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Inflammatory Phenotypes, Early Clinical Trajectories, and Outcomes in Acute Respiratory Distress Syndrome: A Multicenter Derivation and Validation Study

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Research Article

Keywords:

Posted Date: October 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9466623/v1

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Additional Declarations:
No competing interests reported.

Tables are available in the Supplementary Files section.

Figures are available in the Supplementary Files section.

Inflammatory Phenotypes, Early Clinical Trajectories, and Outcomes in Acute Respiratory Distress Syndrome: A Multicenter Derivation and Validation Study

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Abstract

Background: Acute respiratory distress syndrome (ARDS) is biologically and clinically heterogeneous. Whether baseline inflammatory phenotypes correspond to distinct early clinical trajectories, how those trajectories evolve during the first 72 hours, and whether trajectory information adds prognostic value beyond baseline inflammatory phenotype alone remain unclear.

Methods: We conducted a multicenter study of clinically recognized ARDS across three Chinese centers. Ruijin Hospital served as the derivation cohort, Fujian Provincial Hospital as the retrospective validation cohort, and The Second People's Hospital of Fuyang City as the prospective validation cohort. Baseline inflammatory phenotypes were defined in biomarker-rich cohorts using a prespecified parsimonious biomarker framework based on interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), procalcitonin, and lactate. Early clinical trajectories over the first 72 hours were derived in the Ruijin cohort and assigned in the validation cohorts using a fixed-reference framework. We examined associations between baseline phenotype and trajectory class, phenotype transition and speed of early change, clinical outcomes across trajectories, and the incremental prognostic value of trajectory beyond phenotype alone.

Results: A total of 995 patients with clinically recognized ARDS were included, including 150 in the Ruijin derivation cohort, 505 in the Fujian retrospective validation cohort, and 340 in the Fuyang prospective validation cohort. In the derivation cohort, three early trajectory classes were identified: rapid improvers (54/150, 36.0%), intermediate resolvers (53/150, 35.3%), and refractory patients (43/150, 28.7%). In Ruijin, PaO_2/FiO_2 changed from 238.4 ±54.1 to 320.4 ±70.3 mmHg in rapid improvers, from 186.3 ±41.8 to 192.1 ±49.7 mmHg in intermediate resolvers, and from 103.1 ± 28.5 to 87.0 ±24.1 mmHg in refractory patients over the first 72 hours (all $P < 0.001$ across groups). Compared with the lower-inflammatory phenotype, the higher-inflammatory phenotype was associated with lower odds of a rapid improver trajectory (adjusted OR 0.54, 95% CI 0.29–0.98) and higher odds of a refractory trajectory (adjusted OR 2.81, 95% CI 1.59–4.95). In transition analyses, persistent lower-inflammatory status was associated with improving oxygenation ($\Delta PaO_2/FiO_2$ +78.4 ±64.2 mmHg) and lower ICU mortality (11.9%), whereas persistent higher-inflammatory status was associated with worsening oxygenation ($\Delta PaO_2/FiO_2$ -8.7 ±58.6 mmHg) and higher ICU mortality (32.2%). Rapid improvers consistently had the most favorable outcomes across cohorts. Addition of baseline inflammatory phenotype improved discrimination for 28-day mortality from 0.71 to 0.78, and further addition of early trajectory improved discrimination to 0.84.

Conclusions: In ARDS, baseline inflammatory phenotype captures only part of clinically relevant heterogeneity. Early 72-hour clinical trajectory, including the direction and speed of change, provides complementary dynamic information and improves prognostic stratification beyond baseline phenotype alone.

Keywords: acute respiratory distress syndrome; inflammatory phenotype; clinical trajectory; heterogeneity; prognosis

Introduction

Acute respiratory distress syndrome (ARDS) remains a biologically and clinically heterogeneous syndrome, and this heterogeneity is widely considered a major reason for repeated failures of interventional trials and for inconsistent treatment responses across apparently similar patients.1–4 Patients meeting the same diagnostic criteria may differ substantially in the biology of lung injury, the degree of systemic inflammation, the severity of extrapulmonary organ dysfunction, and their subsequent clinical course.3–5

Among available enrichment frameworks, hyperinflammatory and hypoinflammatory phenotypes represent the most reproducible and biologically interpretable approach to ARDS heterogeneity.5–9 These phenotypes have consistently shown differences in biomarker profiles, severity of illness, prognosis, and, in some studies, treatment responsiveness.5–10 However, most prior work has relied on baseline single-time-point classification. Although this framework is an important starting point, it does not fully capture the dynamic nature of ARDS. Recent work has shown that inflammatory phenotypes in ARDS may be temporally unstable, with clinically important transitions occurring over time, further underscoring the limitations of baseline single-time-point classification alone.11–13

ARDS is fundamentally an evolving syndrome. Even among patients assigned to the same baseline inflammatory phenotype, the early clinical course may diverge substantially, with some patients showing rapid physiologic recovery and others remaining persistently hypoxemic or deteriorating despite apparently similar baseline biology. Early trajectory heterogeneity may therefore help explain why baseline phenotype alone does not fully account for subsequent prognosis. In this sense, phenotype may define the biological starting point, whereas early trajectory reflects how that biology is expressed clinically during the critical first days of illness. This concept is also aligned with emerging work emphasizing ARDS recovery and resolution as dynamic processes rather than static disease states.14,15

We therefore conducted a multicenter derivation-and-validation study in clinically recognized ARDS to address three related questions. First, are baseline inflammatory phenotypes associated with distinct 72-hour early clinical trajectories? Second, what are the direction and speed of early phenotype and physiologic change? Third, does early trajectory provide incremental prognostic information beyond a conventional baseline phenotype model? We hypothesized that the higher-inflammatory phenotype would be associated with a lower likelihood of rapid early improvement and a greater likelihood of a refractory trajectory, and that incorporating 72-hour trajectory information would improve prognostic stratification beyond baseline severity and baseline inflammatory phenotype alone.

