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Bronchoalveolar Fluid and Plasma Inflammatory Biomarkers in Contemporary ARDS Patients

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Abstract

Purpose: Bronchoalveolar fluid (BALF) and plasma biomarkers are often endpoints in early phase randomized trials (RCTs) in acute respiratory distress syndrome (ARDS). With ARDS mortality decreasing, we analyzed baseline biomarkers in samples from contemporary ARDS patients participating in a prior RCT and compared these to historical controls.

Materials and Methods: Ninety ARDS adult patients enrolled in the parent trial. BALF and blood were collected at baseline, day 4±1, and day 8±1. Interleukins−8/−6/−1β/−1 receptor antagonist/−10; granulocyte colony stimulating factor; monocyte chemotactic protein-1; tumor necrosis factor-α; surfactant protein-D; vonWillebrand factor; leukotriene B_4; receptor for advanced glycosylation end products; soluble Fas ligand; and neutrophil counts were measured.

Results: Compared to historical measurements, our values were generally substantially lower, despite our participants being similar to historical controls. For example, our BALF IL-8 and plasma IL-6 were notably lower than in a 1999 RCT of low tidal volume ventilation and a 2007 biomarker study, respectively.

Conclusions: Baseline biomarker levels in current ARDS patients are substantially lower than 6–20 years before collection of these samples. These findings, whether from ICU care changes resulting in less inflammation or from variation in assay techniques over time, have important implications for design of future RCTs with biomarkers as endpoints.

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Keywords

Acute respiratory distress syndrome (ARDS); Inflammatory mediators; Respiratory failure; Critical illness; Bronchoalveolar lavage; Plasma

INTRODUCTION

The incidence of the acute respiratory distress syndrome (ARDS) in the US is approximately 200,000 cases annually, and the current case fatality is 30–40%, but has been as high as 60% historically.(Rubenfeld *et al.*, 2005, Stapleton *et al.*, 2005, Maca *et al.*, 2017) Although low tidal volume ventilation and prone positioning improve survival, there are no other known treatments that reduce ARDS mortality, and therefore there is an ongoing need for trials of novel therapeutic agents.(Network, 2000, Guerin *et al.*, 2013)

ARDS pathogenesis is thought to result from massive activation of the proinflammatory response, with release of multiple mediators of inflammation and injury into the alveolar space and the bloodstream.(Ware and Matthay, 2000, Matthay and Zemans, 2011) Increased levels of many of these biomarkers, including interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, are not only present in patients with ARDS but are associated with greater mortality.(Donnelly *et al.*, 1996, Goodman *et al.*, 1996, Ranieri *et al.*, 1999, Ware *et al.*, 2001, Goodman *et al.*, 2003, Bhatia and Moochhala, 2004, Krupa *et al.*, 2004, Parsons *et al.*, 2005, Mukhopadhyay *et al.*, 2006, Allen and Kurdowska, 2014, Aisiku *et al.*, 2016) Furthermore, plasma IL-8 and IL-6 have been found to be reduced in patients receiving lung protective ventilation compared to higher tidal volumes.(Parsons *et al.*, 2005) Therefore, several prior early phase trials of novel therapies in ARDS have utilized bronchoalveolar fluid (BALF) or plasma biomarker measurements as endpoints.(Gadek *et al.*, 1999, Ranieri *et al.*, 1999, Stapleton *et al.*, 2011)

We conducted a phase II randomized controlled trial (RCT) of fish oil versus placebo in patients with ARDS that did not find evidence of efficacy.(Stapleton *et al.*, 2011) This RCT utilized a few selected BALF and plasma biomarkers as endpoints, and power calculations for the trial were performed using historical controls (namely, two 1999 phase II RCTs comparing lung protective versus conventional ventilation and an enteral formula containing fish oil versus a control high fat enteral formula, respectively, where BALF sampling had occurred).(Gadek *et al.*, 1999, Ranieri *et al.*, 1999, Pacht *et al.*, 2003) We found not only that the baseline BALF concentrations of those selected biomarkers in our recent RCT were notably lower than the historical controls receiving lung protective ventilation in the 1999 study,(Ranieri *et al.*, 1999) but also that plasma concentrations of key inflammatory mediators Il-6 and IL-8 were lower than a study measuring plasma cytokines from samples obtained from patients participating in the NIH ARDS Network RCT of low tidal volume versus conventional ventilation.(Parsons *et al.*, 2005) We hypothesized that these decreased levels may be explained by various processes of care leading to reduced lung inflammation in patients with ALI, especially in the era of low tidal volume ventilation.

