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Early View

Original research article

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Factor H preserves alternative complement function during ARDS linked to improved survival

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"Take home" summary: Factor H, a key regulator of the alternative complement pathway (AP), associates with restrained AP activation and preserved AP factor levels and function in ARDS with improved survival. Alternative pathway regulation may be a therapeutic target in ARDS.

Abstract (247 of 250 words)

Background: Effective regulation of complement activation may be crucial to preserving complement function during ARDS. Factor H (FH) is the primary negative regulator of the alternative pathway (AP) of complement. We hypothesized that preserved FH levels are associated with decreased complement activation and reduced mortality during ARDS.

Methods: Total AP function was measured by serum hemolytic assay (AH50) using available samples from the ARDSnet LARMA trial (N=218). Factor B (FB) and FH levels were quantified by ELISA using samples from the ARDSnet LARMA and SAILS (N=224) trials. Meta-analyses included previously quantified AH50, FB, and FH values from an observational registry (ALIR). Complement C3, and complement activation products C3a and Ba plasma levels were measured in SAILS.

Results: AH50>median was associated with reduced mortality in meta-analysis of LARMA and ALIR [hazard ratio (HR)=0.66, 95% confidence interval (CI) 0.45-0.96]. In contrast, patients in the lowest AH50 quartile demonstrated relative deficiency of both FB and FH. Relative deficiency of FB [HR 1.99, CI 1.44-2.75], or FH [HR 1.52, CI 1.09-2.11], were associated with increased mortality in meta-analysis of LARMA, SAILS, and ALIR. Relative FH deficiency was associated with increased factor consumption as evidenced by lower FB and C3 levels and higher Ba:FB and C3a:C3 ratios. Higher FH levels associated with lower inflammatory markers.

Conclusions: Relative FH deficiency, higher Ba:FB and C3a:C3 ratios, and lower FB and C3 levels suggest a subset of ARDS with complement factor exhaustion, impaired AP function, and increased mortality that may be amenable to therapeutic targeting.

Keywords: complement Factor H, complement Factor B, complement C3, alternative complement pathway, acute respiratory distress syndrome

Word count: 2,997 words (limit 3,000)

Introduction

The complement system is a major component of the blood proteome[1] with key roles in host immunity including recognition and clearance of pathogens.[2, 3] Complement functions through intricate proteolytic cascades that require tight regulation to prevent exhaustion of complement factors and excessive inflammation.[4] In contrast to the target-based classical and lectin pathways of activation,[3] the alternative complement pathway is unique because it is constitutively active and it can amplify all complement activity, including its own, through a complement component 3 (C3) feedback loop.[5] We have previously demonstrated in a heterogenous single-center prospective observational cohort that preserved alternative complement function is associated with improved survival during acute hypoxemic respiratory failure.[6] However, two key knowledge gaps remain. First, it is unclear whether the association between preserved alternative pathway function and improved survival is generalizable to patients with the acute respiratory distress syndrome (ARDS). Second, the role of alternative pathway regulation in determining alternative pathway function during ARDS remains poorly characterized. Because ARDS is defined by life-threatening biological stress that may cause widespread complement activation, effective complement regulation may be crucial to prevent exhaustion of complement factors and thereby preserve complement function. Factor H is the primary negative regulator of the alternative pathway. Genetic deficiency of Factor H protein or function is marked by exhaustion of complement factors including Factor B and C3 and increased risk of infection and organ impairment.[7, 8] Therefore, we hypothesized that lower blood levels of Factor H are associated with impaired alternative pathway function, lower levels of key alternative complement pathway factors Factor B and C3, and worse clinical outcomes in ARDS patients.

Methods

Patient biospecimens

Biospecimens were obtained from the National Heart, Lung, and Blood Institute Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC).[9] Serum collected at the time of enrollment into the Lisofylline And Respiratory Management of Acute lung injury (LARMA) multi-center randomized clinical trial was provided by BioLINCC.[10] EDTA plasma collected at the time of enrollment into the Statins for Acutely Injured Lungs from Sepsis (SAILS) multi-center randomized clinical trial was provided by BioLINCC.[11] Notably, serum biospecimens were not available from SAILS. Previously quantified data using serum collected at the time of enrollment into the prospective, observational University of Pittsburgh Acute Lung Injury Registry and Biospecimen Repository (ALIR) study were utilized in meta-analyses.[6] Only patients classified as ARDS (N=107) in ALIR were included in analyses. Factor H and Factor B values were only available for a subset of patients with ARDS in ALIR (N=69). Inclusion and exclusion criteria have been described previously for the LARMA[10], SAILS[11], and ALIR[6] patient cohorts and are summarized in Supplemental Table 1. Only those patients for whom biospecimens were available are included herein. Informed patient consent was obtained by the relevant study teams prior to transfer of de-identified biospecimens and/or data.

Clinical characteristics

Patient clinical characteristics associated with de-identified biospecimens were provided by BioLINCC for LARMA and SAILS cohorts and by study investigators for the ALIR cohort. Modified sequential organ failure assessment (mSOFA) scores were calculated in LARMA and

SAILS using the unimputed score for the six components. Vasopressor dosage was not available in LARMA, therefore a modified hemodynamics score was utilized for both SAILS and LARMA cohorts as follows: 0=mean arterial pressure (MAP)>70, 1=MAP<70 not on vasopressors or MAP >70 on vasopressors, and 2=MAP<70 on vasopressors. The modified SOFA in ALIR does not include the neurological component, providing a maximum score of 20.[6] Ventilator-free-days (VFD) and organ-support-free days (OSFD) were calculated to day 28.

Alternative complement pathway function

Alternative complement pathway function was quantified in serum from LARMA using a previously described micro-scaled AH50 protocol.[6] Pooled serum from healthy adults (Complement Tech) was utilized as a reference control. Briefly, serially-diluted subject serum was incubated in a 96-well plate with rabbit erythrocytes in the presence of gelatin-veronal buffer (GVB) with 5 mM magnesium chloride and EGTA. Samples were incubated for 60 minutes at 37°C with re-suspension every 10 minutes. After incubation, plates were transferred immediately to ice, and ice-cold GVB-EDTA was added. The samples were centrifuged at 800 *g* for 3 minutes and the optical density (412 nm) of the supernatant was measured. A simple linear equation of the subject OD412 compared to a 100% lysis control was used to determine the AH50.

