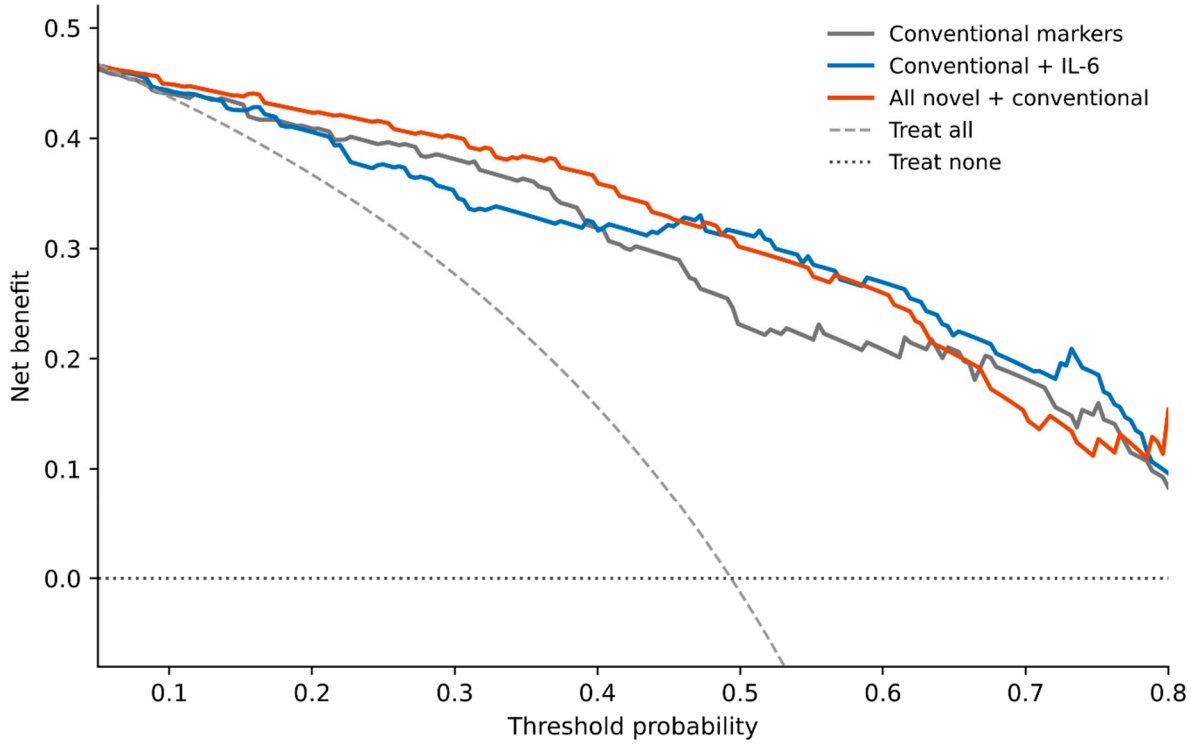
Supplementary Figure legends



Supplementary Figure S1. Decision-curve analysis of the exploratory multivariable models. Net benefit is plotted against threshold probability for the conventional biomarker model, the conventional model with IL-6 added, and the model containing all six investigational biomarkers together with the conventional markers, alongside the treat-all and treat-none strategies. All curves were computed from out-of-fold predicted probabilities obtained by stratified five-fold cross-validation, so they reflect internally validated rather than apparent performance. Net benefit is expressed relative to the clinician-assigned reference classification (Section 2.4), to which C-reactive protein and procalcitonin themselves contributed in a minority of patients; the analysis therefore describes agreement with clinician-assigned categories and does not establish clinical utility. Estimates above a threshold probability of approximately 0.65 are based on few predictions and are unstable.

Supplementary Table S1. Coefficients, conditional effects, and standardization constants of the exploratory multivariable models.

