

MMP8⁺ cells in peripheral blood mononuclear cells as an independent prognostic marker of 60-day mortality in sepsis: a prospective observational cohort study

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Running title: PBMC MMP8⁺ cells and sepsis prognosis

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Abstract

Background. Sepsis remains a leading cause of death in intensive care units, and circulating biomarkers that capture host immune dysregulation are still needed. Matrix

21 metalloproteinase-8 (MMP8) has immunomodulatory functions beyond matrix
22 degradation, but the prognostic value of MMP8-expressing cells in peripheral blood
23 mononuclear cells (PBMCs) is unknown.

24 **Methods.** In this prospective single-center cohort study, 85 adults with sepsis admitted to
25 the emergency intensive care unit (EICU) were enrolled. PBMCs were isolated within 24 h
26 of EICU admission, and MMP8⁺ cells (quantified as the percentage of MMP8-positive cells
27 within the monocyte (FSC/SSC) light-scatter gate), together with CD11c⁺MMP8⁺ cells,
28 CD11c⁺HLA-DR⁻MMP8⁺ cells (Q3) and the ln-transformed MMP8⁺/HLA-DR⁺ ratio, were
29 quantified by flow cytometry. Associations with 60-day all-cause mortality were assessed
30 using Cox regression on multiply imputed datasets (m = 20), with incremental value
31 evaluated by receiver operating characteristic analysis, decision curve analysis, net
32 reclassification improvement (NRI) and integrated discrimination improvement (IDI).
33 Model interpretability was examined using SHapley Additive exPlanations (SHAP).

34 **Results.** Twenty-two patients (25.9%) died within 60 days. MMP8⁺ cell proportion was
35 independently associated with 60-day mortality after adjustment for age, SOFA score and
36 CD4/CD8 ratio (hazard ratio [HR] per 1% increment 1.026, 95% CI 1.003–1.048, P = 0.025;
37 HR per SD 1.453, 95% CI 1.048–2.015); the Q3 subset showed the strongest standardized
38 association (HR per SD 1.612, 95% CI 1.129–2.300, P = 0.009). Adding MMP8⁺ cells to the
39 baseline model did not significantly improve discrimination (AUC 0.788 vs. 0.731, DeLong P
40 = 0.090) but improved reclassification (continuous NRI 0.579, P = 0.023; IDI 0.106, P =
41 0.026). In SHAP analysis, MMP8⁺ cells ranked second only to SOFA score in feature
42 importance. The CD11c⁺MMP8⁺ and Q3 subsets and the MMP8⁺/HLA-DR⁺ ratio were also

43 associated with persistent organ dysfunction (day 1-to-7 SOFA decrease < 2 points),
44 whereas associations with 30-day mortality did not remain significant after adjustment.

45 **Conclusions.** MMP8⁺ cell proportion within the monocyte scatter gate was independently
46 associated with 60-day mortality and improved risk reclassification beyond clinical
47 variables, supporting its candidacy as a cell-level prognostic marker pending external
48 validation.

49 **Trial registration.** Chinese Clinical Trial Registry, ChiCTR2200063908. Registered 20
50 September 2022 (prospective registration).

51 **Keywords:** sepsis; matrix metalloproteinase-8; flow cytometry; peripheral blood
52 mononuclear cells; prognosis; 60-day mortality

53 **Background**

54 Sepsis, defined as life-threatening organ dysfunction caused by a dysregulated host
55 response to infection [1], remains one of the leading causes of death in critical care
56 worldwide. The Global Burden of Disease study estimated approximately 48.9 million
57 incident cases and 11 million sepsis-related deaths in 2017, accounting for nearly 20% of
58 all global deaths [2]. Despite ongoing advances in early recognition, antimicrobial therapy,
59 fluid resuscitation and organ support [3], mortality from sepsis and septic shock remains
60 high. Accurate early risk stratification is essential for allocating resources, guiding
61 individualized intervention and improving outcomes. The Sequential Organ Failure
62 Assessment (SOFA) score [4] and the Acute Physiology and Chronic Health Evaluation II
63 (APACHE II) score [5] are widely used, but they mainly reflect organ dysfunction and global

64 physiological derangement rather than the underlying immune disturbance that
65 characterizes sepsis [6,7]. Biomarkers that capture host immune status are therefore an
66 active focus of sepsis research [8], and reduced monocyte HLA-DR expression, a hallmark
67 of sepsis-induced immunosuppression, has repeatedly been linked to adverse outcomes
68 [9].

69 Matrix metalloproteinase-8 (MMP8; collagenase-2 or neutrophil collagenase) is a zinc-
70 dependent endopeptidase classically stored in the specific granules of neutrophils and
71 released upon activation [10]. By degrading extracellular matrix components and
72 processing chemokines and cytokines, MMP8 participates in inflammatory cell recruitment
73 and tissue injury [11]. Monocytes and macrophages can also express MMP8, and monocyte
74 subsets undergo profound remodeling during sepsis in relation to disease severity [12].
75 More recent work has extended the biological scope of MMP8 beyond matrix turnover:
76 circulating myeloid-derived MMP8 can cross the blood–brain barrier and modulate
77 neuroimmune signaling [13]; clinical cohorts have shown that serum MMP-8
78 concentrations are elevated in sepsis and correlate with organ failure and death [14,15];
79 and a recent mechanistic study demonstrated that MMP8 regulates dendritic cell tolerance
80 in late polymicrobial sepsis via the nuclear factor kappa-B p65/ β -catenin pathway,
81 indicating an immunoregulatory role in sepsis-induced immune dysfunction [16].

82 Notably, these two lines of evidence converge on the same antigen-presenting
83 compartment but have rarely been examined together. Down-regulation of monocytic HLA-
84 DR reflects failure of antigen presentation and is an established marker of sepsis-induced
85 immunosuppression [9], whereas MMP8 has recently been shown to actively induce a

86 tolerogenic dendritic-cell phenotype via NF- κ B p65/ β -catenin signaling [16]. Whether
87 MMP8 expression and HLA-DR loss co-localize within the same circulating myeloid cells —
88 a putative “double-hit” tolerogenic phenotype combining impaired antigen presentation
89 with an immunoregulatory protease — and whether such co-localization carries prognostic
90 information, remain unknown. Addressing this question requires cell-level, rather than
91 serum-level, measurement.

92 Previous studies have focused almost exclusively on circulating protein levels of MMP8.
93 Whether MMP8-expressing cell subsets within peripheral blood mononuclear cells
94 (PBMCs) carry prognostic information in sepsis has, to our knowledge, not been
95 investigated. We therefore conducted a prospective single-center cohort study using flow
96 cytometry to quantify, within 24 h of EICU admission, the percentage of MMP8-positive
97 cells within the monocyte forward/side-scatter gate (hereafter MMP8⁺ cells), together with
98 three related phenotypes pre-specified to interrogate this co-localization (CD11c⁺MMP8⁺
99 cells, CD11c⁺HLA-DR⁻MMP8⁺ cells and the MMP8⁺/HLA-DR⁺ ratio). CD11c, rather than the
100 monocyte marker CD14, was used to define the myeloid compartment for quadrant
101 analysis: CD11c (integrin α X) marks monocytes and dendritic-lineage antigen-presenting
102 cells — the compartment in which loss of HLA-DR defines sepsis-induced
103 immunosuppression [9] and in which MMP8-driven tolerogenic reprogramming has been
104 mechanistically demonstrated [16] — whereas CD14 would have largely recapitulated the
105 light-scatter monocyte gate without adding orthogonal information. We systematically
106 evaluated their association with 60-day all-cause mortality, their predictive performance,
107 their incremental value over clinical severity scores, and model interpretability, aiming to
108 identify a candidate cell-level marker for early risk stratification in sepsis.