Factor B and Ba split-product

Factor B was quantified in plasma and serum using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Abcam ab137973). The Ba split-product was quantified in plasma by ELISA (Quidel #A033).

Factor H

Factor H was quantified in plasma from the SAILS cohort using a sandwich ELISA kit (Abcam ab137975). Factor H was quantified in serum from the LARMA cohort using a custom sandwich ELISA similar to prior studies.[6] A 96-well plate was coated with 1 µg/mL anti-human Factor H monoclonal antibody (Biolegend #518402) in PBS overnight at 4°C then blocked with PBS with 3% BSA for 2 hours at 37°C. Varying dilutions of the patient samples or of purified Factor H (0-3 ng/ml; Complement Tech #A137) were added and the plate was then incubated for 1 hour at 37°C. Anti-Factor H polyclonal antibody (#5793) was used as a detection antibody with incubation at 37°C for 1 hour followed by horseradish peroxidase-conjugated IgG. Finally, ABTS substrate and enhancer were added and the absorbance of each well at 405nm was measured. The amount of Factor H in the samples was calculated using a 4-parameter model applied to the standard curve.

Complement C3 and C3a

Quantification of complement C3 and C3a in EDTA plasma from the SAILS cohort was performed by Exsera BioLabs (Aurora, CO). C3 levels were quantified by nephelometry and C3a levels were quantified by ELISA (Quidel #A301) per standard operating procedure.

Host Biomarkers

Previously quantified interleukin-8 (IL-8),[12] plasminogen activator inhibitor-1 (PAI-1),[13] soluble tumor necrosis factor receptor-1 (sTNFR-1),[14] and surfactant protein-A (SP-A)[15] in LARMA were obtained from BioLINCC. Select host biomarkers previously quantified in ALIR using a customized Luminex assay were utilized.[6, 16–18]

Statistical analysis and rigor

All assays were performed on de-identified biospecimens by personnel blinded to patient clinical data. The associations of AH50, Factor B, and Factor H with survival were performed with Cox regression analysis after adjustment for age, gender, and mSOFA. Meta-analyses were performed using fixed effects inverse variance models.[19] Kruskal-Wallis test with Dunn's test for multiple comparisons was applied to compare serum levels of Factor B and Factor H by AH50 quartile. We applied a mean-centering method to remove batch effect on Factor H levels in the LARMA cohort. Incidence rate ratios for the association between Factor B, Factor H, and VFD or OSFD were calculated by negative binomial regression after adjustment for age, gender, and mSOFA. For plasma Ba concentrations greater than the upper limit of detection (6% of samples), we utilized the Ba distribution in all samples (shape and scale parameters of a gamma distribution) to re-assign an imputed value higher than the upper limit of detection. Correlations between complement factors and biomarkers were assessed using Spearman rank test. All analyses were performed after adjusting for confounders, which were selected among those variables considered plausible confounders.

Results

Patient clinical characteristics

Table 1 describes key clinical characteristics of patients included in the LARMA (N=218), SAILS (N=224), and ALIR (N=107) cohorts. Notably, patients with sepsis comprised a larger portion of the SAILS cohort (n=188, 84%), for which the presence of known or suspected infection was an inclusion criterion, than the more heterogenous LARMA (n=76, 35%) cohort. Median ventilator-free days (VFD) were higher in LARMA (median 19, IQR 0-25) and SAILS (median 20, IQR 0-25) than ALIR (median 6, IQR 0-14). However, 28- and 60-day mortality were similar between LARMA (29% and 35% respectively), SAILS (25% and 29%), and ALIR (30% and 37%) patient cohorts.

Preserved alternative complement pathway function is associated with reduced mortality in LARMA and ALIR cohorts

We quantified alternative complement pathway function in available LARMA serum samples (N=218) with a micro-scaled hemolytic AH50 assay.[6] We noted a broad distribution of AH50 values in LARMA with median 131 U/mL (IQR 102-175), which was comparable to AH50 values in the ALIR cohort (Supplemental figure S1) despite 12 or more additional years of cryopreservation. Median AH50 in pooled reference serum from healthy adults was 187 U/mL (IQR 180-197). Patients with AH50>median in the LARMA cohort showed reduced mortality (hazard ratio, or HR, for 90-day mortality = 0.64 with 95% confidence interval, or CI, = 0.39-1.04) after adjustment for age, gender, and mSOFA (Figure 1A). Adjustment for randomization to tidal volume strategy (i.e., 6-8 vs. 10-12 cc/kg) was tested in LARMA but was discarded as there was no change in results. Given the heterogeneity of the LARMA cohort, we performed a

sub-analysis for patients with sepsis, which strengthened the findings (HR 0.36, CI 0.14-0.89) compared to the entire cohort. Strikingly, the reduction in mortality with AH50>median in the entire LARMA cohort is nearly identical to patients with ARDS in ALIR (N=107; HR for 1-year mortality = 0.69, 95% CI = 0.37-1.26).[6] Meta-analysis of LARMA and ALIR cohorts demonstrated a consistent relationship between AH50>median and reduced mortality (HR = 0.66, 95% CI = 0.45-0.96, I^2 for heterogeneity=0.00%) (Figure 1B), which was replicated using a random effects model for meta-analysis (Supplemental Table 2). We assessed serum levels of Factor B and Factor H in the LARMA cohort stratified by AH50 quartile to better understand the relationship between alternative pathway function and levels of key complement factors.