Because information on BALF and plasma biomarkers may be critical for the design of phase II RCTs in ARDS patients, we therefore measured additional biomarkers of

inflammation and injury in samples obtained during this trial so that these data from a more contemporary cohort of patients with ARDS could be publicly reported and made available to the scientific community for future use. Additionally, we compared our findings to historical controls.

MATERIALS AND METHODS

Participants and Study Design

This study used BALF and plasma samples obtained from 90 patients with ARDS during a phase II RCT of fish oil versus placebo (NCT00351533); a detailed description of that study has been published.(Stapleton *et al.*, 2011) Because that RCT did not find any differences between the intervention and placebo groups, we report measurements here by combining the entire study population. Briefly, adult participants meeting acute lung injury (ALI) criteria as defined by the American-European Consensus Conference (AECC) (Bernard *et al.*, 1994) (ratio of partial pressure of arterial oxygen to fraction of inspired oxygen [PaO_2/FiO_2] ≤300, chest radiograph demonstrating bilateral infiltrates consistent with pulmonary edema, and no evidence of left heart failure causing the infiltrates) were enrolled from 2006–2008 at 5 North American hospitals. Potential participants were excluded if they 1) had met ARDS criteria for ≥ 48 hrs; 2) had an expected ICU length of stay ≤48 hrs or expected survival ≤ 28 days from an underlying pre-ICU condition; 3) were unable to undergo BAL at enrollment (PaO_2/FiO_2 <80 on 100% FiO_2, endotracheal tube too small, marked cardiovascular instability, or intracranial pressure ≥20mmHg); 4) were not able to receive enteral access; 5) were pregnant; 6) were receiving recombinant human activated protein C (rh-APC); 7) had received an enteral formula containing Ω−3 fatty acids during the ICU stay; or 8) had any of the following conditions: metastatic cancer, AIDS with CD4 < 200, post-cardiac arrest with suspected significant anoxic brain injury, past bone marrow or solid organ transplant (lung, heart, liver, pancreas, or kidney), platelets < 30,000/mm^3, active bleeding, International normalized ratio (INR) > 3.0, history of ventricular tachycardia or fibrillation, or known allergy to fish. The research was approved by human subjects committees at all enrolling sites.

After informed consent and within 48 hours of ARDS onset, participants were randomized to receive enteral fish oil or 0.9% saline. Baseline demographic, laboratory, and physiologic data were recorded, and all subjects were ventilated according to local protocols mimicking the ARDS Network low tidal volume ventilation protocol.(Network, 2000)

Procedures

BALF and plasma were obtained at study entry (day 0) and on days 4±1 and 8±1. The 2nd and 3rd BALs did not occur if participants were extubated or were clinically unstable (PaO_2/FiO_2 <80 on 100% FiO_2, hypotensive with systolic blood pressure <90mmHg, ongoing or new uncontrolled dysrhythmia, acute coronary ischemia, or intracranial pressure ≥20mmHg) for bronchoscopy. BAL was performed by passing a fiberoptic bronchoscope through the endotracheal tube. Patients were preoxygenated with 100% oxygen for 15 minutes before and during the procedure. After the bronchoscope was wedged in the right middle lobe or lingula (unless both areas were frankly purulent on inspection in which case

another segment was selected), five separate 30 mL aliquots (total of 150 mL) of normal saline were instilled and recovered by hand-suction in all participants.

Measurements

The following biomarkers were measured in both BALF and plasma: IL-8, IL-6, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-10, granulocyte colony stimulating factor (G-CSF), monocyte chemotactic protein-1 (MCP-1), TNF-α, surfactant protein-D (SP-D), vonWillebrand factor (vWF), and leukotriene B_4 (LTB_4). Neutrophil counts (PMNs) were also performed on BALF samples. BALF IL-8 was the primary endpoint for the parent RCT. Other selected biomarkers were chosen *a priori* as secondary endpoints for the RCT (BALF IL-6, MCP-1, LTB_4, and PMNs; and plasma IL-8, IL-6, SP-D, vWF, and LTB4), and these results have been reported previously in the main RCT manuscript.(Stapleton *et al.*, 2011) The results of other biomarkers (BALF IL-1 β, IL-1ra, IL-10, G-CSF, SP-D, vWF; and TNF-α; and plasma G-CSF, IL-1β, IL-1ra, IL-10, MCP-1, and TNF- α) have not been reported previously.

