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ORIGINAL

Inflammatory subphenotypes in patients at risk of ARDS: evidence from the LIPS-A trial

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Abstract

Purpose: Latent class analysis (LCA) has identified hyper- and non-hyper-inflammatory subphenotypes in patients with acute respiratory distress syndrome (ARDS). It is unknown how early inflammatory subphenotypes can be identified in patients at risk of ARDS. We aimed to test for inflammatory subphenotypes upon presentation to the emergency department.

Methods: LIPS-A was a trial of aspirin to prevent ARDS in at-risk patients presenting to the emergency department. In this secondary analysis, we performed LCA using clinical, blood test, and biomarker variables.

Results: Among 376 (96.4%) patients from the LIPS-A trial, two classes were identified upon presentation to the emergency department (day 0): 72 (19.1%) patients demonstrated characteristics of a hyper-inflammatory and 304 (80.9%) of a non-hyper-inflammatory subphenotype. 15.3% of patients in the hyper- and 8.2% in the non-hyper-inflammatory class developed ARDS ($p = 0.07$). Patients in the hyper-inflammatory class had fewer ventilator-free days (median [interquartile range, IQR] 28[23–28] versus 28[27–28]; $p = 0.010$), longer intensive care unit (3[2–6] versus 0[0–3] days; $p < 0.001$) and hospital (9[6–18] versus 5[3–9] days; $p < 0.001$) length of stay, and higher 1-year mortality (34.7% versus 20%; $p = 0.008$). Subphenotypes were identified on day 1 and 4 in a subgroup with available data ($n = 244$). 77.9% of patients remained in their baseline class throughout day 4. Patients with a hyper-inflammatory subphenotype throughout the study period ($n = 22$) were at higher risk of ARDS (36.4% versus 10.4%; $p = 0.003$).

Conclusion: Hyper- and non-hyper-inflammatory subphenotypes may precede ARDS development, remain identifiable over time, and can be identified upon presentation to the emergency department. A hyper-inflammatory subphenotype predicts worse outcomes.

Keywords: Respiratory distress syndrome, Latent class analysis, Inflammation, Aspirin

Introduction

The acute respiratory distress syndrome (ARDS) remains a life-threatening syndrome, resulting in high morbidity and mortality [1]. In patients with ARDS and in mechanically ventilated critically ill patients, two distinct subphenotypes, presenting hyper- and non-hyper-inflammatory characteristics, have been identified using routine patient blood tests as well as assessments of inflammatory biomarkers, and were shown to be stable over the course of the disease [2–5]. These subphenotypes have been associated with differential outcomes [2–10]. Further, a reanalysis of the HARP-2 trial investigating the effect of statins on ARDS showed that statin treatment was associated with increased survival in patients presenting a hyper-inflammatory subphenotype

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[9]. This suggests that inflammatory subphenotypes may have important therapeutic implications.

It remains unclear whether inflammatory subphenotypes can be identified in patients at risk of ARDS, as early as upon presentation to the emergency department. Such early identification could serve as a predictive or prognostic enrichment strategy for future trials leading to early intervention and individualization of care [11, 12].

We, therefore, hypothesized that inflammatory subphenotypes are present before ARDS development in at-risk patients presenting to the emergency department and remain identifiable over time.

Take-home message

Subphenotypes exhibiting hyper- and non-hyper-inflammatory characteristics can be identified in at-risk patients upon presentation to the emergency department, prior to the development of acute respiratory distress syndrome (ARDS). A hyper-inflammatory subphenotype identified patients with worse clinical outcomes. Subphenotyping of patients at risk could serve as a potential predictive or prognostic enrichment strategy in clinical trials, allowing for individualized prophylactic treatment of ARDS.

Methods

Study design and population of LIPS-A

In this secondary analysis, we analyzed data prospectively collected as part of LIPS-A, a double-blind, placebo-controlled, randomized trial conducted at 16 academic hospitals in the United States (US) between January 2012 and November 2014 [13]. The trial assessed whether early administration of aspirin could prevent ARDS in patients at elevated risk [14]. 390 patients presenting to the emergency department with a Lung Injury Prediction Score [LIPS] ≥ 4 [14] were included. Following inclusion, patients were randomized (1:1) to receive either aspirin or placebo treatment. Patients assigned to the intervention group received an initial loading dose of aspirin (325 mg) within 24 h and subsequent daily doses of 81 mg aspirin up to study day 7, hospital discharge, or death, whichever came first. Patients diagnosed with ARDS upon screening were excluded. Data were collected on day 0 (upon presentation to the emergency department, before treatment initiation), day 1, and day 4. This secondary analysis was approved by the institutional review board at Beth Israel Deaconess Medical Center (protocol number: 2022P001145), and the requirement for informed consent was waived.