Diminished alternative complement pathway function is associated with lower levels of Factor B and Factor H

We found significantly decreased levels of both Factor B (Figure 2A) and Factor H (Figure 2B) in the lowest AH50 quartile, which is similar to previously published results in the ALIR cohort.[6] Notably, the distribution of circulating levels of Factor B and Factor H were grossly similar across the LARMA, SAILS, and ALIR cohorts (Supplemental Figure S2; Factor B and Factor H values were only available for 69 patients in ALIR). Functional complement assays such as AH50 are typically applied in clinical settings to assess for complement factor deficiencies.[20, 21] Therefore, we investigated whether relative deficiency of Factor B or Factor H is associated with increased mortality during ARDS.

Relative Factor B deficiency is associated with increased mortality during ARDS

Patients with circulating Factor B levels <25th percentile in LARMA were more likely to die (HR for 90-day mortality = 1.70, 95% CI = 1.02-2.82) after adjustment for age, sex, and mSOFA (Figure 3A). Similarly in SAILS, patients with Factor B<25th percentile were more likely to die (HR for 90-day mortality = 2.61, 95% CI = 1.57-4.35) after adjustment for age, sex, and mSOFA (Figure 3B). Meta-analysis of LARMA, SAILS, and ALIR cohorts demonstrated a strong relationship with Factor B<25th percentile and mortality (overall HR = 1.99, 95% CI = 1.44-2.75, I^2 =0.0%)(Figure 3C). Despite this relationship, we were unable to find an association between Factor B<25th percentile and ventilator free-days in LARMA (adjusted incidence rate ratio, or IRR =1.06, p=0.51) or SAILS (IRR=1.05, p=0.30) cohorts (Table 2). Furthermore, we were unable to find an association between Factor B<25th percentile and organ-support-free-days to day 28 (adjusted IRR=0.79, p=0.15) in the SAILS cohort (Table 2). Given the strong association between relative Factor B deficiency and deficits in alternative pathway function, we hypothesized that alternative pathway activation could lead to consumption of complement factors such as Factor B.

Alternative complement pathway activation is higher in ARDS non-survivors and Factor H levels inversely correlate with both alternative and total complement activation.

EDTA plasma quenches proteolytic complement reactions, which enables measurement of complement split-products such as Ba and C3a, which are produced by proteolysis of Factor B and C3, respectively. We measured Ba, C3a, and C3 in EDTA plasma from patients in the SAILS cohort (N=224). We calculated the Ba:Factor B and C3a:C3 ratios as indices of alternative complement and total complement activation, respectively.[22–25] The Ba:Factor B ratio was significantly higher in non-survivors compared to survivors (Figure 4A). The C3a:C3

ratio was higher in non-survivors compared to survivors but this result did not reach statistical significance. As Factor H is the primary negative regulator of the alternative pathway, we hypothesized that Factor H could restrain complement activation by preventing exhaustion of complement factors. Indeed, we found a weak, inverse relationship between Factor H levels and both the Ba:Factor B (Figure 4B; Spearman Rho -0.28, p,0.01) and C3a:C3 ratios (Figure 4C; Spearman rho -0.21, $p<0.01$). Moreover, restraint of alternative pathway activation by Factor H would preserve key complement factors such as C3. Consistent with this notion, there was a moderate, positive association between C3 and Factor H levels (Figure 4C; Spearman rho 0.62, $p<0.001$). Because Factor H restrains alternative pathway activation that preserves key complement factors such as Factor B and C3, we hypothesized that relative Factor H deficiency would be associated with increased mortality during ARDS.

Relative Factor H deficiency is associated with increased mortality during acute respiratory distress syndrome

Patients with circulating Factor H levels $<25^{th}$ percentile showed a trend toward increased probability of death in both LARMA (HR for 90-day mortality = 1.36, 95% CI 0.81-2.30) and SAILS (HR for 90-day mortality = 1.62, 95% CI 0.97-2.75) (Figures 5A-B). Similar to Factor B, we noted a significantly increased risk of death for Factor H$<25^{th}$ percentile (overall HR = 1.52, 95% CI = 1.09-2.11, I^2 =0.00%) by meta-analysis of LARMA, SAILS, and ALIR cohorts (Figure 5C). Congruent with Factor B, we were unable to find an association between Factor H$<25^{th}$ percentile and VFD in LARMA (adjusted IRR=1.10, p=0.22) or SAILS cohorts (adjusted IRR=1.10, p=0.24), or OSFD to day 28 in SAILS (adjusted IRR=0.93, p=0.62) (Table 2). Because over-exuberant complement activation can lead to both exhaustion of complement

factors and unrestrained inflammation, we investigated the relationship between circulating Factor H levels and previously quantified biomarkers of host inflammatory response in the LARMA and ALIR cohorts.

Lower circulating Factor H levels are associated with higher circulating levels of inflammatory biomarkers

We noted weak, inverse correlations of Factor H, as well as Factor B and AH50, with circulating levels of PAI-1, IL-8, and soluble tumor necrosis factor receptor-1 (sTNFR1) in LARMA (Figure 6A). In ALIR, we found weak, inverse correlations between Factor H and circulating levels of angiopoietin-2, interleukin-8, ST2, soluble receptor for advanced glycation end-products (sRAGE), sTNFR-1, interleukin-10, fractalkine, and procalcitonin (Figure 6B).

Discussion

In two independent cohorts of patients with ARDS, we demonstrate that preserved circulating alternative complement pathway function is associated with reduced mortality. We note that impaired alternative pathway function is associated with lower levels of both Factor B and Factor H. Furthermore, relative deficiency of Factor B is associated with increased mortality across three cohorts. Deficiency of Factor H, the primary negative regulator of the alternative pathway, can lead to over-consumption and exhaustion of key complement factors such as Factor B and C3[7, 8]. Consistent with its regulatory role, we show that a relative deficiency of Factor H was weakly associated with both increased alternative pathway and total complement activation as measured by the Ba:Factor B and C3a:C3 ratios. Furthermore, Factor H levels were strongly associated with circulating levels of the central complement component, C3, and relative deficiency of Factor H was associated with increased mortality. Furthermore, we find a weak inverse relationship between Factor H levels and biomarkers of host inflammatory response. Taken together, these data suggest a key role for Factor H in restraint of over-exuberant complement activation, which preserves C3 and Factor B to sustain alternative complement function, limit inflammation, and protect the host during ARDS.

