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Inflammatory Markers Preceding the Microbiological Result in 53,033 Blood Cultures

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Abstract

Inflammatory biomarkers are read at the bedside as one value against one threshold, although serial determinations can be summarized in several ways, and the summary being tested is seldom stated. We measured the extent to which the discrimination attributed to a biomarker depends on the summary is read. We analyzed 53,033 blood cultures from 16,431 patients at one hospital between January 2020 and August 2026, anchoring each culture to its microbiological result and deriving, for eight biomarkers, the first value before the result, the maximum before it, the value nearest it and the amplitude between determinations. The outcomes were pathogen growth, excluding coagulase-negative staphylococci, and bacterial group. In the 35,150 cultures with a procalcitonin determination preceding the result, procalcitonin provided the best discrimination on every metric: area under the curve, 0.766 (95% CI 0.756–0.776) for the maximum before the result, 0.738 (0.728–0.748) for the first value, and 0.773 (0.764–0.784) nearest the result. The advantage of the maximum grew once sampling density and interval length were held constant. The neutrophil-to-lymphocyte ratio performed better before the result than near it, 0.707 versus 0.620, and fell from 0.738 on surgical wards to 0.512 in hematology, whereas procalcitonin varied little between services. Sensitivity at 0.5 ng/mL ranged from 88.0% for Enterobacterales to 55.6% for other Gram-positive organisms. Combining biomarkers improved the area by, at most, 0.015. Which number is read changes the answer as much as which marker is chosen. A procalcitonin level below 0.5 ng/mL argues strongly against Gram-negative bacteremia and far more weakly against enterococcal, other Gram-positive, and fungal bloodstream infection.

Keywords: procalcitonin; blood culture; bacteremia; neutrophil-to-lymphocyte ratio; biomarker kinetics; diagnostic accuracy; bloodstream infection

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1. Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. The Global Burden of Disease program estimated 166 million cases and

21.4 million sepsis-related deaths worldwide in 2021, 31.5% of all deaths recorded that year, with the decline achieved between 1990 and 2019 reversed during the pandemic [2]. Bacteremia is the subset in which the organism is recovered from blood. Every decision that matters is made, including whether to admit, which antimicrobial to start, and how broad its spectrum should be, is made before anyone knows whether the culture will grow.

Blood culture is the reference standard, but its results take time. In a contemporary automated system, the interval from venipuncture to a positive signal averaged close to 25 h, roughly half of this time spent before incubation had even begun [3]. A negative result is not issued until five days of incubation have elapsed, so the interval the clinician is required to cover is longer in the cultures that will not grow. Yield is low, between 2% and 20% of cultures drawn, and contaminants may account for 30–50% of what grows [4]. In septic shock, each hour of delay in effective antimicrobial therapy over the six hours after the onset of hypotension was associated with an average 7.6% decline in survival [5].

Inflammatory biomarkers have been proposed for three decades as the bridge across that interval. A systematic reappraisal counted 258 sepsis biomarkers published between 2009 and 2019 and concluded that adding more biomarkers without a rigorous framework is futile [6]. A bedside guide covering more than 250 candidates advises that kinetics carry more information than single values [7]. Neither statement has been tested, analyte by analyte, against a common comparator, and the published estimates show why that matters. For procalcitonin and bacteremia alone, reported areas under the curve range from 0.55 to 0.98 across settings [4,8,9]; for the neutrophil-to-lymphocyte ratio, they range from 0.59 to 0.73 [10,11], and differences by organism have been described but never examined alongside either [12,13]. A range that wide is not a property of the marker. It is what happens when each study fixes one value, in one population, at one moment, and reports the result as though those choices were incidental.

We took the biomarkers already ordered by a general hospital every day and read them several ways in a single cohort. Every blood culture processed over six and a half years was anchored to its microbiological result, and for each analyte, we derived the first value available before that result, the highest value before it, the value nearest it, and the amplitude of movement between them. The aim was to determine how much of the discrimination attributed to an inflammatory biomarker depends on which of its numbers is read and whether that dependence holds when the marker, the organism and the clinical setting change. The primary outcome was growth of a pathogen. We then asked, among the cultures that grew, how far the same readings distinguish one group of organisms from another, and whether the answers hold across levels of severity, across the wards where samples were drawn, and against a recognized contaminant.

2. Materials and Methods

2.1. Study Design and Setting

This was a retrospective cohort study of routinely collected clinical and laboratory data. It was conducted at Hospital Universitari Son Llàtzer, a 400-bed university hospital in Palma de Mallorca, Spain, serving a reference population of approximately 300,000. The hospital runs a hospital-wide sepsis code managed by a multidisciplinary sepsis unit, whose activation patterns and outcomes over sixteen years have been reported previously [14]. All microbiological samples processed by the hospital laboratory between 2 January 2020 and 28 August 2026 were retrieved, together with every determination of the inflammatory biomarkers of interest recorded in the laboratory information system over the same period. The cohort was not limited to critical care units: it included every blood culture processed by the hospital laboratory over the study period, from all clinical settings (Section 3.7). Reporting follows the STARD 2015 statement for studies of diagnostic accuracy.

2.2. Cohort Assembly

All 64,620 blood cultures processed by the hospital laboratory during the study period were eligible, and all were included consecutively; there was no sampling. Cultures without a determination of any of ten analytes inside the analysis window were excluded; the ten were the eight analyzed here, total cholesterol, and interleukin-6. This left 53,033 blood cultures from 16,431 patients (Figure 1). Each blood culture was treated as one observation; patients contributing more than one culture appear more than once, and all inference accounts for this.

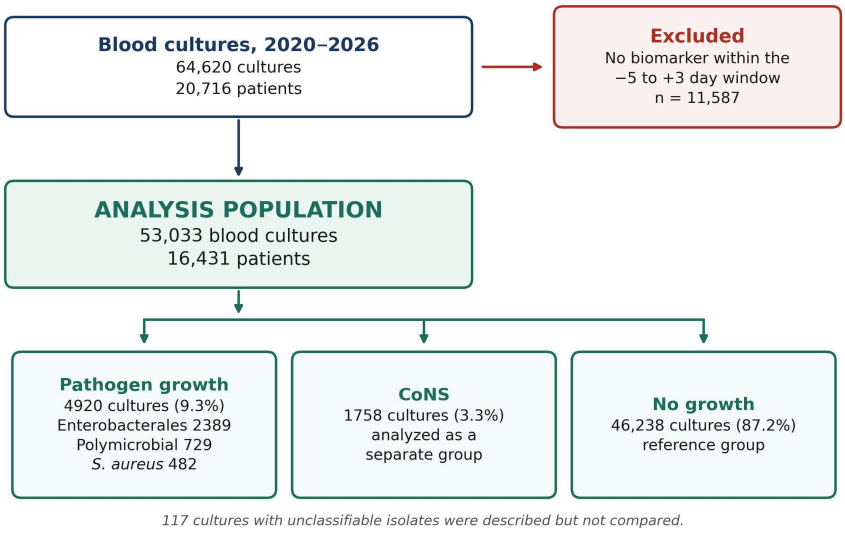

Figure 1. Study flow. Blood cultures processed during the study period, cultures excluded, and the three outcome groups of the analysis population. CoNS, coagulase-negative staphylococci.

2.3. Reference Standard

The reference standard was the final report of the hospital microbiology laboratory for each blood culture. A set comprised four bottles, two aerobic and two anaerobic, so anaerobic organisms were recovered routinely rather than on request. Bottles were incubated for five days, and a set was reported as negative only after five days without a positive signal. Bottles that flagged positive underwent Gram staining and subculture; organisms were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and susceptibility was determined on an automated system (VITEK, bioMérieux, Marcy-l'Étoile, France). The analysis used the organism named in the final report. No culture result was reclassified by clinical adjudication, and no organism was designated a contaminant on clinical grounds; coagulase-negative staphylococci were handled by the rule stated in Section 2.5 and by no other criterion. The same reference standard was applied to every culture in the cohort.

2.4. Anchoring and Analysis Window

Every culture was anchored to the date and time at which its final microbiological report was issued. In cultures with growth, that report follows the positive signal, subculture and identification of the organism; in cultures without growth, it follows the completion of five days of incubation. The interval before the anchor is therefore longer in cultures without growth; it is reported by arm in Section 3.1 and examined in the sensitivity analyses described in Section 2.8. Biomarker determinations were collected in a window running from five days before to three days after the anchor, spanning the incubation period of the culture and the three days following the report.