Primary analysis

To assess whether inflammatory subphenotypes can be identified before the development of ARDS in at-risk patients presenting to the emergency department, we applied latent class analysis (LCA) [15] to data obtained in the emergency department on day 0. Patient demographics (age, sex, race, and body mass index [BMI]), clinical variables (respiratory rate, systolic blood pressure [SBP], and body temperature), blood tests (creatinine, hemoglobin [Hb], white blood cells [WBC], platelets, and blood glucose), and inflammatory biomarkers (interleukin [IL]-1β, IL-2, IL-4, IL-6, IL-8, IL-10, and tumor necrosis factor α [TNF-α]), as well as angiopoietin-2 (Ang-2), and surfactant protein D (SP-D), were considered as class-defining variables. An overview of class-defining variables considered in this study as well as in comparable studies can be found in electronic supplemental material (ESM), Fig. S1 and further details on LCA are presented in ESM, Sects. S1.1–S1.2. Highly correlated variables (IL-6, IL-8, and IL-10) were excluded from the primary model (ESM, Fig. S2) [16].

Secondary analyses

Identification of subphenotypes over time

We tested for the presence of inflammatory subphenotypes upon reassessments on day 1 and 4 in a subgroup of patients with complete data for inflammatory biomarkers on all reassessments (n = 244) by repeating LCA at the corresponding time point (ESM, Sects. S1.1–S1.2).

Association between subphenotypes and outcomes of the LIPS-A trial

We investigated whether the outcomes assessed in LIPS-A, including the primary outcome (development of ARDS within seven days of presenting to the emergency department), and secondary outcomes (i.e., ventilator-free days on day 28, intensive care unit (ICU) and hospital length of stay, as well as 28-day and 1-year mortality), differed between subphenotypes identified on day 0.

Exploratory analyses

In an exploratory attempt, we tested whether the treatment effect of aspirin was modified by the subphenotype identified on day 0. No differences in outcomes between aspirin and placebo treatment were observed in the original trial. To explore whether subphenotypes over time, as identified through complex LCA models, may be identified in the clinical setting using only a single variable, we used group-based trajectory modeling (GBTM), as previously published (ESM, Sect. S1.3) [8, 17, 18]. We then evaluated whether the trajectories identified through GBTM were associated with outcomes assessed in LIPS-A. To assess overlap of subphenotypes identified using our primary LCA-model with those described in patients with established ARDS, we performed a comparison with

a previously published and validated clinical classifier model [10].

Sensitivity analyses

IL-6 was excluded from LCA models due to high correlation with other parameters (ESM, Sects. S1.1–S1.2 and Fig. S2) [16]. Considering the clinical relevance of IL-6 [19, 20], we conducted a sensitivity analysis, including IL-6 as an additional class-defining variable. Further, in the primary LCA model, we included 18 class-defining variables. To address potential bias due to overfitting, we performed a parsimonious LCA-model using a small number of variables (i.e., TNF-α, IL-1β, Ang-2, IL-2, and creatinine), which were chosen based on their contribution to class-separation in the primary LCA-model as well as their central role in the pathogenesis of ARDS and association with clinical outcomes [2, 19, 21–23].