Preserved alternative pathway function is protective in pre-clinical models of disseminated pneumonia[6] and is associated with improved survival across multiple human research cohorts. Because the alternative pathway is spontaneously and constitutively active and able to amplify all complement activity including its own, effective regulation of alternative pathway activity is crucial to limit exhaustion of complement factors and restrain excessive inflammation. Building upon this understanding of complement biology to highlight potential pathophysiology during ARDS, we propose that relative deficiency of Factor H leads to

inadequate alternative pathway regulation and exhaustion of the key complement factor C3, yielding a functional disarray or “complementopathy" of the alternative pathway.[26] Key findings in support of this model include the positive association between Factor H levels and AH50, the inverse association between Factor H levels and complement activation, and the positive association between FH levels and levels of C3. Conceptually, complementopathy during ARDS, which is most commonly caused by overwhelming infections such as pneumonia,[27, 28] may be analogous to exhaustion of coagulation factors that can occur during the massive tissue injury of polytrauma.[26, 29] Coagulopathy during polytrauma can increase hemorrhage whereas the potential consequences of complementopathy during ARDS may be wide-ranging with implications for susceptibility to infection, host immune function, and lung epithelial health.[6, 30–33] Therefore, we propose that adequate regulation of the alternative pathway by Factor H is crucial to prevent exhaustion of complement factors such as C3 and Factor B and preserve complement function during ARDS. One limitation of our study is that only baseline samples were available from the LARMA and SAILS cohorts, so the kinetics of alternative pathway function and Factor H levels during ARDS remain unclear. Furthermore, we are unable to study the potential role of cell-membrane-bound regulators that may also influence complement function.

In conclusion, we demonstrate that relative deficiency of Factor H is associated with increased complement activation and consumption of Factor B and C3, impaired alternative complement function likely due to complement factor exhaustion, and increased risk of mortality during ARDS. We interpret these data to suggest that Factor H restraint of complement activation prevents complementopathy and preserves alternative pathway function to promote survival during ARDS. We speculate that alternative complement function may be a targetable

biological pathway and we propose that a subset of ARDS patients with lower Factor H levels, increased Ba:Factor B and C3a:C3 ratios, and lower Factor B and C3 levels may be a rational target for future therapeutic investigation. Further work is necessary to investigate whether Factor H may be modulated for therapeutic benefit during ARDS.

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Author contributions: W.B. conceived, designed, analyzed, and interpreted the data, performed the experiments, and wrote the manuscript. M.T. and X.A. performed the experiments, analyzed and interpreted the data, and revised the work for important intellectual content. S.R.M., G.D.K., P.R., A.R., R. K.M., and V.P.F. designed and interpreted the data and revised the work for important intellectual content. J.S.L. conceived, designed, analyzed, interpreted the data, and wrote the manuscript. S.M.N. provided critical statistical expertise and conceived, designed, analyzed, interpreted the data, and wrote the manuscript.

Tables

	LARMA N = 218	**SAILS N = 224**	**ALIR N = 107**
Age, median (IQR)	49 (37-66)	57 (43-64)	56 (44-65)
Male, n (%)	135 (62)	107 (48)	57 (53)
Self-reported race, n (%)			
White	168 (77)	171 (76)	103 (96)
Black	36 (17)	38 (17)	4 (4)
Other	14 (6)	11 (5)	0
Unknown	0	4 (2)	0
Medical history			
AIDS, n (%)	13 (6.0)	7 (3.1)	--
Immune suppression, n (%)	26 (11.9)	41 (18.3)	19 (17.8)
Cirrhosis	4 (1.8)	16 (7.2)	10 (9.3)
Leukemia, n (%)	2 (0.9)	16 (7.1)	--
Solid tumor malignancy, n (%)	3 (1.4)	10 (4.5)	4 (3.7)
Baseline severity of illness			
Sepsis, n (%)	76 (35)	188 (84)	93 (87)
mSOFA score, median (IQR)*	8 (6-10)	9 (7-11)	8 (5-9)
Outcomes			
VFD, median (IQR)	19 (0-25)	20 (0-25)	6 (0-14)
28-day mortality, n (%)	61 (29)	57 (25)	32 (30)
60-day mortality, n (%)	69 (35)	66 (29)	40 (37)

Table 1. Clinical characteristics and outcomes of LARMA, SAILS, and ALIR cohorts.
Abbreviations: AIDS = acquired immune-deficiency syndrome; ALIR = University of Pittsburgh Acute Lung Injury Registry and Biospecimen Repository cohort; ARDS = acute respiratory distress syndrome; IQR = inter-quartile range; LARMA = Lisofylline And Respiratory Management of Acute lung injury clinical trial cohort; mSOFA= modified sequential organ failure assessment score; SAILS = Statins for Acutely Injured Lungs from Sepsis clinical trial cohort; VFD = ventilator free days.
* Modified SOFA (mSOFA) in LARMA and SAILS reflects the unimputed score for the 6 components comprising the SOFA score. There was no data on vasopressor dosage in the LARMA cohort, therefore a modified hemodynamics score (0= MAP>70, 1= MAP<70 on no vasopressors or MAP > 70 and on vasopressors, 2= MAP<70 and on vasopressors) was utilized for both LARMA and SAILS cohorts. The mSOFA score in ALIR does not include the neurologic component – therefore, the maximum score is 20.

