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Beyond Hemoglobin: Estimating Erythrocyte Circulatory Value

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Abstract

Background:

Red blood cell transfusion decisions in critically ill patients remain largely protocol-driven, as conventional models fail to integrate volume resuscitation with oxygen delivery dynamics. We developed a novel circulatory model to assess erythrocyte value across hemodynamic states and evaluated its generalizability.

Methods

From this model, we derived two key indices: cyclic score (P_{life}) and relative lactate level (R_{lac}). Using retrospective data from a local hospital system, we examined their association with all-cause mortality via Cox proportional hazards models, Kaplan–Meier analysis, and restricted cubic splines. Joint latent class trajectory modeling characterized longitudinal P_{life} and R_{lac} patterns. Machine learning identified key predictors, and an online dynamic risk calculator was developed. External validation used the MIMIC-IV database.

Results

Higher R_{lac} was associated with increased mortality risk (HR = 1.062, 95% CI: 1.035–1.090, $p < 0.001$), while higher P_{life} was protective (HR = 0.962, 95% CI: 0.942–0.982, $p < 0.001$). Using cutoffs of $R_{lac} > 1$ and $P_{life} > 5$, four risk groups were identified; the "low P_{life} & high R_{lac} " and "high P_{life} & high R_{lac} " phenotypes showed markedly elevated mortality. Three distinct joint trajectories were identified; transition to the "Persistent decompensation" phenotype marked poor prognosis. The parsimonious model ($R_{lac} + P_{life}$) demonstrated good discriminative ability in internal (AUC = 0.770) and external (AUC = 0.805–0.912) validation.

Conclusion:

This circulatory framework links erythrocyte dynamics to mortality, with P_{life} and R_{lac} providing complementary long- and short-term risk stratification to guide individualized transfusion decisions in critically ill patients.

Keywords:

Red blood cell transfusion; Fluid therapy; Trajectory analysis; ICU mortality

1. Introduction

Red blood cell transfusion (RBCT) remains a cornerstone of supportive care for critically ill patients, serving to correct anemia, maintain oxygen delivery, and preserve organ function^[1]. For decades, transfusion guidelines have centered on hemoglobin (Hb) thresholds, with restrictive strategies (typically 7–8 g/dL) established as the standard of care^[2-5]. Yet a growing body of pathophysiological evidence now exposes a widening disconnect between this Hb-centric paradigm and the complex physiological reality of critically ill patients—a gap that increasingly calls into question the prevailing approach to transfusion practice.

Oxygen delivery and consumption are dynamic processes governed by circulatory state, tissue perfusion, and metabolic demand^[6,7]. Reducing this physiological complexity to a single Hb threshold inherently misaligns guidelines with clinical reality^[8]. Reducing this physiological complexity to a single Hb threshold inherently misaligns guidelines with clinical reality^[3,9] — substitute diagnostic labels for oxygen balance, overlooking both the shared oxygen transport deficits that cut across conditions and the substantial inter-individual variability within diseases. Meanwhile, key physiological markers of systemic oxygen balance—oxygen extraction ratio and central venous oxygen saturation—are critical for guiding RBCT decisions, yet they remain

conspicuously absent from major trials and are rarely incorporated into bedside practice^[10].

A further flaw lies in conflating "hemodynamic stability" with "absence of active bleeding" as the prerequisite for restrictive transfusion^[11]. The 2023 AABB guideline recommends restrictive transfusion only for hemodynamically stable patients, but fails to distinguish these concepts on physiological grounds^[12]. In reality, many critically ill patients without overt bleeding still require vasopressors or exist in a precarious state of oxygen-delivery compensation—they are not truly stable. Consequently, applying restrictive transfusion thresholds derived from such populations to all critically ill patients risks undertransfusion in those with compensated hypoperfusion and overtreatment in those without.

Landmark trials mirror this mismatch. In TRICC (n=838), vasopressor use was common^[13]; TRISS (n=998) mandated vasoactive therapy^[14]; and in cardiac surgery trials (TRACS, TRICS-III), vasopressor support was frequent, with nearly one-quarter of RBC events occurring during hemodynamic fluctuations^[15,16]. Collectively, 30–50% of patients in the studies that established current Hb thresholds received circulatory support in the absence of overt bleeding^[8]. Thus, the restrictive strategy—ostensibly designed for "stable non-bleeding" patients—was derived from populations that were largely circulatory-compromised. Routine application of a uniform Hb threshold therefore extrapolates evidence from a specific population to all critically ill patients, risking diminished precision and potential organ injury from unrecognized hypoperfusion.

Current hemodynamic models fail to adequately capture the interplay between circulatory state, time-dependent physiological variability, and transfusion requirements^[17,18]. To bridge this gap, we derived two bedside indices from a novel integrative circulatory model—the cyclic score (P_{life}), which reflects macrocirculatory-to-microcirculatory effects, and the relative lactate level (R_{lac}),

which captures microcirculation-metabolism coupling. Using local real-world data for derivation and the MIMIC-IV database for external validation, we assessed the mortality association of P_{life} and R_{lac} , characterized their longitudinal trajectories, and explored their potential to guide individualized RBCT decisions in critically ill patients.