Statistical analysis

Class-defining variables were selected based on clinical reasoning, published literature [2, 6], and availability from the LIPS-A trial. Variables considered relevant but not available from LIPS-A data are highlighted in Fig. S1 of the ESM. Variables were pre-processed, including log-transformation of non-normally distributed data and subsequent z-transformation, as previously published [16]. No outcomes of the LIPS-A trial were included in LCA models. At each time point (day 0, 1, and 4), we fitted four models, yielding one to four classes, respectively. The appropriate number of classes was determined based on the Bayesian Information Criterion (BIC) [24] and the number of patients assigned to each class. We further assessed class separation through entropy [16, 25]. An exploratory Vuong-Lo-Mendell-Rubin test was performed to provide comprehensive assessment of model selection methods. However, it was not considered for decision on number of classes due to the controversy on its appropriateness to determine the ideal number of classes (ESM, Sect. S1.2) [16, 26]. If multiple models demonstrated similar performance, the lowest number of classes was selected [8]. We used model-generated probabilities to partition the cohort into the identified classes [2]. Details are provided in ESM, Sects. S1.1–S1.2. Primary and secondary analyses were defined a priori. A two-sided p value < 0.05 was deemed statistically significant. Class-differences in patient characteristics and outcomes were assessed using the Pearson's χ^2, Fisher's exact test, or Mann–Whitney *U* test, where appropriate. Interaction of subphenotypes and aspirin treatment was tested using regression models. Analyses were performed in Stata (version MP 16.0, StataCorp LLC, College Station, TX, USA, including *traj* package) [18] and R Statistical Software (version 4.2.0, R Foundation for Statistical Computing, Vienna, Austria).

Results

Study population

376 (96.4%) of 390 patients in the LIPS-A trial were included in this analysis after exclusion of 14 patients with missing data on all inflammatory biomarkers (ESM, Table S1). 189 (50.3%) patients included received aspirin. Patient characteristics at study enrollment are presented in the original publication [13] and summarized in the ESM, Table S2. 36 (9.6%) patients developed ARDS within seven days.

Primary analysis

Using data obtained on day 0, with LCA models yielding one to four classes, the two-class model demonstrated the best fit. Partitioning into two classes resulted in better model fit compared to the one-class model, with the largest improvement in BIC (ESM, Fig. S3), excellent class separation, and adequate class sizes, while more classes did not yield relevant improvements in performance (Table 1). In the two-class model, the average probability for the most likely class was 0.98 in patients assigned to class 1 and 0.92 for class 2. Class 1 consisted of 304 patients (80.9%), while class 2 included 72 patients (19.1%), with both classes demonstrating distinct patterns of biomarkers. Specifically, IL-1β, TNF-α,

Table 1 Model fit of 1-, 2-, 3-, and 4-class model on day 0

Number of classes	Number of patients assigned to each class				Bayesian information criterion	Entropy
	1	2	3	4		
1	376 (100%)	–	–	–	17,705	–
2	304 (80.9%)	72 (19.1%)	–	–	17,313	1
3	281 (74.7%)	85 (22.6%)	10 (2.7%)	–	17,211	0.97
4	188 (50%)	148 (39.4%)	30 (8%)	10 (2.7%)	17,208	0.95

Using data obtained on day 0, four latent class models were fitted, yielding one to four classes, respectively. The Bayesian information criterion (BIC) was used to identify the best model fit. The lower the BIC, the better the model fit. Entropy was used to assess class separation. Entropy ranges from 0 to 1, with higher values indicating better class separation

IL-2, Ang-2, IL-4, body temperature, and creatinine were markedly increased in class 2, while glucose, SP-D, SBP, WBC, platelets, and Hb were decreased, when compared to class 1 (Fig. 1A, Table 2). Based on the between-class distribution of class-defining variables, class 2 exhibited characteristics of a hyper-inflammatory subphenotype, while patients assigned to class 1 were characterized by lower levels of inflammatory biomarkers (non-hyper-inflammatory subphenotype).

Secondary analyses

Subphenotypes over time

Presence of two classes was confirmed on day 1 and 4 after presentation to the emergency department in the subgroup with complete data (n = 244). Two classes yielded relevant improvements in model fit over single-class models, while additional classes did not further improve the models (ESM, Fig. S3 and Tables S3–S5). On both day 1 and 4, the class profiles exhibited qualitatively overlapping patterns of inflammatory biomarkers and laboratory values compared to those observed on day 0 (Fig. 1B and ESM, Tables S6–S8). Class assignment of patients on day 0 remained mostly stable throughout day 1 (89.3%, 218/244) (Fig. 2). From day 1 to 4, 18% (44/244) were assigned to a different class, with 95.5% (42/44) of these class-switches representing changes from the hyper-inflammatory to non-hyper-inflammatory class. This resulted in a hyper-inflammatory class size of 25 patients on day 4 (versus 57 on day 0 and 65 on day 1). 77.9% (190/244) of patients consistently remained in their class throughout the study period. Among patients that developed ARDS with available data on inflammatory biomarkers on all reassessments (n = 31), the hyper-inflammatory class increased by 18.2% from day 0 to day 1 (from 11 to 13), while it decreased by 38.5% (to 8) from day 1 to day 4 (ESM, Table S9).