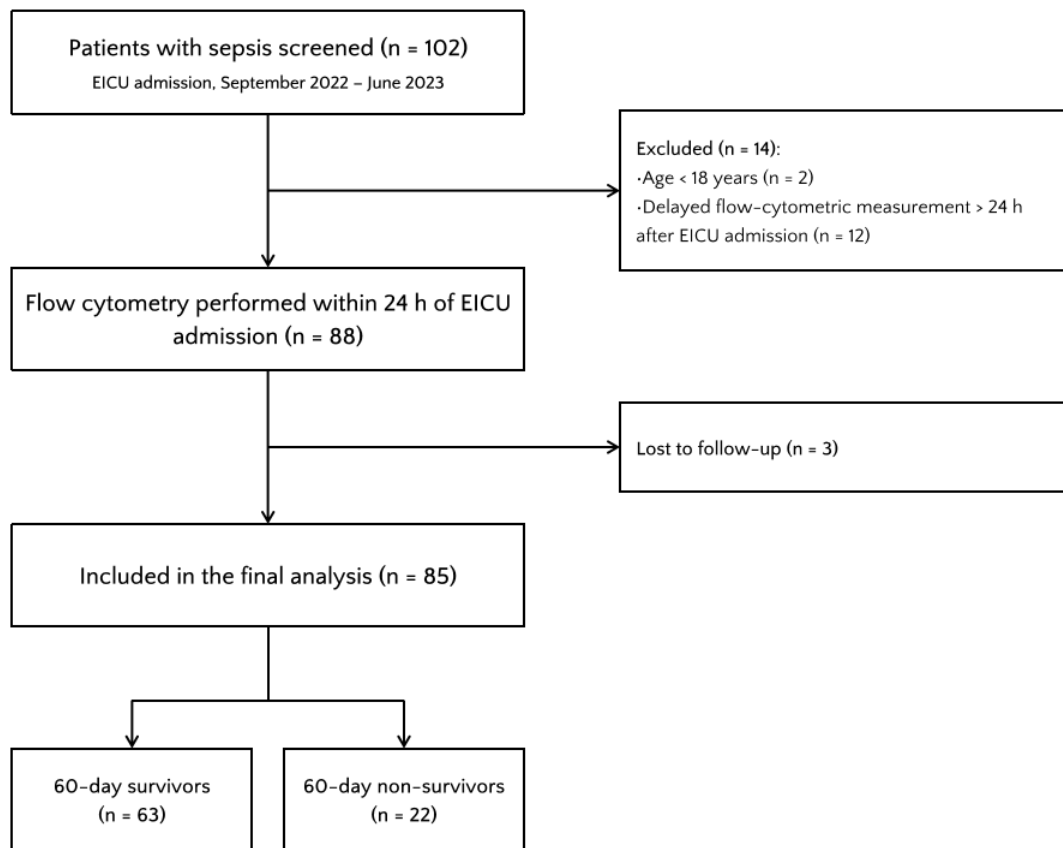
109 **Methods**

110 **Study design and setting**

111 This was a single-center prospective observational cohort study reported in accordance
112 with the STROBE statement [17]. Consecutive patients diagnosed with sepsis and admitted
113 to the EICU of the First Affiliated Hospital of Wenzhou Medical University between
114 September 2022 and June 2023 were screened for eligibility. The study protocol was
115 reviewed and approved by the institutional medical ethics committee (approval
116 No. 2022051) and was registered with the Chinese Clinical Trial Registry (registration
117 No. ChiCTR2200063908). Written informed consent was obtained from all patients or their
118 legal representatives.

119 **Participants**

120 Eligible patients were adults (≥ 18 years) meeting the Sepsis-3 diagnostic criteria [1] who
121 underwent peripheral blood flow cytometry within 24 h of EICU admission. Exclusion
122 criteria were: (i) age < 18 years; (ii) hematological disease; (iii) recent immunosuppressive
123 therapy; (iv) pregnancy; and (v) incomplete clinical data. A total of 102 patients with sepsis
124 were screened; 2 were excluded because of age < 18 years and 12 because of delayed flow-
125 cytometric measurement (> 24 h after EICU admission). Of the 88 patients who underwent
126 flow cytometry, 3 were lost to follow-up, leaving 85 patients in the final analysis (Fig. 1).



127

128 **Figure 1.** Flow diagram of patient screening, exclusions and final inclusion. A total of 102
 129 patients with sepsis were screened; 14 were excluded (age < 18 years, n = 2; delayed flow-
 130 cytometric measurement > 24 h after EICU admission, n = 12); 88 patients underwent flow
 131 cytometry, of whom 3 were lost to follow-up, leaving 85 patients in the final analysis (63
 132 survivors and 22 non-survivors at day 60).

133 Clinical data and outcome definition

134 Baseline data collected within 24 h of EICU admission included demographics (age, sex,
 135 body mass index), comorbidities (hypertension, diabetes mellitus, coronary heart disease,

136 chronic kidney disease, chronic liver disease, malignancy), infection site, circulating
137 cytokines (IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ and the IL-6/IL-10 ratio), the CD4/CD8 ratio,
138 routine laboratory tests (white blood cell count, C-reactive protein, procalcitonin, lactate),
139 disease severity scores (SOFA [4] and APACHE II [5]) and glucocorticoid use. Serial SOFA
140 scores from day 1 to day 7 were collected for the full cohort. Septic shock was defined
141 according to Sepsis-3 [1]. The primary outcome was 60-day all-cause mortality. All patients
142 were followed until death or day 60; all survivors completed the full 60-day follow-up with
143 no censoring. Thirty-day all-cause mortality was analyzed as a secondary outcome.
144 Persistent organ dysfunction (POD) was defined as a decrease in the SOFA score from day 1
145 to day 7 of less than 2 points, consistent with definitions based on early SOFA trajectories
146 in sepsis trials and cohorts [36–38]; for patients without a day-7 score (early discharge or
147 transfer), the last available score was carried forward, and patients who died before day 7
148 were classified as having POD [38].

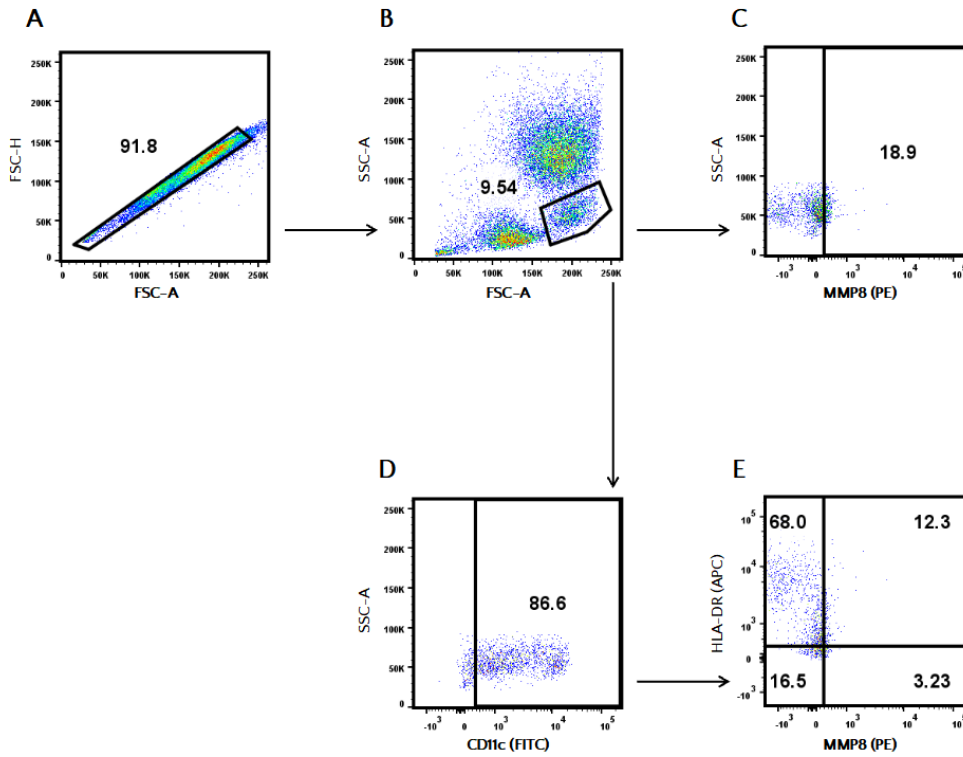
149 **Flow cytometry and nomenclature**

150 Peripheral venous blood was drawn within 24 h of EICU admission, and PBMCs were
151 isolated by Ficoll density-gradient centrifugation using a high-speed refrigerated centrifuge
152 (Thermo Fisher Scientific, USA); cell counts were obtained with a Countess 3FL automated
153 cell counter (Thermo Fisher Scientific). Cells were stained with a three-color antibody
154 panel: PE-conjugated anti-human MMP8 (clone EP1252Y; Abcam, cat. ab211967), APC-
155 conjugated anti-human HLA-DR (clone TU36; BD Pharmingen, cat. 559868) and FITC-
156 conjugated anti-human CD11c (clone B-LY6; BD Pharmingen, cat. 561355). Positivity
157 thresholds were set using fluorescence-minus-one (FMO) controls. Samples were acquired

158 on a BD FACSCanto II flow cytometer (BD Biosciences, USA), and data were analyzed with
159 FlowJo 10.8.1 (BD Biosciences). CD11c was selected as the myeloid/dendritic-lineage
160 marker because the analysis focused on the antigen-presenting compartment defined by
161 HLA-DR co-expression; within the three-color panel, CD14 was not included, as it would
162 have largely duplicated the scatter-based monocyte gate. Flow cytometry practice and
163 reporting followed international guidelines [18].

164 A gate was set on the monocyte region according to forward scatter (FSC) and side scatter
165 (SSC) characteristics, as previously described for scatter-based pre-gating [19–22]
166 (representative gating strategy in Fig. 2), and the percentage of MMP8⁺ cells within this
167 gate was recorded. Because scatter gates have limited purity and may contain large
168 lymphocytes, dendritic cells and residual granulocytes [19–21], and because our gating
169 strategy did not include lineage markers such as CD14, cells within the gate cannot be
170 strictly equated with monocytes. The primary indicator is reported as the percentage of
171 MMP8-positive cells within this gate and is hereafter referred to simply as MMP8⁺ cells.

172 Four flow-cytometric indicators were pre-specified: (i) MMP8⁺ cells (primary indicator;
173 percentage of MMP8-positive cells within the monocyte scatter gate); (ii) CD11c⁺MMP8⁺
174 cells (percentage of CD11c⁺ cells within the gate that expressed MMP8); (iii) CD11c⁺HLA-
175 DR⁻MMP8⁺ cells (quadrant Q3 of the HLA-DR × MMP8 plot within CD11c⁺ gated cells); and
176 (iv) the ratio of MMP8⁺ to HLA-DR⁺ cells (ln-transformed owing to right skewness).