Each biomarker was measured based on specific biologic rationale. Interleukin (IL)-8 is a neutrophil chemoattractant(Oppenheim *et al.*, 1991, Goodman *et al.*, 2003) and is decreased in the BALF of patients receiving low tidal volume ventilation,(Ranieri *et al.*, 1999) an intervention known to improve ARDS.(Network, 2000) IL-6 activates leukocytes, affects the acute phase response, and levels correlate with severity of ARDS.(Bhatia and Moochhala, 2004) LTB_4 is also a potent neutrophil chemoattractant.(Martin *et al.*, 1989, Peters-Golden and Henderson, 2007) MCP-1 is a monocyte chemoattractant, and increasing levels have been associated with both worse lung injury and persistent inflammation in ARDS. (Goodman *et al.*, 1996, Bautista *et al.*, 2013) SP-D is a biomarker of alveolar epithelial injury and vWF is a marker of endothelial activation and injury; both have been associated with increased mortality in ARDS.(Ware *et al.*, 2001, Eisner *et al.*, 2003, Calfee *et al.*, 2007) G-CSF may play a role in ARDS pathogenesis and baseline levels are associated with mortality in a bimodal fashion.(Suratt *et al.*, 2009) IL-1β stimulates production of other inflammatory cytokines and plays a prominent role in the early and fibroproliferative phases of ARDS,(Goodman *et al.*, 2003) and increased levels of IL-1β in BALF of ARDS patients are also associated with death.(Goodman *et al.*, 1996) Low concentrations of IL-1ra in BALF from ARDS patients are associated with increased mortality.(Donnelly *et al.*, 1996) TNF-α plays a key role in the development and progression of inflammation in ARDS. (Goodman *et al.*, 1996, Mukhopadhyay *et al.*, 2006) Increased plasma levels of the anti-inflammatory cytokine IL-10 are associated with death in ARDS.(Parsons *et al.*, 1997, Parsons *et al.*, 2005) RAGE is a marker of alveolar type I cell injury and increasing baseline levels are strongly associated with greater mortality in patients with ARDS.(Calfee *et al.*, 2008) Finally, sFasL is involved in the regulation of epithelial cell apoptosis, is released into the airspaces of patients with ARDS, and higher BALF concentrations are significantly associated with death.(Matute-Bello *et al.*, 1999, Lee *et al.*, 2008)

BAL and plasma samples were transported to the laboratory immediately for analysis. BALF was filtered through sterile 100 μM filters to remove mucus. Total cell counts were performed in a hemocytometer, and differential cell counts were performed on cytospin

preparations using standard techniques.(Sittipunt *et al.*, 2001) The remaining BAL fluid was spun at 200 × *g* for 30 min, and the supernatant was removed and stored at −80°C.

Inflammatory biomarker assays were performed in duplicate on stored BALF and plasma samples in a central research laboratory in Seattle, Washington. Concentrations of IL-8, IL-6, IL-1β, IL-1ra, IL-10, TNF-α, G-CSF, and MCP-1 were determined using Luminex bead-based immunoassays (R&D Systems).(Haegens *et al.*, 2007) With these assays, small amounts of sample are added to color-coded beads that are pre-coated with antibodies against each analyte of interest. Biotinylated detection antibodies against each analyte are then added, followed by phycoerythrin-conjugated streptavidin, creating an antibody-antigen sandwich. This mixture is then passed through a laser flow-based detection instrument to detect the color-coded beads and quantify the magnitude of the signal.

BAL and plasma for LTB_4 analysis were treated with 4 volumes of methanol with formic acid, and LTB4 was measured by enzyme immunoassay (Cayman Chemical) after solid phase extraction on an Oasis HLB column (Waters, Milford, Mass).(Debley *et al.*, 2007) SP-D (BioVendor), vWF (Diagnostica Stago), and RAGE and sFasL (R&D Systems) were all measured by standard enzyme-linked immunosorbent assays.

RESULTS

Participants and Baseline Characteristics

Ninety participants were enrolled in the parent RCT.(Stapleton *et al.*, 2011) One patient was extubated before the baseline BAL was performed, leaving 89 baseline BALF samples (Figure 1). As BALs were not performed in extubated patients, and no samples were obtained from patients who had either died or were discharged, the number of BALF and plasma samples obtained on days 4±1 (n=66) and 8±1 (n=42) appropriately decreased. Hospital mortality in the trial was 21.1%.