Association between subphenotypes and outcomes

A higher proportion of patients in the hyper-inflammatory class on day 0 developed ARDS compared to the non-hyper-inflammatory class (15.3% [n = 11/72] versus 8.2% [n = 25/304], respectively), although this did not reach statistical significance (p = 0.07). However, patients consistently assigned to the hyper-inflammatory subphenotype throughout the study period were at increased risk of developing ARDS, when compared to the rest of the cohort (36.4% versus 10.4%, respectively, p = 0.003). Patients presenting with the hyper-inflammatory class on day 0 had fewer ventilator-free days, longer ICU and hospital lengths of stay, as well as higher 1-year mortality (Table 3).

Exploratory analyses

Subphenotypes identified on day 0 did not modify the treatment effect of aspirin (ESM, Table S10).

Trajectories of IL-1β and Ang-2 identified through GBTM most closely mimicked the results of LCA, with temporal patterns similar to the non-hyper- (trajectory A) and hyper-inflammatory (trajectory B) subphenotype (ESM, Fig. S4). The hyper-inflammatory trajectory of IL-1β (trajectory B) was associated with longer ICU (median [interquartile range, IQR] 3 days [0–5] versus 2 days [0–5], p = 0.035), and hospital length of stay (9 days [16–18] versus 7 days [4–10.5], p = 0.004), compared to the non-hyper-inflammatory trajectory (trajectory A). Similarly, patients assigned to the hyper-inflammatory trajectory of Ang-2 (trajectory B) had longer ICU (3 days [0–6] versus 1 day [0–4], p = 0.008), and hospital length of stay (9 days [6–18] versus 7 days [4–10], p = 0.001), compared to the non-hyper-inflammatory trajectory (trajectory A). Further, higher risk of ARDS was observed in patients with the hyper-inflammatory trajectory (trajectory B) of Ang-2 (19.8% versus 7.8%, p = 0.006). IL-1β and Ang-2 levels at baseline were predictive of subphenotypes identified through the primary LCA-model, however adequate class-assignment was more frequent for the non-hyper-inflammatory than for the hyper-inflammatory class (95.7% versus 51.4% for IL-1β, and 94.1% versus 38.9% for Ang-2, ESM Table S11). Trajectories of the other class-defining variables did not overlap with results from LCA and thus, were not considered further as prognostic indicator (ESM, Fig. S5).

There was overall good agreement (area under the receiver operating characteristic (AU-ROC) of 0.92) between the primary LCA-model and a previously used and validated clinical classifier model in patients with ARDS [10]. 95.4% of patients in the non-hyper-inflammatory subgroup and 63.9% of patients in the hyper-inflammatory subgroup were identified through the clinical classifier model (ESM, Table S12).

Sensitivity analyses

IL-6 was highly correlated with TNF-α ($r = 0.67$), IL-8 ($r = 0.66$), IL-10 ($r = 0.62$), and IL-1β ($r = 0.54$) on day 0. When additionally including IL-6 as class-defining variable, the best model fit was achieved with two classes (ESM, Table S13): 296 (78.7%) patients were assigned to the non-hyper-inflammatory and 80 (21.3%) patients to the hyper-inflammatory subphenotype, with an overall class-agreement of 96.8%, when compared to the primary model. In the parsimonious model, 298 (79.3%) patients were assigned to the non-hyper-inflammatory and 78 (20.7%) patients to the hyper-inflammatory subphenotype, with an overall class-agreement of 95.2%, when compared to the primary model (ESM, Table S14).

