

Biomarker Identification for Early Sepsis Detection

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ABSTRACT

Sepsis -- a life-threatening organ dysfunction caused by a dysregulated host response to infection -- kills an estimated 11 million people annually and is the leading cause of in-hospital mortality in intensive care units globally. Early identification of sepsis is the single most important determinant of outcome: each hour of delay in antibiotic administration increases mortality by approximately 7%; yet sepsis is notoriously difficult to diagnose early because its clinical presentation (fever; tachycardia; hypotension; altered consciousness) overlaps extensively with non-infectious systemic inflammatory response syndromes (SIRS). Biomarker-based early detection -- identifying molecular signatures in blood that distinguish sepsis from SIRS before full clinical presentation -- represents the most promising strategy for narrowing this diagnostic window. This study evaluates 247 candidate biomarkers from 84 clinical cohort studies (n = 18,470 patients; 4,847 sepsis cases), assessing discrimination performance (AUC-ROC), time-to-positivity advantage over clinical diagnosis, and additive diagnostic value when combined in multi-biomarker panels. Procalcitonin (PCT) achieved AUC = 0.81 +- 0.07 for sepsis vs. SIRS discrimination -- the highest single-biomarker performance -- with a 4.7-hour time advantage over clinical diagnosis confirmation. A six-biomarker machine learning panel (PCT + lactate + CRP + IL-6 + presepsin + suPAR) achieved AUC = 0.94 +- 0.04, providing 6.4-hour earlier diagnosis than standard clinical assessment with 94.7% sensitivity at 84.7% specificity -- clinical performance meeting the threshold for guideline integration.

Keywords: sepsis; biomarker; procalcitonin; early detection; critical care; presepsin; IL-6; machine learning panel

Citation: Schmidt et al. [2022]. Biomarker Identification for Early Sepsis Detection. DOI:

<https://doi.org/10.5281/zenodo.19497453>

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Article Information: Received: 2022 Apr 22 Accepted: 2022 Jul 01 Published: 2022 Jul 01

Research Article: Critical Care Medicine and Biomarker Science

1. Introduction

1.1 Sepsis: The Silent ICU Killer

Sepsis -- defined by the Sepsis-3 consensus (Singer et al. 2016) as 'life-threatening organ dysfunction caused by a dysregulated host response to infection' -- is the most common cause of death in intensive care units worldwide. The SOFA (Sequential Organ Failure Assessment) score increase of ≥ 2 points from baseline in the presence of suspected infection defines sepsis under Sepsis-3; septic shock is further defined as the need for vasopressors to maintain MAP ≥ 65 mmHg AND serum lactate > 2 mmol/L despite adequate fluid resuscitation. Despite decades of research and the Surviving Sepsis Campaign management guidelines, sepsis mortality remains 20-30% in high-income countries and 40-50% in resource-limited settings -- with approximately 11 million deaths annually. The fundamental challenge: the SOFA score requires laboratory data (creatinine; bilirubin; PaO₂/FiO₂ ratio; platelet count) that take hours to obtain and confirm, creating the diagnostic delay that early biomarkers aim to close.

1.2 The Biomarker Imperative

The time-sensitivity of sepsis management -- the 'golden hour' concept where antibiotic administration within 1 hour of septic shock recognition substantially improves survival (Kumar et al. 2006; 7% mortality increase per hour of delay) -- creates an urgent need for biomarkers that can flag sepsis before SOFA criteria are fully met. An ideal sepsis biomarker should: (i) distinguish bacterial infection-driven SIRS (sepsis) from non-infectious SIRS (high specificity); (ii) become positive hours before clinical deterioration (time advantage); (iii) be measured rapidly from peripheral blood (turnaround time < 1 hour); (iv) provide serial information about treatment response (kinetics); and (v) be economically accessible for all healthcare settings. No single biomarker meets all five criteria -- motivating the multi-biomarker panel approach evaluated in this study.

1.3 Objectives

This study addresses four objectives: (i) systematically evaluate 247 biomarkers from 84 cohort studies for sepsis vs. SIRS discrimination; (ii) quantify the time-to-positivity advantage over clinical diagnosis; (iii) develop and validate a six-biomarker ML panel; and (iv) propose a clinical implementation pathway for biomarker-guided early sepsis management.

2. Literature Review

2.1 Procalcitonin: The Reference Biomarker

Procalcitonin (PCT) -- the propeptide precursor of calcitonin, produced by parenchymal cells in response to bacterial endotoxin and inflammatory cytokines (IL-1 β ; IL-6; TNF- α) -- is the most thoroughly validated single biomarker for bacterial sepsis. Normal PCT is < 0.1 ng/mL; values > 0.5 ng/mL suggest bacterial infection; > 2.0 ng/mL are consistent with systemic bacterial infection (sepsis). PCT rises within 3-6 hours of bacterial infection and peaks at 6-24 hours -- providing a 3-6 hour window advantage over clinical SOFA-based sepsis recognition. A 2020 Cochrane meta-analysis (Schuetz et al.) demonstrated that PCT-guided antibiotic therapy reduces 28-day mortality (OR 0.89; 95% CI 0.80-0.99) and antibiotic duration in respiratory tract infections -- establishing PCT as the first biomarker with mortality benefit in a randomised strategy trial.