	LARMA		SAILS	
Ventilator-Free Days	Unadjusted IRR (P value)	Adjusted IRR (P value)	Unadjusted IRR (P value)	Adjusted IRR (P value)
Factor B <25%	1.04 (0.59)	1.06 (0.51)	1.01 (0.80)	1.05 (0.30)
Factor H <25%	1.10 (0.24)	1.10 (0.22)	0.98 (0.68)	1.01 (0.89)
Organ-Support-Free Days	Unadjusted IRR (P value)	Adjusted IRR (P value)	Unadjusted IRR (P value)	Adjusted IRR (P value)
Factor B <25%	N/A	N/A	0.74 (0.06)	0.79 (0.15)
Factor H <25%	N/A	N/A	0.89 (0.45)	0.93 (0.62)

Table 2. Relative deficiency of circulating Factor B or Factor H levels does not associate with ventilator-free days or organ-support-free days. Ventilator-free and organ-support-free days are calculated to day 28. Organ-support-free days are not available for LARMA cohort. Incident rate ratios are displayed both before and after adjustment for age, sex, and mSOFA score.

Abbreviations: IRR = incident rate ratio; LARMA = Lisofylline And Respiratory Management of Acute lung injury clinical trial cohort; mSOFA= modified sequential organ failure assessment score; N/A = not applicable; SAILS = Statins for Acutely Injured Lungs from Sepsis clinical trial cohort.

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Figures

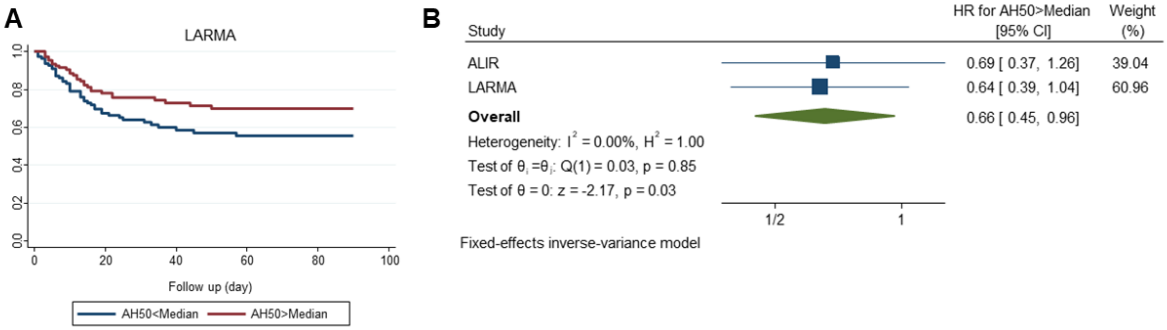

Figure 1. Preserved alternative complement pathway function is associated with decreased mortality during acute respiratory distress syndrome.

(A) Patient survival in LARMA cohort by AH50 stratified by relationship to median (hazard ratio 0.64 [95% confidence interval 0.39-1.04]). (B) Meta-analysis of AH50>median and survival. Survival estimates in both cohorts are adjusted for age, sex, and modified sequential organ failure assessment (mSOFA) score and are presented as hazard ratios (HR) with 95% confidence intervals (95% CI).

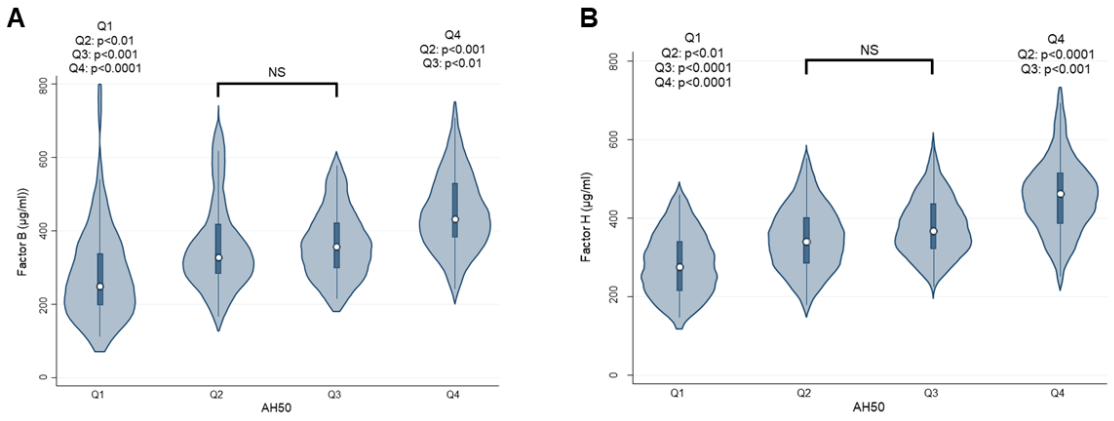

Figure 2. Diminished alternative pathway function is associated with relative deficiency of complement Factor B and Factor H.

Sera from 218 patients in LARMA were characterized for (A) Factor B (µg/mL; Q1 median, inter-quartile range: 249, 197-339, Q4: 432, 382-531) and (B) Factor H (µg/mL; Q1 median, inter-quartile range: 276, 214-341, Q4: 462, 384-515) stratified by quartile of AH50. P values for inter-quartile post-hoc comparisons are displayed in each figure panel (NS=non-significant). Statistical analysis was by Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons.