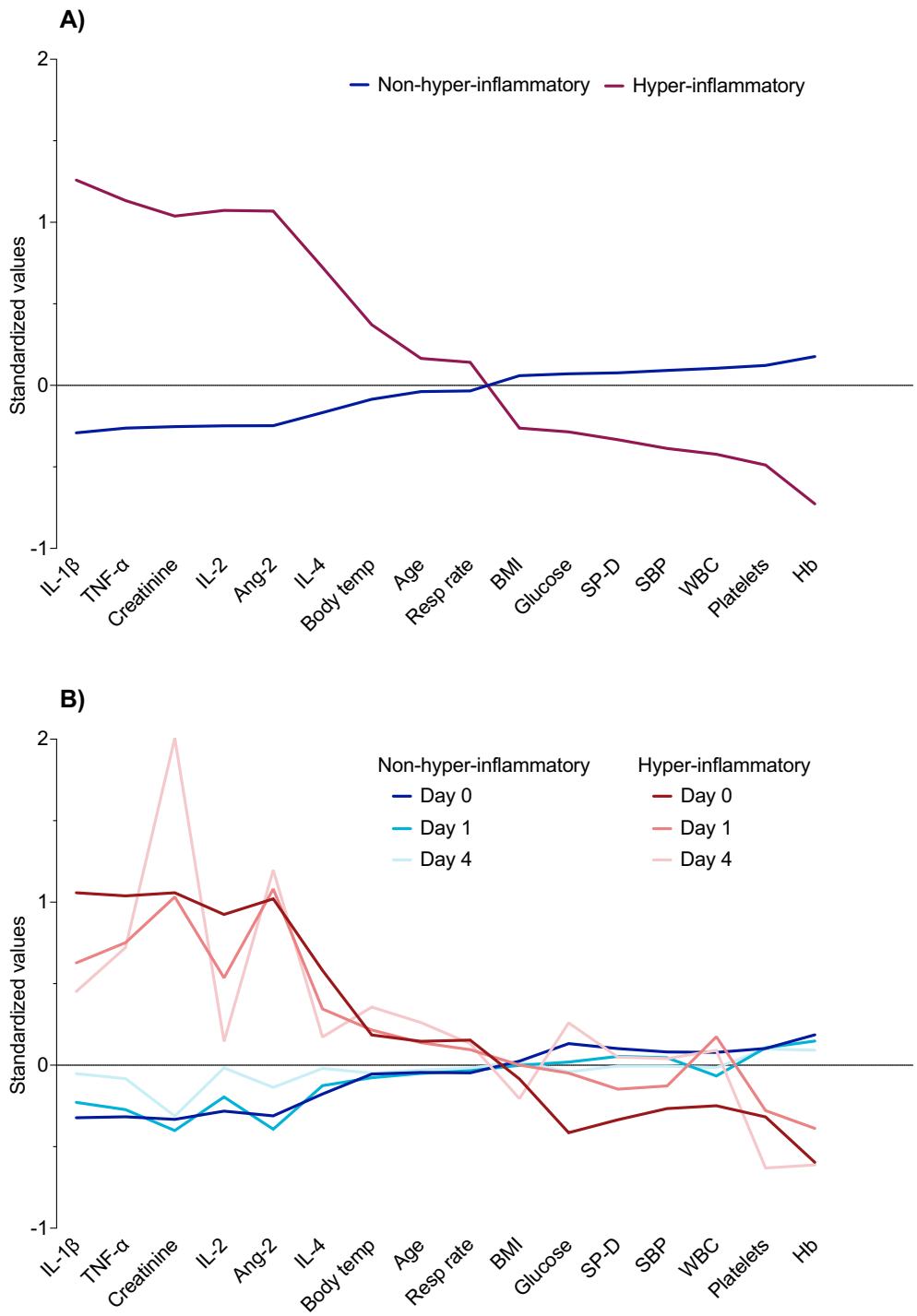

Fig. 1 Subphenotypes identified in patients at risk of ARDS presenting to the emergency department. Continuous class-defining variables are presented as standardized values, with a mean of 0 and a standard deviation of 1. To illustrate the class profiles, individual standardized values of the variables are connected by lines. **A** Identified subphenotypes in the full cohort (n = 376), upon presentation to the emergency department at day 0. The order of variables reflects the degree of class separation between the two classes, with the highest standardized values in the hyper-inflammatory subphenotype on the left. **B** In a subgroup of patients with available data for inflammatory biomarkers on all assessments (n = 244), we repeated LCA on day 0, 1, and 4 to assess whether subphenotypes remained identifiable over time. The variables were arranged based on their level of class separation in the primary analysis of the full cohort on day 0 (as depicted in Panel **A**) to facilitate better comparison. *IL*, Interleukin; *TNF*, Tumor necrosis factor; *Ang*, Angiopoietin; *Temp*, Temperature; *Resp*, Respiratory; *BMI*, Body mass index; *SP*, Surfactant protein; *SBP*, Systolic blood pressure; *WBC*, White blood cells; *Hb*, Hemoglobin