177

178 **Figure 2.** Representative flow-cytometric gating strategy. (A) Singlet gate on the FSC-A ×
 179 FSC-H plot. (B) Monocyte scatter gate (P1) on the FSC-A × SSC-A plot. (C) MMP8 × SSC-A
 180 plot within the monocyte gate; the MMP8 positivity threshold was defined by the
 181 fluorescence-minus-one (FMO) control, and the percentage of MMP8-positive cells within
 182 the gate (MMP8⁺ cells) was the primary indicator. (D) CD11c × SSC-A plot within the
 183 monocyte gate, used to identify CD11c⁺ cells. (E) HLA-DR × MMP8 quadrant plot within
 184 CD11c⁺ cells: Q1 = HLA-DR⁺MMP8⁻, Q2 = HLA-DR⁺MMP8⁺, Q3 = HLA-DR⁻MMP8⁺, Q4 =
 185 HLA-DR⁻MMP8⁻. Antibody panel: PE-MMP8 (EP1252Y), APC-HLA-DR (TU36), FITC-CD11c
 186 (B-LY6); samples were acquired on a BD FACSCanto II.

187 **Statistical analysis**

188 The statistical analysis plan followed the TRIPOD recommendations for prognostic model
189 studies [23]. Continuous variables, all non-normally distributed, are summarized as median
190 (interquartile range) and were compared between survivors and non-survivors using the
191 Wilcoxon rank-sum test; categorical variables are reported as n (%) and were compared
192 using the chi-square or Fisher exact test. Between-group comparisons in Table 1 are
193 descriptive and were not adjusted for multiplicity.

194 The four flow-cytometric indicators were complete for all 85 patients. Missing baseline
195 covariates (CD4/CD8 ratio 5.9%, cytokines 7.1%, lymphocyte subset absolute counts 12.9–
196 16.5%) were handled by multiple imputation with chained equations (20 imputations,
197 predictive mean matching; mice package), and model estimates were pooled using Rubin's
198 rules [24]. Kaplan–Meier analyses used the original (unimputed) data because the grouping
199 variables had no missing values.

200 Cox proportional hazards regression was used to estimate hazard ratios (HRs) for 60-day
201 mortality per 1% increment of each indicator and per standard deviation (SD), under four
202 adjustment sets: unadjusted; Model 1 (age); Model 2 (age + SOFA); and Model 3 (age +
203 SOFA + CD4/CD8 ratio). Because MMP8⁺ and CD11c⁺MMP8⁺ cells were almost perfectly
204 correlated (Spearman $\rho = 0.99$), correlated indicators were never entered into the same
205 model; each indicator was modeled separately. For the three secondary indicators,
206 Benjamini–Hochberg false discovery rate (FDR) adjusted P values are reported for Model 3
207 [25]. The proportional hazards assumption was examined with scaled Schoenfeld residuals
208 (cox.zph) across imputations [26], and linearity of the exposure–log-hazard relationship

209 was assessed with restricted cubic splines (4 knots). Firth-penalized Cox models (coxphf)
210 and nested likelihood-ratio tests were fitted as sensitivity analyses given the modest
211 number of events. Associations with 30-day mortality were estimated with the same Cox
212 models with follow-up censored at day 30. Associations with POD were estimated by
213 logistic regression (odds ratios per 1% increment) under the same adjustment sets, with
214 baseline (day 1) SOFA included in adjusted models, and were pooled across imputations; as
215 a supporting analysis, Spearman correlations between the indicators and the day 1-to-7
216 SOFA change were computed. Analyses of secondary outcomes are exploratory and were
217 not adjusted for multiplicity.

218 For visualization, optimal cutoffs for MMP8⁺ cells and the Q3 subset were determined by
219 maximally selected rank statistics (maxstat package, minprop = 0.1) [27,28], and Kaplan–
220 Meier curves were compared with the log-rank test. Because data-driven cutpoint selection
221 inflates the type I error and the apparent separation [29,30], the dichotomized analyses
222 were regarded as exploratory.

223 Predicted 60-day risks averaged over imputations were used for receiver operating
224 characteristic (ROC) analysis; areas under the curve (AUCs) with 95% confidence intervals
225 (CIs) were computed with pROC, and AUCs were compared using the DeLong test [31].
226 Because follow-up was complete (no censoring before day 60), ROC, decision curve and
227 reclassification analyses were based on the binary 60-day outcome. Calibration of the
228 combined model was assessed with calibration curves (500 bootstrap resamples). Decision
229 curve analysis [32] quantified net benefit across threshold probabilities. Incremental value
230 was evaluated by adding MMP8⁺ cells to a baseline model (age + SOFA + CD4/CD8 ratio):

231 categorical NRI (risk thresholds 0.2 and 0.4; nricens, 1000 bootstrap resamples),
232 continuous NRI and integrated discrimination improvement (IDI) were computed [33].
233 Internal validation used 500 bootstrap resamples (rms) to estimate the optimism-
234 corrected concordance index.

235 Pre-specified subgroup analyses (age, sex, septic shock, SOFA, APACHE II, glucocorticoid
236 use; medians as stratification cutoffs) estimated subgroup-specific HRs with multiplicative
237 interaction tests on imputed datasets. For interpretability, SHAP values [34] were
238 computed for the final Cox model (age, SOFA, CD4/CD8 ratio, MMP8⁺ cells) on the linear
239 predictor (log-hazard) scale using kernel SHAP with MI-averaged predictions; feature
240 importance was summarized as mean |SHAP|, and the stability of the top-ranked feature
241 was quantified by bootstrap resampling. SHAP was used as an interpretive aid, not for
242 causal inference.

243 With 22 events and four predictors, the events-per-variable ratio was approximately 5.5,
244 below the conventional threshold of 10 [35]; this limitation was addressed through
245 penalized (Firth) sensitivity analyses and bootstrap internal validation and is discussed
246 below. Two-sided $P < 0.05$ was considered statistically significant. Analyses were
247 performed in R 4.5.2 (survival 3.8-9, mice 3.19.0, rms 8.1-1, pROC 1.19.0.1, maxstat, coxphf
248 1.13.4, nricens 1.6, kernelshap 0.9.1, shapviz 0.10.3).

249 Results

250 Baseline characteristics

251 Eighty-five patients were included; 22 (25.9%) died within 60 days and 63 survived.
 252 Baseline characteristics are summarized in Table 1. Non-survivors had higher SOFA
 253 (median 11.0 vs. 8.0, $P = 0.005$) and APACHE II scores (24 vs. 18, $P = 0.001$), and more
 254 chronic kidney disease (40.9% vs. 12.7%, $P = 0.011$). Infection site distribution also
 255 differed between groups ($P = 0.011$; Table S1). All four pre-specified flow-cytometric
 256 indicators were significantly higher in non-survivors: MMP8⁺ cells (median 18.40%
 257 vs. 8.23%, $P = 0.023$), CD11c⁺MMP8⁺ cells (21.70% vs. 10.26%, $P = 0.014$), CD11c⁺HLA-
 258 DR⁻MMP8⁺ (Q3) cells (17.75% vs. 7.20%, $P = 0.012$) and the MMP8⁺/HLA-DR⁺ ratio (1.98
 259 vs. 0.81, $P = 0.027$).

260 **Table 1.** Baseline characteristics of the study population according to 60-day outcome.

Characteristic	Overall (n = 85)	Survivors (n = 63)	Non-survivors (n = 22)	P
Age, years	66.00 [55.00, 75.00]	65.00 [52.50, 76.00]	72.00 [59.75, 74.00]	0.164
Female sex, n (%)	31 (36.5)	22 (34.9)	9 (40.9)	0.806
Body mass index, kg/m ²	22.90 [19.50, 26.00]	23.40 [19.08, 26.05]	21.75 [20.32, 25.05]	0.684
Hypertension, n (%)	43 (50.6)	29 (46.0)	14 (63.6)	0.240
Diabetes mellitus, n (%)	33 (38.8)	28 (44.4)	5 (22.7)	0.122
Coronary heart disease, n (%)	15 (17.6)	11 (17.5)	4 (18.2)	1.000
Chronic kidney disease, n (%)	17 (20.0)	8 (12.7)	9 (40.9)	0.011
Chronic liver disease, n (%)	16 (18.8)	10 (15.9)	6 (27.3)	0.389
Malignancy, n (%)	8 (9.4)	7 (11.1)	1 (4.5)	0.628