Demographic and physiologic characteristics of the 90 participants are demonstrated in Table 1. Average age of participants was approximately 50 years and the majority were Caucasian men. Trauma, shock, aspiration, and sepsis were the most common ARDS risk factors. This population was quite ill with an average APACHE II score of 21.8.(Knaus *et al.*, 1985)

Biomarker Results

Results of BALF and plasma biomarker measurements are shown in Table 2, as mean ± standard deviation (SD). Even though most biomarker values are non-normally distributed, we chose not to depict them as median and intraquartile range because power calculations are much easier to perform with mean ± standard deviation. As indicated by the large standard deviations, variability was quite high for many of the biomarkers. In general, biomarker concentrations tended to decrease over the 3 sampling times, as would be expected.

DISCUSSION

This study reports concentrations of BALF and plasma biomarkers of inflammation and injury in a contemporary population of patients with ARDS who were cared for in an era in which low tidal volume ventilation is standard, and other process of care that could reduce inflammation are also common. The data both improve our understanding of the alveolar and circulating inflammatory milieu in today's patients with ARDS and should also be useful to the scientific community when performing power calculations for future early phase studies of ARDS.

In general, our results indicate decreased baseline levels of alveolar and circulating inflammation when compared to results of prior studies (see comparisons in Table 3), all of which defined ALI according to the 1994 AECC definition (Bernard *et al.*, 1994). BALF concentrations of the early response cytokine IL-1β in our study are approximately 40-fold lower than those measured in a population of ARDS patients from 1988–1991(Goodman *et al.*, 1996). Patients in this prior study by Goodman, *et al.* were similar to those in our study, as approximately 60% were men, mean age was 45.5 years, APACHE II score was 21.6±7.2, and trauma and sepsis were the ALI risk factor in 39.1% and 34.8%, respectively. BALF IL-1β in our study was also 4-fold lower than values from a 1999 phase II RCT of low tidal volume ventilation (Ranieri *et al.*, 1999), where 61% were men, mean age was 51 years, mean APACHE II Score was 15, and trauma and sepsis were the ALI risk factor in 44.4% and 22.2%, respectively. Alveolar levels of its naturally occurring antagonist, IL-1ra, of which decreased levels are associated with higher mortality, were substantially increased in our study compared with a small cohort of 28 patients with ARDS in the mid-1990s (Donnelly *et al.*, 1996) where mean age was 53 years old and sepsis was the ARDS risk factor in 79% of patients. Our study found concentrations of IL-6, IL-8, and TNF-α in BALF were reduced by several fold compared to levels in both the intervention and control groups in the 1999 phase II RCT of low tidal volume versus conventional mechanical ventilation in 37 patients with ARDS described above.(Ranieri *et al.*, 1999) Plasma concentrations of IL-8 and TNF-α, but not IL-6, were also lower in our trial than in that same 1999 RCT. Plasma IL-6 concentrations in our study, however, were lower than those measured in a more recent study comparing levels of inflammatory mediators in trauma and non-trauma patients with ARDS.(Calfee *et al.*, 2007) This study analyzed samples from two large NIH NHLBI ARDS Network trials (Network, 2000, Brower *et al.*, 2004). Some characteristics of this large cohort were similar to our study including mean age of approximately 50 years, 59% women, and mean APACHE III score of approximately 85. However, only 9.7% of patients in the Calfee et al. 2007 study had trauma as their ALI risk factor, compared with 40% in our study. Our BALF concentrations of LTB_4 and TNF-α were also lower than a 1999 phase II RCT (n=146) of an enteral feeding formula containing fish oil versus another high fat enteral formula in patients with ARDS.(Gadek *et al.*, 1999), where the n=98 participants had a mean age of 51 years, 47% were women, and 23.5% had trauma as their ALI risk factor. Increased plasma IL-10 is associated with greater mortality, and our plasma levels were lower than a 1997 cohort of 77 ARDS patients (Parsons *et al.*, 1997), who were approximately 40 years old, had APACHE II of 11.0, and 31.6% had trauma as their risk factor. Plasma SP-D levels in our study are also several fold lower than