Methods

Study Design

We conducted a multicenter study of clinically recognized ARDS across three Chinese centers. The study used a staged design with one derivation cohort, one retrospective validation cohort, and one prospective validation cohort. The analytic framework was prespecified to examine the relationship between baseline inflammatory phenotype, early 72-hour clinical trajectory, and clinical outcomes. The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.16

Ruijin Hospital (Shanghai, China) served as the derivation cohort and included consecutive patients enrolled from January 2022 through December 2023. Fujian Provincial Hospital (Fuzhou, China) served as the retrospective validation cohort and included consecutive patients enrolled from January 2020 through December 2024. Fuyang Second People's Hospital (Fuyang, China) served as the prospective validation cohort and included consecutively enrolled patients from January 2025 through March 2026.

Ethics Approval

The study was approved by the ethics committees of all participating centers before analysis. Approval was granted by the Ruijin Hospital Ethics Committee, Shanghai Jiao Tong University School of Medicine (Reference No. 20250518), by the Ethics Committee of The Second People's Hospital of Fuyang City (Approval No. 20250729099), and by the Fujian Provincial Hospital Ethics Committee (Approval No. K202601041). The study was conducted in accordance with the Declaration of Helsinki and relevant institutional requirements. Given the observational nature of the retrospective cohorts, informed consent was waived where permitted by local ethics committees; prospective data collection in the Fuyang cohort followed local ethics requirements for consent and oversight.

Study Population

The analytic population consisted of adults aged 18 years or older with clinically recognized ARDS. Because complete retrospective adjudication of all Berlin criteria was not uniformly feasible across centers, we defined the study population using a pragmatic clinician-recognized ARDS framework based on acute hypoxemic respiratory failure requiring respiratory support together with documented clinical diagnosis and/or structured registry coding for ARDS. This approach was prespecified before analysis and applied consistently within each cohort, with the intention of reflecting real-world clinician-recognized ARDS while minimizing misclassification arising from incomplete retrospective capture of radiographic findings, timing, and ventilator settings.1,2,17

Eligible patients were required to have sufficient serial clinical data during the first 72 hours after cohort qualification for trajectory analysis. Patients were excluded if they were younger than 18 years, lacked adequate longitudinal oxygenation data during the early observation window, or had missing

essential information precluding phenotype or outcome analysis. The detailed screening process is shown in Figure 1 and Supplementary Figure S1.

Cohort Roles

The Ruijin cohort was used to derive inflammatory phenotypes, define early trajectory classes, and establish the reference trajectory framework. The Fujian cohort was used as an independent retrospective validation cohort to test reproducibility of phenotype-trajectory mapping and trajectory-outcome associations in a large real-world historical dataset. The Fuyang cohort was used as an independent prospective validation cohort to test transportability of the framework in a temporally distinct, prospectively assembled dataset.

Data Collection and Harmonization

For each center, baseline demographic characteristics, severity indices, laboratory variables, inflammatory biomarkers, respiratory support variables, longitudinal oxygenation measurements, and clinical outcomes were collected using standardized definitions established before pooled analysis. A common data dictionary was used for variable harmonization across centers. Quality-control procedures included checks for unit consistency, temporal consistency, biologically implausible values, duplication, and missingness patterns. Variable-level missingness is summarized in Supplementary Table S1.

Baseline Inflammatory Phenotyping

Baseline inflammatory phenotypes were defined in biomarker-rich cohorts using a prespecified parsimonious biomarker framework based on interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), procalcitonin, and lactate, informed by prior ARDS subphenotype literature.5,6 Patients were classified into lower-inflammatory and higher-inflammatory phenotype groups using prespecified decision rules established before outcome analysis. In the present study, this baseline inflammatory classification served as the reference conventional phenotype framework against which the added prognostic value of early 72-hour clinical trajectory was evaluated.

Early Clinical Trajectory Assessment

The primary trajectory window was the first 72 hours after cohort qualification. This window was chosen a priori because it captures the interval during which early divergence in disease course is most likely to emerge while limiting bias introduced by longer observation periods, including survivorship bias and informative missingness.11,12,13

Trajectory assessment focused on serial, routinely available, and clinically interpretable variables. The principal longitudinal variable was PaO_2/FiO_2, supplemented where available by nonrespiratory organ dysfunction and respiratory support intensity. In addition to classifying trajectory patterns, we quantified the direction and speed of early change, including change in oxygenation over the first 72 hours and, in biomarker-rich subsets, change in inflammatory phenotype over time.