Characteristic	Overall (n = 85)	Survivors (n = 63)	Non-survivors (n = 22)	P
Septic shock, n (%)	62 (72.9)	46 (73.0)	16 (72.7)	1.000
SOFA score	9.00 [6.00, 12.00]	8.00 [6.00, 11.00]	11.00 [8.00, 14.75]	0.005
APACHE II score	20.00 [15.00, 25.00]	18.00 [12.00, 23.50]	24.00 [20.00, 29.00]	0.001
White blood cell count, $\times 10^9/L$	19.26 [12.53, 27.97]	20.29 [12.64, 30.53]	15.35 [12.03, 21.88]	0.118
C-reactive protein, mg/L	190.70 [117.10, 265.60]	212.90 [136.45, 279.75]	126.70 [88.47, 186.60]	0.005
Procalcitonin, ng/mL	30.20 [6.29, 100.00]	35.50 [9.27, 100.00]	22.85 [3.62, 67.38]	0.111
Lactate, mmol/L	4.00 [2.30, 6.30]	3.40 [2.00, 5.40]	5.40 [3.90, 8.40]	0.020
CD4/CD8 ratio ^a	1.50 [0.91, 2.60]	1.37 [0.96, 2.42]	2.04 [0.82, 2.75]	0.673
IL-6/IL-10 ratio ^b	11.34 [2.37, 45.90]	11.34 [2.41, 44.31]	10.53 [2.33, 45.87]	0.660
MMP8 ⁺ cells, %	9.80 [5.67, 20.80]	8.23 [5.12, 19.00]	18.40 [8.32, 27.73]	0.023
CD11c ⁺ MMP8 ⁺ cells, %	11.58 [5.91, 23.18]	10.26 [5.31, 20.65]	21.70 [10.37, 40.29]	0.014
CD11c ⁺ HLA-DR ⁺ MMP8 ⁻ (Q1) cells, %	73.70 [59.50, 85.50]	75.20 [62.80, 85.60]	66.75 [51.25, 74.55]	0.053
CD11c ⁺ HLA-DR ⁺ MMP8 ⁺ (Q2) cells, %	1.91 [0.93, 5.40]	1.91 [0.93, 5.51]	2.28 [0.80, 5.30]	0.968
CD11c ⁺ HLA-DR ⁻ MMP8 ⁺ (Q3) cells, %	8.40 [3.75, 19.40]	7.20 [3.15, 13.75]	17.75 [8.38, 23.58]	0.012
CD11c ⁺ HLA-DR ⁻ MMP8 ⁻ (Q4) cells, %	7.67 [2.56, 18.20]	8.48 [3.94, 19.10]	3.32 [1.61, 17.12]	0.081
MMP8 ⁺ /HLA-DR ⁺ ratio	1.04 [0.50, 3.07]	0.81 [0.44, 2.68]	1.98 [0.89, 7.57]	0.027

261 Data are median [interquartile range] or n (%). P values are from Wilcoxon rank-sum, chi-
262 square or Fisher exact tests and are descriptive (not multiplicity-adjusted). SOFA,
263 Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health
264 Evaluation; NK, natural killer. Missing data: ^a 5.9%, ^b 7.1%; all other variables and all flow-

cytometric indicators were complete. Quadrant definitions (within CD11c⁺ gated cells), numbered in the default FlowJo order (upper-left, upper-right, lower-right and lower-left quadrants): Q1 = HLA-DR⁺MMP8⁻, Q2 = HLA-DR⁺MMP8⁺, Q3 = HLA-DR⁻MMP8⁺, Q4 = HLA-DR⁻MMP8⁻.

Correlations among flow-cytometric indicators and clinical variables

Spearman correlations are shown in Fig. S1. MMP8⁺ cells were almost perfectly correlated with CD11c⁺MMP8⁺ cells ($\rho = 0.99$, $P < 0.001$) and strongly correlated with the Q3 subset ($\rho = 0.89$, $P < 0.001$) and the MMP8⁺/HLA-DR⁺ ratio ($\rho = 0.59$, $P < 0.001$), confirming that these indicators must not be entered into the same regression model. Correlations of MMP8⁺ cells with clinical severity scores were weak (SOFA $\rho = 0.18$; APACHE II $\rho = 0.10$) and the correlation with C-reactive protein was essentially nil ($\rho = -0.01$), whereas a moderate correlation with IFN- γ was observed ($\rho = 0.38$, $P < 0.01$).

Association of flow-cytometric indicators with 60-day mortality

Cox regression results on pooled multiply imputed datasets are presented in Table 2. The primary indicator, MMP8⁺ cell proportion, was associated with 60-day mortality in the unadjusted analysis (HR per 1% 1.037, 95% CI 1.016–1.059, $P < 0.001$) and remained independently associated after adjustment for age (Model 1), age and SOFA score (Model 2), and age, SOFA score and CD4/CD8 ratio (Model 3: HR per 1% 1.026, 95% CI 1.003–1.048, $P = 0.025$; HR per SD 1.453, 95% CI 1.048–2.015, $P = 0.025$). Among secondary indicators, the Q3 subset showed the strongest standardized association (Model 3: HR per SD 1.612, 95% CI 1.129–2.300, $P = 0.009$, FDR-adjusted $P = 0.026$), and CD11c⁺MMP8⁺ cells likewise remained significant after FDR adjustment (Model 3: HR per 1% 1.021, 95% CI

1.002–1.041, P = 0.029, FDR-adjusted P = 0.043). The ln-transformed MMP8⁺/HLA-DR⁺ ratio also remained independently associated after full adjustment (Model 3: HR 1.331 per unit, 95% CI 1.010–1.755, P = 0.043, FDR-adjusted P = 0.043).

Table 2. Cox proportional hazards models for 60-day mortality (pooled across 20 multiply imputed datasets).

Indicator	Model	HR per 1% (95% CI)	P	HR per SD (95% CI)
MMP8 ⁺ cells	Unadjusted	1.037 (1.016–1.059)	< 1 0.001	.721 (1.265–2.341)
	Model 1 (age)	1.039 (1.017–1.060)	< 1 0.001	.751 (1.292–2.372)
	Model 2 (age + SOFA)	1.025 (1.003–1.048)	0.029	1.441 (1.039–1.999)
	Model 3 (age + SOFA + CD4/CD8)	1.026 (1.003–1.048)	0.025	1.453 (1.048–2.015)
CD11c ⁺ MMP8 ⁺ cells	Unadjusted	1.033 (1.016–1.051)	< 1 0.001	.729 (1.296–2.306)
	Model 1 (age)	1.034 (1.016–1.051)	< 1 0.001	.749 (1.317–2.323)
	Model 2 (age + SOFA)	1.020 (1.001–1.040)	0.037	1.402 (1.020–1.927)
	Model 3 (age + SOFA + CD4/CD8)	1.021 (1.002–1.041)	0.029 ^a	1.425 (1.036–1.959)
CD11c ⁺ HLA-DR ⁺ MMP8 ⁺ cells (Q3)	Unadjusted	1.048 (1.025–1.072)	< 1 0.001	.903 (1.400–2.586)
	Model 1 (age)	1.052 (1.028–	< 1 0.001	.990 (1.449–2.732)

Indicator	Model	HR per 1% (95% CI)	P	HR per SD (95% CI)
ln(MMP8 ⁺ /HLA-DR ⁺ ratio) ^c		1.076)		
	Model 2 (age + SOFA)	1.036 (1.009– 1.063)	0.008	1.615 (1.132– 2.303)
	Model 3 (age + SOFA + CD4/CD8)	1.036 (1.009– 1.063)	0.009 ^b	1.612 (1.129– 2.300)
	Unadjusted	1.483 (1.124– 1.957)	0.005	1.919 (1.214– 3.035)
	Model 1 (age)	1.454 (1.099– 1.923)	0.009	1.857 (1.169– 2.949)
	Model 2 (age + SOFA)	1.348 (1.016– 1.789)	0.038	1.640 (1.027– 2.618)
	Model 3 (age + SOFA + CD4/CD8)	1.331 (1.010– 1.755)	0.043 ^d	1.605 (1.016– 2.536)

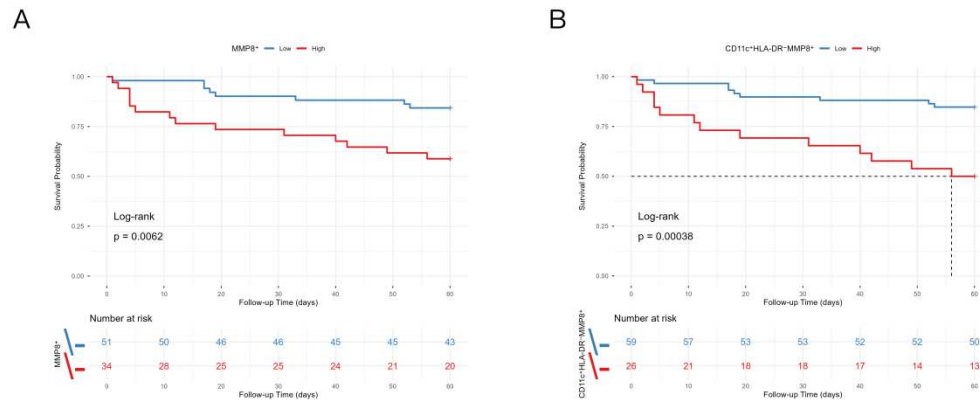
HR, hazard ratio; CI, confidence interval; SD, standard deviation. Indicators were modeled separately owing to collinearity (MMP8⁺ vs. CD11c⁺MMP8⁺, $\rho = 0.99$). ^a FDR-adjusted P = 0.043; ^b FDR-adjusted P = 0.026; ^c HR per unit of the ln-transformed ratio; ^d FDR-adjusted P = 0.043. Benjamini–Hochberg FDR adjustment was applied across the three secondary indicators for Model 3.