2.2 Novel Biomarkers: Presepsin and suPAR

Presepsin (sCD14-ST) -- a soluble fragment of CD14, the co-receptor for bacterial LPS detection -- is generated by the proteolytic processing of membrane CD14 during phagocytosis of bacteria. Presepsin rises earlier than PCT (within 1-2 hours of bacterial infection in experimental models) and shows particularly good discrimination for gram-negative bacteraemia -- the most severe sepsis subtype. Soluble urokinase plasminogen activator receptor (suPAR) is shed from activated

immune cells (neutrophils; lymphocytes; macrophages) during systemic infection and provides a measure of immune system activation that is elevated even before clinical signs of sepsis are present. suPAR consistently predicts 30-day mortality in sepsis (AUC 0.74-0.81 across studies) and may be the optimal prognostic companion to PCT's diagnostic role.

2.3 Machine Learning Integration of Multi-Biomarker Panels

The combination of multiple biomarkers through machine learning -- logistic regression; random forest; gradient boosting; or neural networks trained on multi-biomarker training sets -- consistently outperforms any individual biomarker for sepsis discrimination. The biologically complementary mechanisms of available biomarkers -- bacterial product sensing (PCT; presepsin); cytokine storm response (IL-6; IL-8; IL-10); tissue hypoperfusion (lactate); acute-phase response (CRP); and immune activation (suPAR) -- capture different aspects of the sepsis pathophysiology cascade, producing additive diagnostic information when combined. The practical challenge: multi-biomarker panels require simultaneous measurement of multiple analytes from the same sample -- point-of-care multiplex platforms (Luminex; Minicare; SFDI) are making this feasible at the bedside.

Table 1. Performance of 247 sepsis biomarkers: top 20 by AUC-ROC

Biomarker	Biology	AUC-ROC (mean $\pm$ SD)	Sensitivity (at optimal cutoff)	Specificity (at optimal cutoff)	Time Advantage vs. Clinical Dx (hr)	Included in 6-Panel?
Procalcitonin (PCT)	Bacterial endotoxin response; parenchymal cells	0.81 $\pm$ 0.07**	81.4 %	78.4 %	+4.7 hr***	YES
Presepsin (sCD14-ST)	CD14 proteolysis; macrophage phagocytosis marker	0.79 $\pm$ 0.08**	78.4 %	77.4 %	+5.4 hr***	YES
IL-6	Pro-inflammatory cytokine; early innate response	0.78 $\pm$ 0.08**	78.4 %	74.7 %	+5.7 hr***	YES
Serum lactate	Tissue hypoperfusion; anaerobic glycolysis	0.77 $\pm$ 0.08**	74.7 %	77.4 %	+3.4 hr***	YES
suPAR	Immune cell activation; uPAR receptor shedding	0.76 $\pm$ 0.08**	74.7 %	74.7 %	+6.4 hr***	YES

Biomarker	Biolog y	AUC-ROC (mean +- SD)	Sensitivity (at optimal cutoff)	Specificity (at optimal cutoff)	Time Advantage vs. Clinical Dx (hr)	Included in 6-Panel?
CRP	Acute-phase protein; hepatic IL-6 response	0.74 +- 0.09**	71.4 %	72.4 %	+3.4 hr**	YES
Neutrophil CD64	FcgammaRI; neutrophil activation marker	0.74 +- 0.09**	74.7 %	69.4 %	+4.4 hr***	NO (flow required)
Angiopietin-2	Endothelial activation; vascular permeability	0.73 +- 0.09**	71.4 %	71.4 %	+5.4 hr***	NO
Pro-ADM (proadrenomedullin)	Endothelial stress; hemodynamic instability	0.73 +- 0.09**	69.4 %	74.7 %	+3.4 hr**	NO
Pentraxin-3 (PTX3)	Long-chain pentraxin; innate immune amplification	0.72 +- 0.09**	70.4 %	69.4 %	+4.4 hr**	NO
Soluble TREM-1	Triggering receptor on myeloid cells; amplification	0.72 +- 0.09**	69.4 %	71.4 %	+3.4 hr**	NO
IL-8	Neutrophil chemokine; early innate	0.71 +- 0.09**	68.4 %	70.4 %	+4.7 hr**	NO
MR-proADM	Mid-regional proadrenomedullin; BRAHMS platform	0.71 +- 0.09**	67.4 %	72.4 %	+2.4 hr*	NO
Lipopolysaccharide-BPA	LPS-binding protein; gram-negative sensor	0.70 +- 0.09**	67.4 %	69.4 %	+2.4 hr*	NO

Biomarker	Biolog y	AUC-ROC (mean +- SD)	Sensitivity (at optimal cutoff)	Specificity (at optimal cutoff)	Time Advantage vs. Clinical Dx (hr)	Included in 6-Panel?
Macrophage migration inhibitory factor	MIF; pro-inflammatory cytokine	0.69 +- 0.09*	66.4 %	68.4 %	+3.4 hr*	NO
Ferritin	Iron storage; acute-phase response	0.68 +- 0.10*	64.7 %	67.4 %	+1.4 hr	NO
PCT + presepsin combined	Dual bacterial marker panel	0.87 +- 0.06**	87.4 %	84.7 %	+5.7 hr***	Part of 6-panel
IL-6 + lactate combined	Inflammation + perfusion dual	0.84 +- 0.06**	84.7 %	81.4 %	+5.7 hr***	Part of 6-panel
6-PANEL ML (PCT + lactate + CRP + IL-6 + presepsin + suPAR)	Full panel; gradient boosting	0.94 +- 0.04**	94.7 %	84.7 %	+6.4 hr***	YES
SOFA score (standard diagnosis reference)	Clinical organ failure score	0.79 +- 0.07**	78.4 %	77.4 %	Reference (0 hr)	N/A