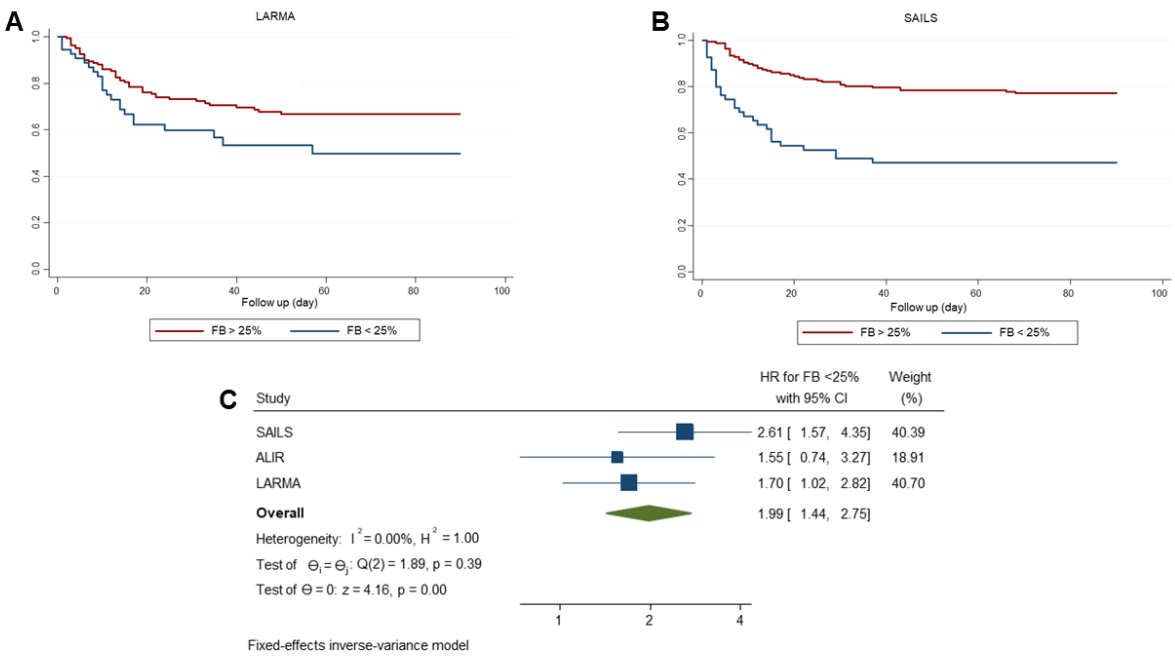

Figure 3. Patients in lowest quartile of circulating Factor B levels demonstrate increased mortality during acute respiratory distress syndrome

(A) Patient survival at 90 days from ICU admission date by Factor B grouped by relationship to 25th percentile (<25th percentile hazard ratio 1.70 [95% confidence interval 1.02-2.82]) in the LARMA cohort. (B) Patient survival at 90 days from ICU admission date by Factor B grouped by relationship to 25th percentile (<25th percentile hazard ratio 2.61 [95% confidence interval 1.57-4.35]) in the SAILS cohort. (C) Meta-analysis of Factor B stratified by relationship to 25th percentile. Survival estimates in all cohorts are adjusted for age, sex, and modified sequential organ failure assessment (mSOFA) score and are presented as hazard ratios (HR) with 95% confidence interval (95% CI).

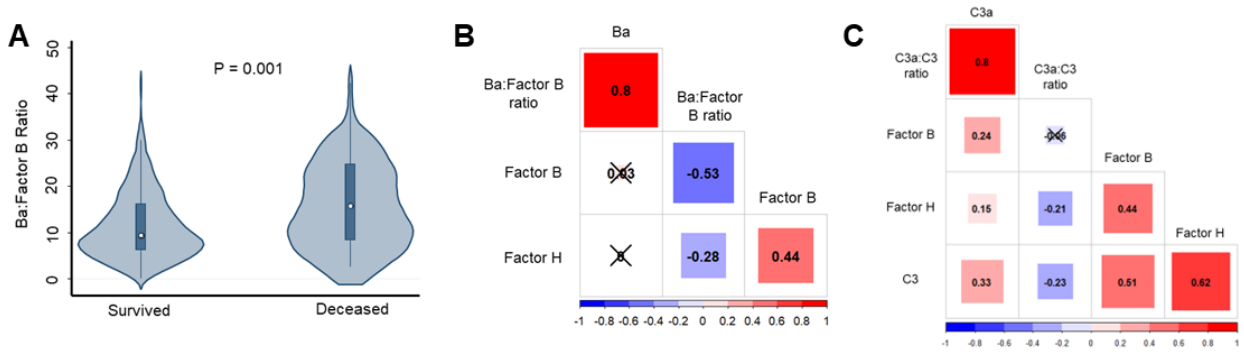

Figure 4. Alternative complement pathway activation is higher in ARDS non-survivors and Factor H levels inversely correlate with both alternative and total complement activation.

(A) Ratio of Factor Ba (Ba) split-product to Factor B levels, a marker of alternative complement pathway activation, is significantly higher in SAILS non-survivors (N=57) compared to survivors (N=167). P value is displayed in the figure. (B) Correlogram of ratio of Ba:Factor B, Ba, Factor B, and Factor H in the SAILS cohort (N=224). (C) Correlogram of ratio of C3a, C3, C3a:C3 ratio (C3aC3), Factor B (FB), and Factor H (FH) in the SAILS cohort (N=224). In both panels B and C, Spearman rho are displayed in each box and p value≥0.05 is marked through with "x." Red boxes indicate positive correlation and blue boxes indicate negative correlation as displayed in figure legend. The size of the box corresponds to magnitude of correlation.

