Elevated Plasma Interleukin-18 Identifies High-Risk Acute Respiratory Distress Syndrome Patients not Distinguished by Prior Latent Class Analyses Using Traditional Inflammatory Cytokines: A Retrospective Analysis of Two Randomized Clinical Trials

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OBJECTIVES: Interleukin-18 (IL-18) plasma level and latent class analysis (LCA) have separately been shown to predict prognosis and treatment response in acute respiratory distress syndrome (ARDS). IL-18 is a measure of inflammasome activation, a pathway potentially distinct from inflammation captured by biomarkers defining previously published LCA classes. We hypothesized that elevated IL-18 would identify distinct "high-risk" patients not captured by prior LCA classifications.

DESIGN: Statins for acutely injured lungs from sepsis (SAILS) and hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in acute lung injury to reduce pulmonary dysfunction trial (HARP-2) are two large randomized, controlled trials in ARDS in which both LCA assignments and IL-18 levels were shown to predict mortality. We first evaluated the overlap between high IL-18 levels (≥ 800 pg/mL) with prior LCA class assignments using McNemar's test and then tested the correlation between IL-18 and LCA biomarkers using Pearson's exact test on log-2 transformed values. Our primary analysis was the association of IL-18 level with 60-day mortality in the hypoinflammatory LCA class, which was assessed using the Fisher exact test and Cox proportional hazards modeling adjusting for age, Acute Physiology and Chronic Health Evaluation score, and gender. Secondary analyses included the association of IL-18 and LCA with mortality within each IL-18/LCA subgroup.

SETTING: Secondary analysis of two multicenter, randomized controlled clinical trials of ARDS patients.

SUBJECTS: Six hundred eighty-three patients in SAILS and 511 patients in HARP-2.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: We found that 33% of patients in SAILS and HARP-2 were discordant by IL-18 level and LCA class. We further found that IL-18 level was only modestly correlated (0.17–0.47) with cytokines used in the LCA assignment. A substantial subset of individuals classified as hypoinflammatory by LCA (14% of SAILS and 43% of HARP-2) were classified as high risk by elevated IL-18. These individuals were at high risk for mortality in both SAILS (42% 60-d mortality, odds ratio [OR] 3.3; 95% CI, 1.8–6.1; $p < 0.001$) and HARP-2 (27% 60-d mortality, OR 2.1; 95% CI, 1.2–3.8; $p = 0.009$).

CONCLUSIONS: Plasma IL-18 level provides important additional prognostic information to LCA subphenotypes defined largely by traditional inflammatory biomarkers in two large ARDS cohorts.

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KEY POINTS

Question: Both latent class analysis (LCA) using traditional inflammatory biomarkers and interleukin-18 (IL-18) (a measure of inflammasome activation) have been shown to subphenotype patients with differential mortality and response to treatments. The overlap between these subphenotypes is unclear. Can IL-18, a marker of inflammasome activation, identify distinct high-risk patients not defined by hypo-inflammatory LCA assignment?

Findings: In this secondary analysis of two large, multicenter, randomized controlled trials in patients with acute respiratory distress syndrome (ARDS), we found that a third of patients were discordant by IL-18 level and LCA classification. Elevated IL-18 identified a subgroup of patients in both statins for acutely injured lungs from sepsis and hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in acute lung injury to reduce pulmonary dysfunction trial within the hypoinflammatory (low-risk) LCA subphenotype who experienced higher mortality.

Meaning: Inflammasome activation, defined by plasma IL-18, may define a high-risk subgroup of patients not identified as hyperinflammatory by LCA. Incorporating measures of inflammasome activation may enhance predictive and prognostic enrichment in ARDS clinical trials.