the recent Calfee, et al. 2007 study described above that analyzed samples from two large ARDS Network RCTs of patients with ARDS conducted between 1996 and 2002.(Network, 2000, Brower *et al.*, 2004, Calfee *et al.*, 2007) Similarly, we found that concentrations of plasma G-CSF were four- to five-fold lower than values in another recent study that measured G-CSF in samples from the same two large RCTs (Suratt *et al.*, 2009); participants in this study were a mean of 53 years old, 40% women, 31% sepsis as ALI risk factor, and had mean APACHE III score of 76. Baseline levels of plasma RAGE in our study were more than 10-fold lower than levels obtained from participants in the NIH ARDS Network RCT of low tidal volume ventilation who were randomized to the 6cc/kg group.(Network, 2000, Calfee *et al.*, 2008) Finally, compared with a 1999 study analyzing plasma sFasL in patients with ARDS (mean age 42.7 years, 68.8% men, 37.8% trauma, mean APACHE II score 22.4), our values were approximately 30% lower.(Matute-Bello *et al.*, 1999) Interestingly, our concentrations of plasma vWF in our study were slightly higher than in 2 prior studies in the literature.(Ware *et al.*, 2001, Calfee *et al.*, 2007) The Calfee 2007 study has been described above. The Ware 2001 study included 51 patients with ALI who were 51% men, mean age 48 years, and had a SAPS II score of 49±15 (Le Gall *et al.*, 1993); none of the participants in this study had trauma as their ALI risk factor. Taken as a whole, the baseline characteristics of historical controls from many of these studies are quite similar to those in our study, although some cohorts had varying gender and ALI risk factor proportions.

There are several possible explanations as to why our results indicate that alveolar and circulating concentrations of mediators of inflammation and injury in contemporary ARDS patients are generally decreased, in some cases several fold, compared with historical studies. Perhaps most notable is that the parent RCT occurred in an era of relatively improved care for ARDS patients. Evidence suggests that survival in ARDS patients has increased over the past 30 years.(Stapleton *et al.*, 2005, Erickson *et al.*, 2009, Phua *et al.*, 2009) One reason for this improved mortality is early delivery of lung protective ventilation, an intervention know to decrease both lung and systemic inflammation as well as mortality. (Ranieri *et al.*, 1999, Network, 2000, Parsons *et al.*, 2005) Additional reasons for the increased survival are not entirely clear, but there has been speculation that other processes of care including early fluid resuscitation and antibiotic administration, prophylaxis against deep venous thrombosis and stress ulcer bleeding, and prevention of nosocomial infections may have lead to reduced lung and systemic inflammation in patients with ARDS.(Hudson, 1995, Cook *et al.*, 1996, Rivers *et al.*, 2001, Pronovost *et al.*, 2006, Cook *et al.*, 2011)

Other explanations for our findings include different populations or assay methods between studies. While most of our measurements were performed with Luminex bead-based immunoassays (R&D Systems), a more recent technology where multiple biomarkers can be measured simultaneously, prior studies used enzyme-linked immunosorbent assays or other methods.(Haegens *et al.*, 2007) However, results from bead-based immunoassays have been shown to correlate well with results from current ELISAs.(Kofoed *et al.*, 2006, Codorean *et al.*, 2010, Wang *et al.*, 2012) ELISAs used in the past, though, may have had different sensitivities and detection limits. Indeed, a few investigations do suggest that cytokine concentrations obtained with two different techniques, while proportionally equivalent, may not necessarily be interchangeable due to differences in the concentration levels.(Loo *et al.*, 2011) If this is the case, there remains an argument to power future ARDS trials where

cytokine concentrations is an outcome using data obtained utilizing the same assay methodology that will be used in the planned future trial. Additionally, as described above, our study population contained a higher proportion of patients with trauma as their risk factor for ARDS than in other recent RCTs.(Network, 2000, Brower *et al.*, 2004, Rice *et al.*, 2012) Clinical outcomes in patients with trauma-associated ARDS are better than in patients with sepsis or another non-trauma ARDS risk factor, and evidence suggests that this may be partially explained by less severe endothelial and alveolar epithelial lung injury.(Calfee *et al.*, 2007) Therefore, a higher proportion of trauma patients in our cohort could result in lower levels of inflammatory mediators. However, levels of plasma IL-6, a key inflammatory mediator in ARDS, in our study were notably decreased compared to values obtained in both trauma and non-trauma patients with ARDS from 1996–2002.(Calfee *et al.*, 2007)

This study does have some limitations. First, generalizability of our results may be limited due to the population of patients enrolled in the parent RCT.(Stapleton *et al.*, 2011) Additionally, the sample size was modest (n=90).

The fact that our study demonstrates reduced baseline concentrations of biomarkers of alveolar and systemic inflammation and injury in patients with ARDS, compared to prior studies, indicates that we must be very careful when using data from historical controls to perform power calculations for clinical trials. Our results reveal the importance of using biomarker data from a population of patients not only temporally close to the proposed clinical trial, but also similar in terms of ARDS risk factor and receipt of ICU processes of care.