Trajectory Derivation and Fixed-Reference Validation

Trajectory classes were derived in the Ruijin derivation cohort using a parsimonious and clinically interpretable framework. Candidate trajectory structures were evaluated according to clinical coherence, separation of longitudinal patterns, and internal robustness. The final trajectory framework comprised three classes: rapid improvers, intermediate resolvers, and refractory patients.

Rapid improvers were characterized by marked early improvement in oxygenation, generally approaching or exceeding the recently proposed consensus threshold for ARDS resolution, whereas refractory patients exhibited persistently poor or worsening oxygenation with limited early recovery.14,15 To preserve consistency across cohorts, trajectory classes were derived in Ruijin and then assigned to Fujian and Fuyang using a fixed-reference framework rather than being re-derived independently in each cohort.

Phenotype Transition and Speed of Change

In biomarker-rich subsets with repeated inflammatory measurements, we examined early phenotype transition patterns. Patients were categorized as persistent lower-inflammatory, persistent higher-inflammatory, transition from higher- to lower-inflammatory, or transition from lower- to higher-inflammatory. We additionally quantified the speed of early physiologic change, primarily using change in PaO_2/FiO_2 across the first 72 hours and, where feasible, change in nonrespiratory organ dysfunction.

Outcomes

The primary outcome was 28-day mortality. Secondary outcomes included ICU mortality, hospital mortality, duration of mechanical ventilation, ICU length of stay, hospital length of stay, and ventilator-free days at day 28 where available. Ventilator-free days were calculated according to established critical care trial conventions. For prognostic model comparisons, 28-day mortality was used consistently across all hierarchical and supplementary model-performance analyses.

Statistical Analysis

Baseline characteristics are presented using standard descriptive methods. Associations between baseline inflammatory phenotype and trajectory class were examined using multivariable regression models adjusted for prespecified baseline covariates. Associations between trajectory class and clinical outcomes were assessed using models adjusted for baseline severity and, where applicable, baseline inflammatory phenotype.

To evaluate whether early trajectory added prognostic information beyond conventional baseline inflammatory phenotyping, we prespecified a hierarchical nested-model framework for 28-day mortality. Model 1 included baseline clinical severity variables alone; Model 2 represented a conventional baseline phenotype model and included Model 1 plus baseline inflammatory phenotype; and Model 3 included Model 2 plus early 72-hour trajectory class. Model comparisons focused on

discrimination, calibration, reclassification, and overall model fit. In this framework, the incremental value of trajectory was specifically interpreted relative to the classical baseline phenotype model rather than relative to clinical severity alone.

To further compare the relative contributions of baseline inflammatory phenotype and early trajectory, we performed an additional supplementary analysis comparing three alternative prognostic strategies: baseline severity plus inflammatory phenotype, baseline severity plus trajectory class, and baseline severity plus inflammatory phenotype plus trajectory. These analyses were prespecified as comparative model-performance analyses intended to assess complementary prognostic information, rather than to claim replacement of baseline phenotyping by trajectory alone.

Prespecified sensitivity analyses included restriction to biomarker-rich cohorts, analyses stratified by direct and indirect ARDS where available, complete-case analyses, and alternative trajectory specifications. Statistical analyses were performed using R (version 4.0 or later). A two-sided P value less than 0.05 was considered statistically significant.

Results

Cohort construction and phenotype availability

A total of 995 patients with clinically recognized ARDS were included in the primary analysis, including 150 in the Ruijin derivation cohort, 505 in the Fujian retrospective validation cohort, and 340 in the Fuyang prospective validation cohort (Figure 1). Across the overall cohort, median age was 62 [51–71] years, 640 patients (64.3%) were male, median APACHE II score was 22 [18–28], median SOFA score at enrollment was 10 [7–12], and baseline PaO_2/FiO_2 was 179 [120–232] mmHg (Table 1). Baseline higher-inflammatory phenotype was present in 417 patients (41.9%), whereas 578 (58.1%) were classified as lower-inflammatory.

Biomarker availability was greatest in the Ruijin and Fuyang cohorts, enabling inflammatory phenotyping and phenotype-transition analyses in those cohorts. The Fujian cohort served as the larger retrospective validation cohort for assessment of trajectory reproducibility and trajectory–outcome associations.

Identification of early trajectory classes

In the Ruijin derivation cohort, three clinically interpretable early trajectory classes were identified over the first 72 hours: rapid improvers (54/150, 36.0%), intermediate resolvers (53/150, 35.3%), and refractory patients (43/150, 28.7%). These classes showed clear separation in serial oxygenation, organ dysfunction, and support intensity during the early observation window (Table 2; Figure 2).

At day 1, mean PaO_2/FiO_2 was 238.4 ±54.1 mmHg in rapid improvers, 186.3 ±41.8 mmHg in intermediate resolvers, and 103.1 ±28.5 mmHg in refractory patients ($P < 0.001$). By day 3, these values had changed to 320.4 ±70.3, 192.1 ±49.7, and 87.0 ±24.1 mmHg, respectively ($P < 0.001$). A similar graded pattern was observed for organ dysfunction and support intensity. Median SOFA

score at day 3 was 6 [4–8] in rapid improvers, 9 [7–11] in intermediate resolvers, and 13 [10–15] in refractory patients (P < 0.001). Vasopressor use at day 3 was 16.7%, 35.8%, and 60.5%, respectively (P < 0.001), while renal replacement therapy at day 3 was required in 5.6%, 13.2%, and 25.6%, respectively (P = 0.02).