Most clinical trials in acute respiratory distress syndrome (ARDS) failed to show the efficacy of the studied intervention, which may relate to disease heterogeneity (1). Latent class analysis (LCA) is a statistical methodology that subclassifies patients using baseline characteristics irrespective of outcomes. This methodology has identified two classes of patients in several independent ARDS cohorts: a lower-risk, hypoinflammatory subgroup and a high-risk, hyperinflammatory subgroup with higher mortality and preferential response to therapies (2–4). LCA biomarkers have varied by study but included interleukin-6 (IL-6), IL-8, soluble tumor necrosis factor receptor-1 (sTNFR-1), soluble intercellular adhesion molecule-1 (ICAM-1), plasminogen activator inhibitor-1 (PAI-1), and protein C (2–4).

IL-18 is a cytokine within the IL-1 family that is activated through the inflammasome pathway and has been associated with several disease states (5). The study by Rogers et al (6) showed that a baseline plasma IL-18 level greater than or equal to 800 pg/mL was associated with mortality in sepsis-induced ARDS in the statins for acutely injured lungs from sepsis (SAILS) trial. The study by Boyle et al (7) recently validated this finding in ARDS from all etiologies in the hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in acute lung injury to reduce pulmonary dysfunction (HARP-2) trial and further showed that patients with elevated IL-18 benefited from simvastatin treatment. To date; however, IL-18 has rarely been included in measures of inflammation in ARDS and has not been used as a class-defining variable in the previously published LCA algorithms (3, 4). We hypothesized that markers of inflammasome activation are distinct from inflammation defined by other proinflammatory cytokines used in LCA assignment and that IL-18 level would identify a distinct high-risk subgroup of patients not captured by prior LCA assignment. In this study, we used previously analyzed plasma samples in the HARP-2 and SAILS trials to evaluate whether IL-18 provides additive and/or distinct information to previous LCA subphenotype analyses in these cohorts. Preliminary data from this study were previously presented as an American Thoracic Society abstract in 2021.

MATERIAL AND METHODS

We performed a secondary analysis of the SAILS and HARP-2 multicenter, randomized controlled clinical trials. SAILS evaluated rosuvastatin versus placebo in patients with sepsis-induced ARDS (8), whereas HARP-2 evaluated simvastatin versus placebo in patients with ARDS from any cause (9). LCA assignments and IL-18 measurements were previously performed for patients in SAILS and HARP-2 (3, 4, 6, 7). Biomarkers in SAILS included IL-6, IL-8, ICAM-1, sTNFR-1, PAI-1, protein C, and C-reactive protein. Biomarkers used in HARP-2 included IL-6 and sTNFR-1.

We included patients in SAILS and HARP-2 with complete baseline IL-18 and LCA data. Baseline characteristics were described. In accordance with prior analyses, patients were categorized based on low

(< 800 pg/mL) versus high (≥ 800 pg/mL) IL-18 (6, 7) and hypoinflammatory versus hyperinflammatory LCA class (3, 4). We tested the agreement between high IL-18 and LCA classes using McNemar's test. We evaluated the difference in log-2 transformed IL-18 levels by LCA class using Wilcoxon rank sum test. We assessed 60-day survival using Cox proportional hazards models adjusting for age, sex, and Acute Physiology and Chronic Health Evaluation (APACHE) II score (HARP-2) or APACHE III score (SAILS).

Given our hypothesis that IL-18 reflects a distinct inflammatory pathway, we performed a post hoc analysis to evaluate the relationship between IL-18 and inflammatory markers used in LCA. We calculated Pearson's correlation coefficient of log-2 transformed IL-18 level and each cytokine used in LCA assignment in the SAILS and HARP-2 studies.

All statistical analyses were performed using R Statistical Software v4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Packages used for analysis included survival (v3.5-5), tidyverse (v2.0.0), and survminer (v0.4.9). This study was a post hoc secondary analysis of two prospective studies (NCT00979121 and ISRCTN88244364). Due to this study's de-identified nature, institutional review board (IRB) approval was waived by the Stanford University Administrative Panel for Protection of Human Subjects (Stanford University IRB53611). Procedures were followed in accordance with the ethical standards of the responsible committee and with the Helsinki Declaration of 1975.