CONCLUSION

In conclusion, this study has reported BALF and plasma levels of many biomarkers of inflammation and injury in a contemporary population of patients with ARDS that can be used to perform accurate power calculations for future early phase clinical trials with biologic endpoints. Furthermore, we have also demonstrated that concentrations of these biomarkers in our population are generally decreased compared with prior studies. If alveolar and systemic inflammation is indeed decreased in today's ARDS patients, conducting phase II clinical trials of novel therapies with primary biologic endpoints then becomes more difficult as much larger sample sizes are required.

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CLINICAL SIGNIFICANCE:

- Bronchoalveolar fluid (BALF) and plasma biomarkers are frequent endpoints in studies of human acute respiratory distress syndrome (ARDS)
- Data from contemporary ARDS patients are not available, thus making power calculations for future studies difficult
- ARDS mortality has decreased over time
- We therefore measured baseline biomarkers in 90 ARDS patients by analyzing BALF and blood samples from a recent RCT and compared these values to historical controls
- Compared to historical measurements, our biomarker values were generally substantially lower
- These findings may result from changes in ICU processes of care over time
- Proper design of contemporary ARDS studies is now improved with these newer data

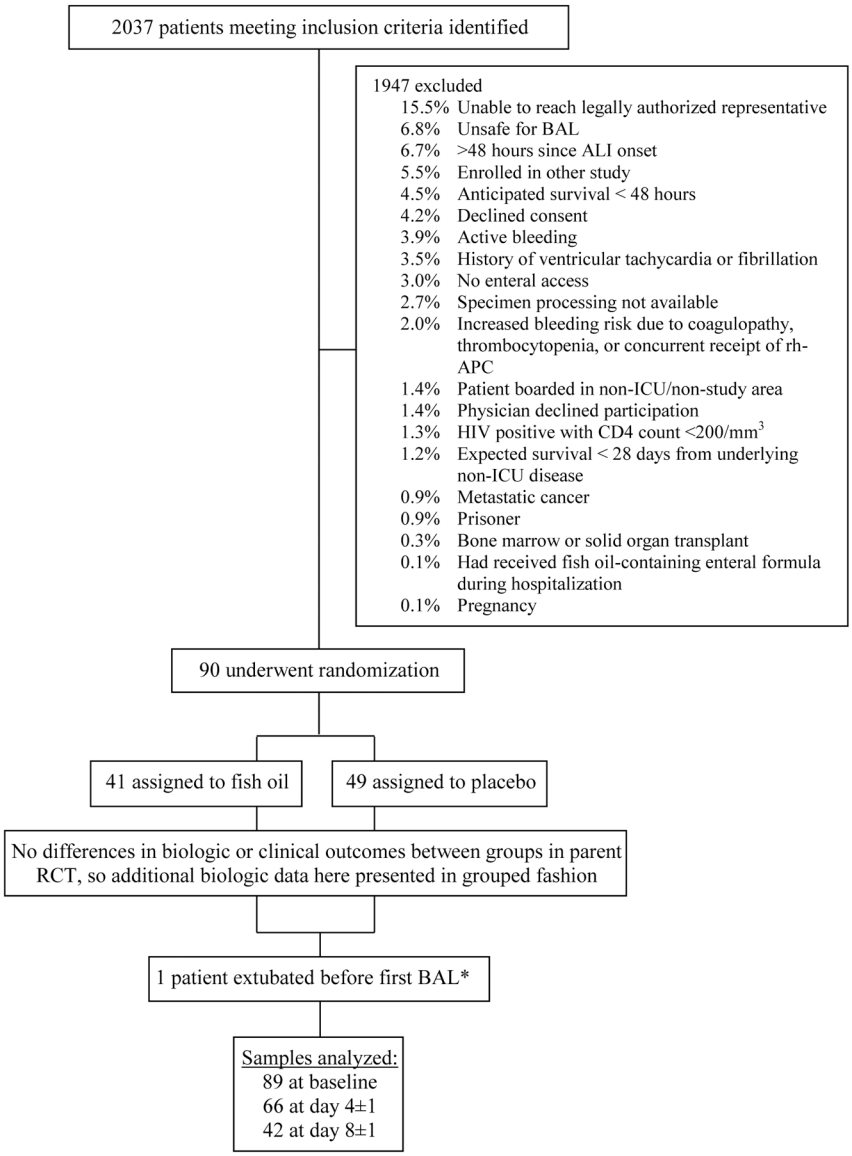

Figure 1.

Diagram of Participant Flow and Sample Collection in Parent RCT. Patients may have had more than one reason for exclusion. *Baseline biologic available on 40 participants in the fish oil group and all 49 patients in placebo group.

Table 1.