Sensitivity analyses supported the robustness of the primary finding. Firth-penalized Cox regression yielded essentially unchanged estimates (Model 3: MMP8⁺ HR per 1% 1.026, 95% CI 1.004–1.049, P = 0.021; Q3 HR per 1% 1.036, 95% CI 1.009–1.063, P = 0.008). Nested likelihood-ratio tests comparing Model 3 with and without the indicator favored inclusion of MMP8⁺ cells (median P = 0.030 across imputations, range 0.019–0.049) and the Q3 subset (median P = 0.010, range 0.006–0.020). Restricted cubic spline analysis

303 found no evidence of non-linearity for MMP8⁺ cells (P for non-linearity = 0.474),
304 supporting the per-1% linear specification (Fig. S2).
305 Examination of scaled Schoenfeld residuals indicated no violation of the proportional
306 hazards assumption for the exposure itself (MMP8⁺, P = 0.210), although the global test
307 was borderline (median P = 0.026 across imputations), driven by age (P ≈ 0.05) rather than
308 by the exposure (SOFA P = 0.109; CD4/CD8 P = 0.710; Table S2). The exposure HR was
309 stable across all sensitivity analyses above.

310 **Kaplan–Meier analysis at data-driven cutoffs**

311 The maxstat procedure identified a cutoff of 15% for MMP8⁺ cells: 60-day mortality was
312 15.7% (8/51) in the low group versus 41.2% (14/34) in the high group (log-rank P =
313 0.006; Fig. 3A). For the Q3 subset (cutoff 15.5%), 60-day mortality was 15.3% (9/59) in the
314 low group versus 50.0% (13/26) in the high group (log-rank P < 0.001; Fig. 3B). Because
315 both cutoffs were data-driven, these dichotomized analyses should be regarded as
316 exploratory, and any proposed threshold requires external validation.



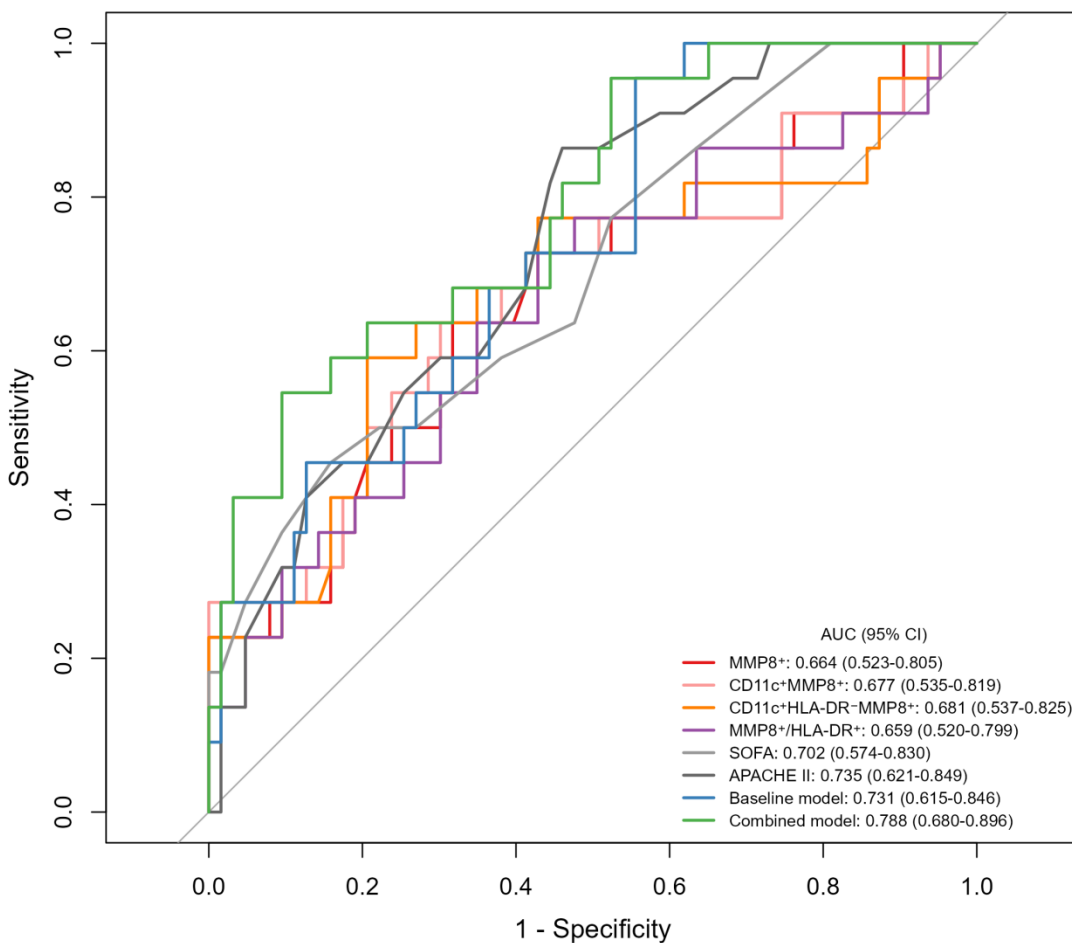
317

318 **Figure 3.** Kaplan–Meier curves of 60-day survival. (A) Patients stratified by MMP8⁺ cell
 319 proportion at the maxstat cutoff of 15% (low n = 51, 8 deaths; high n = 34, 14 deaths; log-
 320 rank P = 0.006). (B) Patients stratified by CD11c⁺HLA-DR⁺MMP8⁺ (Q3) cell proportion at
 321 the cutoff of 15.5% (low n = 59, 9 deaths; high n = 26, 13 deaths; log-rank P < 0.001).
 322 Number-at-risk tables are shown below each panel. The cutoffs were data-driven;
 323 dichotomized comparisons are exploratory.

324 Discrimination, calibration and reclassification

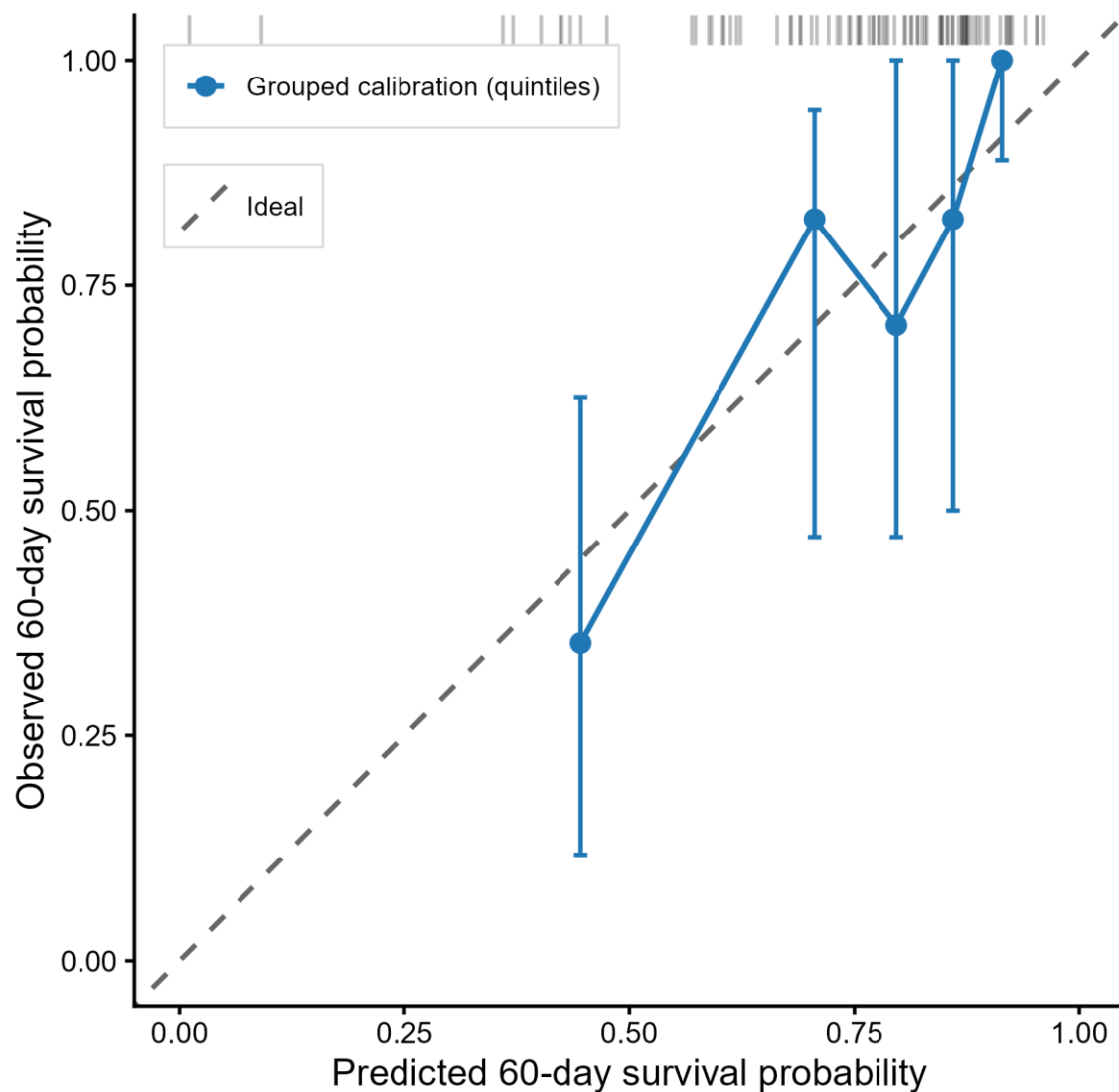
325 Discrimination of individual indicators and models is summarized in Fig. 4. The AUC of
 326 MMP8⁺ cells alone was 0.664 (95% CI 0.523–0.805), comparable to that of CD11c⁺MMP8⁺
 327 cells (0.677, 95% CI 0.535–0.819; DeLong P = 0.074 vs. MMP8⁺), the Q3 subset (0.681, 95%
 328 CI 0.537–0.825; P = 0.498) and the MMP8⁺/HLA-DR⁺ ratio (0.659, 95% CI 0.520–0.799; P =
 329 0.945), and numerically lower than SOFA (0.702, 95% CI 0.574–0.830; P = 0.649) and
 330 APACHE II (0.735, 95% CI 0.621–0.849; P = 0.385), although none of these pairwise

331 differences reached statistical significance. The baseline model (age + SOFA + CD4/CD8)
 332 achieved an AUC of 0.731 (95% CI 0.615–0.846), and adding MMP8⁺ cells increased the
 333 AUC to 0.788 (95% CI 0.680–0.896); this improvement was not statistically significant
 334 (DeLong P = 0.090). The bootstrap optimism-corrected concordance index of the combined
 335 model was 0.725 (range across the 20 imputed datasets, 0.711–0.735). Calibration of the
 336 combined model showed good agreement between predicted and observed 60-day
 337 mortality (Fig. 5), and decision curve analysis demonstrated higher net benefit of the
 338 combined model than the baseline model across a clinically relevant range of threshold
 339 probabilities (Fig. 6).