RESULTS

Of the 745 patients in SAILS and the 540 patients in HARP-2, 683 (92%) and 511 (95%), respectively, had complete LCA and IL-18 data. Baseline characteristics are outlined in **Table 1**. Despite similar mortality, the HARP-2 cohort had higher illness severity than the SAILS cohort, with a higher percentage of moderate-severe ARDS and vasopressor use. The median value of IL-18 in the SAILS cohort was 554 pg/mL (interquartile range [IQR] 383–763 pg/mL). HARP-2 had higher IL-18 levels with a median value of 845 pg/mL (IQR 485–1,538 pg/mL). IL-18 levels were significantly higher in the hyperinflammatory LCA class relative to the hypoinflammatory LCA class in both cohorts (Wilcoxon rank sum test $p < 0.001$, **Supplemental Fig. 1**, http://links.lww.com/CCM/H412). Clinical characteristics and outcomes by LCA class and IL-18 level are outlined in Table 1. Of the patients in the SAILS cohort, 151 (22%) had elevated IL-18 levels (≥ 800 pg/mL), compared with 265 patients (52%) in the HARP-2 cohort. In SAILS, 255 patients (37%) were classified as hyperinflammatory by LCA compared with 177 patients (35%) in the HARP-2 cohort.

There was significant agreement between IL-18 and LCA classifications. IL-18 and LCA class were concordant (i.e., IL-18 < 800 and hypoinflammatory LCA class or IL-18 ≥ 800 and hyperinflammatory LCA class) in 66% of patients in SAILS (McNemar's $p < 0.001$) and 60% of patients in HARP-2 (McNemar's $p < 0.001$). Despite this overlap, IL-18 was elevated (≥ 800) in 14% and 43% of cases classified as hypoinflammatory by LCA in SAILS and HARP-2, respectively. Importantly, IL-18 was only weakly to moderately correlated with biomarkers used to determine LCA assignment, with a correlation coefficient ranging from 0.17 to 0.47 across all biomarkers (**Supplemental Fig. 2**, http://links.lww.com/CCM/H412).

Regarding our primary hypothesis, patients in the previously described lower-risk hypoinflammatory LCA subgroup with IL-18 levels greater than or equal to 800 pg/mL had a higher 60-day mortality in both SAILS (42% vs 18% mortality; OR 3.3; 95% CI, 1.8–6.1; $p < 0.001$) and HARP-2 (27% vs 15%; OR 2.1; 95% CI, 1.2–3.8; $p = 0.009$). Cox regression confirmed the independent association of IL-18 in the hypoinflammatory subgroup in both SAILS (hazard ratio = 2.78, $p < 0.001$) and HARP-2 (hazard ratio = 1.9, $p = 0.02$) with mortality (**Fig. 1**; and **Supplemental Fig. 3, c** and **d**, http://links.lww.com/CCM/H412). Overall, this suggests that IL-18 identifies high-risk subgroups of patients not captured by the inflammatory markers in prior LCA assignments.

Among those patients classified as hyperinflammatory by prior LCA, patients with elevated IL-18 (≥ 800) experienced higher 60-day mortality (OR 1.8; 95% CI, 1.0–3.1; $p = 0.04$) in SAILS but not in HARP-2 (OR 0.9; 95% CI, 0.48–1.88; $p = 0.88$). Cox regression adjusting for age, sex, and APACHE II (HARP-2) or APACHE III (SAILS) also showed reduced survival in SAILS patients with elevated IL-18 levels ($p = 0.03$; **Supplemental Fig. 3e**, http://links.lww.com/CCM/H412) but not in HARP-2 patients ($p = 0.72$; **Supplemental Fig. 3f**, http://links.lww.com/CCM/H412).