Baseline Participant Characteristics

	n=90
Age (years)[a]	49.9±16.4
Male gender, n (%)	57 (63.3)
Race, n (%)	
African-American	3 (3.3)
American Indian	2 (2.2)
Asian	2 (2.2)
Pacific Islander	3 (3.3)
White	80 (88.9)
Ethnicity, n (%)	
Hispanic	3 (3.3)
Non-Hispanic	84 (93.3)
Unknown	3 (3.3)
ALI risk factor, n (%) (not mutually exclusive)	
Trauma	36 (40.0)
Shock	36 (40.0)
Aspiration	17 (18.9)
Sepsis	14 (15.6)
Pneumonia	12 (13.3)
Massive transfusion	2 (2.2)
Moderate transfusion	8 (8.9)
Overdose	3 (3.3)
Near drowning	1 (1.1)
Inhalation injury	1 (1.1)
Admission Diagnosis, n (%)	
Trauma	34 (37.8)
Sepsis	13 (14.4)
Pneumonia	9 (10.0)
Respiratory failure	9 (10.0)
ALI/ARDS	5 (5.6)
Intracranial hemorrhage	5 (5.6)
Other	15 (16.7)
APACHE II Score[a]	21.8±16.4
P_aO_2/FiO_2[a]	164.4±58.2
Tidal volume (mL/kg predicted body weight)[a]	7.2±1.7
Plateau pressure (cm H_20)[a]	24.6±5.5
PEEP (cm H_20)[a]	9.1±4.0

Lung Injury Score(Murray *et al.*, 1988) [a]	2.6±0.6

[a]Data presented as mean ± standard deviation

Abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome; APACHE = Acute Physiologic and Chronic Health Evaluation; P_aO_2/FiO_2 = ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen; PEEP = positive end expiratory pressure.

Table 2.

Bronchoalveolar Lavage Fluid and Plasma Biomarker Measurements

Biomarker* (mean±SD)	Site	Study Entry (baseline) n=89	Day4±1 n=66	Day 8±1 n=42
G-CSF	BALF	611.6± 1967.3	173.2±349.8	168.2±360.1
	Plasma	2213.6±14658.4	209.4±1472.1	27.8±33.9
IL-1β	BALF	238.5±1370.4	193.2±838.4	301.8±736.2
	Plasma	9.0±1.8	8.7±0.4	8.8±0.01
IL-1ra	BALF	3905.6±7336.4	3682.3±7395.1	2909.7±5006.3
	Plasma	6727.0±17427.5	3799.9±6193.0	3189.9±3077.7
IL-6	BALF	796.0±2320.5	245.6±753.8	151.7±353.6
	Plasma	403.5±1829.8	103.1±407.5	48.9±57.4
IL-8	BALF	2407.6±8870.3	1909.9±3687.7	3511.9±8116.9
	Plasma	86.1±419.2	42.0±l 12.5	43.7±133.9
IL-10	BALF	7.4±13.4	4.8±5.0	10.6±38.9
	Plasma	39.6±159.2	16.2±13.7	15.0±7.5
LTB_4	BALF	126.9±496.2	37.6±43.5	71.0±108.5
	Plasma	47.6±61.2	41.3±25.9	44.5±38.7
MCP-1	BALF	1122.9±2094.4	643.2±1259.5	333.7±523.4
	Plasma	318.8±333.7	205.5±180.8	163.7±115.5
PMNs†	BALF	52.8±31.5	50.2±31.0	41.7±34.8
	Plasma	52.8±31.5	47.3±32.3	40.7±35.0
RAGE	BALF	14509.2±51201.2	1237.2±2958.8	451.0±510.7
	Plasma	386.8±630.9	170.0±202.4	108.4±150.1
sFasL	BALF	39.3±2.0	40.1±5.7	40.1 ±4.7
	Plasma	129.1±381.2	60.2±31.1	56.2±28.9
SP-D	BALF	371.4±414.9	312.7±366.6	252.7±572.0
	Plasma	186.9±253.9	230.4±201.5	166.2±124.8
TNF-α	BALF	17.3±55.1	23.0±80.9	14.1±28.6
	Plasma	15.4±2.0	15.2±2.8	15.0±2.2
vWF‡	BALF	0.8±1.5	0.3±0.6	0.2±0.4
	Plasma	453.0±1058.2	524.3±686.8	694.7±881.5

*Data are presented as mean ± standard deviation in pg/mL, except PMN

†data are presented as % of total cells, and vWF

‡data are presented as % control.

Table 3.