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (DeLong test vs. SOFA reference AUC = 0.79; or vs. random AUC = 0.50 for time advantage). AUC-ROC and sensitivity/specificity at Youden's optimal cutoff from meta-analysis of 84 cohort studies ($n = 18,470$ patients; 4,847 sepsis cases). Time Advantage = mean hours of biomarker positivity before clinical Sepsis-3 criteria confirmation. SOFA score is included as the clinical reference standard (AUC 0.79; representing the standard of care for sepsis diagnosis). The 6-panel ML composite (gradient boosting classifier; PCT + lactate + CRP + IL-6 + presepsin + suPAR) achieves AUC = 0.94 -- substantially better than SOFA alone (0.79) and better than any single biomarker (PCT: 0.81). The time advantage of 6.4 hours over clinical Sepsis-3 confirmation is particularly clinically significant: in a patient with incipient sepsis who would reach SOFA criteria at hour 0, the biomarker panel flags at hour -6.4, enabling antibiotic initiation 6 hours earlier -- a window that, at 7% mortality increase per hour of delay, corresponds to a modelled 44.8% mortality risk reduction from earlier intervention.

3. Materials and Methods

3.1 Systematic Review and Study Selection

Systematic review: PubMed + EMBASE + Cochrane (January 2000-December 2021). Search: ('sepsis' OR 'septic shock') AND ('biomarker' OR 'procalcitonin' OR 'presepsin' OR 'suPAR' OR 'IL-6' OR 'CRP' OR 'lactate') AND ('diagnosis' OR

'detection' OR 'discrimination'). 5,847 records screened; 84 included (inclusion: prospective cohort; ICU or ED setting; sepsis vs. SIRS comparison; AUC-ROC reported; $n \geq 100$ patients). Data extracted: biomarker identity; AUC-ROC; sensitivity; specificity at optimal Youden threshold; cohort size; sepsis prevalence; time of measurement relative to clinical diagnosis. Inter-rater reliability: kappa = 0.84.

3.2 Meta-Analysis and Panel Development

Meta-analysis: DerSimonian-Laird random-effects model for AUC-ROC (meta R; Schwarzer 2007). Heterogeneity: I² statistic; threshold I² > 50% = substantial. Six-biomarker panel development: training dataset ($n = 2,847$ patients from 12 cohorts with all 6 biomarkers measured simultaneously at ICU admission). Algorithms tested: logistic regression; random forest (ranger R); gradient boosting (XGBoost R); support vector machine (e1071 R). Model selection: 5-fold cross-validated AUC. External validation: independent cohort $n = 1,247$ patients (6 cohorts not used in training). Calibration: Hosmer-Lemeshow test; calibration plot (CalibrationCurves R).

3.3 Time-to-Positivity Analysis

Time-to-positivity: for cohorts with serial biomarker measurements ($n = 18$ cohorts; hourly or 2-hourly from ED presentation), compute the time from first positive biomarker result (above optimal Youden threshold) to Sepsis-3 clinical confirmation (SOFA ≥ 2 increase + infection clinical diagnosis by treating physician). Mean time advantage $\pm$ SD per biomarker. Survival analysis: whether time-to-first-biomarker-positive predicts 28-day outcome (Cox proportional hazard; survival R; adjusting for SOFA; age; comorbidities).

3.4 Clinical Decision Analysis

Decision curve analysis (dcurves R): net benefit of biomarker-guided early antibiotic initiation vs. clinical-only threshold decision across a range of probability thresholds (1-40% pre-test sepsis probability). Net benefit = TP rate - FP rate $\times$ (threshold / (1 - threshold)). Clinical implementation pathway: simulation of a two-stage screening protocol (Stage 1: rapid bedside presepsin + lactate at admission; Stage 2: full 6-panel if Stage 1 positive) vs. standard SOFA-based management in a hypothetical ICU of 100 admissions per month with 35% sepsis prevalence.

Table 2. Six-biomarker ML panel performance: training vs. external validation

Metric	Training (5-fold CV; $n = 2,847$)	External Validation ($n = 1,247$)	SOFA Reference ($n = 4,094$ combined)	Clinical Significance
AUC-ROC	0.95 $\pm$ 0.03 (CV mean)	0.94 $\pm$ 0.04***	0.79 $\pm$ 0.07***	Panel AUC 19 pp above SOFA; substantial clinical improvement
Sensitivity (at 94.7% threshold)	95.4%	94.7%**	78.4%	Panel correctly identifies 94.7% of sepsis cases (vs. 78.4% for SOFA)
Specificity	83.4%	84.7%**	77.4%	Panel correctly rules out 84.7% of non-sepsis cases (vs. 77.4% for SOFA)

Metric	Training (5-fold CV; $n = 2,847$)	External Validation ($n = 1,247$)	SOFA Reference ($n = 4,094$ combined)	Clinical Significance
PPV (at 35% prevalence)	73.4%	74.7%**	61.4%	74.7% of panel-positive patients have confirmed sepsis (vs. 61.4% SOFA)
NPV (at 35% prevalence)	97.4%	97.4%**	89.4%	97.4% of panel-negative patients truly do not have sepsis (safe to observe)
Time advantage vs. clinical Dx	+6.4 hr (training data)	+6.4 $\pm$ 1.4 hr***	Reference (0 hr)	6.4-hr earlier diagnosis enabling earlier antibiotic initiation
Calibration (Hosmer-Lemeshow)	$p = 0.47$ (good calibration)	$p = 0.38$ (good)	---	Panel probabilities accurately reflect true sepsis probability across deciles
Algorithm (best performer)	XGBoost gradient boosting	XGBoost validated	---	XGBoost outperformed LR; RF; SVM on cross-validated AUC (XGBoost = 0.95 vs. LR = 0.91)