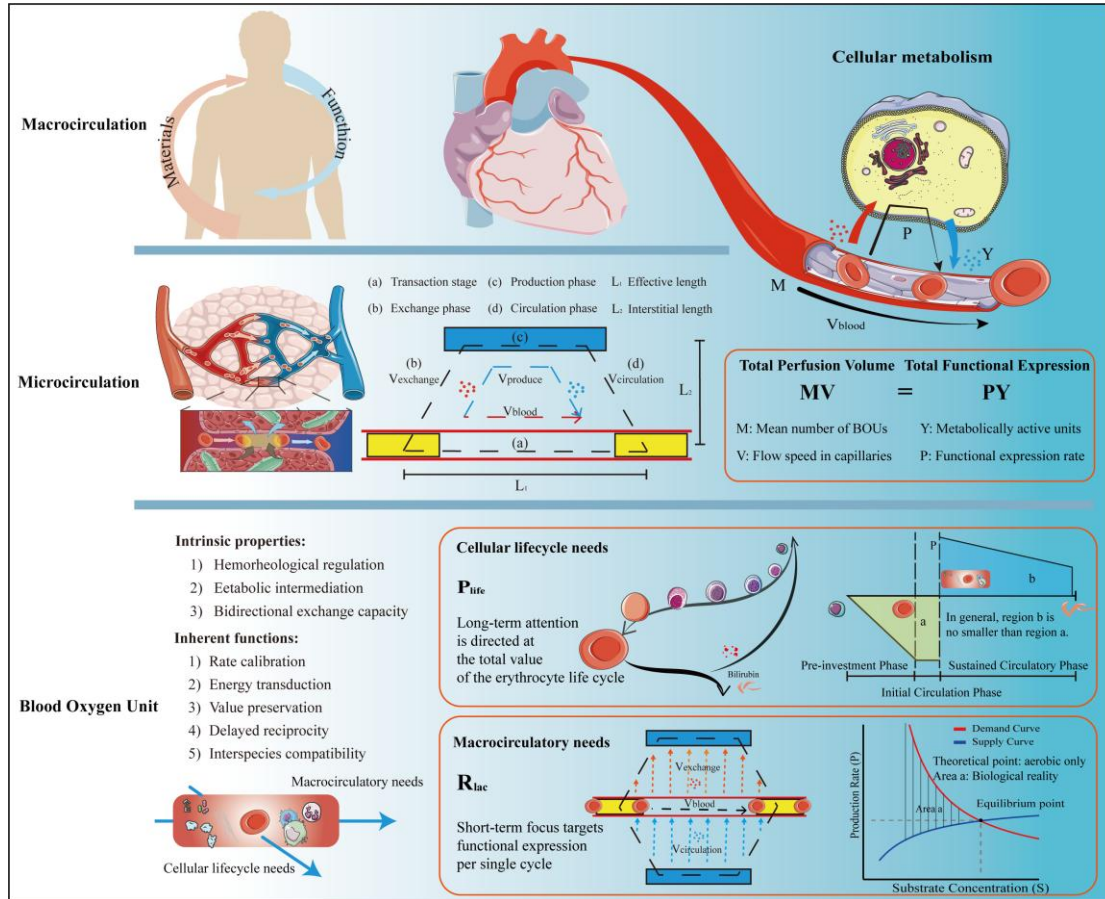


Figure 1. Conceptual framework of the multi-stage circulatory model, integrating macrocirculation, microcirculation, and cellular metabolism. The blood-oxygen unit (BOU) serves as the functional interface, facilitating nutrient delivery and waste removal. Because BOU composition and lifecycle are modulated by macrocirculatory dynamics, long-term production costs must be balanced against cumulative physiological benefit.

2. Hypothesis Development

Extending our prior circulatory model, we define the blood-oxygen unit (BOU) as a functional microcirculatory segment between adjacent erythrocyte centers, comprising one red blood cell and its associated plasma volume (Appendix 1). The BOU forms the basis for the perfusion–metabolism

coupling equation (Figure 1; Appendix 2):

$$M \times V = P \times Y$$

where M is the mean number of BOUs exchanged in the capillaries per unit time, V is the mean capillary flow velocity, Y denotes the number of metabolically active functional units, and P represents the functional expression rate.

2.1. Holding Cost and Steady-State Constraint

Analogous to Keynes's concept of interest^[19], the holding cost of a BOU is defined as the metabolic expenditure required to maintain a fixed number of functionally intact units—encompassing clearance and resynthesis of inactivated proteins, as well as compensatory synthesis from hemodilution. This parameter quantifies the continuous metabolic burden of sustaining dynamic homeostasis.

At steady state, the system's functional parameters remain constant across consecutive macrocirculatory cycles. Given total circulating blood volume (Vol), cardiac output (CO), and average solute concentration per BOU (Cx), we express:

$$\begin{cases} M = HCT \times Vol / MCV \\ Y = (1 - HCT) \times Vol \times Cx \end{cases}$$

Substituting these expressions into the perfusion–metabolism equation and rearranging gives:

$$P_{BOU} = \frac{1}{MCV} \times \frac{1}{Cx} \times CO \times \frac{HCT}{(1 - HCT)}$$

Because Cx includes non-quantifiable components, we focus on its theoretical minimum — the metabolic load required to preserve BOU integrity and hemoglobin content (Appendix 2.1). Let MCHC denote mean corpuscular hemoglobin concentration. Normalizing per unit hemoglobin mass provides an upper bound for the unit holding cost:

$$P_{unit} = \frac{1}{MCV} \times \frac{1}{MCHC} \times CO \times \frac{HCT}{(1 - HCT)}, \text{ where } P_{BOU} \leq P_{unit}$$

Using the previously defined inflation rate (Appendix 3.1):

$$Inflation Rate = \frac{1}{HCT} - 1$$

Rearranging gives:

$$P_{unit} = \frac{1}{MCH} \times \frac{CO}{Inflation Rate}$$

2.2. Life-Cycle Discounting

Erythropoiesis constitutes a “pre-investment”: resources are committed to produce RBCs that generate returns via oxygen delivery over their lifespan. Assuming constant MCH and n cycles, life-cycle return offsets synthesis cost:

$$P_{life} = \sum_{k=1}^n \left(\frac{1}{MCH} \times \frac{CO}{Inflation Rate} \right)_k$$

where n is the total number of macrocirculatory cycles in the RBC lifespan, measured in the same time units as k.

2.3. Estimating Erythrocyte Mortality

Given contributions to total bilirubin from senescent RBCs (80–85%), immature erythroid cells (10–15%), and hepatic heme turnover (1–5%)^[20,21], we derive the hemoglobin turnover rate (HTR) as a proxy for the metabolic cost of hemoglobin degradation per cycle (Appendix 3.2):

$$HTR = Total\ Bilirubin / Hemoglobin$$

Each gram of degraded hemoglobin yields approximately 35 mg of bilirubin based on heme stoichiometry (Appendix 3.3)^[22].