2.5. Outcomes

The primary outcome was pathogen growth, defined as growth of any organism other than coagulase-negative staphylococci; cultures growing coagulase-negative staphylococci were removed from both arms of that comparison and analyzed as a separate group. The secondary outcome was the bacterial group of the isolate. Isolates were assigned to Enterobacterales, non-fermenting Gram-negative bacilli, other Gram-negative organisms, *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus* spp., *Streptococcus* spp., other Gram-positive organisms, anaerobes, fungi, or polymicrobial growth. Cultures whose isolate could not be assigned to any of these groups were described but not compared. Groups contributing fewer than 30 cultures are not reported separately. Within the cultures that grew a pathogen, discrimination between Gram-negative and Gram-positive isolates was additionally evaluated as a binary comparison, with fungi, anaerobes and polymicrobial growth excluded from it.

2.6. Index Tests

The extraction retrieved thirteen analytes from the laboratory information system. Eight were analyzed here: procalcitonin, C-reactive protein, total leukocyte count, absolute neutrophil count, absolute lymphocyte count, platelet count, fibrinogen and albumin. Procalcitonin was measured by immunoassay on an Abbott platform (Abbott Laboratories, Abbott Park, IL, USA), which did not change during the study period. The neutrophil-to-lymphocyte ratio was computed within each blood draw from the absolute neutrophil and lymphocyte counts carrying the same timestamp.

Determinations with a timestamp preceding the result were used to derive two primary metrics: the first value before the result, defined as the earliest such determination, and the maximum before the result, defined as the highest. A third metric, the value nearest the result in time, was retained as secondary; it may fall on either side of the result. Amplitude was defined as the base-ten logarithm of the ratio of the maximum to the minimum among determinations preceding the result, and required at least two determinations. Platelets and lymphocytes are additionally summarized by their minimum before the result. For every culture and analyte, the number of determinations preceding the result and the interval between the earliest of these and the result were recorded.

Units were harmonized across the study period before analysis. Procalcitonin, C-reactive protein and albumin results reported in alternative units were converted to a single unit per analyte; leukocyte results reported in units incompatible with a blood count were discarded; and lymphocyte percentages were converted to absolute counts using the total leukocyte count from the same draw. No determination was treated as indeterminate on any other ground. No biomarker value was used to define eligibility for the cohort or to define either outcome. All determinations were produced by the hospital's clinical laboratory as part of routine care.

Biomarker determinations and culture results were extracted from the laboratory information system after both had been issued in the course of care. Biomarker samples were processed and reported before the culture result existed, and microbiological processing was carried out by laboratory staff who had no role in this study and no access to its analyses. Neither test was read or interpreted by an observer for the purposes of the study, so no further blinding was applied.

2.7. Covariates

Age, sex, Sequential Organ Failure Assessment (SOFA) score, National Early Warning Score 2 (NEWS2), Charlson comorbidity index and the admitting clinical service were retrieved for each culture. The hospital sepsis code is activated on modified Sepsis-2 crite-

ria [14]; SOFA and NEWS2 are not part of that protocol and were calculated retrospectively, without imputation, for the cultures with the required data available at the time of the culture. SOFA was available for 68.2% of cultures, NEWS2 for 54.2% and the Charlson index for 82.8%. The clinical service and the admission diagnosis are both linked to the episode identifier that is active when a sample is registered. When a patient is subsequently admitted, the hospital system issues a new episode identifier and keeps the patient identifier, so a sample registered before that reassignment has neither field, nor do samples drawn outside any admission episode. A clinical service was recorded for 22,901 of the 53,033 cultures, 22,783 of them from services that could be grouped into critical care, internal medicine, surgical, other medical, oncology and hematology. Service codes changed format during 2024 and were harmonized before grouping. Antimicrobial exposure and vasoactive drug administration are recorded in prescribing systems whose coverage varied across the study period and which under-capture treatment in critical care; neither of these was used.

2.8. Statistical Analysis

No sample size calculation was performed. The analysis used every eligible culture recorded over the study period, and the resulting size was fixed by the extraction rather than chosen.

Continuous variables were summarized as median and interquartile range (IQR) and were compared by non-parametric methods throughout; no distributional assumption was made for any biomarker. Discrimination of pathogen growth is reported as the area under the receiver operating characteristic curve, computed as the Mann–Whitney statistic over all case and non-case pairs, with ties assigned half weight. The 95% confidence interval (CI) was obtained by bootstrap resampling at the patient level with 2000 replicates so that all cultures from a resampled patient enter or leave the sample together. Areas below 0.5 indicate lower values in cultures with pathogen growth. Sensitivity, specificity and likelihood ratios were computed at seven prespecified procalcitonin thresholds from 0.25 to 20 ng/mL, none of them derived from these data; predictive values were derived from the likelihood ratios at the prevalence observed in the corresponding population. Sensitivity and negative predictive value at 0.5 ng/mL were also computed within each bacterial group against the arm without growth.

The effect of bacterial group on each biomarker was quantified by the epsilon-squared measure, i.e., the proportion of rank variance attributable to group, derived from the Kruskal–Wallis statistic. Groups contributing fewer than 20 available values of an analyte were excluded from that comparison, and comparisons with fewer than three remaining groups were not made.

Statistical significance for the association between each biomarker and pathogen growth was assessed by permutation, with group labels permuted at patient level over 20,000 replicates and the Benjamini–Hochberg procedure applied within each family of comparisons.

Two analyses addressed the unequal pre-result interval between arms. The first restricted the comparison of the first value with the maximum to cultures with two or more pre-result determinations and repeated it within strata of the determination count. The second derived both metrics within fixed pre-result windows of 24, 48 and 72 h, identical in both arms. Differences between areas on the same cultures were estimated by paired bootstrapping, resampled at the patient level. The subset of cultures with a clinical service recorded was compared with the remainder in prevalence of growth, severity and comorbidity, overall and within strata of the number of cultures per patient.

Further sensitivity analyses restricted the cohort to the first blood culture of each patient, analyzed the 2020–2021 and 2022–2026 periods separately, and computed the

operating characteristics of procalcitonin within each calendar year. Combinations of biomarkers were evaluated in two ways: all two-way and three-way products of the pre-result metrics, formed without fitted parameters, and logistic models fitted with five-fold cross-validation allocated at patient level. Analyses used Python 3.11.15 with pandas 3.0.2, NumPy 2.4.4, SciPy 1.17.1 and scikit-learn 1.8.0-10.24.

The two outcomes, the analysis window, the three metrics, the procalcitonin thresholds, and the first-culture and period analyses were fixed before the comparisons reported here were run. The analyses by clinical service, by severity and comorbidity stratum, and by species within the coagulase-negative staphylococci, as well as the evaluation of biomarker combinations, were exploratory. The analyses added at revision, namely the two examinations of the pre-result interval, the operating characteristics by bacterial group, the discrimination between Gram-negative and Gram-positive isolates, the neutrophil-to-lymphocyte ratio by clinical service within each period and on the first culture of each patient within each service, the comparison of the subset with a clinical service recorded and the operating characteristics of procalcitonin by calendar year, were carried out in response to peer review and are post hoc.

2.9. Use of Generative Artificial Intelligence

Generative artificial intelligence (Claude Opus 5, Anthropic, San Francisco, CA, USA) was used for translation, for improving the wording and readability of the manuscript text, and for improving the visual elements of the article. It was not used to generate or interpret the data. All authors reviewed the final text and figures and take responsibility for their content.

3. Results

3.1. Cohort

Of the 64,620 blood cultures processed during the study period, 53,033 from 16,431 patients had at least one biomarker determination inside the window and entered the analysis (Figure 1). A pathogen grew in 4920 (9.3%) cultures, coagulase-negative staphylococci in 1758 (3.3%), and 46,238 (87.2%) showed no growth. In 117 cultures, the isolate could not be assigned to a group.

Age and sex distributions were similar across groups (Table 1). Severity was not: SOFA reached 2 or more in 47% of cultures growing Enterobacterales and in 23% of cultures without growth.

At least one determination before the microbiological result was available in 94.9% of cultures for total leukocytes and platelets, 94.8% for neutrophils and lymphocytes, 85.3% for C-reactive protein, 69.0% for procalcitonin, 62.7% for fibrinogen and 62.1% for albumin, with a median of two determinations per culture for each analyte, except albumin, for which there was one.