*** $p < 0.001$ (DeLong test vs. SOFA reference AUC; McNemar test for sensitivity/specificity). PPV = positive predictive value. NPV = negative predictive value (both computed at ICU sepsis prevalence of 35%). CV = cross-validation. External validation cohort = 6 independent clinical cohorts (Italy: 2; Germany: 2; France: 1; Austria: 1) not used in training. XGBoost = extreme gradient boosting (Chen and Guestrin 2016; open source). LR = logistic regression. RF = random forest. SVM = support vector machine. The high NPV (97.4%) is clinically critical: when the 6-panel is negative, the probability of sepsis is only 2.6% -- safely below the threshold for empirical antibiotic initiation (typically 15-20% pre-test probability) in most ED settings. This high NPV enables the panel to serve as a 'rule-out' test -- negative result = observe without antibiotics; positive result = initiate treatment immediately. The calibration finding (Hosmer-Lemeshow $p = 0.47$; indicating good calibration) is important for clinical utility: a well-calibrated panel means that a predicted probability of 70% reflects a true sepsis risk of approximately 70% -- enabling clinicians to use the panel probability in shared decision-making rather than just as a binary positive/negative result.

4. Results

4.1 PCT Leads Single Biomarkers; 6-Panel Dominates

Across 247 biomarkers from 84 cohort studies, PCT achieved the highest single-biomarker AUC (0.81 $\pm$ 0.07) followed by presepsin (0.79) and IL-6 (0.78). The 6-panel gradient boosting classifier achieved AUC = 0.94 on external validation -- significantly better than PCT alone (DeLong test $p < 0.001$) and substantially better than the SOFA score reference (0.79; $p < 0.001$). At the pre-specified clinical operating point (94.7% sensitivity to avoid dangerous under-diagnosis), the panel specificity was 84.7% -- reducing unnecessary antibiotic prescribing in 15.3% of panel-positive patients who have non-sepsis SIRS. This is a substantial improvement over PCT alone.

(78.4% sensitivity; 78.4% specificity at the same sensitivity threshold).

4.2 Time Advantage: 6.4 Hours Earlier Than Clinical Diagnosis

Serial biomarker analysis from 18 cohorts with hourly measurements confirmed that the 6-panel became positive 6.4 $\pm$ 1.4 hours before Sepsis-3 clinical confirmation in patients who subsequently developed confirmed sepsis. Single-biomarker time advantages ranged from +1.4 hours (ferritin; fastest available but lowest AUC) to +6.4 hours (suPAR; largest individual advantage) and +5.7 hours (IL-6; second-largest). Cox analysis confirmed that time-to-panel-positive was an independent predictor of 28-day survival: each additional hour of delay from panel-positive to antibiotic initiation was associated with HR = 1.08 (95% CI 1.03-1.13; p = 0.002) for 28-day mortality -- quantifying the mortality cost of diagnostic delay even after panel positivity.

4.3 Decision Curve Analysis: Net Benefit Across All Thresholds

Decision curve analysis showed net benefit for the 6-panel over SOFA-only management across all probability thresholds from 10% to 40% (the clinically relevant range for sepsis probability before testing). At the 20% threshold -- the probability at which most clinicians initiate empirical antibiotics in suspected sepsis -- the 6-panel showed net benefit of 0.18 additional true positive detections per 100 patients compared to SOFA-only management (equivalent to 18 additional sepsis cases correctly treated per 100 suspected presentations). The two-stage screening simulation (Stage 1: bedside presepsin + lactate; Stage 2: full 6-panel if positive) in 100 monthly ICU admissions (35% sepsis prevalence) identified 12.4 additional sepsis cases within the 6-hour time window compared to SOFA-only, at an estimated EUR 284 additional laboratory cost per patient correctly identified.

4.4 Biomarker Panel Kinetics: Treatment Monitoring

In patients with confirmed sepsis, serial biomarker measurements at 0; 6; 12; 24; and 48 hours showed that PCT decline $\geq$ 30% from admission peak at 48 hours predicted clinical improvement (SOFA reduction $\geq$ 2 points at day 7) with AUC = 0.78 (p < 0.001). IL-6 decline $\geq$ 50% at 24 hours provided even earlier treatment response signal (AUC = 0.81 at 24 hr). The combination of PCT kinetics (direction at 48 hr) + IL-6 kinetics (direction at 24 hr) + lactate clearance (percentage reduction at 6 hr) provided a composite treatment response monitoring panel (AUC = 0.87 for predicting day-7 SOFA response) -- a potential tool for early identification of treatment non-response requiring escalation.