2.4. Assessment of Circulatory Function in BOUs

RBCs serve as the core carriers of the BOU, delivering oxygen via the microcirculation to sustain aerobic metabolism. Oxygen deprivation triggers anaerobic respiration and systemic lactate accumulation. Based on this mechanism, we define the following metric (Appendix 3.4):

$$R_{Lac} = Lac / CaO_2$$

where CaO_2 is the arterial oxygen content (expressed in units of 10 mL/L, with gas molecules calculated using the ideal gas law at body temperature (37°C), 22.7 L/mol), and Lac is the lactate concentration (mmol/L).

3. Study Design and Methods

3.1. Data Sources

We conducted a two-database retrospective cohort study: (1) the Hospital Information System of Zhejiang Provincial People's Hospital (2018–2026), approved by the local ethics committee, with waived informed consent for retrospective use of anonymized data; and (2) the MIMIC-IV database (v3.1, PhysioNet ID: 66212733), which contains de-identified clinical data from Beth Israel Deaconess Medical Center (2008–2022), approved by MIT and BIDMC IRBs with waived consent^[23]. All procedures complied with the Declaration of Helsinki.

3.2. Study Population and Data Collection

Eligible patients were adults (aged ≥ 18 years) with comprehensive hemodynamic monitoring data. The study population consisted of patients monitored with the PiCCO system. For individuals with multiple hospital admissions, only the first eligible ICU stay was retained. Exclusion criteria

were: (i) fewer than 1 valid measurement of the primary biomarkers (P_{life} or R_{lac}) within the first 7 ICU days; and (ii) receipt of either left ventricular assist device (LVAD) or extracorporeal membrane oxygenation (ECMO), as these mechanical support devices fundamentally alter circulatory dynamics and may confound the relationship between endogenous biomarkers and mortality (Supplementary Figure 1).

Baseline demographic characteristics, APACHE II score, and comorbidities (hypertension, diabetes mellitus, hepatic dysfunction, and New York Heart Association functional class) were recorded at ICU admission. Time zero was defined as the first documented cardiac output measurement after ICU admission.

For daily data resampling, the first routine complete blood count of each calendar day served as the observation time point, chosen because the complete blood count is routinely collected at fixed intervals (typically daily) and is directly relevant to P_{life} calculation. For continuous variables (e.g., total bilirubin), the first measurement recorded within a 24-hour window of the CBC time point was selected. For other variables (e.g., hemodynamic parameters, lactate), the first measurement recorded within a ± 3 -hour window of the blood count was selected.

In the primary analysis, patients with missing data for any key variable were excluded (complete case analysis); no imputation was performed for missing items, and such entries were simply deleted.

The primary outcome was all-cause mortality during hospitalization. Follow-up continued until the earliest occurrence of death, hospital discharge, or the study cut-off date (December 20, 2025). Survival time was calculated as the interval from time zero to death, hospital discharge, or last known follow-up, whichever occurred first. Weaning from circulatory support was not considered

a censoring event; patients were followed for vital status regardless of support status.

3.3. Statistical Analysis

3.3.1 Association with Mortality

Cox proportional hazards models were used to estimate the association of each indicator with all-cause mortality, reported as hazard ratios (HRs) with 95% confidence intervals (CIs). Three sequentially adjusted models were constructed: Model 1 (unadjusted); Model 2 (adjusted for age, sex, APACHE II score, and BMI); and Model 3 (fully adjusted for all covariates, including diabetes mellitus, cardiopulmonary arrest, peripheral vascular disease, cardiac dysfunction, hypertension, and other comorbidities).

For trend analysis, quartiles of each indicator were treated as continuous variables, and the HR per 25-percentile increment was estimated. Kaplan–Meier survival curves with log-rank tests compared survival across quartiles. Nonlinear dose–response relationships were explored using restricted cubic splines (RCS) with four knots, selected by comparing AIC and BIC. After identifying optimal cutoffs ($R_{lac} > 1$, $P_{life} > 5$), additive interaction metrics — including the relative excess risk due to interaction (RERI), attributable proportion (AP), and synergy index (SI) — were calculated to quantify potential synergistic or antagonistic effects.

The proportional hazards assumption was tested using Schoenfeld residuals. For variables violating the PH assumption (e.g., P_{life} , P_{lac} and their combined groups), restricted mean survival time (RMST) analysis was performed as a complementary approach, with $\tau = 168$ hours (7 days). Results are reported as RMST differences and ratios.

Subgroup analyses evaluated effect modification by baseline characteristics, with interaction

p -values calculated using likelihood ratio tests.

3.3.2 Longitudinal Trajectories and Interaction Analysis

To identify heterogeneous longitudinal patterns of P_{life} and R_{lac} , joint latent class mixed models (JLCMM) were fitted^[24]. These models allowed fixed-effect coefficients to vary across latent classes and included random intercepts and random slopes for individuals. Models with one to five latent classes were compared. The optimal number of classes was determined by BIC, with each class comprising >5% of the sample, a mean posterior class-membership probability >0.7, and concordance between longitudinal and survival classifications.

Based on the identified trajectories, each patient was assigned a latent class label. This class variable, together with the four-level risk group (derived from $R_{lac} > 1$ and $P_{life} > 5$), was incorporated into logistic regression models to predict 1-, 3-, and 7-day mortality. Model performance was assessed using AUC, net reclassification improvement (NRI), and integrated discrimination improvement (IDI). The incremental predictive value of trajectory classification and risk grouping was evaluated by comparing nested models using likelihood ratio tests.

3.3.3 Machine Learning and External Validation

The extreme gradient boosting (XGBoost) algorithm was used to identify predictors of short-term mortality. Participants were randomly split into training and testing sets at a 6:4 ratio. Variables were ranked by their contribution to model prediction using SHapley Additive exPlanations (SHAP) analysis. Model performance was evaluated using AUC. To assess robustness to feature selection, three alternative XGBoost models were trained using 2, 3, and 9 clinically accessible features.

External validation was performed using the MIMIC-IV database. For the primary analysis, patients with missing data for any variable were excluded. Given substantial differences in data collection frequency between databases, a sensitivity analysis was performed using forward filling for missing values: if P_{life} or R_{lac} was missing at a given time point, the most recent valid measurement within the prior 3 days was used for imputation. Analyses were then repeated on the imputed dataset.