The interval available before the result was longer in cultures without growth: for procalcitonin, the median was 99 h (IQR 74 to 117) versus 71 h (45 to 107). The number of determinations was slightly higher in cultures with growth, with a mean of 2.40 versus 2.21. C-reactive protein and the blood count showed the same pattern (Table 2).

A pathogen grew in 11.7% of the cultures with a procalcitonin determination before the result and in 5.0% of those without. A SOFA score was recorded in 76.7% versus 49.2%, and where recorded, it was 2 or more in 39.8% versus 28.6%. The proportion with a prior determination was stable across years, between 67% and 70%.

Table 1. Characteristics and procalcitonin concentrations by bacterial group.

Group	Cultures	Patients	Age, y	Male, %	SOFA $\geq$ 2, %	Charlson	n with PCT	PCT First, ng/mL	PCT Maximum, ng/mL
Enterobacterales	2389	1223	73 (63–82)	56	47	4 (3–5)	2025	4.02 (0.80–16.54)	7.19 (1.47–29.23)
Polymicrobial	729	554	74 (64–82)	65	40	4 (3–6)	624	2.15 (0.34–12.88)	5.07 (0.68–23.52)
Streptococcus spp.	413	260	70 (59–80)	62	35	4 (3–5)	337	2.13 (0.52–8.79)	3.63 (0.71–10.90)
Non-fermenting GNB	201	126	72 (63–80)	66	37	4 (4–5)	174	2.84 (0.76–9.33)	3.31 (0.89–11.81)
Other Gram-negative	57	36	72 (60–80)	61	33	4 (3–5)	40	1.13 (0.19–12.63)	1.16 (0.31–13.11)
Fungi	107	52	70 (63–75)	68	46	5 (4–6)	88	0.61 (0.28–2.97)	1.17 (0.30–6.19)
Staphylococcus aureus	482	200	64 (53–78)	73	45	3 (2–5)	395	1.54 (0.41–6.49)	1.98 (0.68–8.71)
Anaerobes	149	110	68 (56–78)	64	30	4 (3–5)	122	1.27 (0.32–7.69)	1.38 (0.32–10.28)
Enterococcus spp.	245	122	73 (64–81)	73	55	5 (4–6)	205	0.43 (0.19–1.12)	0.76 (0.28–2.24)
Other Gram-positive	148	131	70 (55–81)	65	31	4 (3–5)	108	0.40 (0.13–3.71)	0.59 (0.16–4.09)
Coagulase-negative staphylococci	1758	1273	72 (60–80)	59	33	4 (3–5)	1364	0.28 (0.10–1.18)	0.39 (0.12–2.10)
No growth	46,238	15,470	70 (58–80)	60	23	4 (3–5)	31,032	0.29 (0.10–1.27)	0.34 (0.11–1.65)
All blood cultures	53,033	16,431	70 (59–80)	60	25	4 (3–5)	36,602	0.35 (0.11–1.69)	0.43 (0.12–2.43)

Values are median (interquartile range) unless stated. SOFA was available for 68.2% of cultures and the Charlson index for 82.8%. Groups with fewer than 30 cultures are not shown; 117 cultures whose isolate could not be assigned to a group are included in the last row only. Metrics are defined inside the window running from five days before to three days after the microbiological result. "First value before result" is the earliest determination with a timestamp preceding the result; "maximum before result" is the highest such determination; "nearest the result" is the determination closest in time to the result and may fall on either side of it. Concentrations of the remaining biomarkers by group are given in Table S2. Antimicrobial exposure and vasoactive drug administration are recorded in prescribing systems whose coverage varied across the study period and which under-capture treatment in critical care; neither is reported. "PCT first" and "PCT maximum" are the first and the highest procalcitonin determination preceding the microbiological result; "n with PCT" is the number of cultures with at least one such determination. SOFA $\geq$ 2 is given as a percentage of all cultures in each group, including those without a recorded SOFA. The anaerobe group comprised mainly *Bacteroides* (72 isolates), *Clostridium* (21), *Fusobacterium* (14) and *Propionibacterium* (14).

Table 2. Biomarker discrimination of pathogen growth in blood cultures.

Biomarker	n Pathogen	n No Growth	Patients	First Value Before Result	Maximum Before Result	Nearest the Result	Determinations Before Result, Growth/No Growth	Hours Before Result, Growth/No Growth
Procalcitonin	4118	31,032	11,591	0.738 (0.728–0.748)	0.766 (0.756–0.776)	0.773 (0.764–0.784)	2 (1–3)/ 2 (1–3)	71 (45–107)/ 99 (74–117)
C-reactive protein	4153	39,415	13,895	0.571 (0.557–0.583)	0.651 (0.640–0.663)	0.675 (0.664–0.686)	2 (2–3)/ 2 (1–3)	85 (47–116)/ 100 (75–117)
Neutrophil-to-lymphocyte ratio	4832	43,639	15,579	0.680 (0.667–0.693)	0.707 (0.695–0.719)	0.620 (0.608–0.632)	2 (2–3)/ 2 (1–3)	82 (48–114)/ 99 (74–117)
Absolute lymphocyte count	4832	43,652	15,582	0.338 (0.326–0.349)	0.374 (0.362–0.386)	0.382 (0.370–0.394)	2 (2–3)/ 2 (1–3)	82 (48–114)/ 99 (74–117)
Platelet count	4832	43,692	15,595	0.403 (0.391–0.415)	0.389 (0.377–0.401)	0.358 (0.346–0.370)	2 (2–4)/ 2 (1–3)	84 (48–114)/ 99 (74–117)
Absolute neutrophil count	4832	43,652	15,582	0.598 (0.585–0.611)	0.630 (0.618–0.644)	0.562 (0.549–0.575)	2 (2–3)/ 2 (1–3)	82 (48–114)/ 99 (74–117)
Total leukocyte count	4832	43,673	15,598	0.564 (0.550–0.577)	0.600 (0.586–0.613)	0.533 (0.520–0.547)	2 (2–3)/ 2 (1–3)	82 (48–114)/ 99 (74–117)
Fibrinogen	4016	27,777	10,248	0.531 (0.517–0.546)	0.559 (0.544–0.573)	0.585 (0.571–0.599)	2 (1–3)/ 2 (1–3)	72 (46–110)/ 100 (74–117)
Albumin	3329	28,313	10,433	0.472 (0.455–0.488)	0.470 (0.453–0.486)	0.417 (0.403–0.431)	2 (1–2)/ 1 (1–2)	71 (44–97)/ 94 (69–114)

Pathogen growth excludes coagulase-negative staphylococci, which are analyzed separately. Cells give the area under the receiver operating characteristic curve with its 95% confidence interval; areas below 0.5 indicate lower values in cultures with pathogen growth. Metrics are defined inside the window running from five days before to three days after the microbiological result. "First value before result" is the earliest determination with a timestamp preceding the result; "maximum before result" is the highest such determination; "nearest the result" is the determination closest in time to the result and may fall on either side of it. Confidence intervals are from a bootstrap resampled at patient level with 2000 replicates because cultures are correlated within patients. Counts refer to the two metrics computed before the result; the nearest-the-result metric has slightly larger denominators because a few cultures have determinations only after the result. The last two columns give the median (interquartile range) number of determinations preceding the result and the interval in hours between the earliest of them and the result in cultures with pathogen growth and in cultures without growth. Permutation of outcome labels at patient level over 20,000 replicates, applied to the first value before the result, gave $p < 0.001$ for every biomarker.

3.2. Bacterial Group and Biomarker Concentration

Procalcitonin showed the greatest separation of the bacterial groups of any analyte on every metric (Table S1). ε^2 was 0.074 for the first value before the result, 0.090 for the maximum before the result and 0.096 for the value nearest the result. Enterobacterales had the highest medians, and cultures without growth the lowest: 4.02 versus 0.29 ng/mL on the first value, and 7.19 versus 0.34 on the maximum (Table 1, Figure 2a). Cultures growing coagulase-negative staphylococci had values close to those of cultures without growth on both metrics, 0.28 and 0.39 ng/mL. Concentrations of the remaining biomarkers by group are given in Table S2. The largest effect obtained for any other biomarker on any metric was 0.048 for the maximum neutrophil-to-lymphocyte ratio before the result.