When the fixed-reference framework was applied to Fujian and Fuyang, the same three longitudinal oxygenation patterns were preserved (Figure 2). In Fujian, PaO_2/FiO_2 changed from 250.9 to 312.2 mmHg in rapid improvers, from 194.8 to 196.2 mmHg in intermediate resolvers, and from 126.9 to 94.6 mmHg in refractory patients. In Fuyang, the corresponding values were 230.5 to 330.3 mmHg, 164.6 to 193.3 mmHg, and 101.1 to 92.0 mmHg.

In biomarker-rich subsets with repeated measurements, early transition between inflammatory states was observed. Patients transitioning from a higher- to a lower-inflammatory state generally showed faster physiologic improvement than those with persistent higher-inflammatory status (Table 4; Figure 4). Higher-inflammatory phenotype and refractory trajectory were characterized by higher concentrations of IL-6, TNFR1, procalcitonin, and lactate than lower-inflammatory phenotype and rapid improver trajectory groups (Supplementary Table S4; Supplementary Figure S3).

Association between baseline phenotypes and trajectory class

Baseline inflammatory phenotype was associated with early trajectory assignment but did not fully determine it (Table 3; Figure 3). Compared with the lower-inflammatory phenotype, the higher-inflammatory phenotype was associated with lower odds of a rapid improver trajectory relative to the intermediate trajectory (adjusted OR 0.54, 95% CI 0.29–0.98; P = 0.043) and higher odds of a refractory trajectory relative to the intermediate trajectory (adjusted OR 2.81, 95% CI 1.59–4.95; P < 0.001). Higher baseline APACHE II score and lower baseline PaO_2/FiO_2 were also associated with refractory trajectory assignment.

Substantial within-phenotype heterogeneity remained. Although higher-inflammatory phenotype was enriched in refractory trajectories and lower-inflammatory phenotype was enriched in rapid improver trajectories, favorable and unfavorable early courses were observed within both phenotype groups.

Where direct versus indirect ARDS classification was available, the association between higher-inflammatory phenotype and refractory trajectory was directionally consistent in both direct ARDS (adjusted OR 2.63, 95% CI 1.51–4.58) and indirect ARDS (adjusted OR 2.94, 95% CI 1.42–6.06), with no significant interaction by ARDS etiology (interaction P = 0.71) (Supplementary Table S3).

Early phenotype transition patterns and speed of change

In transition analyses, persistent lower-inflammatory status was the most frequent pattern (402/995, 40.4%), followed by persistent higher-inflammatory status (286/995, 28.7%), lower-to-higher conversion (176/995, 17.7%), and higher-to-lower conversion (131/995, 13.2%) (Table 4).

Transition pattern was strongly associated with the direction and speed of early physiologic change. Persistent lower-inflammatory status was associated with improving oxygenation ($\Delta PaO_2/FiO_2$ +78.4 ±64.2 mmHg; +39.2 ±32.1 mmHg/day) and improving organ dysfunction (ΔSOFA -2.1 ±2.4; -1.05 ±1.20 points/day). In contrast, persistent higher-inflammatory status was associated with worsening oxygenation ($\Delta PaO_2/FiO_2$ -8.7 ±58.6 mmHg; -4.4 ±29.3 mmHg/day) and worsening organ dysfunction (ΔSOFA +0.9 ±2.8; +0.45 ±1.40 points/day). Patients converting from a higher- to a lower-inflammatory state showed favorable changes in both oxygenation ($\Delta PaO_2/FiO_2$ +64.6 ±61.5 mmHg) and SOFA score (ΔSOFA -1.8 ±2.3), whereas lower-to-higher conversion was associated with worsening oxygenation ($\Delta PaO_2/FiO_2$ -21.4 ±54.9 mmHg) and worsening SOFA score (ΔSOFA +1.2 ±2.6) (all overall $P < 0.001$).

These differences were clinically meaningful. ICU mortality was lowest in the persistent lower-inflammatory group (48/402, 11.9%) and in the higher-to-lower conversion group (19/131, 14.5%), whereas mortality was higher in the lower-to-higher conversion group (46/176, 26.1%) and highest in the persistent higher-inflammatory group (92/286, 32.2%) ($P < 0.001$).

Clinical outcomes across trajectory classes

Early trajectory class was strongly associated with clinically important outcomes across cohorts (Table 5; Figure 5). In the Ruijin derivation cohort, ICU mortality was 20.4% in rapid improvers, 32.1% in intermediate resolvers, and 53.5% in refractory patients ($P = 0.002$). Corresponding 28-day mortality rates were 22.2%, 34.0%, and 55.8%, respectively ($P = 0.001$). Rapid improvers also had more ventilator-free days at day 28 (16 [0–22] vs 9 [0–18] vs 0 [0–8], $P < 0.001$), shorter duration of mechanical ventilation (8 [5–14] vs 12 [7–19] vs 17 [10–25] days, $P < 0.001$), and shorter ICU length of stay (13 [8–21] vs 19 [12–28] vs 28 [17–39] days, $P < 0.001$).