3.3.4 Additional Analyses

Time-dependent analyses were performed to evaluate the prognostic value of R_{lac} and P_{life} over time. Univariable Cox regression identified optimal prediction windows, time-dependent AUC analysis evaluated discriminative ability (calculated at days 1, 3, 7, 10, and 14), and time-dependent Cox models estimated the association of dynamic biomarker changes with mortality risk.

Mediation analysis was conducted to evaluate whether the protective effects of Hb and RBC count on mortality were mediated by P_{life} and R_{lac} . Joint model mediation analysis estimated indirect, direct, and total effects, along with the proportion mediated. Bayesian Markov chain Monte Carlo (MCMC) methods were used with 20,000 iterations, 5,000 burn-in, and 2 chains; convergence was assessed using effective sample size. Results are reported as means, standard deviations, 95% credible intervals, and posterior p-values.

3.3.5 Descriptive Statistics and Software

Continuous variables are presented as mean $\pm$ standard deviation (SD) or median with interquartile range (IQR); categorical variables as counts (percentages). Group comparisons were performed using one-way ANOVA, the Wilcoxon rank-sum test, or the chi-square test, as

appropriate. All statistical analyses were conducted using R software version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Additional R packages used are listed in Appendix 5.

4. RESULTS

4.1. Study Population

A total of 161 patients were included, comprising 71 survivors and 90 non-survivors. Non-survivors were significantly older (73.5 vs. 64.9 years, $p < 0.001$), had higher APACHE II scores (27.0 vs. 22.5, $p = 0.001$), and exhibited worse metabolic profiles, including elevated lactate (4.20 vs. 2.10 mmol/L, $p < 0.001$) and reduced hemoglobin (8.15 vs. 9.25 g/dL, $p = 0.027$). Hemodynamically, non-survivors had higher CVP (12.0 vs. 10.0 mmHg, $p = 0.025$), lower GEF (13.0% vs. 17.0%, $p = 0.003$), and increased SVV (17.0% vs. 12.5%, $p = 0.002$). Notably, non-survivors demonstrated significantly lower P_{life} (6.26 vs. 13.44, $p = 0.001$) and higher R_{lac} (0.87 vs. 0.42, $p < 0.001$), along with greater vasoactive support requirements (VIS scores: 91.9 vs. 26.5, $p = 0.006$) (Supplementary Table 1). Among patients with only a single measurement (16 survivors, 29 non-survivors), similar trends were noted, though cross-sectional data limit causal interpretation (Supplementary Table 2).

Longitudinal analysis revealed divergent trajectories between groups. Among 56 survivors, blood pressure and lactate improved significantly, vasoactive support decreased markedly (34.8 to 8.6, $p = 0.007$), and P_{life} remained stable ($p = 0.929$). In contrast, among 60 non-survivors, P_{life} deteriorated progressively (7.10 to 4.65, $p = 0.038$), accompanied by worsening SOFA scores (8.0 to 10.0, $p = 0.022$), with no significant hemodynamic improvement (sTable 3,4).

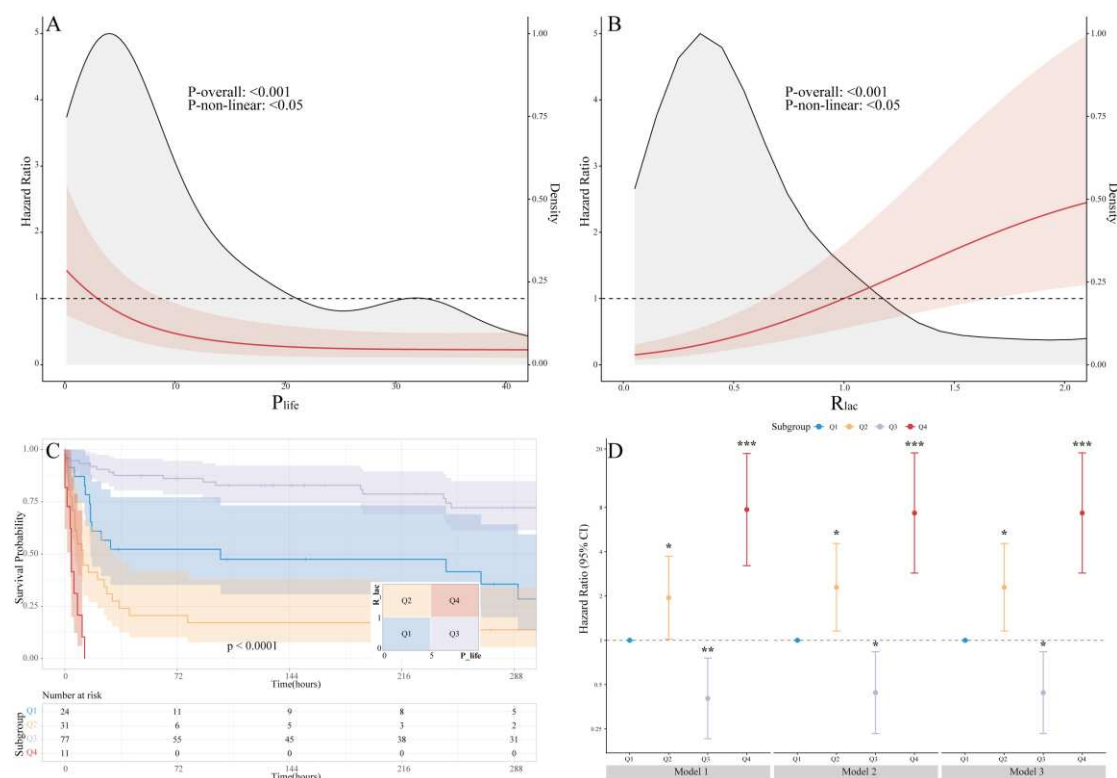


Figure 2. Dose-response and joint effects of P_{life} and R_{lac} on mortality. (A) RCS analysis of P_{life} showing a nonlinear inverse association with mortality ($p < 0.001$). (B) RCS analysis of R_{lac} showing a nonlinear positive association ($p < 0.001$), with density plots on margins. (C) Kaplan-Meier curves for four composite risk strata (Q1–Q4) defined by R_{lac} (>1) and P_{life} (>5). (D) Joint effect analysis depicting the association between composite risk strata and all-cause mortality. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4.2. Integrated Analysis of the Association Between Scores and Mortality

The prognostic value of R_{lac} and P_{life} differed by circulatory support modality, as consistently demonstrated by Cox regression, Kaplan-Meier, and RCS analyses. Both biomarkers exhibited opposite dose-response associations with all-cause mortality (both p for trend < 0.001) (Figure 2). Higher P_{life} was associated with lower mortality (Q4 vs. Q1: adjusted HR 0.259, 95% CI: 0.125–0.537; mortality: 83.3% vs. 36.1%), whereas higher R_{lac} was associated with higher mortality (Q4 vs. Q1: adjusted HR 9.438, 95% CI: 4.216–21.126; mortality: 33.3% vs. 86.1%; both log-rank $p < 0.001$) (sTable 5,6; sFigure 2,3).