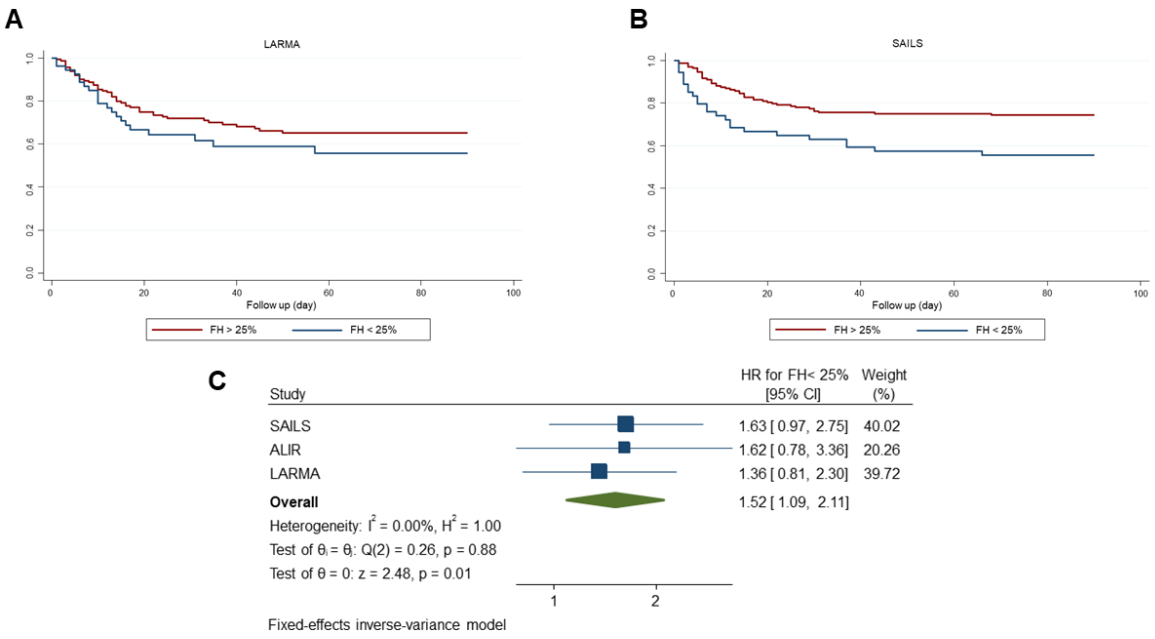

Figure 5. Patients in the lowest quartile of circulating Factor H levels demonstrate increased mortality during acute respiratory distress syndrome

(A) Patient survival at 90 days from ICU admission date by Factor H grouped by relationship to 25th percentile (<25th percentile hazard ratio 1.36 [95% confidence interval 0.81-2.30]) in the LARMA cohort. (B) Patient survival at 90 days from ICU admission date by Factor H grouped by relationship to 25th percentile (<25th percentile hazard ratio 1.63 [95% confidence interval 0.97-2.75]) in the SAILS cohort. (C) Meta-analysis of Factor H stratified by relationship to 25th percentile. Survival estimates in all cohorts are adjusted for age, sex, and modified sequential organ failure assessment (mSOFA) score and are presented as hazard ratios (HR) with 95% confidence interval (95% CI).

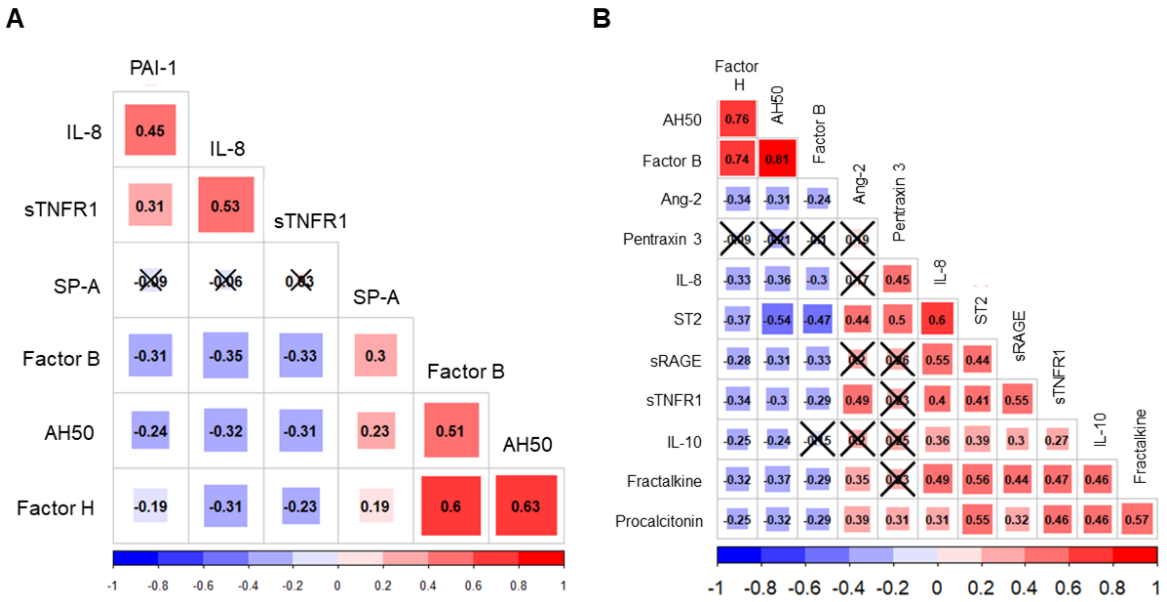

Figure 6. Circulating Factor H levels are associated with preserved complement function and inversely correlated with circulating markers of host inflammatory response during ARDS

(A) Correlogram of plasminogen activator inhibitor-1 (PAI-1), interleukin-8 (IL-8), soluble tumor necrosis factor-1 (sTNFR1), surfactant protein A (SP-A), Factor B, AH50, and Factor H in the LARMA cohort (N=224). (B) Correlogram of AH50, Factor B, Factor H, interleukin-10 (IL-10), interleukin-8 (IL-8), pentraxin 3, soluble suppressor of tumorigenicity-2 (ST2), angiopoietin-2 (Ang-2), procalcitonin, fractalkine, soluble receptor for advanced glycation end products (sRAGE), and soluble tumor necrosis factor receptor-1 (TNFR1) in the ALIR cohort (N=107). Spearman rho are displayed in each box and p values>0.05 are marked through with "x." Red boxes indicate positive correlation and blue boxes indicate negative correlation as displayed in figure legend. The size of the box corresponds to magnitude of correlation.

Factor H preserves alternative complement function during ARDS linked to improved survival

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Supplemental Materials

Supplemental Table 1: Summary of key characteristics of included cohorts

Cohort	Dates of enrollment	Key Inclusion Criteria	Key Exclusion Criteria	Biospecimens
LARMA	February 1998 - June 1999	ARDS[1]	Chronic medical conditions including respiratory failure; estimated 6-month mortality>50%; age<18	Serum
SAILS	March 2010 – September 2013	ARDS[1] with known or suspected infection	Chronic medical conditions ; >5 times ULN of CK/AST/ALT	EDTA Plasma
ALIR	October 2011 - December 2017	ARDS[2,3]; Age 18-90 years	Chronic respiratory failure; tracheostomy; hemoglobin <8 g/dL	Serum

Abbreviations: ALIR = University of Pittsburgh Acute Lung Injury Registry and Biospecimen Repository cohort; ALT = alanine transaminase; ARDS = acute respiratory distress syndrome; AST = aspartate transaminase; CK = creatine kinase; LARMA = Lisofylline And Respiratory Management of Acute lung injury clinical trial cohort; SAILS = Statins for Acutely Injured Lungs from Sepsis clinical trial cohort; ULN = upper limit of normal.