In the Fujian retrospective validation cohort, a similar pattern was observed. ICU mortality was 6.4% in rapid improvers, 9.7% in intermediate resolvers, and 25.3% in refractory patients; 28-day mortality was 7.4%, 11.5%, and 28.6%, respectively (both $P < 0.001$). Ventilator-free days at day 28 were 19 [12–24], 15 [7–21], and 6 [0–15], respectively ($P < 0.001$).

In the Fuyang prospective validation cohort, rapid improvers again had the most favorable outcomes, whereas both intermediate and refractory trajectories were associated with substantially worse outcomes. ICU mortality was 20.0% in rapid improvers, 61.5% in intermediate resolvers, and 68.8% in refractory patients ($P < 0.001$), while 28-day mortality was 21.4%, 70.8%, and 64.1%, respectively ($P < 0.001$). Ventilator-free days at day 28 were 14 [0–21], 3 [0–10], and 2 [0–9], respectively ($P < 0.001$).Across cohorts, rapid improvers consistently showed the most favorable short-term outcomes, whereas the adverse outcome burden was concentrated in the intermediate and refractory trajectory groups, with the relative rank order of these two higher-risk groups varying by cohort.

Incremental prognostic value of early trajectory beyond the conventional baseline phenotype model

We next examined whether early trajectory improved prognostic assessment beyond a conventional baseline phenotype framework (Table 6; Figure 6). In the hierarchical nested-model analysis for 28-day mortality, Model 1 included baseline clinical severity variables alone, Model 2 represented the conventional baseline phenotype model by adding baseline inflammatory phenotype, and Model 3 further incorporated early 72-hour trajectory class.

Relative to baseline clinical severity alone, addition of baseline inflammatory phenotype improved prognostic discrimination, with the C-statistic increasing from 0.71 (95% CI, 0.67–0.75) to 0.78 (95% CI, 0.74–0.82), together with improved calibration and reclassification (Table 6). Importantly, addition of early trajectory to this conventional baseline phenotype model further increased the C-statistic to 0.84 (95% CI, 0.81–0.88), with a ΔC-statistic of +0.06 versus Model 2 (likelihood ratio test $P < 0.001$). Calibration slope improved from 0.98 in Model 2 to 1.01 in Model 3, and the Brier score decreased from 0.161 to 0.138.

Reclassification analyses showed a similar pattern. Compared with Model 1, the conventional baseline phenotype model (Model 2) yielded a continuous net reclassification improvement (NRI) of 0.24 and an integrated discrimination improvement (IDI) of 0.041. Addition of early trajectory beyond the baseline phenotype model (Model 3 vs Model 2) yielded a further NRI of 0.29 and IDI of 0.056 (all $P < 0.001$). Taken together, these findings indicate that baseline inflammatory phenotype provided meaningful prognostic information, but early trajectory captured additional risk heterogeneity not explained by baseline phenotype alone.

Figure 6 illustrates this hierarchical comparison visually, showing progressive improvement in discrimination and calibration from baseline severity alone, to the conventional baseline phenotype model, and finally to the combined phenotype-plus-trajectory model.

Sensitivity analyses

Prespecified sensitivity analyses were broadly consistent with the primary findings (Supplementary Table S7; Supplementary Figure S5). Relative to intermediate resolvers, rapid improvers remained associated with lower 28-day mortality across complete-case analysis (adjusted OR 0.61, 95% CI 0.37–1.00), after multiple imputation (0.56, 95% CI 0.35–0.88), after excluding transferred patients (0.60, 95% CI 0.37–0.97), among invasively ventilated patients (0.54, 95% CI 0.33–0.89), and among patients with Berlin moderate-to-severe ARDS (0.52, 95% CI 0.31–0.86). Refractory trajectory remained associated with higher 28-day mortality across the same analyses, with adjusted ORs ranging from 2.29 to 2.71 (all $P < 0.001$).

Development of the Web-based Calculator

To translate our findings into a clinically actionable tool, we developed a web-based dynamic phenotype-plus-trajectory calculator using HTML and JavaScript. The baseline inflammatory phenotype is determined based on the prespecified parsimonious biomarker framework. The 72-hour clinical trajectory is assigned using a nearest centroid classification algorithm derived from the Ruijin derivation cohort's Day 1 and Day 3 PaO_2/FiO_2 coordinates. The tool provides real-time prognostic assessment based on the hierarchical nested-model results. The calculator is freely accessible at [https://mcp.edgeone.site/share/o7M7zZIOXO0B9bTKMni8i].

Supplementary comparative model analysis

In an additional comparative analysis evaluating alternative prognostic strategies (Supplementary Table S8), the combined phenotype-plus-trajectory model showed the best overall performance. A model including baseline severity plus trajectory class also showed favorable prognostic performance relative to the model including baseline severity plus inflammatory phenotype alone, whereas the model incorporating both inflammatory phenotype and trajectory performed best overall. These findings suggest that early trajectory carries prognostic information complementary to conventional baseline inflammatory phenotyping.

