event-related depiction of PSP and routine inflammatory biomarkers. PSP serum levels tripled within 72 h and doubled within 48 h.

In addition, interim data from the recently completed clinical trial (AB-PSP-001, NCT03474809[23]) reveals PSP levels to increase significantly earlier in patients developing sepsis when compared to PCT, CRP and WBCs (manuscript in preparation). These initial observations are currently being validated in four hospitals throughout Europe, where the Global COVID19 pandemic enabled a rapid implementation of Abionic's PSP test into the standard of care to assist in the early diagnosis of sepsis. If confirmed, this could prove ground-breaking for the standard of care by allowing an earlier diagnosis of sepsis and immediate optimal clinical management.

To illustrate this, two patient case reports are summarized in Figures 7 and 8.

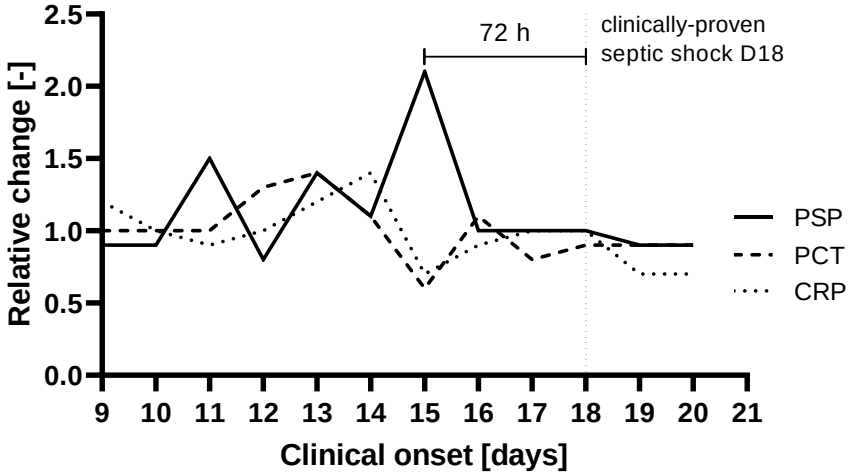
Case report 1 shows the kinetics of PSP and PCT measured on a daily basis shown together with the moment the clinicians first identified an ongoing infection. The concentration of PSP was raised before the clinical diagnosis of an infection, suggesting that clinicians may have acted differently if they had had access to the data. Of note, the PCT increased much later than the PSP did.



Case report#1: a 71-year-old patient admitted in ICU for a brain trauma, put under invasive mechanical ventilation from day 1. No microbiological culture ordered until day 10, when a clinically documented infection was identified. The patient developed a sepsis on day 12 and died that day.

Figure 7 | Early identification of infection and sepsis by real-time monitoring of PSP at the bedside. The graph depicts the PSP values (left axis) and the PCT values (right axis), measured daily. Clinicians were blinded to the PSP and PCT results. PSP = pancreatic stone protein; PCT = procalcitonin; ICU = intensive care unit.

Case report 2 shows the daily PSP measurements on critically ill COVID19 patients in the ICU. Fortunately, these patients did not present a lot of bacterial superinfections, however a small subset of these patients did go on to develop bacterial infections, and the daily PSP measurement was able to detect this event up to 72 hours in advance (Figure 8).



Case report#2: a 71-year-old patient admitted to ICU for interstitial pneumonia and COVID19 positive 9 days after the first onset of symptoms. Bronchoalveolar lavage on day 18, in the context of septic shock, revealed a bacterial superinfection. PSP levels doubled on day 15, 72 hours in advance.

Figure 8 | Early identification of bacterial superinfection and septic shock in critically ill COVID19 patients by real-time monitoring of PSP at the bedside. The graph depicts the relative

change of biomarkers per day after ICU admission. PSP = pancreatic stone protein; PCT = procalcitonin; CRP = C-reactive protein; ICU = intensive care unit.

4. Conclusion

Sepsis, the second cause of mortality worldwide, is a medical emergency where every hour that passes is critical. Physicians will benefit greatly from any solutions that aid them to identifying sepsis earlier, thus substantially improving the chances of patient survival. The combination of the newly CE certified abioSCOPE and PSP test, which unites the fastest technology for immunoassay and a highly sensitive and specific biomarker, is positioned to achieve this ambitious and important mission.

- PSP is a reliable, highly specific and sensitive biomarker for the early diagnosis of sepsis.
- Data show that PSP raises earlier than PCT and CRP in case of infection and sepsis.
- Besides the diagnostic accuracy of PSP for sepsis, its capacity to correctly predict sepsis complications such as in-hospital mortality, makes it a valuable prognostic marker in the ICU setting.
- PSP is secreted from the pancreas in response to remote infection-induced organ dysfunction and participates in the activation of neutrophil granulocytes.
- The analytical performances of the PSP test on the abioSCOPE device are in line with the clinical needs.
- Recent clinical data demonstrates that PSP raises up to 72 hours before a bacterial infection is detected by standard methods of care in ICU patients and in critically ill COVID19 patients.
- A multi-centre clinical trial in currently ongoing in the US with the purpose of obtaining FDA approval.
- Altogether, the data presented here support the disruptive potential of PSP to reduce the burden of sepsis worldwide.

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