Using cutoffs of high R_{lac} (>1) and high P_{life} (>5), patients were stratified into four risk groups: Q1 (low R_{lac} & low P_{life} ; reference), Q2 (high R_{lac} & low P_{life}), Q3 (low R_{lac} & high P_{life}), and Q4

(high R_{lac} & high P_{life}) (Figure 4). Compared with Q1, adjusted HRs for Q2, Q3, and Q4 were 3.18 ($p = 0.002$), 0.45 ($p = 0.024$), and 8.67 ($p < 0.001$), respectively. High P_{life} was protective only in the absence of high R_{lac} (Q3), whereas high R_{lac} dominated risk regardless of P_{life} level (Q2, Q4), indicating that metabolic debt is the primary driver of mortality (sTable 7).

Mediation analysis revealed that the protective effect of high P_{life} was largely mediated by suppression of R_{lac} elevation (80.4%), whereas the harmful effect of high R_{lac} was predominantly direct (95.8%) (sTable 8). Additive interaction was present ($RERI = 6.035$; $AP = 0.696$), yet high R_{lac} remained dominant (Q4 HR 8.67) (sTable 9).

Given violations of the proportional hazards assumption for P_{life} and R_{lac} identified by the Schoenfeld residual test (sTable 10), RMST analysis was performed to further evaluate survival differences. RMST analysis demonstrated opposite dose-response relationships for P_{life} and R_{lac} . Higher P_{life} was associated with progressively longer survival (Q4 vs Q1: +15.26 days, $p < 0.001$; RMST ratio 4.25), whereas higher R_{lac} was associated with progressively shorter survival (Q4 vs Q1: -5.19 days, $p < 0.001$; RMST ratio 0.18) (sTable 11).

Subgroup analyses showed consistent effects of both biomarkers across all strata (all HRs < 1 for P_{life} , all > 1 for R_{lac}). Significant effect modification was observed for sex and hepatic dysfunction with P_{life} (P for interaction = 0.024 and 0.008), and for cardiac dysfunction, arrhythmia, and hepatic dysfunction with R_{lac} (P for interaction = 0.043, 0.049, and 0.043). Notably, hepatic dysfunction was the only common modifier: patients with hepatic dysfunction derived stronger protection from high P_{life} (HR 0.13 vs 0.45) but faced greater risk from high R_{lac} (HR 7.36 vs 4.24). No other significant modifications were detected (all P for interaction > 0.05) (sFigure 4).

4.3. Longitudinal Dynamics and Joint Trajectories of P_{life} and R_{lac}

We compared joint latent class models with 1 to 5 classes (Figure 3). The BIC decreased from 1 to 2 classes, reaching its minimum at the 2-class solution, and increased thereafter. However, the 3-class solution (BIC = 3386.91, entropy = 0.604) was selected as optimal among acceptable models (all classes >5% of the sample), as it demonstrated acceptable classification quality (the smallest class had 66.7% of patients with posterior probability >0.7) and perfect concordance between longitudinal and survival classifications. Models with 4 or more classes were excluded due to higher BIC, presence of classes <5%, or classification discordance (sTable 12).

Three distinct latent classes with distinct temporal patterns were identified (Figure 3). The classes were characterized as follows: Class 2, "Stable compensatory" (n=50.0%; mortality 27.8%), featuring persistently high P_{life} with declining R_{lac} ; Class 3, "Protracted exhaustion" (n=6.1%; mortality 40.0%), featuring late-stage decline in P_{life} and late-stage elevation of R_{lac} ; and Class 1, "Persistent decompensation" (n= 43.9%; mortality 68.3%), featuring persistently low and deteriorating P_{life} with sustained R_{lac} elevation (sTable 13). A clear prognostic gradient was observed across the three phenotypes(log-rank $p < 0.001$) (sFigure 3).

Trajectory classification and the four-level risk group significantly improved 7-day mortality prediction ($p < 0.001$) but did not improve 1-day or 3-day prediction (likelihood ratio tests, both $p > 0.05$) (sTable 15). The baseline model ($R_{lac} + P_{life}$) had test AUCs of 0.770 (1-day), 0.762 (3-day), and 0.750 (7-day). For 7-day mortality, the combined model achieved the best performance (AUC = 0.759; $\Delta AIC = -16.01$; NRI = 0.175; IDI = 0.011). However, for 1-day and 3-day mortality, model improvements were minimal ($\Delta AUC \leq +0.010$) and statistically non-significant, suggesting that the added predictive value of trajectory and risk group is specific to longer-term outcomes (sTable 14).