[1]Acute respiratory distress syndrome was defined in the LARMA and SAILS trials by the 1994 American-European Consensus Conference on ARDS as described in "Bernard GR et al. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med*. 1994 Mar;149(3 Pt 1):818-24. doi: 10.1164/ajrccm.149.3.7509706."

[2]Acute respiratory distress syndrome was defined in ALIR by the 2012 Berlin Definition as described in "Acute Respiratory Distress Syndrome: The Berlin Definition**,** JAMA. 2012;307(23):2526-2533. doi:10.1001/jama.2012.5669."

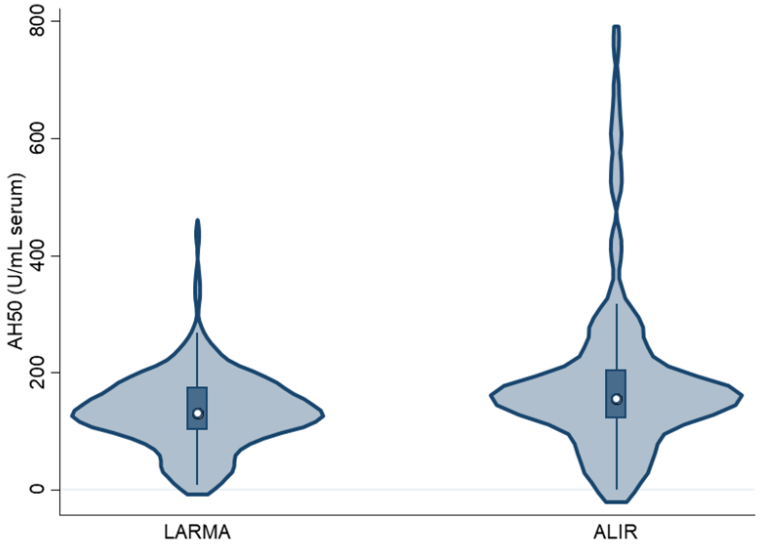
[3]ALIR inclusion criteria are not limited to ARDS. However, the analyses in this paper are limited to those subjectsnin ALIR diagnosed with ARDS, N=107.

Supplemental Table 2: Comparison of meta-analyses using random and fixed effects models

Meta-Analysis	Fixed Effect HR (95% CI)	I^2	Random Effect HR (95% CI)	I^2
AH50>Median	0.65 (0.45-0.96)	0.00%	0.66 (0.45-0.96)	0.00%
Factor B<25% percentile	1.99 (1.44-2.75)	0.00%	1.98 (1.42-2.77)	4.56%
Factor H<25 percentile	1.52 (1.09-2.11)	0.00%	1.52 (1.09-2.11)	0.00%

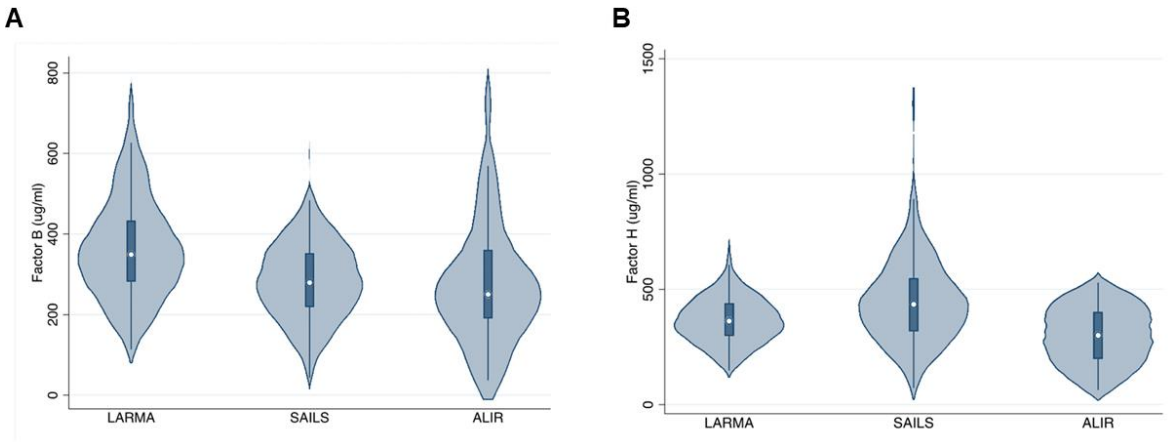
Abbreviations: CI = confidence interval; HR = hazard ratio for mortality; I^2 = heterogeneity statistic

Supplemental Figures



Supplemental Figure S1. Distribution of AH50 values in the ALIR and LARMA cohorts.

In the LARMA cohort (N=218), median AH50 was 131 U/mL with inter-quartile range 102-175 U/mL. In the ALIR cohort (N=107), median AH50 was 155 U/mL with inter-quartile range 123-206 U/mL.



Supplemental Figure S2. Distribution of circulating levels of Factor B and Factor H in LARMA, SAILS, and ALIR cohorts.

(A) Factor B levels in LARMA (N=218, µg/mL; median 349, IQR 282-433), SAILS (N=224, µg/mL; median 280, IQR 219-353), and ALIR (N=69, µg/mL; median 250, IQR 190-361). The median Factor B level in pooled reference serum from healthy adults was 366 µg/mL (IQR 285-378).

(B) Factor H levels in LARMA (N=218, µg/mL; median 363, IQR 298-440), SAILS (N=224, µg/mL; median 435, IQR 318-549), and ALIR (N=69, µg/mL; median 301, IQR 199-402). The median Factor H level in pooled reference serum from healthy adults was 454 µg/mL (IQR 401-482).