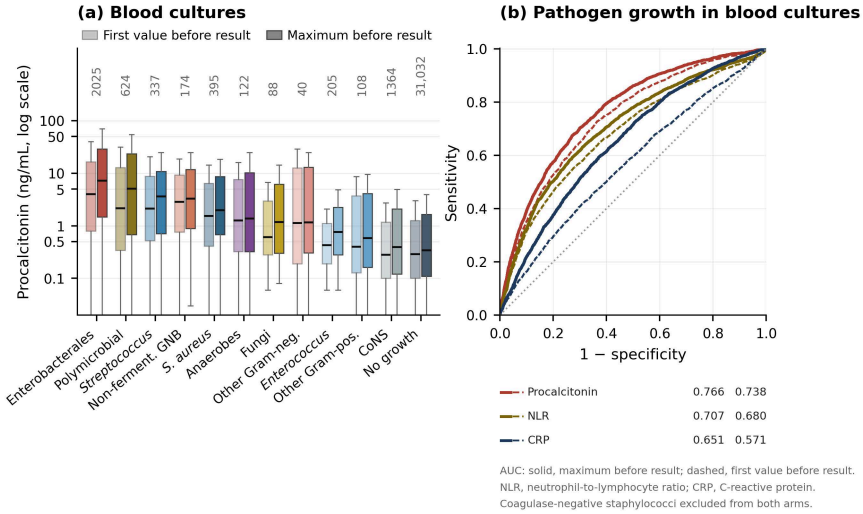

Figure 2. Procalcitonin and blood culture result. (**a**) Procalcitonin by bacterial group, first value before the result (pale) and maximum before the result (solid), on a logarithmic scale; the number of cultures with a procalcitonin determination before the result is printed above each group. (**b**) Receiver operating characteristic curves for pathogen growth; the areas are point estimates, and their 95% confidence intervals are given in Table 2. The dotted diagonal line corresponds to no discrimination (area under the curve of 0.5). CoNS, coagulase-negative staphylococci; CRP, C-reactive protein; GNB, Gram-negative bacilli; NLR, neutrophil-to-lymphocyte ratio.

Among the cultures that grew a pathogen, procalcitonin separated Gram-negative from Gram-positive isolates with an area under the curve of 0.657 (95% CI 0.626–0.687) on the maximum before the result, computed on 2239 Gram-negative and 1045 Gram-positive cultures. The lymphocyte count ranked next, with lower values in Gram-negative isolates and an area of 0.422 (0.395–0.449) on the first value before the result; no other biomarker departed from 0.5 by more than 0.06 on any metric (Table S10).

3.3. Prediction of Pathogen Growth

Coagulase-negative staphylococci were excluded from the positive arm. Procalcitonin gave the largest area under the curve on all three metrics: 0.766 (95% CI 0.756–0.776) for the maximum before the result, 0.738 (0.728–0.748) for the first value and 0.773 (0.764–0.784) for the value nearest the result (Table 2, Figure 2b). The neutrophil-to-lymphocyte ratio showed the opposite pattern, with 0.707 (0.695–0.719) for the maximum before the result and 0.620 (0.608–0.632) for the value nearest it. C-reactive protein fell from 0.675 (0.664–0.686) nearest the result to 0.571 (0.557–0.583) on the first value before it. Platelet count, lymphocyte count and albumin were lower in cultures with pathogen growth, and their areas fell below 0.5 accordingly.

Operating characteristics of both pre-result metrics of procalcitonin at seven thresholds are given in Table 3, with the cross-tabulation of each threshold against the culture result. They were computed on the 35,150 cultures, from 11,591 patients, in which procalcitonin had been measured before the result, 4118 with pathogen growth and 31,032 without, with a prevalence of 11.7%. At 0.5 ng/mL, the maximum before the result gave a sensitivity of 81.5% and a negative predictive value of 95.8%, with a specificity of 56.8%; the first value gave 75.0%, 94.8% and 60.2%. The highest positive likelihood ratio across the whole range was 4.84 (95% CI 4.37–5.38) at 20 ng/mL.

Sensitivity at 0.5 ng/mL differed by organism (Table S8). It reached 88.0% (86.2–89.9) for Enterobacterales and 86.2% (80.0–91.2) for non-fermenting Gram-negative bacilli, compared with 61.0% (51.7–70.4) for enterococci, 62.5% (47.8–75.9) for fungi and 55.6% (44.4–65.8) for other Gram-positive organisms. Negative predictive value was 98.6% or higher for every group taken separately, compared with 95.8% for pathogen growth as a whole.

3.4. Effect of the Metric

The effect of bacterial group was compared across the four metrics of each biomarker (Table S1, Figure 3). For procalcitonin and C-reactive protein, ε^2 was the largest for the value nearest the result and the smallest for the first value before it: 0.096 versus 0.074 for procalcitonin and 0.032 versus 0.007 for C-reactive protein. The neutrophil-to-lymphocyte ratio and the lymphocyte count showed the reverse pattern: 0.048 for the maximum before the result versus 0.017 for the value nearest it and 0.029 for the first value before the result versus 0.016 for the value nearest it. Amplitude, the ratio of maximum to minimum among determinations preceding the result, reached 0.020 for lymphocytes and did not exceed 0.013 for any other analyte.

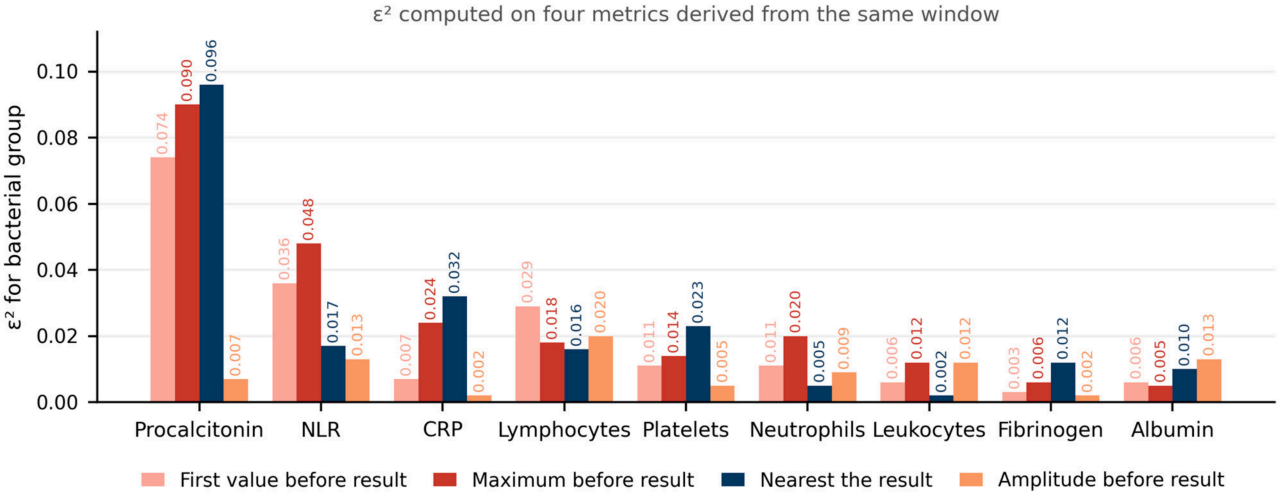

Figure 3. Effect of bacterial group by metric. ε^2, the proportion of rank variance attributable to bacterial group, for each biomarker on each of the four metrics. The same values are tabulated in Table S1. CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio.

Table 3. Operating characteristics of procalcitonin for pathogen growth, with cross-tabulation against the culture result.