Discussion

These findings extend prior work on ARDS subphenotypes. Hyperinflammatory and hypoinflammatory phenotypes have consistently been associated with differences in biomarker profiles, severity of illness, and mortality, and they remain the most biologically interpretable stratification framework in the field.5–10 However, most previous studies have treated phenotype as a fixed baseline attribute. Our results suggest that this static view is incomplete. Patients with the same initial phenotype may follow substantially different early courses, and some patients with initially higher-inflammatory status may transition toward a more favorable state. In this sense, phenotype may define the biological starting point, whereas early trajectory determines how that biology is expressed clinically.

Our findings also complement recent longitudinal work showing that inflammatory phenotypes in ARDS may be temporally unstable. Pensier and colleagues reported substantial phenotype transition over time and demonstrated that such temporal evolution may influence corticosteroid responsiveness.11 Rather than focusing primarily on treatment interaction, our study addresses a distinct but related question: whether early 72-hour clinical trajectories help explain the clinical meaning of baseline inflammatory phenotype and provide additional prognostic information beyond baseline phenotyping alone. Together, these studies support the view that ARDS heterogeneity cannot be adequately characterized by a single baseline label.

A key message of the present study is therefore not that baseline inflammatory phenotyping should be replaced, but that it should be longitudinally contextualized. In our hierarchical model comparisons, the conventional baseline phenotype model clearly improved prognostic performance relative to baseline severity alone, confirming that inflammatory phenotype remains clinically informative. However, the additional gain observed after incorporation of early trajectory indicates that baseline phenotype alone remains insufficient to capture the full extent of clinically relevant early heterogeneity. Trajectory and phenotype should therefore be understood as complementary rather than competing dimensions of ARDS stratification.

Our study was designed to address three related questions. First, do different inflammatory phenotypes correspond to different early trajectories, and are those trajectories associated with outcome? The answer appears to be yes, but only partially. Compared with the lower-inflammatory phenotype, the higher-inflammatory phenotype was associated with lower odds of a rapid improver trajectory and higher odds of a refractory trajectory, although the relationship was not deterministic. Second, what are the direction and speed of those changes? We found that transition toward a lower-inflammatory state and more rapid physiologic improvement were associated with more favorable recovery, whereas persistent higher-inflammatory status was associated with worsening oxygenation, worsening organ dysfunction, and higher mortality. Third, does trajectory provide prognostic

information beyond phenotype alone? The addition of trajectory to baseline phenotype improved discrimination, calibration, and reclassification, indicating that early trajectory captured clinically meaningful heterogeneity not fully available from baseline classification alone.

Our findings also align with recent efforts to frame ARDS recovery more explicitly. The Delphi consensus definition proposed by Weir and colleagues emphasizes both oxygenation recovery and de-escalation of respiratory support [14]. The accompanying editorial argued that identifying when ARDS resolves is essential if the field is to move beyond onset-based classification alone [15]. Our data provide empirical support for that perspective. The rapid improver trajectory reflected early movement toward physiologic recovery, whereas the refractory trajectory remained biologically and clinically distant from resolution. Early trajectory analysis may therefore provide a practical bridge between baseline phenotype classification and clinically meaningful recovery states.

An important implication is that the future of phenotype-based stratification may lie not only in identifying who is high risk at baseline, but also in identifying who has the capacity to improve, and how quickly. Persistent higher-inflammatory status was associated with refractory trajectory and poor outcome, whereas patients showing transition toward a lower-inflammatory state and faster early improvement were more likely to recover favorably. This supports the concept that phenotype plasticity and the speed of early change may be as important as baseline phenotype itself.

The clinical outcome analyses reinforce this interpretation. Across cohorts, rapid improvers consistently showed the most favorable short-term outcomes. In contrast, the adverse outcome burden was concentrated in the intermediate and refractory trajectory groups, with some variation in the relative rank order of these two higher-risk groups across cohorts. This pattern suggests that baseline phenotype alone is insufficient to distinguish patients who will rapidly recover from those who will remain clinically unstable or deteriorate during the first several days of illness.

This complementary interpretation is reinforced by the comparative prognostic analyses. When we explicitly framed the nested models as a comparison between baseline severity alone, a conventional baseline phenotype model, and a phenotype-plus-trajectory model, each step yielded meaningful improvement in discrimination, calibration, and reclassification. Notably, the gain from adding early trajectory on top of baseline phenotype was not trivial, suggesting that longitudinal characterization captures prognostic information not reducible to the initial inflammatory state. If confirmed in future studies, this framework may be useful not only for risk stratification but also for cohort enrichment and adaptive treatment assignment in ARDS trials.

This study has several strengths. First, the three-center design—incorporating a derivation cohort, a retrospective external validation cohort, and an independent prospective external validation cohort—substantially strengthens the reproducibility and transportability of our findings. Second, the prespecified analytic framework followed a coherent sequence: from baseline phenotyping to early

clinical trajectory, transition dynamics, and incremental prognostic value. Third, our trajectory framework is intentionally parsimonious and clinically interpretable, avoiding the opacity that often limits the usefulness of more complex analytic approaches. Crucially, we leveraged this interpretability to translate our dynamic phenotype-plus-trajectory framework into an open-access, web-based prediction tool. While previous subphenotype classifications often rely on complex latent class analyses or machine learning models that are difficult to implement in real time, our tool requires only a parsimonious biomarker panel and serial PaO_2/FiO_2 measurements. By inputting these parameters, clinicians can instantly identify whether a patient is experiencing favorable phenotype plasticity (e.g., transition from a higher- to lower-inflammatory state) or alarming clinical deterioration (e.g., persistent higher-inflammatory status), thereby facilitating personalized monitoring and timely intervention at the bedside.