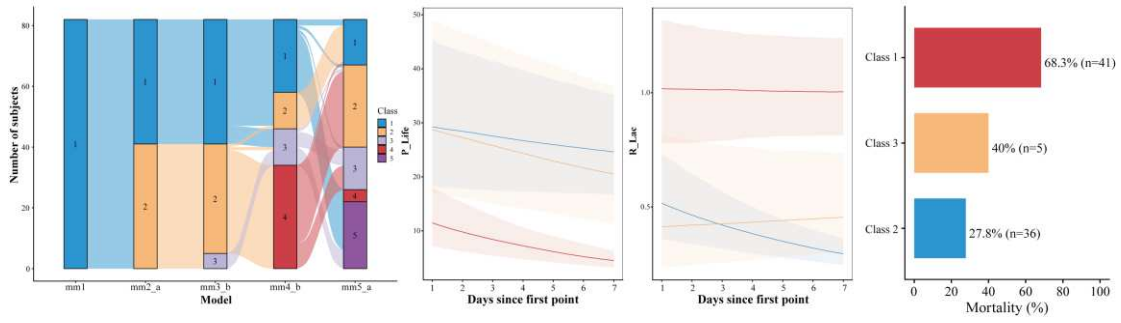


Figure 3. Joint latent class trajectories. Three distinct phenotypes were identified based on longitudinal P_{life} and R_{lac} patterns. Class 1 (Persistent decompensation, mortality 68.3%) showed persistently low and deteriorating P_{life} with sustained R_{lac} elevation. Class 2 (Stable compensatory, mortality 27.8%) showed persistently high P_{life} with declining R_{lac} . Class 3 (Protracted exhaustion, mortality 40.0%) showed late-stage decline in P_{life} and late-stage elevation of R_{lac} . Shaded areas indicate 95% CIs.

4.4. Machine Learning and External Validation

XGBoost with SHAP identified R_{lac} , P_{life} , and VIS score as key predictors (Figure 4). In internal validation (sTable 16), XGBoost outperformed the baseline logistic model for 1-day (AUC 0.813 vs. 0.770, $p = 0.044$) and 3-day (0.777 vs. 0.762) mortality. To assess feature selection robustness, we trained XGBoost models using 2, 3, and 9 clinically accessible features. The 9-feature XGBoost achieved the highest 1-day AUC (0.850, $p = 0.033$ vs. baseline). However, for 7-day mortality, all XGBoost variants performed worse than the baseline logistic model, indicating that logistic regression may be preferred for longer-term prediction (sTable 17; sFigure 5).

Due to data completeness constraints, external validation was performed using the MIMIC-IV database for logistic regression models (sTable 18; sFigure 6). The baseline model achieved AUCs of 0.905 (1-day), 0.912 (3-day), and 0.805 (7-day). Models incorporating trajectory classification and the combined model performed comparably, with 1-day AUCs of 0.901 and 0.906, 3-day AUCs of 0.895 and 0.899, and 7-day AUCs of 0.803 and 0.786, respectively. None of the more complex models showed meaningful improvement over the baseline model, as reflected by ΔAUC values ranging from -0.019 to +0.001 and NRI/IDI values close to zero (sTable 19). The parsimonious

baseline model therefore demonstrated good generalizability for early mortality risk stratification in an external ICU population.

Sensitivity analysis using 3-day forward imputation of missing values resulted in decreased AUCs for all models. Nevertheless, all models retained acceptable discrimination, and the baseline model remained the best performer, supporting its robustness for external validation (sTable 19).

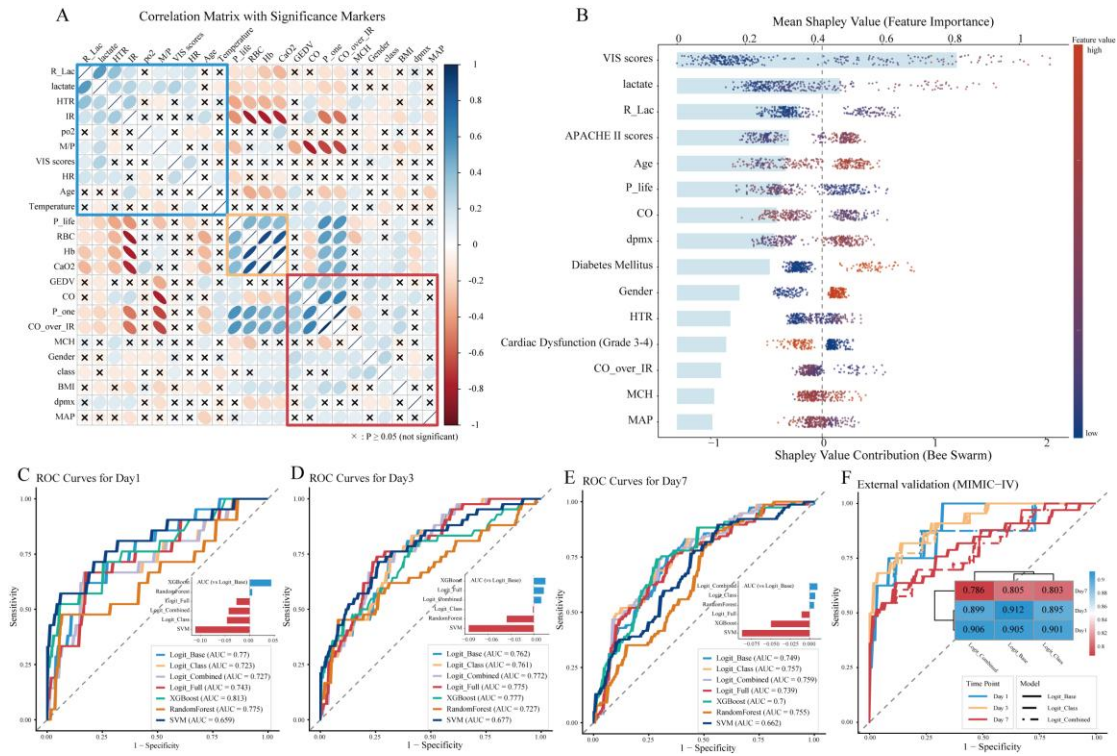


Figure 4. Model evaluation and validation. (A) Correlation heatmap of all variables. (B) SHAP analysis: bar plot (mean absolute SHAP) and bee-swarm plot of feature importance. (C–E) ROC curves for 1-, 3-, and 7-day mortality in internal validation, with Δ AUC vs. baseline annotated. (F) External validation AUC heatmap for three models at three time points (MIMIC-IV cohort).

4.5. Additional Analyses

Univariable Cox regression identified optimal prediction windows of 1–3 days for R_{lac} and 7–14 days for P_{life} . Time-dependent AUC analysis showed that R_{lac} peaked at day 1 (AUC = 0.733) and declined thereafter, while P_{life} peaked at day 10 (AUC = 0.785) and remained moderate through day 14 (AUC = 0.613). Time-dependent Cox modeling revealed that R_{lac} was associated with increased mortality risk (HR = 1.217, 95% CI: 1.189–1.237), while P_{life} was associated with

decreased risk (HR = 0.989, 95% CI: 0.987–0.990) (sTable 20).