Conventional biomarker model (n = 156); intercept = +0.0175

Predictor	Coefficient	Univariable OR (95% CI)	Conditional OR (95% CI)	p value	Mean of log1p(x)	SD of log1p(x)
CRP, mg/L	+1.5604	4.73 (2.85–7.85)	4.76 (2.76–8.21)	<0.001	2.7723	1.1288
PCT, ng/mL	+0.8523	2.13 (1.47–3.10)	2.35 (1.51–3.65)	<0.001	0.8981	0.7562
WBC, $\times 10^9$/L	+0.1298	1.80 (1.16–2.77)	1.14 (0.73–1.78)	0.571	2.6743	0.3738

Predictors were log1p-transformed and then standardized as z = (log1p(x) − mean) / SD, using the constants given below. The linear predictor equals the intercept plus the sum of each coefficient multiplied by its corresponding z score, and the predicted probability is obtained by the logistic transformation of the linear predictor. These constants are reported so that each prediction rule can be applied unchanged to an external cohort without re-estimation.

All novel + conventional biomarker model (n = 156); intercept = +0.0022

Predictor	Coefficient	Univariable OR (95% CI)	Conditional OR (95% CI)	p value	Mean of log1p(x)	SD of log1p(x)
IL-6, ng/L	+1.1048	2.66 (1.81–3.91)	3.02 (1.57–5.79)	0.001	3.5815	1.0206
TNF-α, ng/L	+0.9824	1.92 (1.34–2.74)	2.67 (1.55–4.61)	<0.001	5.0953	0.5468
IL-1β, ng/L	+0.7662	2.03 (1.42–2.91)	2.15 (1.23–3.75)	0.007	7.0292	0.6984
α-Defensin 1, ng/mL	−0.6012	0.70 (0.51–0.98)	0.55 (0.33–0.91)	0.019	5.5349	0.6354
β-Defensin 1, ng/L	+0.2115	1.26 (0.91–1.74)	1.24 (0.71–2.14)	0.450	4.2584	0.5677
β-Defensin 2, ng/L	+0.0523	0.98 (0.71–1.34)	1.05 (0.65–1.72)	0.834	7.1662	0.5698
CRP, mg/L	+1.5633	4.73 (2.85–7.85)	4.77 (2.45–9.30)	<0.001	2.7723	1.1288
PCT, ng/mL	+0.9829	2.13 (1.47–3.10)	2.67 (1.54–4.62)	<0.001	0.8981	0.7562
WBC, $\times 10^9$/L	+0.0145	1.80 (1.16–2.77)	1.01 (0.52–1.96)	0.966	2.6743	0.3738

Coefficients are on the standardized log1p scale and odds ratios are per one standard deviation of the log-transformed concentration. The univariable odds ratio is from a model containing that predictor alone; the conditional odds ratio and the p value are from the multivariable model shown, that is, adjusted for every other predictor in the same model. Comparing the two columns identifies predictors carrying information not already present in the others: IL-6 and TNF-α strengthened on adjustment, whereas the white blood cell count lost its association once CRP and procalcitonin were present. All estimates are apparent, in-sample values obtained at 8.6 events per variable in the nine-predictor model, so individual coefficients are imprecise and are reported for transparency and reproduction rather than as estimates of independent biological effect; the cross-validated discrimination of these models, with calibration and a paired comparison between them, is given in Table 5. All estimates refer to the clinician-assigned reference classification (Section 2.4). CI, confidence interval; CRP, C-reactive protein; IL, interleukin; OR, odds ratio; PCT, procalcitonin; SD, standard deviation; TNF-α, tumor necrosis factor alpha; WBC, white blood cell count.

Supplementary Table S2. Admission diagnoses of the 79 patients classified as having noninfectious systemic inflammation.

Admission diagnosis	**n**	**%**
Cardiac arrest (post-resuscitation syndrome)	22	27.8
Postoperative inflammation	19	24.1
Exacerbation of chronic obstructive pulmonary disease	10	12.7
Hypertensive or cardiogenic pulmonary oedema	9	11.4
Cerebrovascular accident	3	3.8
Decompensated heart failure	2	2.5
Epilepsy or status epilepticus	2	2.5
Lung cancer	2	2.5
Intracranial haemorrhage	1	1.3
Encephalopathy	1	1.3
Neuroleptic malignant syndrome	1	1.3
Pulmonary embolism	1	1.3
Diabetic ketoacidosis	1	1.3
Acute kidney injury	1	1.3
Anaphylactic shock	1	1.3
Lymphoedema	1	1.3
Poisoning	1	1.3