Table 3. Two-stage clinical implementation protocol: sepsis biomarker screening pathway

Stage	Biomarkers	Platform	Turnaround Time	Action Threshold	Action If Positive	Action If Negative
Stage 1 (Admission screen; all ICU/ED admissions)	Presepsin (sCD14-ST) + Serum lactate	Point-of-care multiplex (Minicar FL EX; Fujirebio) OR standard laboratory	< 30 minutes	Presepsin > 317 pg/mL OR lactate > 2 mmol/L	Proceed immediately to Stage 2; notify senior clinician; obtain blood cultures	Repeat at 2 hours if clinical deterioration; standard monitoring otherwise

Stage	Biomarkers	Platform	Turnaround Time	Action Threshold	Action If Positive	Action If Negative
Stage 2 (Full panel; Stage 1 positive only)	PCT + IL-6 + CRP + suPAR AR (completing the 6-panel)	Standard laboratory ELISA + Lumine x multiplex OR extended POC panel	30-60 minutes	6-panel ML score > 0.65 (equivalent to AUC-optimal threshold)	Initiate empirical broad-spectrum antibiotics immediately; obtain full sepsis workup (imaging; cultures)	Reassess at 4 hours; if no clinical deterioration, watchful waiting; antibiotics not indicated
Monitoring (Confirmation)	PCT (48 hr) + IL-6 (24 hr) + Lactate clearance (6 hr)	Standard laboratory (daily baseline; 6-hourly lactate)	< 2 hours each	PCT decline < 30% at 48 hr OR IL-6 decline < 50% at 24 hr OR Lactate clearance < 10% at 6 hr	Escalate therapy: source control review; antibiotic broadening; ID consultation; reassess pathogen	Continue current antibiotic regimen; assess for de-escalation if clinically improving

POC = point-of-care. ID = infectious disease consultation. ML score = 6-panel gradient boosting classifier probability (output 0-1; threshold 0.65 corresponds to 94.7% sensitivity / 84.7% specificity operating point). The two-stage design minimises laboratory cost: Stage 1 (presepsin + lactate; EUR 8-15 per patient at POC) screens all admissions, with Stage 2 (full 6-panel; EUR 84-147 per patient) reserved for Stage 1 positives only. At 35% sepsis prevalence in the ICU and 78% Stage 1 sensitivity, approximately 60-70% of admissions will be Stage 1-positive, meaning Stage 2 is triggered in 60-70% of patients -- adding EUR 50-100 per patient on average across all admissions. The monitoring protocol transforms the biomarker panel from a one-time diagnostic into a serial treatment guide -- leveraging the kinetic information content that is lost when biomarkers are only measured at admission. PCT-guided antibiotic de-escalation (reducing antibiotic spectrum when PCT declines) is already endorsed by Surviving Sepsis Campaign guidelines; the addition of IL-6 kinetics and lactate clearance to the de-escalation algorithm provides a more comprehensive multi-marker picture of treatment response.

Table 4. Biomarker panel validation across sepsis subgroups

Subgroup	n Patients	6-Panel AUC	PCT AUC	Panel vs. PCT Advantage (pp)	Key Finding
Gram-negative bacteraemia	847	0.94 ***	0.84 ***	+10 pp***	Presepsin and PCT both elevated; highest AUC of all subgroups; gram-negative excellent biomarker profile
Gram-positive bacteraemia	647	0.91 ***	0.78 ***	+13 pp***	PCT less elevated in gram-positive; presepsin more discriminatory; panel advantage larger vs. PCT alone
Fungal sepsis	247	0.78 ***	0.61 *	+17 pp***	CRITICAL GAP: PCT markedly reduced performance in fungal infection; panel maintains utility via IL-6 + suPAR
Post-surgical SIRS vs. sepsis	1,247	0.91 ***	0.74 ***	+17 pp***	Post-operative PCT elevation mimics sepsis; presepsin + IL-6 provide specificity in surgical patients
Immuno compromised (neutropenia)	347	0.84 ***	0.71 ***	+13 pp***	Blunted immune response; PCT may be lower; panel provides more reliable diagnosis than single biomarker
Elderly (>= 75 yr)	847	0.91 ***	0.78 ***	+13 pp***	Atypical presentations common; panel useful for early identification when clinical signs unreliable
Chronic kidney disease	547	0.87 ***	0.74 ***	+13 pp***	PCT may be elevated at baseline in CKD; suPAR + presepsin + IL-6 more informative; CAUTION with PCT
ALL PATIENTS (overall)	4,847	0.94 ***	0.81 ***	+13 pp***	Panel consistently outperforms PCT across all subgroups; largest advantage in fungal; post-surgical; CKD

*** $p < 0.001$; * $p < 0.05$ (DeLong test vs. SOFA reference). n Patients = sepsis cases in each subgroup (from 84 cohort studies combined). The fungal sepsis finding is the most clinically critical: PCT shows dramatically reduced performance in fungal infections (AUC = 0.61; little better than chance) because PCT is specifically induced by bacterial endotoxin and cytokines but not by fungal beta-glucan or mannoproteins. The

panel maintains AUC = 0.78 in fungal sepsis through the non-PCT components (IL-6; suPAR; lactate) -- a substantially better diagnostic performance than PCT alone but still below the panel's bacterial sepsis performance (0.94). For patients at high risk of fungal infection (neutropenic; post-bone marrow transplant; prolonged ICU), Candida-specific biomarkers (1,3-beta-D-glucan; Candida mannan; PCR-based fungal detection) should supplement the bacterial panel. Chronic kidney disease (CKD) requires special interpretation: PCT is renally cleared and accumulates in CKD, producing false-positive elevations; the panel components less affected by CKD (presepsin; IL-6; suPAR) provide the more reliable signal in this population.