We further explored the associations of Hb and RBC count with mortality using joint model mediation analysis. The protective effects of Hb and RBC were significantly mediated through P_{life} , but not through R_{lac} . For Hb, the indirect effect via P_{life} was $\beta = -0.188$ (95% CI: -0.402 to -0.035 , $p = 0.008$), accounting for 60.7% of the total effect. For RBC, the indirect effect via P_{life} was $\beta = -0.175$ (95% CI: -0.353 to -0.042 , $p = 0.001$), accounting for 47.1% of the total effect. Indirect effects via R_{lac} were negligible for both Hb ($\beta = -0.0003$, $p = 0.855$) and RBC ($\beta = -0.00007$, $p = 0.949$). These findings suggest that the survival benefit of higher Hb and RBC levels is primarily explained by improved circulatory function as reflected by P_{life} (sTable 21; sFigure 7).

5. Discussion

Building on the BOU framework and the perfusion–metabolism coupling equation, from which we previously derived P_{life} as a functional metric to estimate the circulatory value of RBCs for guiding transfusion and other macro-level interventions, while R_{lac} was developed to assess the association between oxygen delivery and cellular metabolism under varying circulatory conditions.

We now further examine the influence of different therapeutic strategies on P_{life} dynamics in the present study. From the derivation of P_{unit} , the number of BOUs maintainable by a given Hb concentration under the current circulatory state is expressed as (Figure 5C):

$$\frac{M}{P_{unit}} = Hb \times \frac{Inflation\ Rate}{CO}$$

Hb either functions within RBCs or is converted to bilirubin, implying an inverse relationship between M/P_{unit} and HTR. Their product, in turn, defines the reciprocal of P_{life} .

Transfusion transiently increases Hb and HCT, shifting the curve downward and rightward

while reducing oxygen debt via enhanced oxygen-carrying capacity. Yet evidence for transfusion-induced improvement in tissue oxygenation or mitochondrial function is limited^[25]; one study found no increase in mitochondrial oxygen tension in 63 critically ill patients after transfusion^[26]. Our findings similarly indicate that the metabolic benefit of transfusion is mediated through P_{life} rather than directly through R_{lac} .

The mechanisms underlying fluid resuscitation are more complex. When BOUs encounter impaired flow, total perfusion declines, increasing mortality of both tissue cells and erythrocytes—most notably in shock-induced microcirculatory stagnation^[27]. In this setting, fluid infusion enhances erythrocyte perfusion, though its efficacy remains heavily contingent on cardiac pump function; beyond a certain point, continued fluid infusion exerts minimal impact on HTR or Hb. Particular caution is warranted in heart failure, especially right ventricular dysfunction, where the risk of right-left heart mismatch must be considered. Systemic venous congestion is common in ventricular failure, and fluid administration in this context may worsen rather than improve microcirculation^[28]. Conversely, fluid infusion may be beneficial in selected scenarios—for example, cardiogenic shock due to pulmonary embolism or ischemia-induced right ventricular contractile depression^[29-31]. When fluid overload is reached, capillary leakage or intravascular fluid redistribution impairs substrate exchange efficiency, slowing cellular metabolism^[32].

Blood transfusion and fluid resuscitation are only therapeutic means; restoring cellular oxygen metabolism and organ function is the ultimate goal^[33,34]. Whether defining optimal transfusion thresholds or addressing hemodynamic coherence loss, the fundamental issue reflects a persistent gap in integrating oxygen supply–demand balance with circulatory dynamics in current assessment frameworks^[35]. Therefore, while assessing P_{life} , simultaneous attention should be directed to

changes in R_{lac} , as the two metrics together provide a more complete picture of the perfusion–metabolism coupling status.

Risk stratification analysis revealed that patients in Q4 (high R_{lac} & high P_{life}) differed significantly from other groups. This group was classified as highest risk not because of high P_{life} per se, but due to the concurrent presence of high R_{lac} . Multivariable Cox regression confirmed Q4 as the strongest independent predictor of mortality (HR = 8.67, 95% CI: 3.02–24.87, $p < 0.001$). Although this finding appears to contradict the overall negative correlation between P_{life} and R_{lac} , integration with the three-class trajectory model suggests that the Protracted exhaustion phenotype represents a transitional stage toward Persistent decompensation, with R_{lac} elevation preceding and driving subsequent P_{life} deterioration, ultimately leading to the decompensated phenotype.