Table 2 Class-defining variables on day 0

	Non-hyper-inflammatory (n = 304)	Hyper-inflammatory (n = 72)	Standardized difference
Age, years	57 (44–68)	57.5 (49–70)	− 0.231
Female	142 (46.7%)	38 (52.8%)	0.121
Race			− 0.301
White	226 (74.3%)	42 (58.3%)	
American Indian	2 (0.7%)	0 (0%)	
Asian	5 (1.6%)	3 (4.2%)	
Black	47 (15.5%)	19 (26.4%)	
None of the above	9 (3%)	2 (2.8%)	
Unknown	15 (4.9%)	6 (8.3%)	
Body mass index, kg/m^2	28 (22.8–34.4)	25.3 (21.7–30)	0.355
Surfactant protein D, pg/mL	58.1 (35.9–99.9)	50.8 (22.6–83.4)	0.153
Angiopoietin-2, pg/mL	5,525 (3,518–10,066)	16,159 (11,662–27,642)	− 1.107
Interleukin-1β, pg/mL	0.1 (0–0.3)	1.1 (0.5–3.6)	− 0.319
Interleukin-2, pg/mL	0.4 (0.2–0.8)	1.9 (1–4)	− 0.531
Interleukin-4, pg/mL	0.01 (0.01–0.04)	0.03 (0.01–0.42)	− 0.383
Tumor necrosis factor-α, pg/mL	3.6 (2.4–5.9)	15.6 (10.5–29.7)	− 0.291
Hemoglobin, mg/dL	12.6 (10.9–14.1)	10.2 (8.4–11.3)	0.992
Creatinine, mg/dL	0.9 (0.7–1.2)	2.2 (1.3–4.8)	− 1.003
White blood cells, × 1000/mm^3	13 (9.3–17.1)	8.6 (6.2–15.9)	0.293
Platelets, × 1000/mm^3	233 (185–321)	175 (132–260)	0.475
Glucose, mg/dL	123 (103–157)	111 (91–134)	0.069
Respiratory rate, bpm	20 (18–26)	22 (18–28)	− 0.159
Systolic blood pressure, mmHg	124 (108–142)	108 (88–136)	0.395
Body temperature, °C	36.9 (36.5–37.6)	37.6 (36.7–38.7)	− 0.422

Data are presented for subphenotypes identified on day 0. Variables are shown as median (interquartile range), or frequency (prevalence in %), along with their corresponding standardized difference. *Abbreviations: bpm*, Breaths per minute; °C, Degrees Celsius

Discussion

In this secondary analysis of the LIPS-A trial, we identified two distinct latent classes demonstrating hyper-inflammatory and non-hyper-inflammatory characteristics. Inflammatory subphenotypes were present in patients at risk of ARDS, could be identified upon admission to the emergency department, and remained identifiable throughout the study period. While nearly 80% of patients remained in the class assigned on day 0, 20% switched classes, reflecting the dynamic stage of disease development [19]. Patients assigned to the hyper-inflammatory subphenotype on day 0 had worse clinical outcomes. Furthermore, patients consistently assigned to the hyper-inflammatory class were at more than three times higher risk of ARDS, when compared to the rest of the cohort.

Inflammatory subphenotypes have been previously described in mechanically ventilated patients with and without ARDS [2–4, 6–10, 27]. In this study, we found evidence for the presence of two distinct inflammatory subphenotypes in a cohort of patients at risk of ARDS at a very early time, upon presentation to the emergency department.

In previous studies, patients presenting a hyper-inflammatory subphenotype were characterized by elevated levels of inflammatory biomarkers as well as organ dysfunction [2–4, 6–10]. Similar patterns were observed in our cohort (Fig. 1). Over time, we observed mostly stable levels of inflammatory biomarkers in the non-hyper-inflammatory subphenotype, while a downward trend in the hyper-inflammatory subphenotype was present (ESM, Figs. S4–S5).