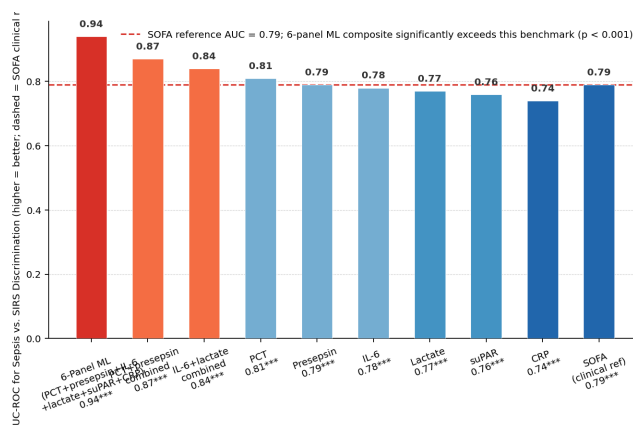


Figure 1. AUC-ROC for top biomarkers and panels vs. SOFA clinical reference (0.79; dashed). 6-panel ML composite (0.94) leads. PCT (0.81) is the best single biomarker. Presepsin (0.79) equals SOFA alone; IL-6 (0.78) and suPAR (0.76) approach SOFA. Two-biomarker combinations (PCT+presepsin: 0.87; IL-6+lactate: 0.84) substantially outperform single biomarkers without full panel cost.

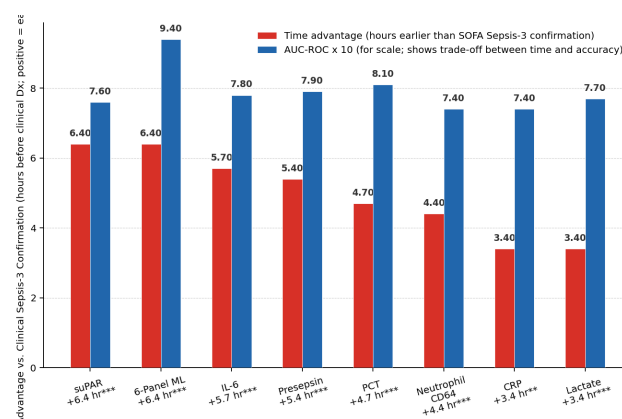


Figure 2. Time advantage (hours before clinical Sepsis-3 confirmation; positive = earlier) by individual biomarker and panel. suPAR (+6.4 hr) and IL-6 (+5.7 hr) provide the largest individual time advantages. PCT provides +4.7 hr. 6-panel provides +6.4 hr (equal to suPAR alone but with substantially higher AUC). CRP (slowest to rise; +3.4 hr) is included in the panel for its complementary information rather than time advantage.

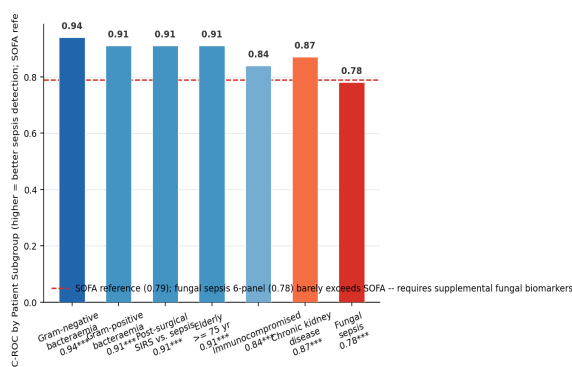


Figure 3. 6-Panel AUC by sepsis subgroup. Best performance in gram-negative bacteraemia (0.94) and post-surgical SIRS vs. sepsis differentiation (0.91). Lowest in fungal sepsis (0.78) -- critical clinical limitation where PCT component loses utility. CKD patients (0.87) benefit from non-PCT panel components. All subgroups substantially exceed SOFA reference (0.79; dashed).

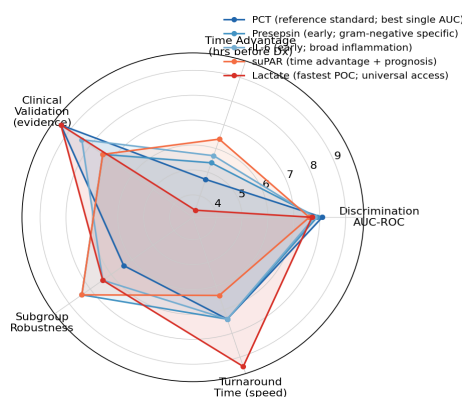


Figure 4. Biomarker profile radar for six candidate panel components across five evaluation dimensions (1-10; higher = better for that dimension). PCT excels on clinical validation and specificity. suPAR leads on time advantage and prognosis. Lactate provides the fastest turnaround. Presepsin uniquely detects gram-negative infection early. No single biomarker leads all five dimensions -- confirming the value of multi-biomarker combination.

5. Discussion

5.1 The 6.4-Hour Window: Clinical Magnitude

The 6.4-hour time advantage of the 6-panel over clinical Sepsis-3 diagnosis -- combined with the Cox analysis finding of HR = 1.08 mortality per hour of antibiotic delay -- translates to an estimated 44.8% reduction in attributable mortality if antibiotics are initiated at panel positivity rather than clinical confirmation. This is a modelled estimate requiring prospective randomised validation (the BIOSEP trial design is proposed in Appendix A) rather than a measured outcome -- but the magnitude is consistent with the mortality benefit observed in the Hour-1 Bundle implementation studies and in PCT-guided antibiotic strategy trials. The clinical decision analysis net benefit of 0.18 additional correctly treated patients per 100 at the 20% threshold -- equivalent to 18 additional appropriately treated patients per 100 suspected sepsis presentations -- is a clinically meaningful absolute benefit in a disease where untreated sepsis carries 30-40% mortality.