PCT Metric	Threshold (ng/mL)	Growth Test +	Growth Test −	No Growth Test +	No Growth Test −	Se, %	Sp, %	LR+	LR−	PPV, %	NPV, %
First value before result	≥0.25	3484	634	16,592	14,440	84.6 (83.1–86.1)	46.5 (45.6–47.4)	1.58	0.33	17.4	95.8
First value before result	≥0.5	3088	1030	12,337	18,695	75.0 (73.1–76.8)	60.2 (59.4–61.1)	1.89	0.42	20.0	94.8
First value before result	≥1	2607	1511	8819	22,213	63.3 (61.2–65.4)	71.6 (70.8–72.4)	2.23	0.51	22.8	93.6
First value before result	≥2	2138	1980	6006	25,026	51.9 (49.7–54.2)	80.6 (80.0–81.4)	2.68	0.60	26.3	92.7
First value before result	≥5	1559	2559	3509	27,523	37.9 (35.7–40.1)	88.7 (88.2–89.2)	3.35	0.70	30.8	91.5
First value before result	≥10	1105	3013	2143	28,889	26.8 (24.9–28.9)	93.1 (92.7–93.5)	3.89	0.79	34.0	90.6
First value before result	≥20	678	3440	1117	29,915	16.5 (14.7–18.2)	96.4 (96.1–96.7)	4.57	0.87	37.8	89.7
Maximum before result	≥0.25	3680	438	17,618	13,414	89.4 (88.1–90.5)	43.2 (42.3–44.1)	1.57	0.25	17.3	96.8
Maximum before result	≥0.5	3355	763	13,412	17,620	81.5 (79.8–83.0)	56.8 (55.9–57.6)	1.89	0.33	20.0	95.8
Maximum before result	≥1	2951	1167	9872	21,160	71.7 (69.7–73.5)	68.2 (67.4–69.0)	2.25	0.42	23.0	94.8
Maximum before result	≥2	2531	1587	7055	23,977	61.5 (59.2–63.6)	77.3 (76.5–78.0)	2.70	0.50	26.4	93.8

Table 3. *Cont.*

PCT Metric	Threshold (ng/mL)	Growth Test +	Growth Test −	No Growth Test +	No Growth Test −	Se, %	Sp, %	LR+	LR−	PPV, %	NPV, %
Maximum before result	≥5	1946	2172	4274	26,758	47.3 (45.1–49.4)	86.2 (85.6–86.8)	3.43	0.61	31.3	92.5
Maximum before result	≥10	1471	2647	2740	28,292	35.7 (33.6–38.0)	91.2 (90.7–91.7)	4.05	0.71	34.9	91.4
Maximum before result	≥20	984	3134	1531	29,501	23.9 (21.9–25.9)	95.1 (94.7–95.4)	4.84	0.80	39.1	90.4

Computed on the 35,150 cultures, from 11,591 patients, in which procalcitonin had been measured before the microbiological result: 4118 with pathogen growth and 31,032 without growth (prevalence 11.7%). These cultures had a higher prevalence of growth and of SOFA ≥ 2 than did the cultures without a determination (Section 3.1), and the operating characteristics apply to them. Coagulase-negative staphylococci were excluded. Both metrics are restricted to determinations preceding the microbiological result. The four count columns give the cross-tabulation of the procalcitonin threshold against the culture result; "test +" is a value at or above the threshold. Predictive values are derived from the likelihood ratios at that prevalence. The 95% confidence intervals below sensitivity and specificity are from a bootstrap resampled at patient level, with 2000 replicates. Se, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR−, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; SOFA, Sequential Organ Failure Assessment.

Two analyses compared the first value with the maximum before the result while holding pre-result sampling constant. Restricted to the 20,670 cultures with two or more procalcitonin determinations before the result, the first value gave 0.715 (0.702–0.729) and the maximum 0.763 (0.752–0.776), a paired difference of 0.048 (0.040–0.057); the difference was 0.052 with two determinations, 0.045 with three and 0.057 with four or more. Derived inside a fixed 48 h pre-result window, again with two or more determinations, the two metrics gave 0.757 (0.736–0.777) and 0.800 (0.781–0.817), a difference of 0.043 (0.032–0.054). In the unrestricted cohort, the difference was 0.028 (0.023–0.034) (Table S7).

3.5. Temporal Behavior

Median procalcitonin by day is shown in Figure S1a. The Gram-negative, streptococcal, staphylococcal, anaerobic and fungal curves lay above the no-growth curve on every day of the window; the enterococcal and other Gram-positive curves crossed it. The coagulase-negative staphylococcal curve tracked the no-growth curve throughout, the two never differing by more than 0.09 ng/mL. The Enterobacterales curve was highest on day −1, at 9.43 ng/mL.

Among cultures with at least three determinations preceding the result, the individual maximum fell on day −2 or −1 in 38.0% of Enterobacterales and in 14.7% of cultures without growth (Figure S1b).

3.6. Severity and Comorbidity

ε^2 for the effect of bacterial group on the maximum procalcitonin before the result rose across SOFA strata, from 0.068 at SOFA 0–1 to 0.161 at SOFA 4 or more, and the Enterobacterales-to-no-growth ratio from 11.8 to 22.5 (Table S3). Across Charlson strata, ε^2 ranged from 0.067 to 0.088. Table S4 repeats Table 2 for the SOFA $\geq$ 2 subgroup, where the maximum before the result gave 0.762 (0.746–0.779) for procalcitonin and 0.673 (0.654–0.693) for the neutrophil-to-lymphocyte ratio.

3.7. Clinical Service

A clinical service was recorded for 22,901 of the 53,033 cultures (43.2%). The field comes from the admission episode and is missing in the same cultures as the admission diagnosis: an admission diagnosis was present in 95.5% of cultures with a service recorded and in none of those without. The Charlson index was more complete in the cultures without a service, 86.4% versus 78.1%.

Growth was more frequent where a service was recorded, 14.2% versus 6.2%, and the difference held within every stratum of the number of cultures per patient, from 14.0% versus 5.7% among patients with two cultures to 13.7% versus 8.3% among those with nine or more (Table S9a). Procalcitonin gave 0.772 (0.758–0.786) where a service was recorded and 0.750 (0.735–0.765) where it was not; the neutrophil-to-lymphocyte ratio gave 0.707 (0.691–0.722) and 0.681 (0.662–0.699).

Discrimination by the maximum procalcitonin level before the result ranged from 0.735 (0.695–0.772) in oncology to 0.788 (0.726–0.843) in hematology, with all intervals overlapping (Table 4). The neutrophil-to-lymphocyte ratio ranged from 0.512 (0.437–0.586) in hematology and 0.623 (0.565–0.677) in oncology to 0.738 (0.709–0.764) on surgical wards and 0.736 (0.699–0.772) in critical care. Hematology contributed the most cultures per patient, 6.6 versus 2.8 to 4.6 elsewhere; on the first culture of each patient within each service, the pattern was unchanged, with the ratio at 0.609 in hematology and 0.632 in oncology (Table S9b).

Table 4. Discrimination of pathogen growth by clinical service.

Clinical Service	Cultures	Patients	Pathogen Growth, %	PCT Maximum Before Result, AUC (95% CI)	NLR Maximum Before Result, AUC (95% CI)	PCT Maximum ≥ 0.5 ng/mL: NPV, %
Critical care	3542	814	12.5	0.785 (0.752–0.817)	0.736 (0.699–0.772)	95.5
Internal medicine	5241	1782	16.0	0.779 (0.754–0.802)	0.722 (0.698–0.745)	94.3
Surgical	3803	1199	19.5	0.761 (0.735–0.786)	0.738 (0.709–0.764)	93.7
Other medical	2050	758	11.1	0.778 (0.735–0.818)	0.706 (0.663–0.751)	95.6
Oncology	1371	411	16.8	0.735 (0.695–0.772)	0.623 (0.565–0.677)	93.6
Hematology	1032	183	11.4	0.788 (0.726–0.843)	0.512 (0.437–0.586)	96.3

Restricted to the 22,783 cultures (43.0% of the analysis population) with a clinical service recorded in one of the six groups, coagulase-negative staphylococci and unclassifiable isolates excluded. Cultures, patients, pathogen growth, the procalcitonin area and the negative predictive value refer to the cultures with a procalcitonin determination before the microbiological result; the area for the neutrophil-to-lymphocyte ratio refers to the cultures with a ratio before the result. Service codes changed format during 2024 and were harmonized before grouping. Confidence intervals are from a bootstrap resampled at patient level with 2000 replicates because cultures are correlated within patients. Areas under the curve and the threshold of 0.5 ng/mL are both applied to the maximum determination preceding the microbiological result, not to the first determination. Negative predictive values are derived from the likelihood ratio at the prevalence observed within each service. NLR, neutrophil-to-lymphocyte ratio; NPV, negative predictive value; PCT, procalcitonin.