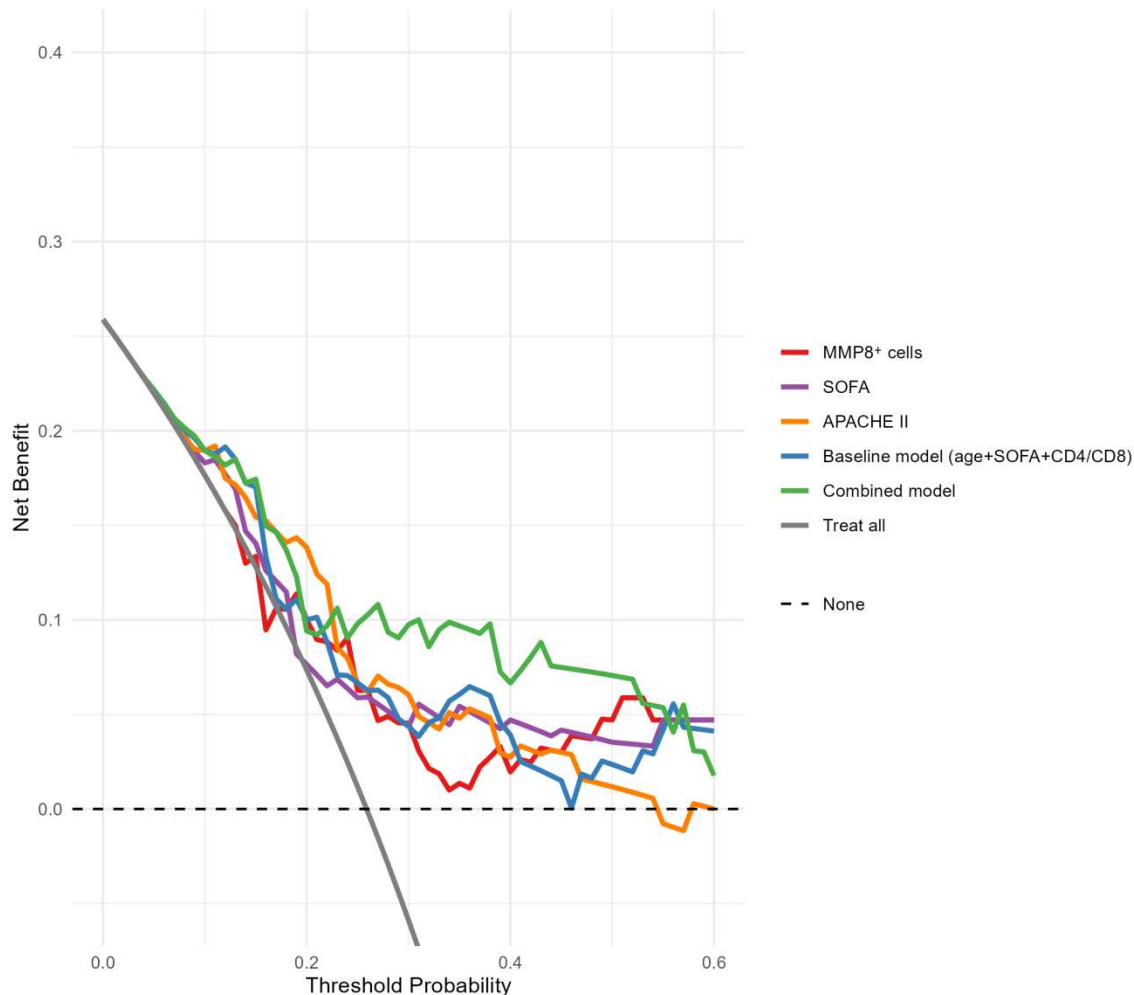
340

341 **Figure 4.** Receiver operating characteristic curves for 60-day mortality: MMP8⁺ cells (AUC
 342 0.664, 95% CI 0.523–0.805), CD11c⁺MMP8⁺ cells (0.677, 0.535–0.819), CD11c⁺HLA-
 343 DR⁻MMP8⁺ (Q3) cells (0.681, 0.537–0.825), MMP8⁺/HLA-DR⁺ ratio (0.659, 0.520–0.799),
 344 SOFA score (0.702, 0.574–0.830), APACHE II score (0.735, 0.621–0.849), the baseline
 345 model (age + SOFA + CD4/CD8; 0.731, 0.615–0.846) and the combined model (baseline +
 346 MMP8⁺ cells; 0.788, 0.680–0.896). Combined vs. baseline, DeLong P = 0.090. Predictions
 347 averaged over 20 multiply imputed datasets.



348

349 **Figure 5.** Calibration curve of the combined model for 60-day mortality (bootstrap-
 350 corrected, 500 resamples; predictions averaged over imputations).



351

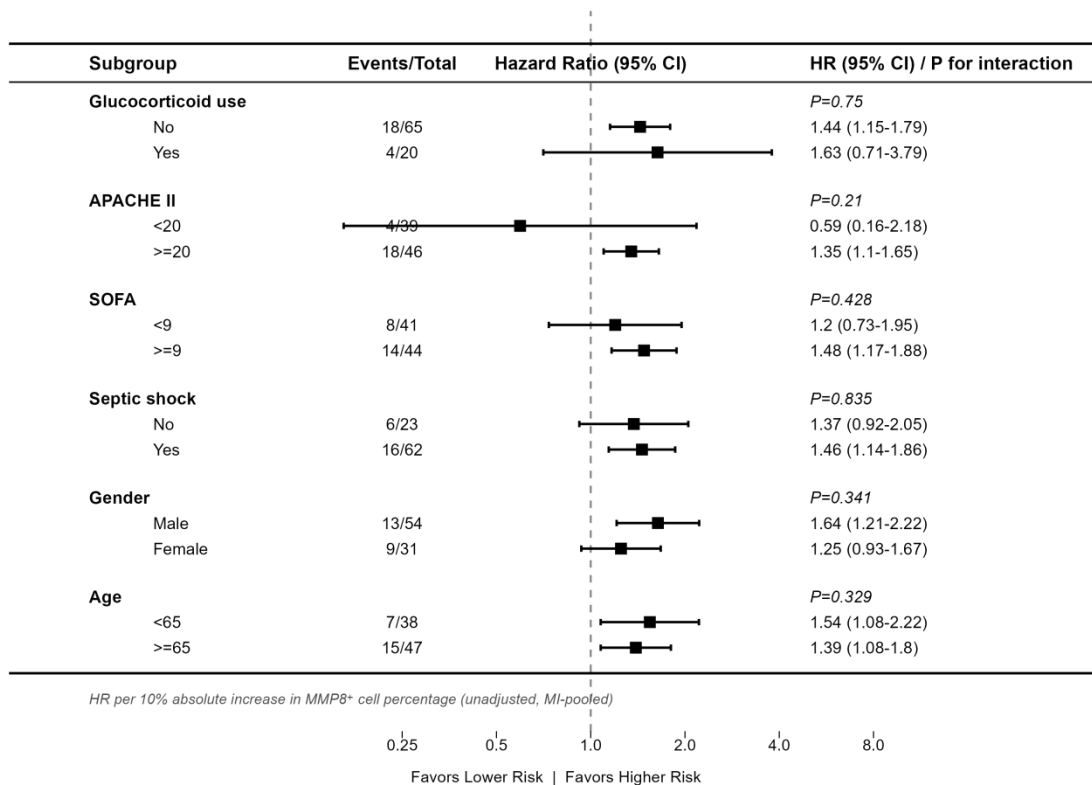
352 **Figure 6.** Decision curve analysis of the baseline and combined models for 60-day
 353 mortality. Net benefit is plotted against threshold probability; the combined model
 354 provides higher net benefit than the baseline model and the treat-all/treat-none strategies
 355 across a clinically relevant range of thresholds.

356 Reclassification analyses provided clearer evidence of incremental value. Compared with
 357 the baseline model, adding MMP8⁺ cells yielded a significant continuous NRI of 0.579 (95%

CI 0.069–1.046, $P = 0.023$) and an IDI of 0.106 (95% CI 0.017–0.199, $P = 0.026$), whereas the categorical NRI at risk thresholds of 0.2 and 0.4 was not significant (-0.030 , 95% CI -0.341 – 0.263 , $P = 0.847$; Fig. S3).