Admission diagnosis	n	%
Multiple trauma	1	1.3
Total	**79**	**100.0**

Diagnoses are the principal noninfectious condition recorded in the final clinical diagnosis for the index episode, that is, the inflammatory episode present at the time of biomarker sampling within the first 24 hours of intensive care unit admission. Every patient is assigned to exactly one category and no category is aggregated as "other", so that the composition of the noninfectious group can be inspected in full. Five of these patients developed a documented intensive care unit-acquired infection more than 48 hours after study blood sampling; because the reference classification refers to the inflammatory state at the time of sampling, they were retained in the noninfectious group, and a sensitivity analysis excluding them is reported in Section 3. Percentages are of the 79 patients in this group and may not sum to 100.0 because of rounding.

Supplementary Table S3. Stability and optimism of the Youden-derived diagnostic thresholds.

Marker	Cutoff	Bootstrap range of reselected cutoff (2.5th–97.5th pct)	Apparent sensitivity / specificity, %	Optimism-corrected sensitivity / specificity, %	Cross-validated sensitivity / specificity, %
Investigational biomarkers					
IL-6, ng/L	≥ 56.01	22.4 – 95.1	59.7 / 81.0	57.3 / 78.9	54.5 / 81.0
TNF-α, ng/L	≥ 132.07	113.8 – 243.4	76.6 / 55.7	74.4 / 53.6	74.0 / 49.4
IL-1β, ng/L	≥ 1218.19	655.7 – 1440.8	62.3 / 69.6	59.9 / 67.3	63.6 / 63.3
α-defensin 1, ng/mL	≤ 300.92	181.6 – 363.7	71.4 / 50.6	68.2 / 48.1	54.5 / 54.4
β-defensin 1, ng/L	≥ 45.61	39.0 – 78.0	90.9 / 25.3	88.0 / 22.3	80.5 / 29.1

Marker	Cutoff	Bootstrap range of reselected cutoff (2.5th–97.5th pct)	Apparent sensitivity / specificity, %	Optimism-corrected sensitivity / specificity, %	Cross-validated sensitivity / specificity, %
β-defensin 2, ng/L	≤ 1220.50	619.9 – 2621.6	50.6 / 59.5	47.2 / 56.2	36.4 / 63.3
Conventional markers					
CRP, mg/L	≥ 11.00	11.0 – 15.3	90.9 / 73.4	89.6 / 72.5	88.3 / 73.4
PCT, ng/mL	≥ 0.43	0.43 – 0.90	88.3 / 54.4	86.6 / 53.2	79.2 / 54.4
WBC, ×10⁹/L	≥ 14.30	12.5 – 14.8	58.4 / 74.7	57.0 / 73.1	61.0 / 68.4
Albumin, g/dL	≤ 3.80	3.10 – 3.90	72.4 / 50.6	70.5 / 48.5	65.8 / 49.4

All estimates refer to the clinician-assigned reference classification (Section 2.4), not to an independently adjudicated standard; n = 156 (77 infectious, 79 noninfectious) except albumin (n = 155). The cutoff is the value maximising the Youden index in the full cohort, as reported in Table 3, and the inequality sign indicates the direction in which the marker was dichotomised. The bootstrap range is the 2.5th to 97.5th percentile of the cutoff reselected independently in each of 2,000 stratified bootstrap resamples and describes how reproducibly the threshold itself can be located; it is not a confidence interval for diagnostic performance. Apparent sensitivity and specificity are those obtained at the full-cohort cutoff in the same data. Optimism-corrected values were obtained by reselecting the cutoff in each bootstrap resample, applying the resulting rule both to that resample and to the original data, and subtracting the mean difference from the apparent estimate. Cross-validated values were obtained by selecting the cutoff within the training folds of a stratified five-fold cross-validation and applying it to the corresponding held-out fold, pooling the resulting classifications; this is the more demanding of the two corrections because the threshold is never evaluated in data that contributed to its selection. Confidence intervals for the apparent estimates are given in Table 3. None of these thresholds was externally validated and none is proposed for clinical use. CRP, C-reactive protein; IL, interleukin; PCT, procalcitonin; TNF-α, tumor necrosis factor alpha; WBC, white blood cell count.