3.8. Sensitivity Analyses

Restricted to the first blood culture of each patient, the maximum procalcitonin before the result gave 0.755 (0.742–0.768) and the maximum neutrophil-to-lymphocyte ratio 0.735 (0.722–0.749). Analyzed separately, the 2020–2021 period gave 0.783 (0.759–0.806) for the maximum procalcitonin before the result and the 2022–2026 period 0.763 (0.752–0.775), at prevalences of 14.5% and 11.2% (Table S5). Across calendar years, the area for the maximum procalcitonin before the result ranged from 0.734 to 0.797, with sensitivity and specificity at 0.5 ng/mL from 78.9% to 84.8% and from 56.0% to 60.4% (Table S11). The neutrophil-to-lymphocyte ratio was stable across the same split, 0.697 (0.671–0.723) versus 0.711 (0.697–0.723), and the pattern across services held within each period: the ratio reached 0.452 and 0.523 in hematology and 0.557 and 0.638 in oncology, compared with 0.63 to 0.76 in the other four services, while procalcitonin stayed between 0.73 and 0.80 throughout (Table S9b). Of 56 combinations of biomarkers formed without fitted parameters, the best improved the area over procalcitonin alone by 0.013, and a logistic model, fitted on seven markers with five-fold cross-validation split at patient level, improved it by 0.015 (Table S6a,b).

3.9. Coagulase-Negative Staphylococci at the Rule-Out Threshold

Staphylococcus epidermidis was the sole isolate in 868 cultures, of which 701 in 522 patients had a procalcitonin determination before the result. The first value reached 0.5 ng/mL in 38.9% of them versus 39.8% of the 31,032 cultures without growth, a difference of −0.8 percentage points (95% CI −5.1 to +3.8); the maximum before the result reached it in 46.2% versus 43.2%, a difference of +3.0 points (−1.3 to +7.4). In 395 cultures growing *Staphylococcus aureus*, the same thresholds were exceeded by 72.9% and 79.0%, differences of +33.2 points (+26.4 to +39.2) and +35.8 points (+30.0 to +41.0).

4. Discussion

Biomarkers of infection are one measurement with one meaning. Serial determinations of a single analyte yield a first value, a maximum, a rate of change and an amplitude, and each of these answers a different question; the same number carries different information, depending on when the sample was drawn, which outcome it is asked about, and which patient it came from. Clinical practice mostly collapses all of this into one threshold applied to one value. In 53,033 blood cultures drawn over six and a half years, we asked what is lost in that collapse, and found that the loss is large and that it differs from marker to marker.

Procalcitonin discriminated pathogen growth from sterile culture better than any other biomarker on every metric, and restricting it to determinations preceding the microbiological result reduced its discrimination only slightly: 0.766 (95% CI 0.756–0.776) for the maximum before the result versus 0.773 (0.764–0.784) for the value nearest it. The neutrophil-to-lymphocyte ratio showed the opposite pattern, 0.707 (0.695–0.719) before the result versus 0.620 (0.608–0.632) near it. C-reactive protein lost most of its apparent performance under the same restriction, from 0.675 (0.664–0.686) to 0.571 (0.557–0.583) on the first value available. Three markers, one outcome, one window, and three different answers, depending on which number is read.

That the maximum outperforms the first value could be an artifact rather than a property of procalcitonin. A negative blood culture is not reported until five days of incubation have elapsed, so the interval before the result is longer in the cultures that will not grow, and a maximum taken over a longer interval has more opportunity to increase. The direction of the resulting bias is against the observed effect rather than toward it, and the data confirm this: with the number of determinations held constant, the gain of the maximum over the first value is 0.048 (0.040–0.057), and within a common 48 h window, it is 0.043 (0.032–0.054), compared with 0.028 (0.023–0.034) in the unrestricted cohort. What dilutes the effect in the whole cohort is the large stratum of cultures with a single determination, in which the two metrics are the same number by construction. The metric matters more, not less, once the sampling is made comparable.

The Surviving Sepsis Campaign suggests clinical evaluation alone over procalcitonin plus clinical evaluation when deciding whether to start antimicrobial therapy [15]. Our data are consistent with that recommendation. No threshold ruled in a bloodstream pathogen: over the whole range, the highest positive likelihood ratio was 4.84 at 20 ng/mL, where sensitivity has already fallen to 23.9%. A marker that cannot rule in infection should not determine whether the first dose is given. An international consensus statement on sepsis code guidelines nonetheless placed procalcitonin among the biomarkers that contribute to early recognition [16], and the recommendations of the Latin American Working Group for Improving Infection Care in Emergency Departments (GT-LATINFURG) position it within the emergency department workup [17]. These positions address different moments in care. The metric that performed best here, the maximum preceding the microbiological result, accumulates over the days that a set is incubated and does not exist when the first dose is due. At that moment, the clinician has the first value, 75.0% sensitivity and a negative predictive value of 94.8%; by the time the culture is reported, the maximum gives 81.5% and 95.8%. What procalcitonin answers well is not whether to start treatment, but which of the patients already treated will turn out to have grown an organism.

Póvoa et al., summarizing more than 250 biomarkers evaluated in sepsis, advise clinicians that biomarker kinetics are more useful than are single values [7]. We tested one kinetic summary, the amplitude between determinations before the result, and our conclusions are restricted to this summary. For procalcitonin, the effect of bacterial group lay almost entirely in the level metrics, $\varepsilon^2 = 0.090$ for the maximum before the result versus 0.007 for the amplitude over the same window, and C-reactive protein and fibrinogen

behaved in the same way. For the white cell counts, the order was reversed, with the amplitude of the lymphocyte count reaching 0.020 versus 0.016 for the value nearest the result, and albumin behaved like the white cell counts. Amplitude does not distinguish a marker that is rising from one that is falling between the same two values; a directional or slope-based summary might behave differently for procalcitonin and C-reactive protein, and we did not test this. Pierrakos et al. counted 258 sepsis biomarkers published between 2009 and 2019 and concluded that producing further reports without a more rigorous framework is futile [6]. Part of that framework is deciding, and stating, which number from a time series is being tested.

As a rule-out test, procalcitonin performed well, and its sensitivity matched that reported in other settings. Julián-Jiménez et al., pooling twenty studies and 18,120 blood cultures from emergency departments, report a sensitivity of 77.6% at the same threshold in their six highest-quality studies [4], within three points of our 75.0% reading on the first value before the result. Their specificity was not similar to ours, at 78% versus our 60.2%. What separates the two settings is the comparator arm. A blood culture is drawn because a clinician suspects bloodstream infection, and in our hospital, that suspicion is accompanied by antimicrobial treatment; the cultures that grew nothing are therefore not from patients without inflammation but represent patients with suspected, treated infections that failed to grow, in wards, intensive care units and oncology services where a raised procalcitonin level has many causes. Ruling in did not work in either setting.

The rule-out did not perform equally across organisms. Sensitivity at 0.5 ng/mL was 88.0% (86.2–89.9) for Enterobacterales and 86.2% (80.0–91.2) for non-fermenting Gram-negative bacilli, and fell to 61.0% (51.7–70.4) for enterococci, 62.5% (47.8–75.9) for fungi and 55.6% (44.4–65.8) for other Gram-positive organisms. Leli et al. described the same ordering of concentrations by organism in 1949 samples from patients with suspected bloodstream infection [12], and Koizumi et al. reported that procalcitonin remains low at the onset of Gram-positive bacteremia, irrespective of severity [13]. A clinician reading a procalcitonin level below 0.5 ng/mL is therefore not ruling out bloodstream infection in general but ruling out Gram-negative bloodstream infection, while missing two of every five enterococcal episodes. Negative predictive value nonetheless stayed at 98.6% or above for every group taken separately because each of them is uncommon; the aggregate 95.8% reflects prevalence rather than uniform sensitivity.

The same asymmetry reappears when the organism itself is the outcome. Among the cultures that grew, procalcitonin separated Gram-negative from Gram-positive isolates with an area of 0.657 (0.626–0.687), and the next biomarker, the lymphocyte count, reached 0.422 (0.395–0.449) in the opposite direction. Yan et al. report a comparable separation in a smaller series [18], and Abbas et al. obtained it prospectively in a tertiary hospital [8]. An area of 0.66 is a real signal and a poor test. It is enough to say that a very high procalcitonin level in a culture that will grow favors a Gram-negative organism and is not enough to guide the choice of antimicrobial.