TABLE 1.
Baseline Demographics and Outcomes by Latent Class Analysis and Interleukin-18 Subgroups

	All Patients	Hypoinflammatory LCA/Low IL-18	Hypoinflammatory LCA/High IL-18	Hyperinflammatory LCA/Low IL-18	Hyperinflammatory LCA/High IL-18
Statins for Acutely Injured Lungs from Sepsis Trial					
n (%)	683	369 (54%)	59 (9%)	163 (24%)	92 (13%)
Median age (IQR)	55 (42–65)	54 (42–65)	58 (43–66)	58 (46–68)	55 (39–65)
% Female	51	48	54	51	60
Median APACHE III score (IQR)	92 (73–112)	80 (65–98)	87 (79–97)	110 (91–126)	120 (100–137)
PF < 200	469 (69%)	252 (68%)	39 (66%)	111 (68%)	67 (73%)
Vasopressor use	309 (45%)	108 (29%)	24 (41%)	112 (69%)	65 (71%)
60-d mortality	184 (27%)	**67 (18%)**[a]	**25 (42%)**[a]	**51 (31%)**[a]	**41 (45%)**[a]
Simvastatin in the Acute Respiratory Distress Syndrome Trial					
n (%)	511	189 (37%)	145 (28%)	57 (11%)	120 (23%)
Median age (IQR)	54 (42–66)	49 (40–61)	50 (39–63)	68 (55–75)	59 (47–67)
% Female	43	41	50	44	38
Median APACHE II score (IQR)	18 (14–24)	15 (12–21)	18 (14–23)	22 (17–27)	22 (16–26)
PF < 200	413 (81%)	146 (77%)	117 (81%)	50 (88%)	100 (83%)
Vasopressor use	332 (65%)	99 (52%)	89 (61%)	47 (82%)	97 (81%)
60-d mortality	146 (29%)	**28 (15%)**[a]	**39 (27%)**[a]	26 (46%)	53 (44%)

APACHE = Acute Physiology and Chronic Health Evaluation, IL = interleukin, IQR = interquartile range, LCA = latent class analysis, PF = Pao_2/Fio_2.

[a]Statistically significant difference between high and low IL-18 level within each LCA class.
Fisher exact test ($p < 0.05$).

DISCUSSION

In the HARP-2 and SAILS trials, elevated plasma IL-18 levels previously identified subgroups with higher mortality and with preferential response to simvastatin in HARP-2 but not rosuvastatin in SAILS (6, 7). In this analysis, we showed that IL-18 provided additive prognostic information to prior LCA classifications defined largely by traditional inflammatory biomarkers. In SAILS and HARP-2, 14% and 43% of patients respectively classified to the hypoinflammatory LCA class had IL-18 levels greater than or equal to 800 pg/mL. These patients, who otherwise would have been classified as "lower-risk" by LCA alone, experienced significantly higher mortality in both SAILS and HARP-2, suggesting that IL-18 adds prognostic information to previously described LCA subphenotyping. Correspondingly, we found that plasma IL-18 level correlates only moderately with the inflammatory biomarkers traditionally used in LCA classification. Overall, this suggests that IL-18 may reflect a complementary but distinct inflammatory pathway in ARDS. Finally, because IL-18 is a single biomarker, this makes it potentially applicable in the prognostic and predictive enrichment of future clinical trials.