Several limitations should be acknowledged. First, the retrospective components of the study remain susceptible to unmeasured confounding and center-level practice variation. Second, inflammatory phenotype analyses were limited to cohorts with sufficient biomarker data, which may affect generalizability. Third, the trajectory framework prioritized clinical interpretability and reproducibility rather than maximal physiologic detail; future work may refine trajectory classification by incorporating dead-space indices, ventilatory ratio, respiratory mechanics, or imaging-derived markers [15,20,21]. Fourth, because clinically recognized ARDS was identified pragmatically, some misclassification remains possible. However, this approach was chosen deliberately to reflect real-world clinician-recognized ARDS while avoiding overreliance on incomplete retrospective reconstruction of all diagnostic elements across multicenter datasets [1,2,17]. Finally, this study is observational and prognostic; it does not imply that modifying phenotype or trajectory would itself improve outcomes.

In summary, our findings support a dynamic framework for ARDS stratification in which baseline inflammatory phenotype and early clinical trajectory are complementary rather than competing constructs. Baseline phenotype identifies an important biological starting point, but early 72-hour trajectory captures how that biology evolves and is translated into clinically observable recovery or nonrecovery. A conventional baseline phenotype framework therefore appears necessary but incomplete. Integrating phenotype with trajectory may provide a more informative approach to understanding dynamic heterogeneity in ARDS and may offer a stronger foundation for future prognostic enrichment and precision-trial design.

Conclusions

In ARDS, baseline inflammatory phenotype is associated with early clinical trajectory but does not fully determine it. Early 72-hour trajectory, including the direction and speed of change, provides complementary dynamic information and improves prognostic stratification beyond baseline

phenotype alone. These findings support a shift from static baseline phenotyping toward a dynamic phenotype-plus-trajectory framework for understanding heterogeneity in ARDS.

Figure Legends

Figure 1. Study design and the dynamic phenotype-plus-trajectory framework.

(A) Multicenter study design incorporating a derivation cohort, a retrospective validation cohort, and a prospective validation cohort (Total n=995). (B) The core analytic framework illustrating the evolution from baseline biological starting points (hyper- vs. lower-inflammatory phenotypes defined by parsimonious biomarkers) to 72-hour clinical expression (rapid, intermediate, and refractory oxygenation trajectories). (C) Translation of the conceptual framework into an open-access, web-based prediction tool, enabling real-time integration of baseline phenotype and early trajectory for bedside prognostic assessment.

Figure 2. Early 72-hour oxygenation trajectories in derivation and validation cohorts.

Longitudinal PaO_2/FiO_2 trajectories from day 1 to day 3 according to assigned trajectory class. Rapid improvers showed marked early improvement in oxygenation, intermediate resolvers showed slower and more limited recovery, and refractory patients showed persistently poor or worsening oxygenation. Lines represent mean PaO_2/FiO_2 values and shaded bands indicate the standard error of the mean. Panel A shows the Ruijin derivation cohort, Panel B the Fujian retrospective validation cohort, and Panel C the Fuyang prospective validation cohort.

Figure 3. Association between baseline inflammatory phenotype and early trajectory class.

(A) Distribution of lower-inflammatory and higher-inflammatory baseline phenotypes across rapid improver, intermediate resolver, and refractory trajectory classes.

(B) Within-trajectory phenotype composition expressed as percentages. Higher-inflammatory phenotypes were enriched in refractory trajectories, whereas lower-inflammatory phenotypes were enriched in rapid improver trajectories. Substantial within-phenotype heterogeneity remained across trajectory classes.

Figure 4. Early phenotype transition patterns and speed of change.

(A) Change in oxygenation from day 1 to day 3 according to inflammatory transition category.

(B) Change in nonrespiratory organ dysfunction, measured by ΔSOFA from day 1 to day 3, according to inflammatory transition category.

Figure 5. Clinical outcomes across early trajectory classes.

(A) Kaplan–Meier survival curves according to trajectory class. Rapid improvers showed the most favorable short-term survival, whereas intermediate and refractory trajectories were associated with substantially worse outcomes. The relative rank order of the two higher-risk groups varied across cohorts.

(B) Adjusted odds ratios for 28-day mortality across trajectory classes, with intermediate resolvers as the reference group.

Figure 6. Incremental prognostic value of early trajectory beyond the conventional baseline phenotype model.

(A) Receiver operating characteristic curves for hierarchical prognostic models predicting 28-day mortality. Model 1 included baseline clinical severity variables alone. Model 2 represented the conventional baseline phenotype model and added baseline inflammatory phenotype to Model 1. Model 3 further incorporated early 72-hour trajectory class. Discrimination improved progressively across models, indicating that early trajectory provided additional prognostic information beyond baseline phenotype alone.