LCA repeated on day 1 and 4 confirmed that subphenotypes demonstrate similar patterns over time and can be identified at different stages of disease development and progression. Most patients (77.9%) remained in the class assigned at baseline. Class-switches were more prevalent between day 1 and 4 (18%), than from day 0 to 1 (10.7%). Notably, 95.5% of patients that switched class from day 1 to 4, improved from the hyper- to the non-hyper-inflammatory subphenotype. Importantly, while we observed a high stability of subphenotypes over time, it remains unclear to what extent subphenotype kinetics

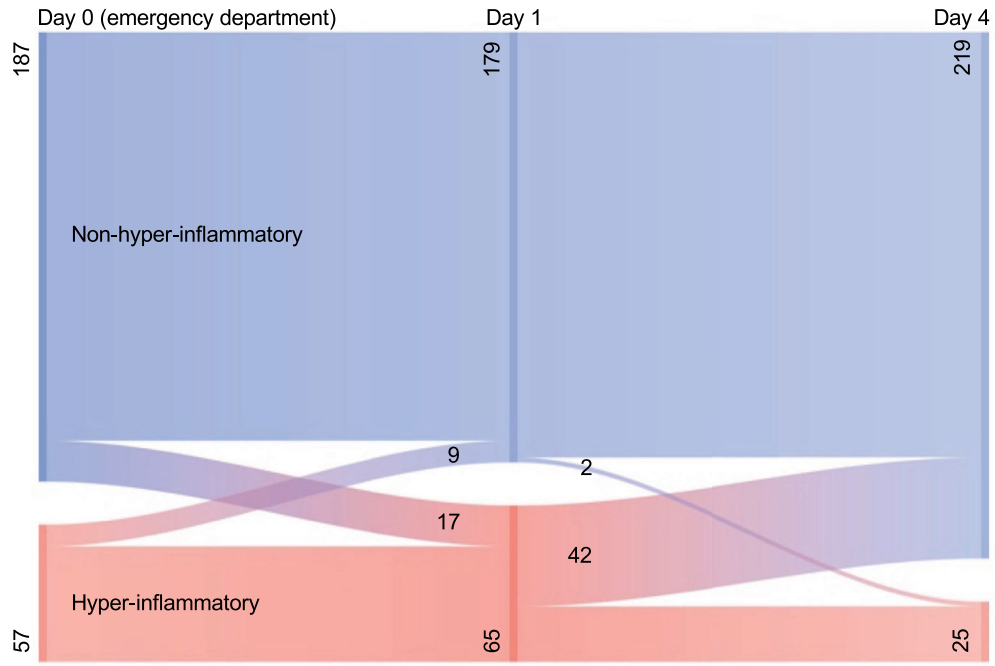

Fig. 2 Identification of subphenotypes over time. Subphenotypes over time were assessed in a subgroup of patients with available data for inflammatory biomarkers on all reassessments (n = 244). The number of patients assigned to the hyper- and non-hyper-inflammatory subphenotype is shown for each respective time point. Class switches are illustrated by connecting lines along with the number of patients that changed class. From day 0 to day 1, 89.3% (218/244) of patients remained in their assigned class, while 18.0% (44/244) switched classes between day 1 and day 4. 95.5% (42/44) of class switches during this period were improvements of patients from the hyper-inflammatory to non-hyper-inflammatory subphenotype. Patients assigned to the hyper-inflammatory subphenotype at all three time points were at increased risk of developing ARDS (36.4% versus 10.4% in the rest of the cohort, $p = 0.003$)

Table 3 Outcomes by subphenotype on day 0

Outcome	Non-hyper-inflammatory (n = 304)	Hyper-inflammatory (n = 72)	*p* value
ARDS	25 (8.2%)	11 (15.3%)	0.07
VFD (days)	28 (27–28)	28 (23–28)	0.010
ICU LOS (days)	0 (0–3)	3 (2–6)	< 0.001
Hospital LOS (days)	5 (3–9)	9 (6–18)	< 0.001
28-day mortality	25 (8.2%)	9 (12.5%)	0.26
1-year mortality	61 (20.1%)	25 (34.7%)	0.008

Comparison of outcomes by subphenotype on day 0. Medians (interquartile range) or frequencies (prevalence in %) are presented with the p value for between-class comparison obtained from the χ^2 Test or Fisher's exact test (binary variables) or Mann–Whitney *U* test (count variables). *Abbreviations: ARDS*, Acute respiratory distress syndrome; *ICU*, Intensive care unit; *VFD*, Ventilator-free days; *LOS*, Length of stay

are influenced by interventions and treatments, or could potentially indicate improvement or deterioration of the clinical course. Patients consistently assigned to the hyper-inflammatory subphenotype had a higher risk of developing ARDS, however, these results must be interpreted with caution due to the small sample size.