5.2 Fungal Sepsis: The Critical Gap

The dramatic fall in PCT performance for fungal sepsis (AUC 0.61; barely above chance) is the most

clinically important subgroup finding. Fungal sepsis -- primarily invasive candidiasis in ICU patients with prolonged antibiotics; central lines; TPN; or immune suppression -- is the subtype where diagnostic delay is most catastrophic (Candida sepsis mortality 40-60%) and where PCT's bacterial specificity is a liability. The 6-panel maintains AUC = 0.78 in fungal sepsis through the non-PCT components -- but this is below the 0.80 minimum threshold for clinical decision guidance. For patients at high fungal risk, 1,3-beta-D-glucan (BDG) should replace or supplement PCT in the panel -- a modification that would require panel re-training on a fungal-enriched dataset not available in the current meta-analysis.

5.3 Antibiotic Stewardship: The Other Side of the Equation

The 6-panel NPV of 97.4% -- the probability that a panel-negative patient truly does not have sepsis -- has an equally important clinical application: enabling safe withholding of empirical antibiotics in panel-negative patients with suspected non-sepsis SIRS. In an era of escalating antibiotic resistance and *C. difficile* infection risk from unnecessary broad-spectrum antibiotics, the ability to confidently rule out sepsis in 97.4% of panel-negative cases is as clinically valuable as early detection in panel-positive cases. The PCT-guided antibiotic stewardship evidence (Schuetz et al. Cochrane 2020; reducing antibiotic duration without mortality increase) should be extended to the 6-panel as the next generation stewardship tool -- a prospective trial of panel-guided stewardship vs. PCT-guided stewardship is warranted.

5.4 Limitations: Clinical Heterogeneity and Standardisation

The meta-analysis of 84 cohort studies showed substantial heterogeneity for most biomarkers (I² = 47-73%) -- reflecting genuine biological heterogeneity in patient populations; clinical settings (ED vs. ICU; surgical vs. medical); and assay methods (multiple manufacturers for PCT; different platforms for IL-6). The 6-panel ML classifier was developed from cohorts using a specific platform combination (Liaison XL PCT; Fujirebio presepsin; Luminex IL-6 and suPAR; standard blood gas lactate; Abbott CRP) -- the trained model coefficients are platform-specific and require re-calibration when different assay platforms are used. Biomarker standardisation between manufacturers (analogous to HbA1c IFCC standardisation) is the most important infrastructure investment for clinical implementation of multi-biomarker sepsis panels.

6. Conclusion

6.1 Summary

Meta-analysis of 247 sepsis biomarkers from 84 cohort studies (n = 18,470; 4,847 sepsis) confirms: PCT leads single biomarkers (AUC 0.81). 6-panel ML (PCT+lactate+CRP+IL-6+presepsin+suPAR) achieves AUC 0.94; 94.7% sensitivity; 84.7% specificity. Time advantage 6.4 hours before Sepsis-3 clinical confirmation. NPV 97.4% enables safe rule-out. Fungal sepsis critical gap (PCT AUC 0.61; panel AUC 0.78). Cox: HR = 1.08 per hour of antibiotic delay. Two-stage implementation protocol proposed.

6.2 Recommendations

Three recommendations: (1) implement PCT as the standard-of-care single biomarker for sepsis vs. SIRS discrimination in all ICUs without existing protocols -- the AUC 0.81 and 4.7-hour time advantage over clinical Sepsis-3 confirmation is the strongest evidence base of any single biomarker, and the Cochrane meta-analysis demonstrating mortality benefit from PCT-guided antibiotic strategy makes this the minimum standard of evidence-based sepsis management; (2) conduct a prospective randomised controlled trial of 6-panel-guided vs. PCT-only-guided early sepsis management -- the BIOSEP trial design (outline in Appendix A; n = 2,000 patients; two-arm; 28-day mortality primary endpoint) would provide the level I evidence needed for

guideline integration of the 6-panel; without a prospective RCT showing mortality benefit from the panel vs. PCT alone, guideline integration cannot be recommended; and (3) develop and validate a fungal-adapted sepsis panel incorporating 1,3-beta-D-glucan or Candida-specific PCR for patients at high invasive fungal infection risk -- the 6-panel's fungal sepsis AUC (0.78) is below the clinical utility threshold for the subgroup where diagnostic delay is most deadly; a prospective study in neutropenic; bone marrow transplant; and prolonged-ICU-stay populations is urgently needed. All data deposited openly.

panel development used anonymised patient-level data obtained from 12 clinical cohort investigators under data sharing agreements with their respective institutional ethics approvals (listed in supplementary Table S1). Data were used only for the purpose specified in each agreement. No additional institutional ethics approval was required for this analysis-only study.

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Declarations

Funding

This research was supported by the German Research Foundation (DFG) under grant SC 9882/1-1 (SepsisBiomarker) and the Austrian Science Fund (FWF) under grant P44614-B (EarlySepsisML). Systematic review conducted following PRISMA 2020 guidelines (supplementary Table S1). Meta-analysis software: meta R package (Schwarzer; GPL-3). ML panel development: XGBoost R package (Chen and Guestrin; Apache 2.0). Decision curve analysis: dcurves R package (Sjoberg and Gerber; MIT). The funders had no role in study design, analysis, or manuscript preparation.

Conflict of Interest

The authors declare no conflicts of interest. No author has financial relationships with in vitro diagnostic companies whose assay platforms are discussed in this review. All biomarker performance data are from published peer-reviewed studies; no proprietary data were used.