Comparison of Our Baseline BALF and Plasma Biomarker Concentrations with Prior Studies

Biomarker*	Site	Baseline concentrations from prior studies§		Mean Baseline Concentration in Our Study mean±SD, n=89	Median Baseline Concentration in Our Study Median (IQR), n=89
		Author	Concentration		
GCSF	Plasma	Suratt (2009)	Survivors: 188(80–540) Non-survivors: 207 (74–887)	2213.6±14658.4	46.5 (20.9–122.7)
IL-1β	BALF	Park (2001)	9.8 (4.4–18.9)	238.5±1370.4	13.8 (2.6–104.9)
		Goodman (1996)	Mean ~ 10,000		
		Ranieri (1999)	Mean ~900		
IL-1ra	BALF	Park (2001)	1829.2 (1124.3–6868.3)	3905.6±7336.4	1729.9 (593.6–3214.0)
		Donnelly (1996)	820 (0–18,900)		
		Ranieri (1999)	16,503±14,935		
	Plasma	Parsons (1997)	~15,000 (1,000–38,000)	6727.0±17427.5	2380.4 (1313.4–4754.6)
		Ranieri (1999)	4,218±3,645		
IL-6	BALF	Park (2001)	1229.5 (263.3–3232.6)	796.0±2320.5	145.3 (33.5–455.9)
		Gadek (1999)	282±145		
		Ranieri (1999)	Mean ~9,000		
	Plasma	Calfee (2007)	Trauma: 300 (128–674) Non-trauma: 259 (100–850)	403.5±1829.8	81.7 (40.9–172.0)
		Ranieri (1999)	Mean −200		
IL-8	BALF	Goodman (1996)	Mean ~ 400	2407.6±8870.3	486.4 (253.5–1390.6)
		Gadek (1999)	2849±1458		
		Ranieri (1999)	Mean ~10,000		
	Plasma	Calfee (2007)	Trauma: 21 (20–59) Non-trauma: 44 (20–103)	86.1±419.2	19.9 (19.8–35.2)
		Ranieri (1999)	Mean ~100		
IL-10	BALF	Park (2001)	4.8 (1.5–9.3)	7.4±13.4	3.6 (3.6–3.6)‡‡
		Donnelly (1996)	100 (0–1600)		
	Plasma	Parsons (1997)	~125,000 (50,000–300,000)	39.6±159.2	14.3 (14.3–14.3)††
LTB_4	BALF	Gadek (1999)	596±495.2	126.9±496.2	20.5 (9.3–31.3)
MCP-1	BALF	Goodman (1996)	Mean ~ 1000	1122.9±2094.4	534.7 (185.2–1318.5)
PMNs†	BALF	Ranieri (1999)	Mean ~ 75	52.8±31.5	54.0 (25.5–82.8)
RAGE	Plasma	Calfee (2008)	Survivors: ~3900 (2000–8000) Non-survivors: ~5500 (2500–10,000)	386.8±630.9	261.5 (137.6–438.4)
sFasL	BALF	Matute-Bello (1999)	Mean ~ 150	39.3±2.0	39.0 (39.0–39.0)‡‡
SP-D	Plasma	Calfee (2007)	Trauma: 58,000 (45,000–78,000) Non-trauma: 95,000 (45,000–211,000)	186.9±253.9	122.4 (56.0–205.6)
TNF-α	BALF	Ranieri (1999)	Mean~750	17.3±55.1	3.8 (3.8–7.9)**

Biomarker*	Site	Baseline concentrations from prior studies§		Mean Baseline Concentration in Our Study mean±SD, n=89	Median Baseline Concentration in Our Study Median (IQR), n=89
		Author	Concentration		
	Plasma	Ranieri (1999)	Mean ~1,000	15.4±2.0	15.4 (15.4–15.4)††
vWF‡	Plasma	Ware (2001)	~250 (150–450)	453.0±1058.2	248.4 (155.5–544.2)
		Calfee (2007)	Trauma: 226 (142–331) Non-trauma: 355 (215–551)		

*Data are presented as pg/mL, except PMN

†data are presented as % of total cells, and vWF

‡data are presented as % control.

§Data presented as mean±SD or median (IQR), as available in prior literature. Use of '~' notes that values are approximated from graphs and charts within that particular prior study. Many prior studies noted means in graphs but did not specifically define SD; this is noted by use of 'mean~'.

‖BALF concentrations of IL-10 and sFasL in 75% of participants in our study were at or below the detection limit or our assay.

**BALF concentrations of TNF-α in at least 50% of participants in our study were at or below the detection limit or our assay.

††Plasma concentrations of IL-10 and TNF-α in 75% of participants in our study were at or below the detection limit or our assay.