Subgroup analyses

The association between MMP8⁺ cells and 60-day mortality was directionally consistent across the 12 pre-specified subgroup strata (Fig. 7): hazard ratios (per 10% increment) exceeded 1 in 11 of the 12 strata, the sole exception being the APACHE II < 20 subgroup (HR 0.59, 95% CI 0.16–2.18, based on only 4 events). No multiplicative interaction reached statistical significance (interaction P values: age 0.329; sex 0.341; septic shock 0.835; SOFA 0.428; APACHE II 0.210; glucocorticoid use 0.750).



368

369 **Figure 7.** Forest plot of subgroup-specific hazard ratios for 60-day mortality per 10%
370 increment in MMP8⁺ cells (multiply imputed Cox models). No multiplicative interaction
371 was significant (all interaction $P > 0.20$).

372 **Secondary outcomes: 30-day mortality and persistent organ dysfunction**

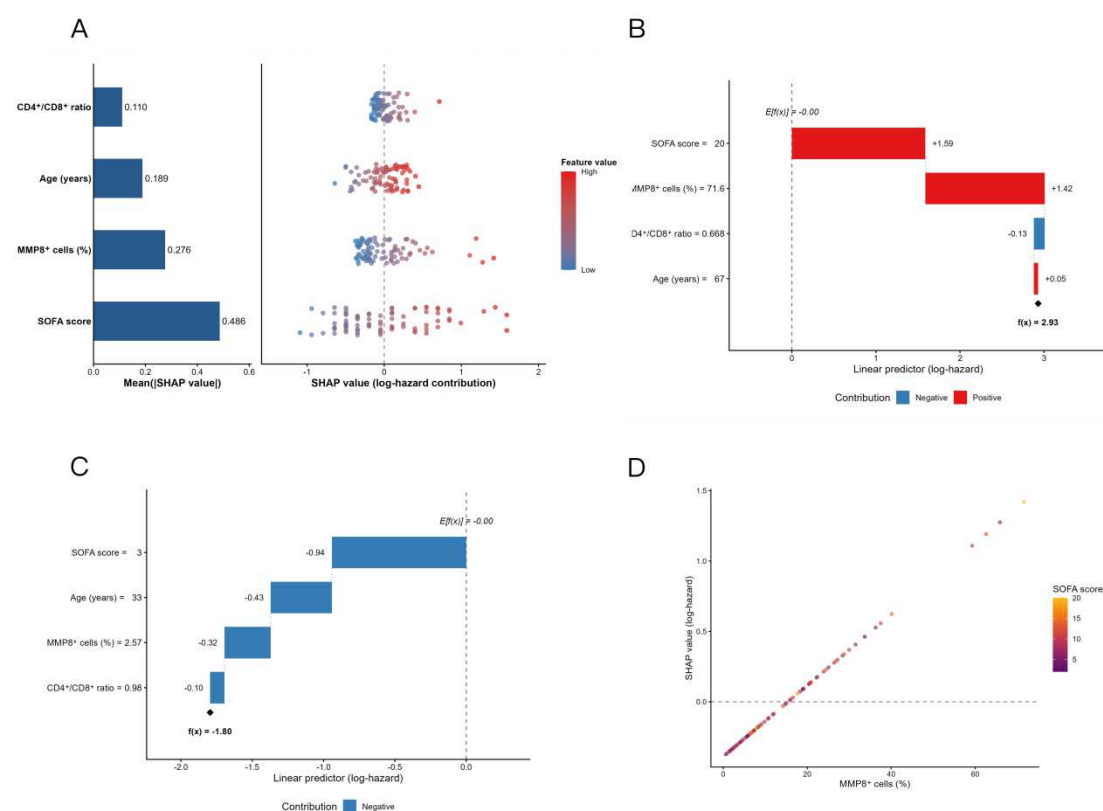
373 Fourteen patients (16.5%) died within 30 days. Associations of the flow-cytometric
374 indicators with 30-day mortality were directionally consistent with the primary analysis
375 but weaker (Table S3). In unadjusted analyses, MMP8⁺ cells showed a borderline
376 association (HR per 1% 1.026, 95% CI 1.000–1.053, $P = 0.052$), and the Q3 subset (HR per
377 1% 1.036, 95% CI 1.008–1.066, $P = 0.012$) and the ln-transformed MMP8⁺/HLA-DR⁺ ratio
378 (HR 1.522, 95% CI 1.093–2.120, $P = 0.013$) were nominally significant; after full adjustment
379 (Model 3), no indicator remained significant (MMP8⁺ cells: HR per 1% 1.010, 95% CI
380 0.983–1.038, $P = 0.47$; Q3: HR per 1% 1.017, 95% CI 0.985–1.049, $P = 0.30$), consistent with
381 limited power at 14 events.

382 Persistent organ dysfunction occurred in 19 of the 85 patients (22.4%). POD was strongly
383 associated with 60-day mortality (57.9% vs. 16.7% in patients with early improvement, $P <$
384 0.001), supporting the prognostic validity of this definition. The Q3 subset was associated
385 with POD (odds ratio [OR] per 1% 1.059, 95% CI 1.018–1.101, $P = 0.005$; adjusted OR
386 1.075, 95% CI 1.018–1.136, $P = 0.010$), as were CD11c⁺MMP8⁺ cells (OR per 1% 1.035, 95%
387 CI 1.005–1.066, $P = 0.022$; adjusted OR 1.038, 95% CI 1.003–1.074, $P = 0.033$) and the ln-
388 transformed MMP8⁺/HLA-DR⁺ ratio (OR 1.772, 95% CI 1.213–2.590, $P = 0.003$; adjusted
389 OR 1.893, 95% CI 1.240–2.890, $P = 0.003$); the primary indicator showed a nominally
390 significant unadjusted association that was borderline after adjustment (OR per 1% 1.034,

391 95% CI 1.001–1.068, $P = 0.042$; adjusted OR 1.038, 95% CI 1.000–1.078, $P = 0.052$; Table
392 S4). Discrimination for POD was moderate for all four indicators (AUC 0.652 for MMP8⁺
393 cells, 0.668 for CD11c⁺MMP8⁺ cells, 0.713 for the Q3 subset and 0.719 for the ratio).
394 Spearman correlations of the indicators with the continuous day 1-to-7 SOFA change were
395 uniformly weak (all $|\rho| \leq 0.16$, all $P > 0.14$), suggesting that the association concentrates at
396 the extreme of absent improvement captured by the POD definition.

397 **Model interpretability (SHAP)**

398 SHAP analysis of the final Cox model (Fig. 8) ranked SOFA score as the most important
399 feature (mean $|\text{SHAP}| = 0.486$ on the log-hazard scale), followed by MMP8⁺ cells (0.276),
400 age (0.189) and the CD4/CD8 ratio (0.110). Bootstrap stability analysis confirmed SOFA as
401 the top-ranked feature in 69.5% of resamples (95% CI 62.6–75.8%), with MMP8⁺ cells top-
402 ranked in 16.0% (95% CI 11.2–21.8%). Waterfall plots for representative patients illustrate
403 the additive contributions: in a non-survivor (MMP8⁺ 71.6%, SOFA 20, age 67 years),
404 MMP8⁺ cells contributed +1.42 and SOFA +1.59 to a model output of +2.93 on the log-
405 hazard scale; in a survivor (MMP8⁺ 2.57%, SOFA 3, age 33 years), low SOFA (−0.94), young
406 age (−0.43) and low MMP8⁺ cells (−0.32) jointly shifted the prediction to −1.80.
407 Dependence plots relating MMP8⁺ percentages to their SHAP contributions, colored by
408 SOFA score, are shown in Fig. 8E.



409

410 **Figure 8.** SHAP interpretability analysis of the final Cox model (age, SOFA, CD4/CD8 ratio,
 411 MMP8⁺ cells; linear predictor scale). (A) Feature importance (mean |SHAP|): SOFA 0.486,
 412 MMP8⁺ cells 0.276, age 0.189, CD4/CD8 0.110. (B) Beeswarm plot of SHAP values. (C)
 413 Waterfall plot of a representative non-survivor (model output +2.93; MMP8⁺ cells 71.6%,
 414 SOFA 20, age 67 years). (D) Waterfall plot of a representative survivor (model output
 415 -1.80; MMP8⁺ cells 2.57%, SOFA 3, age 33 years). (E) SHAP dependence plot of MMP8⁺
 416 cells colored by SOFA score. Top-rank stability over bootstrap resamples: SOFA 69.5%
 417 (95% CI 62.6–75.8%), MMP8⁺ cells 16.0% (11.2–21.8%), age 14.0% (9.5–19.6%), CD4/CD8
 418 0.5% (0.0–2.8%).