Within our own hospital, the comparison across clinical services separated the two biomarkers. Procalcitonin varied little across clinical services, from 0.735 (0.695–0.772) in oncology to 0.788 (0.726–0.843) in hematology, with all intervals overlapping. The neutrophil-to-lymphocyte ratio showed greater variation: 0.738 (0.709–0.764) on surgical wards and 0.736 (0.699–0.772) in critical care, versus 0.623 (0.565–0.677) in oncology and 0.512 (0.437–0.586) in hematology, an interval that includes 0.5. In 2010, de Jager et al. showed that the ratio predicts bacteremia better than do C-reactive protein and the white cell count in an emergency care unit, with an area of 0.73 [10]; on the first blood culture of each patient, we obtained 0.735 (0.722–0.749), with more than eighty times their sample size. Martinez et al. report 0.593 (0.529–0.657) for the same ratio in an oncology center [11],

within our own oncology interval, and their review of the ratio and its variants found the supporting evidence came mostly from patients without cancer, with its accuracy in immunosuppressed cancer patients still unvalidated [19]. What neither study could show, having one setting each, is that the marker moves from useful to uninformative between wards of the same hospital in the same period under the same definitions. Svaldi et al. reported a quarter of a century ago that heavy leukopenia and immunosuppression alter the diagnostic performance of procalcitonin itself [20]; in our hematology patients, procalcitonin retained its performance, and the ratio did not. Cancer, immunosuppression and the treatment already received are among the recognized sources of heterogeneity in how sepsis manifests [21], and here they determined whether a marker derived from the blood count carried any information at all. Where hematopoiesis is intact, the ratio approaches that of procalcitonin and comes at no extra cost, with a blood count already drawn. Where the neutrophil and lymphocyte counts are determined by the disease and its treatment, it carries no information.

Whether procalcitonin was measured at all was informative, and this is a property of the test rather than a defect of the study. A pathogen grew in 11.7% of cultures with a prior determination and in 5.0% of those without, and where a SOFA score was recorded, it was 2 or more in 39.8% versus 28.6%. A clinician who orders procalcitonin testing has already raised the pre-test probability by the act of ordering it, and the marker then operates on that probability rather than on the unconditional prevalence among all blood cultures. Mulherin and Miller drew the distinction that applies here: variation in accuracy across subgroups defined by the clinical reason for testing is a spectrum effect, a real property of the test in that population, and not a bias to be corrected away [22]. That is how a Bayesian modifier is meant to work, and it is how our operating characteristics should be read: they describe the cultures in which a clinician had already chosen to measure procalcitonin, not blood cultures at large.

Coagulase-negative staphylococci were indistinguishable from sterile cultures on every axis we examined, while *Staphylococcus aureus* was separated from them by more than thirty percentage points at the same threshold. Koizumi et al. found that 46% of their *S. epidermidis* isolates reached 0.5 ng/mL and interpreted this as under-detection of a Gram-positive pathogen [13]. Our figure for the same metric is 46.2%, and it is the comparator that changes the reading: 43.2% of the cultures that grew nothing also exceeded that threshold. The 46% is the background rate of a hospital population in which blood cultures are drawn, not a signal from the organism. Truly pathogenic coagulase-negative staphylococci exist, and our data suggest they are rare enough not to move the distribution.

Combining biomarkers did not help with this specific question. The best of 56 parameter-free combinations improved the area by 0.013 over the results for procalcitonin alone, and a logistic model fitted on all seven markers with patient-level cross-validation improved it by 0.015. At the operating point where the test is used, the negative predictive value was identical to one decimal place. Tromp et al. reached the same conclusion in 342 emergency department patients with suspected infection, where neither serial sampling nor a panel of biomarkers improved on the results for a single procalcitonin measurement [23]; our cohort has close to fifty times as many patients, and the answer is the same: three laboratory values instead of one, and the patient who would have been missed is still missed.

This study leaves several questions open. The effect of bacterial group on biomarker concentration exists in our data and is modest, and the comparison of Gram-negative with Gram-positive isolates quantifies how far it can be taken; whether a marker of that discrimination could contribute to an empirical decision, alongside clinical information, is a question for a study designed around that outcome, not for this one. We restricted the

analysis to blood, so nothing in this work speaks to whether the same biomarkers behave differently in urine, respiratory samples or sterile fluids, which we regard as an open rather than answered question. We report discrimination and operating characteristics and not calibration, so predictive values should be read as derived from likelihood ratios at the observed prevalence rather than as validated risk estimates. And we describe associations, not mechanism: why the neutrophil-to-lymphocyte ratio should carry more information three days before a microbiological result than on the day it is obtained is not a question a retrospective cohort can answer.

The study is retrospective and from a single hospital, and the biomarker panel reflected clinical requests rather than a protocol, so availability differs by analyte, from 94.9% of cultures for the leukocyte count to 62.1% for albumin. Procalcitonin was measured on the same platform throughout, and its operating characteristics were stable across calendar years; the other analytes were not examined for drift. Antimicrobial exposure is recorded in a prescribing system whose coverage changed during the study period and which under-captures treatment in critical care, so we could not adjust for it; we report the design-level fact that these cultures were drawn under clinical suspicion of infection, without quantifying the proportion treated. Cultures are correlated within patients, 53,033 in 16,431, and confidence intervals are from a bootstrap resampled at patient level for that reason; restricted to one culture per patient, the estimates were stable; in fact, the ratio improved to 0.735 (0.722–0.749). Blood culturing misses some bloodstream infections, particularly after antimicrobial treatment, and the reference standard is the culture result rather than clinical adjudication, so a treated bacteremia that failed to grow falls in our negative arm. Beyond the stratified analyses of Table S3 and Table S4, we did not model the relation between biomarker concentrations and SOFA or other clinical characteristics. Results were unchanged when 2020–2021 was analyzed separately: 0.783 versus 0.763 for procalcitonin and 0.697 versus 0.711 for the ratio. The analysis by clinical service rests on the cultures for which that field exists, which is a subset defined by how the hospital system assigns episode identifiers rather than by any clinical criterion, and one in which growth is more frequent than in the cohort as a whole; discrimination transported to the remainder for both markers, and hematology rests on 183 patients, so 0.512 should be read as indistinguishable from chance.

5. Conclusions

Across 53,033 blood cultures, the number read from an inflammatory biomarker changes the answer as much as does the biomarker chosen. In the cultures in which it was measured, procalcitonin was the best pathogen growth discriminator on every metric, performed similarly in every clinical service, and never ruled in bloodstream infection. Below 0.5 ng/mL, it argues strongly against Gram-negative bacteremia and much more weakly against enterococcal, other Gram-positive, and fungal bloodstream infection, and any use of it as a rule-out should name the organisms it covers. The neutrophil-to-lymphocyte ratio approached it outside hemato-oncology and was uninformative within it, so it is a reasonable substitute where a blood count is all that is available and the marrow is intact. Combining markers added little. A biomarker result is not a value: it is a value, a metric, a window and a population, and reporting the first without the other three produces the range of published estimates that made this comparison necessary.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms14112444/s1, Table S1, effect of bacterial group on each biomarker, by metric; Table S2, biomarker concentrations by bacterial group; Table S3, effect of bacterial group on the maximum procalcitonin before the result, by severity and comorbidity; Table S4, biomarker discrimination of pathogen growth in the SOFA $\geq$ 2 subgroup; Table S5, sensitivity

analyses; Table S6a, combinations of biomarkers formed without fitted parameters; Table S6b, logistic models with patient-level cross-validation; Table S7, first value and maximum of procalcitonin before the result with the pre-result sampling held constant; Table S8, sensitivity and negative predictive value of procalcitonin at 0.5 ng/mL by bacterial group; Table S9a, cultures with and without a clinical service recorded; Table S9b, discrimination of pathogen growth by clinical service within each period and on the first culture of each patient; Table S10, discrimination of Gram-negative from Gram-positive isolates among cultures with pathogen growth; Table S11, operating characteristics of procalcitonin by calendar year; Figure S1, procalcitonin over the analysis window by bacterial group.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Balearic Islands Ethics Committee for Research with Medicinal Products (Comité de Ética de la Investigación con medicamentos de las Illes Balears, CEIm-IB) within the authorization of the Computerized Protocol for the Integrated Management of Sepsis (Protocolo Informatizado de Manejo Integral de la Sepsis, PIMIS), reference IB 5320/23 PI, with extension approved on 8 July 2026.

Informed Consent Statement: Patient consent was waived by the CEIm-IB because the study was conducted retrospectively on pseudonymized routine clinical and laboratory data, with no intervention and no contact with participants.