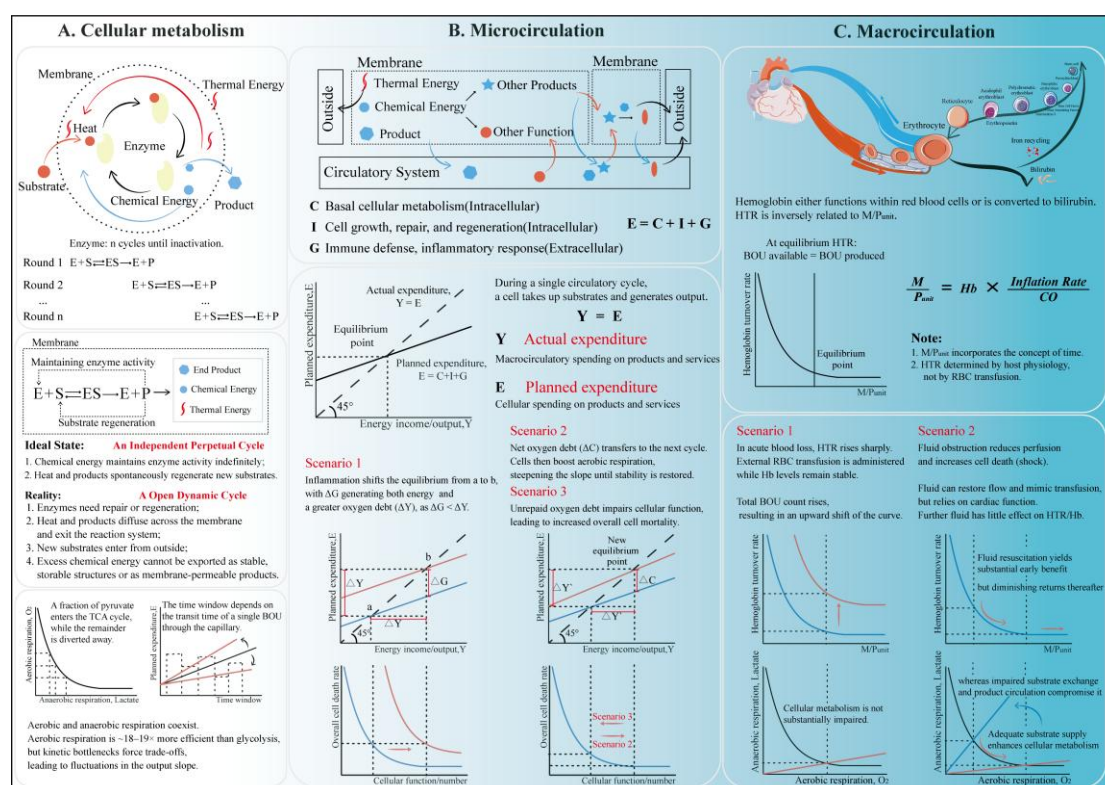


Figure 5. Integrative pathway linking macrocirculation, microcirculation, and cellular metabolism. This schematic illustrates how transfusion, fluid resuscitation, and inflammation exert differential effects on oxygen transport, and metabolic efficiency.

Within the body, both aerobic and anaerobic respiration coexist. Although aerobic respiration has extremely high energy conversion efficiency (approximately 18–19 times that of anaerobic

glycolysis), it is constrained by kinetic bottlenecks such as pyruvate oxidative decarboxylation and pyruvate carrier integrity^[36-38], necessitating dynamic trade-offs between metabolic pathways that cause fluctuations in the expected output slope. Expected output comprises disposable (ATP, etc.) and non-disposable (heat) components, while actual output is determined by the balance between metabolite accumulation and consumption. For example, when muscle or neural activity increases energy demand, metabolites accumulate, and aerobic respiration subsequently converts lactate from the previous cycle back into glucose^[39]. This process has important compensatory value and economy in energy metabolism but exhibits time lag. The disposable portion of expected output can be converted into various metabolites entering different metabolic pathways, contributing to actual output. Maintaining current cellular function and cell count is critical; when disposable output sufficiently covers the energy cost of cell maintenance and regeneration, overall cellular mortality risk declines (Figure 5A). R_{lac} is associated with the relationship between expected output and actual output. This association may be linked to microcirculatory and macrocirculatory changes that correspond to variations in P_{life} (Figure 5B).

Machine learning provided a powerful tool for analyzing complex, high-dimensional data and identifying key predictors. SHAP analysis enhanced model interpretability and identified R_{lac} , P_{life} , and VIS score as key predictors. Changes in vasoactive support intensity correlated with mortality risk gradients. A multicenter cardiac intensive care unit registry showed that early VIS trajectory may improve outcomes in cardiogenic shock^[40]. Similarly, the FROG-ICU study, a prospective, observational, multicenter cohort, confirmed that inotropic and/or vasopressor therapy was a strong predictor of ICU mortality^[41]. However, based on data availability and clinical utility, the baseline model did not differ substantially from more complex models incorporating trajectory or risk

stratification. After internal and external validation, we developed a parsimonious model and built a web-based calculator for clinical use, accessible at: https://tangsiyu.shinyapps.io/erythrocyte_cycle_index_calculator/.

Several limitations should be acknowledged. First, the derivation of P_{life} is based on multiple idealized assumptions. The validity of the hemoglobin turnover index as a surrogate marker for hemoglobin-related short-term mortality risk requires substantial empirical validation; thus, P_{life} represents only an approximate assessment, and missing data further limit its precision. Second, this study did not directly observe microcirculation; we inferred microcirculatory status indirectly through P_{life} and R_{lac} by reflecting the relationship between macrocirculation and cellular metabolism. Future studies incorporating sublingual microcirculatory monitoring^[42] and nailfold capillary refill testing^[43] are warranted.

6. Conclusions

The integrative circulatory model-derived indices P_{life} and R_{lac} provide complementary short-term and long-term risk stratification in critically ill patients. In addition to the "low P_{life} & high R_{lac} " phenotype defining a very-high-risk subgroup, the "high P_{life} & high R_{lac} " phenotype also merits attention. Transfusion and fluid resuscitation decisions may be informed by P_{life} , while incorporating R_{lac} to assess short-term mortality risk. Further studies are needed to confirm their impact on clinical outcomes.

Abbreviations

AIC, Akaike Information Criterion; AP, attributable proportion; APACHE II, Acute Physiology and Chronic Health Evaluation II; BIC, Bayesian Information Criterion; BMI, body mass index; CI,

confidence interval; Hb, hemoglobin; HCT, hematocrit; ICU, intensive care unit; IQR, interquartile range; Lac, lactate; LCMM, latent class mixed model; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PiCCO, pulse index continuous cardiac output; RBC, red blood cell count; RBCT, red blood cell transfusion; RCS, restricted cubic spline; RERI, relative excess risk due to interaction; SD, standard deviation; SI, synergy index.

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Authors' contributions

SY conceived the study, developed the methodology and software, performed validation, formal analysis, investigation, and data curation, wrote the original draft, and administered the project. HJ contributed to data curation, formal analysis, and writing – review & editing. JQ, BX, and YQ provided supervision, validation, and writing – review & editing. XH provided resources, supervision, validation, and writing – review & editing.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its supplementary files. The raw data have been deposited in Figshare with the identifier: 10.6084/m9.figshare.32671452.

Ethics approval and consent to participate

The study was approved by the Institutional Review Committee of Zhejiang Provincial People's Hospital (No. QT2022383) and conducted in accordance with the Declaration of Helsinki. MIMIC-IV was accessed via PhysioNet. Informed consent was waived given the retrospective, de-identified nature of the data.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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