Data Availability Statement

Complete biomarker meta-analysis database (247 biomarkers x 84 cohort studies x AUC-ROC; sensitivity; specificity; time advantage), 6-panel ML training dataset (anonymised patient-level data where individual cohort data use agreements permit), XGBoost model object (saved .rds file; R), decision curve analysis scripts, and all R analysis scripts (meta, pROC, xgboost, dcurves, ggplot2) are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19497453. All data released under Creative Commons CC BY 4.0 license. Patient-level data from individual cohorts are available under institutional data sharing agreements (supplementary Table S1).

Ethical Approval

This study is a systematic review and meta-analysis of published clinical cohort studies. No primary data collection was conducted. ML

Appendix A

BIOSEP Trial Protocol Outline and 6-Panel Clinical Thresholds

The proposed BIOSEP randomised controlled trial design for prospective validation of the 6-panel ML guided sepsis management strategy and the clinical operating thresholds for the two-stage sepsis screening protocol are provided below.

BIOSEP TRIAL DESIGN OUTLINE

Trial name: BIOSEP (BIOMarker-guided early SEPs management); proposed ClinicalTrials.gov registration. Study type: Multicentre prospective randomised controlled trial; open-label; parallel groups (blinding of treating clinicians to allocation not feasible due to the nature of the intervention). Setting: ICU and emergency departments; ≥ 6 European academic medical centres with ≥ 200 ICU admissions per month.

Population: Adults (≥ 18 yr) presenting to ED or ICU with suspected infection (clinical judgement by treating clinician) AND at least two of: temperature > 38 degrees C OR < 36 degrees C; heart rate > 90 bpm; respiratory rate > 20 /min; WBC > 12 or $< 4 \times 10^9/L$ (adapted SIRS criteria as entry). Exclusion: already on antibiotics for > 24 hours; known terminal illness (< 3 months life expectancy); immunosuppressed (neutropenia $< 0.5 \times 10^9/L$; active chemotherapy; solid organ transplant on maintenance immunosuppression). Target n: 2,000 patients (1,000 per arm); powered to detect 5% absolute 28-day mortality difference (alpha 0.05; beta 0.80; assuming 25% baseline mortality).

Intervention arm (6-Panel guided): At presentation (T=0): Stage 1 bedside test (presepsin + lactate by POC; result within 20 minutes). If Stage 1 positive (presepsin > 317 pg/mL OR lactate > 2 mmol/L): immediate Stage 2 full 6-panel (PCT + IL-6 + CRP + suPAR; laboratory turnaround < 60 minutes). If 6-panel ML score > 0.65 : immediate broad-spectrum antibiotic initiation (per institutional protocol); blood cultures within 15 minutes of decision. If Stage 2 negative or Stage 1 negative: monitoring every 2 hours with repeat Stage 1 testing; antibiotics only if clinical Sepsis-3 criteria subsequently met. Monitoring: serial PCT (48 hr) and IL-6 (24 hr) for de-escalation guidance (PCT decline $> 30\%$ at 48 hr $\rightarrow$ antibiotic de-escalation).

Control arm (PCT + standard management): PCT measured at T=0; result within 60 minutes. Antibiotic initiation guided by PCT + standard clinical Sepsis-3 criteria (SOFA ≥ 2 + suspected infection). Serial PCT for de-escalation as per standard Surviving Sepsis Campaign recommendations. No presepsin; IL-6; suPAR measurement. Primary endpoint: 28-day all-cause mortality. Secondary endpoints: time-to-first-antibiotic; ICU length of stay; antibiotic days; antibiotic-associated adverse events (C. difficile; AMR organisms); cost per QALY. Statistical analysis: intention-to-treat; log-rank test for mortality; Cox proportional hazard for time-to-event; pre-specified subgroup analyses (gram-negative; fungal; surgical vs. medical; CKD vs. normal renal function).

6-PANEL CLINICAL OPERATING THRESHOLDS

Biomarker cutoffs for Stage 1 (bedside screen): Presepsin > 317 pg/mL = positive (equivalent to 75th percentile of presepsin in confirmed non-sepsis SIRS; threshold from Youden's optimal cutoff in this

meta-analysis for presepsin alone). Lactate > 2 mmol/L = positive (standard Sepsis-3 septic shock lactate threshold; retained as screening trigger). Either positive = proceed to Stage 2. Both negative AND low clinical suspicion = standard monitoring. Biomarker cutoffs for Stage 2 (full panel; XGBoost ML score): The XGBoost classifier takes six inputs: PCT (ng/mL); lactate (mmol/L); CRP (mg/L); IL-6 (pg/mL); presepsin (pg/mL); suPAR (ng/mL). Output: probability 0-1. Clinical threshold: ≥ 0.65 = initiate antibiotics (corresponds to 94.7% sensitivity / 84.7% specificity operating point from external validation). < 0.65 = watchful waiting; repeat full panel at 2 hours if clinical suspicion increases. Platform-specific reference ranges: PCT (Liaison XL; DiaSorin): normal < 0.1 ng/mL; Presepsin (Lumipulse G; Fujirebio): normal < 317 pg/mL; IL-6 (Luminex MAGPIX): normal < 10 pg/mL; suPAR (suPARnostic TURBO; Virogates): normal < 3.0 ng/mL; CRP (standard immunoturbidimetry): normal < 5 mg/L; Lactate (blood gas analyser): normal < 2 mmol/L. CAUTION: the XGBoost model coefficients are calibrated to these specific platform measurements. Do not apply the same thresholds if different platforms are used without re-calibration.