419 Discussion

420 In this prospective cohort of 85 patients with sepsis, the proportion of MMP8⁺ cells in
421 PBMCs, measured within 24 h of EICU admission, was independently associated with 60-
422 day all-cause mortality. The association persisted after adjustment for age, SOFA score and
423 the CD4/CD8 ratio (HR per 1% 1.026, 95% CI 1.003–1.048), was essentially unchanged
424 under Firth-penalized estimation, was linear on the log-hazard scale, and was directionally
425 consistent in 11 of the 12 subgroup strata, without significant interaction. Among the pre-
426 specified related phenotypes, the CD11c⁺HLA-DR⁺MMP8⁺ (Q3) subset showed the
427 strongest standardized association (HR per SD 1.612) and remained significant after FDR
428 adjustment. Adding MMP8⁺ cells to clinical variables did not significantly improve
429 discrimination (Δ AUC 0.057, DeLong P = 0.090) but did improve risk reclassification
430 (continuous NRI 0.579; IDI 0.106). To our knowledge, this is the first study to evaluate a
431 PBMC-based, cell-level MMP8 phenotype for prognostication in sepsis.

432 Our findings extend previous work on MMP8 in sepsis, which focused on circulating
433 protein concentrations: serum MMP-8 is elevated in sepsis, and high levels have been
434 associated with organ failure and fatal outcome in multicenter cohorts [14,15]. MMP8 is
435 produced predominantly by neutrophils but also by monocytes and macrophages [10,12],
436 and circulating myeloid-derived MMP8 has been implicated in organ-level immune
437 dysregulation beyond the bloodstream [13]. A recent mechanistic study provided a direct
438 link between MMP8 and antigen-presenting cell dysfunction, showing that MMP8 drives
439 dendritic cell tolerance in late polymicrobial sepsis through the NF- κ B p65/ β -catenin
440 pathway [16]. Against this background, our observation that the CD11c⁺HLA-DR⁺MMP8⁺

subset — cells co-expressing the myeloid/dendritic-lineage marker CD11c, lacking HLA-DR, and positive for MMP8 — carried the strongest standardized prognostic association is biologically coherent: loss of monocytic HLA-DR is an established marker of sepsis-induced immunosuppression and predicts death in septic shock [9], and an HLA-DR⁻MMP8⁺ phenotype is compatible with the tolerogenic antigen-presenting cell state described mechanistically [16]. The choice of CD11c deliberately aligned the phenotyping with this dendritic-lineage compartment, in which MMP8 was shown to drive tolerance [16] and in which loss of HLA-DR carries prognostic weight [9]. We emphasize, however, that our data are associational, that CD11c is not specific to dendritic cells, and that flow-cytometric MMP8 positivity cannot distinguish intracellular production from surface-bound or internalized protein; the mechanistic interpretation therefore remains hypothesis-generating.

The quadrant design allows the two alterations to be read jointly. Q3 cells (CD11c⁺HLA-DR⁻MMP8⁺) combine absent antigen-presentation capacity with the presence of an enzyme capable of driving dendritic-cell tolerance [9,16], and this subset showed the strongest standardized association with 60-day mortality (HR per SD 1.612) — stronger than MMP8 positivity irrespective of HLA-DR status (CD11c⁺MMP8⁺ cells) and stronger than the gate-wide primary indicator. This gradient is consistent with the hypothesis that the prognostic signal concentrates in cells bearing both alterations rather than either alone. The MMP8⁺/HLA-DR⁺ ratio, conceived as a single-number summary of this imbalance (tolerogenic enzyme burden relative to preserved antigen-presentation capacity), remained independently associated with 60-day mortality after full adjustment (Model 3: P

463 = 0.043, FDR-adjusted $P = 0.043$), supporting its value as a compact summary of the two-
464 alteration phenotype.

465 The secondary-outcome analyses sharpen this picture. Persistent organ dysfunction during
466 the first week — a patient-centered outcome that was itself strongly associated with 60-
467 day mortality in this cohort — was predicted by the Q3 subset, the $\text{MMP8}^+/\text{HLA-DR}^+$ ratio
468 and $\text{CD11c}^+\text{MMP8}^+$ cells, whereas the primary indicator showed only a borderline
469 association after adjustment ($P = 0.052$). This pattern is consistent with the mortality
470 analyses and further supports the hypothesis that the prognostic information is enriched in
471 the CD11c^+ compartment — particularly in cells combining MMP8 positivity with loss of
472 HLA-DR — rather than in MMP8 positivity per se. In contrast, associations with 30-day
473 mortality, although directionally consistent, did not survive multivariable adjustment; with
474 only 14 events, this most plausibly reflects limited statistical power rather than a genuinely
475 time-restricted effect.

476 Clinically, the incremental value of MMP8^+ cells was expressed mainly through
477 reclassification rather than discrimination. This pattern is expected: the AUC is insensitive
478 to the addition of new markers to already informative models, and reclassification metrics
479 such as NRI and IDI are more responsive to clinically meaningful shifts in predicted risk
480 [33]. The non-significant categorical NRI (-0.030 , $P = 0.847$) alongside a significant
481 continuous NRI suggests that the marker improves risk estimates across the probability
482 continuum rather than moving patients across fixed 0.2/0.4 thresholds in this modest
483 sample. The SHAP analysis placed MMP8^+ cells second only to SOFA score in importance
484 for the model output, and bootstrap resampling showed that its top-rank position was not

485 stable (top-ranked in 16.0% of resamples), which is consistent with a contributory rather
486 than dominant role beside established severity scores. We therefore position MMP8⁺ cell
487 proportion as a candidate adjunct to — not a replacement for — clinical severity
488 assessment, to be tested in larger, preferably multicenter cohorts.

489 Several limitations merit emphasis. First, this was a single-center study with a modest
490 sample (85 patients, 22 events); although penalized sensitivity analyses and bootstrap
491 internal validation yielded stable estimates, the model requires external validation in
492 larger, preferably multicenter cohorts before any clinical application. Second, flow
493 cytometry was performed at a single early time point, and the secondary-outcome analyses
494 are exploratory. Third, residual confounding (e.g., by pre-sampling antibiotics, source
495 control or comorbidity burden) cannot be excluded despite adjustment. Finally, all flow-
496 cytometric indicators, subgroup analyses and SHAP explanations describe associations and
497 model behavior; they do not establish causality.

498 **Conclusions**

499 In patients with sepsis, a higher proportion of MMP8⁺ cells in PBMCs — and particularly of
500 the CD11c⁺HLA-DR⁺MMP8⁺ subset — was independently associated with 60-day mortality
501 and improved risk reclassification beyond age, SOFA score and the CD4/CD8 ratio, without
502 a statistically significant gain in discrimination. These findings nominate PBMC MMP8
503 phenotyping as a feasible cell-level prognostic candidate in sepsis. External validation in
504 larger cohorts, lineage-resolved gating strategies (CD14/CD66b backgating) and serial
505 measurements are warranted before clinical translation.

506 **List of abbreviations**

507 APACHE: Acute Physiology and Chronic Health Evaluation; AUC: area under the receiver
508 operating characteristic curve; CI: confidence interval; DCA: decision curve analysis; EICU:
509 emergency intensive care unit; FDR: false discovery rate; FMO: fluorescence minus one;
510 FSC/SSC: forward/side scatter; HR: hazard ratio; HLA-DR: human leukocyte antigen-DR;
511 IDI: integrated discrimination improvement; IFN- γ : interferon- γ ; IL: interleukin; MI:
512 multiple imputation; MMP8: matrix metalloproteinase-8; NF- κ B: nuclear factor kappa-B;
513 NK: natural killer; NRI: net reclassification improvement; OR: odds ratio; PBMC: peripheral
514 blood mononuclear cell; POD: persistent organ dysfunction; ROC: receiver operating
515 characteristic; SD: standard deviation; SHAP: SHapley Additive exPlanations; SOFA:
516 Sequential Organ Failure Assessment; STROBE: Strengthening the Reporting of
517 Observational Studies in Epidemiology; TNF- α : tumour necrosis factor- α ; TRIPOD:
518 Transparent Reporting of a multivariable prediction model for Individual Prognosis or
519 Diagnosis

520 **Declarations**

521 **Ethics approval and consent to participate.** The study protocol was reviewed and
522 approved by the Medical Ethics Committee of the First Affiliated Hospital of Wenzhou
523 Medical University (Approval No. 2022051) and was registered with the Chinese Clinical
524 Trial Registry (Registration No. ChiCTR2200063908). Written informed consent was
525 obtained from all patients or their legal representatives.

526 **Consent for publication.** Not applicable.

527 **Availability of data and materials.** The datasets used and/or analyzed during the current
528 study are available from the corresponding author on reasonable request.

529 **Competing interests.** The authors declare that they have no competing interests.

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537 **Authors' contributions.** ZC and XC contributed equally to this work and were responsible
538 for study conceptualization, investigation, and writing of the original draft. LC, BW, ZC, YL
539 and YS contributed to data curation, formal analysis, and investigation. LL and GZ were
540 responsible for statistical analysis, supervision, writing—review and editing, and funding
541 acquisition. All authors have read and approved the final manuscript.

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543 **Supplementary Information**

544 **Additional file 1.** File format: DOCX. Title: Supplementary figures and tables. Description:
545 Fig. S1 (Spearman correlation heatmap of flow-cytometric indicators, severity scores,
546 cytokines and lymphocyte subsets), Fig. S2 (restricted cubic spline analysis of MMP8⁺ cells
547 and the 60-day mortality log-hazard), Fig. S3 (risk reclassification scatter plot for the

548 combined vs. baseline model), Table S1 (infection site distribution according to 60-day
549 outcome), Table S2 (proportional hazards assumption diagnostics), Table S3 (Cox
550 regression for 30-day all-cause mortality) and Table S4 (logistic regression for persistent
551 organ dysfunction).

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