In an exploratory intent, we performed GBTM to identify subphenotypes using trajectories of individual variables. Trajectories of IL-1β and Ang-2 closely overlapped with the subphenotypes identified by LCA, and could, thus, be considered for further validation as potential tools for point-of-care identification of subphenotypes. Of note, IL-1β and Ang-2 at baseline were predictive of subphenotypes derived from the primary LCA-model, but resulted in high rates of misclassification for the hyper-inflammatory subphenotype, supporting the use of multivariable models or assessment of single variables over time.

In agreement with previous findings, patients with a hyper-inflammatory subphenotype on day 0 had fewer ventilator-free days, longer ICU and hospital lengths of stay, as well as increased 1-year mortality, when compared to the non-hyper-inflammatory subphenotype [2–4, 6–10]. Moreover, the trajectories of IL-1β and Ang-2 had prognostic value in our cohort, predicting longer ICU and hospital length of stay. Further, Ang-2 was associated with increased risk of ARDS, extending recent evidence highlighting Ang-2 as a potential biomarker of

ARDS development in critically ill patients with sepsis [21].

Secondary analyses of randomized clinical trials in ARDS patients, examining differential responses to treatments across inflammatory subphenotypes, are promising [6, 9], however, we did not find that the effect of aspirin differed between the two identified subphenotypes.

Our findings further corroborate recent findings by Sinha et al. [27], and support the existence of inflammatory subphenotypes in critically ill patients, independently of the presence of ARDS: in a population of patients at risk of ARDS, we observed substantial overlap of subphenotypes identified through our primary LCA-model and those identified in patients with ARDS using a previously published clinical-classifier model [10]. Identification of inflammatory subphenotypes in critically ill patients, based on their biological subphenotype, may facilitate achievement of sufficient outcome incidence in study samples or select a population to investigate targeted treatment.

The present study has limitations. First, this was a secondary analysis of data from a multicentric trial including a variety of locations within the US, thereby representing a large, heterogeneous cohort. Nonetheless, future studies should aim to evaluate our findings in an external cohort, including different geographic locations and settings. Second, we did not find a statistically significant association between the subphenotypes identified on day 0 and the risk of developing ARDS (p value of 0.07), despite large differences in the incidence of ARDS between subphenotypes (15.3% in the hyper-inflammatory versus 8.2% in the non-hyper-inflammatory subphenotype). Considering these findings and the biological plausibility of an inflammatory storm at the onset of ARDS [19], our analysis is likely underpowered due to low overall incidence of ARDS in LIPS-A (37/390, 9.5%). Nonetheless, we studied the largest available cohort of patients enrolled in a randomized control trial investigating the prevention of ARDS. Third, analyses were limited to variables available from the original trial, a common limitation of this type of analysis (ESM, Fig. S1). For example, in our study, serum bicarbonate, a class-defining variable in previous studies of ARDS patients, was not available. Fourth, assessment of subphenotypes over time was restricted to patients with available data on inflammatory biomarkers at all time points, potentially leading to attrition bias.

In conclusion, we identified hyper- and non-hyper-inflammatory subphenotypes in patients at risk of ARDS upon presentation to the emergency department. These subphenotypes remained identifiable over time and a hyper-inflammatory subphenotype was associated with worse clinical outcomes. Future studies on ARDS may benefit from enriching study populations by identifying subphenotypes through methods applicable to the clinical setting.

Supplementary Information
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Author contributions
Study concept and design: SR, DvW, DT, MSS, BJ. Acquisition, analysis, or interpretation of data: SR, DvW, MF, VG. Drafting of the manuscript: SR, DvW, BJ. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: SR, DvW, MF, GC. Study supervision: DT, MSS, BJ.

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Data availability
Requests of qualified researchers trained in human subject research and confidentiality to access additional documents and the dataset may be sent to the corresponding author.

Declarations

Conflicts of interest
MNG has declared funding from the National Heart, Lung, and Blood Institute (NHLBI) and Centers for Disease Control and Prevention. MNG has received consulting fees from Endpoint, honorarium as a visiting professor from Yale University, presentation fees from Washington Health, travel and attendance support for executive board meetings from the American Thoracic Society, and Data Safety Monitoring Board fees. OG has received funding from NHLBI. VG was funded by NIH/NHLBI. DT has declared funding from NHLBI. The other authors declared no competing interests.

Ethics approval
This secondary analysis was approved by the institutional review board at Beth Israel Deaconess Medical Center (protocol number: 2022P001145), and the requirement for informed consent was waived.

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