(B) Calibration plots for the same three models. Predicted probabilities are plotted against observed event rates across risk strata. Calibration improved incrementally from Model 1 to Model 3, supporting the complementary value of combining baseline inflammatory phenotype with early trajectory information.

Abbreviations: ROC, receiver operating characteristic.

Table Titles

Table 1. Baseline characteristics of the derivation and validation cohorts

Table 2. Early oxygenation, organ dysfunction, and support intensity across trajectory classes in the derivation cohort

Table 3. Adjusted association between baseline inflammatory phenotype and early trajectory class

Table 4. Early inflammatory transition patterns and speed of physiologic change

Table 5. Clinical outcomes across early trajectory classes

Table 6. Incremental prognostic performance of early trajectory beyond the conventional baseline phenotype model for 28-day mortality

Supplementary Material Titles

Supplementary Table S1. Missingness and harmonization of candidate variables

Supplementary Table S2. Alternative trajectory specifications and model diagnostics

Supplementary Table S3. Analyses stratified by direct and indirect ARDS

Supplementary Table S4. Biomarker distributions by phenotype and trajectory

Supplementary Table S5. Additional secondary outcomes

Supplementary Table S6. Continuous trajectory analyses

Supplementary Table S7. Sensitivity analyses

Supplementary Table S8. Comparative prognostic performance of conventional baseline phenotype, early trajectory, and combined phenotype-plus-trajectory models for 28-day mortality

Supplementary Figure S1. Detailed eligibility flowchart

Flow diagram showing patient screening, exclusions, and final cohort allocation across the three participating centers. Of 1,248 ICU patients screened, 253 were excluded because they did not meet

prespecified ARDS eligibility criteria, were younger than 18 years, represented duplicate or non-index admissions, lacked core classification variables, or had inadequate early follow-up. The final analytic cohort comprised 995 patients with clinically recognized ARDS, including 150 in the Ruijin derivation cohort, 505 in the Fujian retrospective validation cohort, and 340 in the Fuyang prospective validation cohort. The figure also indicates the biomarker-rich subset used for phenotype derivation and transition analyses and the validation cohorts used for trajectory reproducibility and trajectory–outcome analyses.

Supplementary Figure S2. Trajectory assignment diagnostics

(A) Separation of derived trajectory classes in the Ruijin derivation cohort according to serial PaO_2/FiO_2 values over the first 72 hours. Rapid improvers showed marked early improvement in oxygenation, intermediate resolvers showed minimal change, and refractory patients showed persistently poor or worsening oxygenation.

(B) Mean posterior assignment probability for each trajectory class. High posterior probabilities across all three classes support adequate classification certainty and internal separation of the derived trajectory framework.

Supplementary Figure S3. Biomarker distributions across early trajectory classes in biomarker-rich subsets.

Median baseline concentrations of (A) IL-6, (B) TNFR1, (C) procalcitonin, and (D) lactate according to early trajectory class. Refractory trajectories showed consistently higher biomarker levels than rapid improver trajectories across all four markers.

Supplementary Figure S4. Center-specific validation plots.

(A) Center-specific ICU mortality according to early trajectory class. Mortality increased across trajectory classes in all cohorts, although the relative rank order of intermediate and refractory trajectories differed between centers.

(B) Center-specific change in oxygenation from day 1 to day 3 according to early trajectory class. Rapid improvers showed the greatest improvement in oxygenation, whereas refractory trajectories showed persistently poor or worsening oxygenation.

Supplementary Figure S5. Alternative time-window analyses

(A) Discrimination of the phenotype-plus-trajectory prognostic framework across alternative early observation windows (0–48 h, 0–72 h, and 0–96 h) for 28-day mortality. The 0–72-hour window showed the highest discriminative performance.

(B) Continuous net reclassification improvement across the same alternative time windows. Reclassification performance was greatest for the 0–72-hour window, supporting its selection as the primary trajectory assessment interval.

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Author Contributions Statement

R.Z., X.Z., and L.H. conceived and designed the study. R.Z., J.W., R.T., and L.L. performed patient enrollment and preliminary analyses. R.Z.,L.S., L.H., and P.L. drafted the manuscript. Y.L., H.Q., and L.S. critically revised the manuscript for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Funding

The research funded by Shanghai Science and Technology Commission (23Y11900100), Science and Technology Commission of Shanghai Municipality (24ZR1447000) .

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Ethics approval and consent to participate

No animal studies are presented in this manuscript. No potentially identifiable human images or data are presented in this study. The studies involving human participants were reviewed and approved by an independent institutional review board by Ruijin Hospital Ethics Committee(Approval No. 20250518, Second People's Hospital of Fuyang City (Approval No. 20250729099), and by the Fujian Provincial Hospital Ethics Committee.

Consent for publication

In accordance with legislation, written informed consent was obtained from the patient. Patients who were unable to provide consent prior to enrolment due to orotracheal intubation or other medical conditions were informed as soon as conditions permitted.

Competing interests

The authors report no disclosures of relevance to the manuscript.

Acknowledgements

The authors would like to thank Huaiwu He, Songjin Shi, Fang Wang and Yi Chi for their valuable support and assistance in this work.

Supplementary Files

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