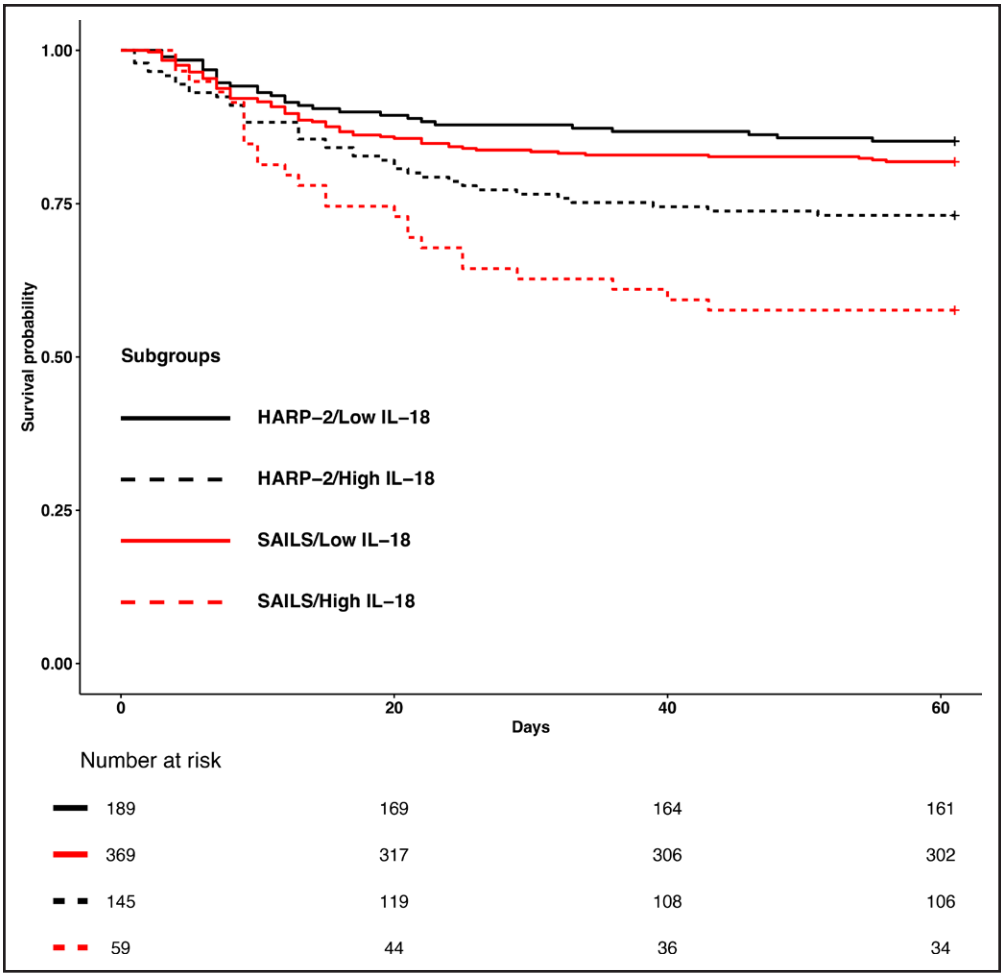

Figure 1. Mortality by interleukin-18 (IL-18) level in patients classified as hypoinflammatory by latent class analysis. Survival analysis shows that elevated IL-18 (*dashed lines*) adds prognostic information in patients classified as hypoinflammatory by latent class analysis in both SAILS (*red*) and HARP-2 (*black*). HARP-2 = hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in acute lung injury to reduce pulmonary dysfunction trial, SAILS = statins for acutely injured lungs from sepsis trial.

This study has several important limitations. This was a post hoc analysis of prior secondary analyses, introducing the risk of type I error. In both trials, data regarding baseline clinical comorbidities were limited and differences in biology by pre-existing conditions could not be assessed. HARP-2 was a pragmatic trial with fewer variables relative to SAILS, and it is unclear how a more complete LCA biomarker and clinical data would affect the interaction between IL-18 and LCA classification in this population. Additionally, IL-18 was quantified using different assays in SAILS and HARP-2. The percentage of patients with elevated IL-18 differed substantially between the 2 cohorts, which could reflect either higher patient acuity in HARP-2, or differences in cohort composition (i.e., SAILS included only sepsis-associated ARDS, whereas HARP-2 included all ARDS etiologies) or differences in IL-18 assays between the two studies. Importantly, the 800 pg/mL cutoff adds to the prognostic value of LCA in both studies despite the different assays, suggesting that these findings are robust to differences in assay and patient characteristics. Further study to determine the ideal IL-18 assay, its correlation with LCA and other biomarkers, and its variation in different patient populations in independent ARDS cohorts may inform the design of future clinical trials.

CONCLUSIONS

These findings suggest that the measurement of IL-18, a marker of inflammasome activation, may identify high-risk ARDS patients not previously captured by prior published LCA subphenotypes defined largely by traditional inflammatory biomarkers. In particular, high IL-18 levels identified a subgroup of patients within the lower-risk hypoinflammatory LCA class with higher 60-day mortality.

The datasets used to generate this article may be made available from the corresponding author upon reasonable request.

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