Data Availability Statement: The data supporting the findings of this study are not publicly available. Spanish data protection legislation does not permit deposition of this dataset in a public repository. The data are available from the corresponding author on reasonable request, subject to the approvals required by the institution and by the applicable data protection framework.

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References

1. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.D.; Coopersmith, C.M.; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **2016**, *315*, 801–810. [CrossRef]
2. GBD 2021 Global Sepsis Collaborators. Global, regional, and national sepsis incidence and mortality, 1990–2021: A systematic analysis. *Lancet Glob. Health* **2025**, *13*, e2013–e2026. Correction in *Lancet Glob. Health* **2026**, *14*, e198. https://doi.org/10.1016/S2214-109X(25)00486-3. [CrossRef]
3. Falconer, K.; Hammond, R.; Parcell, B.J.; Gillespie, S.H. Investigating the time to blood culture positivity: Why does it take so long? *J. Med. Microbiol.* **2025**, *74*, 001942. [CrossRef]
4. Julián-Jiménez, A.; García, D.E.; Merinos-Sánchez, G.; García de Guadiana-Romualdo, L.; González del Castillo, J. Diagnostic accuracy of procalcitonin for bacteremia in the emergency department: A systematic review. *Rev. Esp. Quimioter.* **2023**, *37*, 29–42. [CrossRef]
5. Kumar, A.; Roberts, D.; Wood, K.E.; Light, B.; Parrillo, J.E.; Sharma, S.; Suppes, R.; Feinstein, D.; Zanotti, S.; Taiberg, L.; et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit. Care Med.* **2006**, *34*, 1589–1596. [CrossRef]

6. Pierrakos, C.; Velissaris, D.; Bisdorff, M.; Marshall, J.C.; Vincent, J.L. Biomarkers of sepsis: Time for a reappraisal. *Crit. Care* **2020**, *24*, 287. [CrossRef]
7. Póvoa, P.; Coelho, L.; Dal-Pizzol, F.; Ferrer, R.; Huttner, A.; Conway Morris, A.; Nobre, V.; Ramirez, P.; Rouzé, A.; Salluh, J.I.; et al. How to use biomarkers of infection or sepsis at the bedside: Guide to clinicians. *Intensive Care Med.* **2023**, *49*, 142–153. [CrossRef]
8. Abbas, A.M.; Abdel Aziz, H.S.; Hamed, R.M.R.; Soliman, S.B.; Abd El-Ghani, S.E.; Soliman, N.S. Diagnostic performance of procalcitonin for predicting bloodstream infection and guidance on microbial aetiology: A prospective study at an Egyptian tertiary care hospital. *BMC Infect. Dis.* **2026**, *26*, 1198. [CrossRef]
9. Sun, Q.; Lin, Q.; Lv, Y.; Tian, Z.; Yan, Q.; Yu, Y.; Fu, X.; Yao, H.; Sun, F.; Xia, Y.; et al. Predictive value of serum procalcitonin level for the diagnosis of bloodstream infections in hematological patients. *BMC Infect. Dis.* **2025**, *25*, 162. [CrossRef]
10. de Jager, C.P.C.; van Wijk, P.T.L.; Mathoera, R.B.; de Jongh-Leuvenink, J.; van der Poll, T.; Wever, P.C. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit. Care* **2010**, *14*, R192. [CrossRef]
11. Martínez, J.M.; Espírito Santo, A.; Leite, P.; Pinho, A.; Carneiro, A.R.; Oliveira, A.M.; Ramada, D.; Medeiros, R. Neutrophil–lymphocyte–platelet ratio for predicting bacteremia in immunosuppressed cancer patients: A retrospective diagnostic accuracy study. *Biomedicines* **2026**, *14*, 1170. [CrossRef]
12. Leli, C.; Ferranti, M.; Moretti, A.; Al Dhahab, Z.S.; Cenci, E.; Mencacci, A. Procalcitonin levels in gram-positive, gram-negative, and fungal bloodstream infections. *Dis. Markers* **2015**, *2015*, 701480. [CrossRef]
13. Koizumi, Y.; Sakanashi, D.; Ohno, T.; Nakamura, A.; Yamada, A.; Shibata, Y.; Shiota, A.; Kato, H.; Hagihara, M.; Asai, N.; et al. Plasma procalcitonin levels remain low at the onset of gram-positive bacteremia regardless of severity or the presence of shock: A retrospective analysis of patients with detailed clinical characteristics. *J. Microbiol. Immunol. Infect.* **2021**, *54*, 1028–1037. [CrossRef]
14. Borges-Sa, M.; Giglio, A.; Aranda, M.; Socías, A.; del Castillo, A.; Mena, J.; Franco, S.; Ortega, M.; Nieto, Y.; Nieto Estrada, V.H.; et al. Decoding sepsis: A 16-year retrospective analysis of activation patterns, mortality predictors, and outcomes from a hospital-wide sepsis protocol. *J. Clin. Med.* **2025**, *14*, 5759. [CrossRef]
15. Prescott, H.C.; Antonelli, M.; Alhazzani, W.; Møller, M.H.; Alshamsi, F.; Azevedo, L.C.P.; Belley-Côté, E.; De Waele, J.; Derde, L.; Dionne, J.C.; et al. Surviving Sepsis Campaign: International guidelines for management of sepsis and septic shock 2026. *Intensive Care Med.* **2026**, *52*, 863–936. Correction in *Intensive Care Med.* **2026**, *52*, 1411–1413. https://doi.org/10.1007/s00134-026-08410-9. [CrossRef]
16. Borges-Sa, M.; Giglio, A.; Martín-Loeches, I.; Nates, J.; González del Castillo, J.; Cárdenas, Y.; Mergulhão, P.; Candel, F.J.; Zaragoza, R.; Larrosa-Escartín, M.N.; et al. International multidisciplinary consensus statement on sepsis code guidelines: A Delphi approach. *J. Intern. Med.* **2026**, *early view*. [CrossRef]
17. Julián-Jiménez, A.; García, D.E.; González del Castillo, J.; Escalante-Kanashiro, R.; Candel González, F.J.; Merinos-Sánchez, G.; Estella, Á.; Santillán-Santos, D.A.; Gamazo del Río, J.J.; Fosco, M.J.; et al. Recommendations of the GT-LATINFURG for the identification and initial management of patients with severe infection and sepsis in emergency departments. *Emergencias* **2026**, *38*, 27–63. [CrossRef]
18. Yan, S.T.; Sun, L.C.; Lian, R.; Tao, Y.K.; Zhang, H.B.; Zhang, G. Diagnostic and predictive values of procalcitonin in bloodstream infections for nosocomial pneumonia. *J. Crit. Care* **2018**, *44*, 424–429. [CrossRef]
19. Martínez, J.M.; Espírito Santo, A.; Ramada, D.; Fontes, F.; Medeiros, R. Diagnostic accuracy of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and neutrophil–lymphocyte-to-platelet ratio biomarkers in predicting bacteremia and sepsis in immunosuppressive patients with cancer: Literature review. *Porto Biomed. J.* **2024**, *9*, 254. [CrossRef]
20. Svaldi, M.; Hirber, J.; Lanthaler, A.I.; Mayr, O.; Faes, S.; Peer, E.; Mitterer, M. Procalcitonin-reduced sensitivity and specificity in heavily leucopenic and immunosuppressed patients. *Br. J. Haematol.* **2001**, *115*, 53–57. [CrossRef]
21. Papathanakos, G.; Andrianopoulos, I.; Xenikakis, M.; Papathanasiou, A.; Koulenti, D.; Blot, S.; Koulouras, V. Clinical sepsis phenotypes in critically ill patients. *Microorganisms* **2023**, *11*, 2165. [CrossRef]
22. Mulherin, S.A.; Miller, W.C. Spectrum bias or spectrum effect? Subgroup variation in diagnostic test evaluation. *Ann. Intern. Med.* **2002**, *137*, 598–602. [CrossRef]
23. Tromp, M.; Lansdorp, B.; Bleeker-Rovers, C.P.; Klein Gunnewiek, J.M.; Kullberg, B.J.; Pickkers, P. Serial and panel analyses of biomarkers do not improve the prediction of bacteremia compared to one procalcitonin measurement. *J. Infect.* **2012**, *65*, 292–301. [CrossRef]

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