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Exhaled breath condensate biomarkers in critically ill, mechanically ventilated patients

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Abstract

Pneumonia is a significant risk for critically ill, mechanically ventilated (CIMV) patients. Diagnosis of pneumonia generally requires a combination of clinician-guided diagnoses and clinical scoring systems. Exhaled breath condensate (EBC) can be safely collected non-invasively from CIMV patients. Hundreds of biomarkers in EBC are associated with acute disease states, including pneumonia. We evaluated cytokines in EBC from CIMV patients and hypothesized that these biomarkers would correlate with disease severity in pneumonia, sepsis, and death. EBC IL-2 levels were associated with chest radiograph severity scores (odds ratio = 1.68; 95% confidence interval = 1.09 – 2.60; P = 0.02). EBC TNF-α levels were also associated with pneumonia (odds ratio = 3.20; 95% confidence interval = 1.19 – 8.65; P = 0.02). The techniques and results from this study may be useful for all mechanically ventilated patients.

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Author Contributions:

MD served as primary author of this manuscript and project director of the study. BW, MM, JP, MAW, and CWC contributed performance of experiments, data interpretation, and assistance drafting of this manuscript. CS was the medical director of the study and aided in experimental design, creation of the CXRAY score, and training of staff. AM was PI of the project, designed the study, edited the manuscript, and supervised the work of all team members.

Introduction:

Pneumonia, sepsis, and death are significant risks for critically ill, mechanically ventilated (CIMV) patients(1-7). This population is at greater risk than most due to their weakened immune state, presence of an artificial airway, and exposure to pathogens and vectors present within the hospital. Ventilator-associated pneumonia (VAP), defined as pneumonia occurring after initiation of mechanical ventilation, is the second most common nosocomial infection in the USA and is reported to affect 5-67% of CIMV patients, depending upon facility and reporting techniques(1–3, 5–10). VAP increases the length of the hospital stay for patients by 6-9 days, costing tens of thousands of dollars per patient, and has a reported all cause mortality rate of 20-41%(5, 7). Pneumonia often leads to sepsis in CIMV patients, which is also a leading cause of death in this population(1–6, 11).

Diagnosis of VAP requires the presence of signs and symptoms of infection including fever, increased or purulent respiratory secretions, new infiltrative abnormalities on chest radiographs, and positive bronchoscopic or tracheal aspirate microbiological culture(2, 3, 5, 8, 12, 13). Clinical signs and symptoms have high sensitivity but low specificity related to VAP diagnosis. This can lead to incorrect diagnosis of VAP with inappropriate and untimely administration of antibiotics, worsening patient outcomes and increased healthcare costs(13). Tracheal aspiration and bronchoscopy are also invasive techniques that can interrupt mechanical ventilation, injure airway mucosa, cause transient hypoxia, and de-recruit alveoli. Accordingly, a non-invasive, objective, and specific diagnostic method for detecting VAP would be optimal.

Exhaled breath condensate (EBC) is the water vapor and aerosolized fraction of exhaled breath(14–16). Hundreds of biomarkers have been found in EBC and have been linked to systemic and pulmonary disease states, including pneumonia(14–16). From spontaneously breathing subjects that are not receiving mechanical ventilation, EBC is collected over 10-20 minutes of tidal breathing into a specialized sampling device that condenses liquid in their breath. EBC can be safely collected non-invasively from CIMV patients by placement of the sampling device/condenser into the expiratory limb or at the exhalation port of the ventilator (17–22). EBC is also a minimal-risk biohazard to clinicians and study staff that is only capable of serving as a vector for airborne transmission diseases; even for those diseases, the airborne particles represent less than 0.1% of the EBC sample (14). Furthermore, when collected during mechanical ventilation, EBC is collected without any direct contact to the patient's exhaled breath. Biomarkers within EBC reflect volatile polar organic compounds as well as the aerosol fraction containing semi-volatile metabolites, signaling proteins, viruses, and bacteria. As such, this biological medium is amenable for a variety of different analytical schemes and an ideal candidate for disease monitoring/detection, particularly in vulnerable patient populations such as CIMV patients.

For this work, we focused on the measurement of pro- and anti-inflammatory cytokines in EBC. These compounds represent a family of short-lived "messenger proteins" that regulate immunity, inflammation, and hematopoiesis. Several cytokines have been previously associated with pneumonia and sepsis, including IFN-γ, IL-10, IL-6, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8, and TNF-α(23–29). We have previously studied the presence of cytokines

in human blood, urine, and EBC under less stressful conditions and have found that they are indeed measurable with modern immunochemistry platforms and kits. EBC cytokines were also found to be related to response to external stressors (ambient ozone and diesel exhaust exposures) and involved with the mediation of white blood cells, blood pressure, and pulmonary function(30, 31). The non-invasive and minimally-biohazardous nature of EBC collection for cytokines could make it more clinically preferable than blood and urine. We found we could easily collect EBC from ventilators and hypothesized that cytokines in EBC correlate with pneumonia and sepsis severity in CIMV patients.

Methods:

Study Design –

Following Institutional Review Board (IRB) approval, this study was conducted in a 933-bed tertiary-care urban university medical center. Subjects were recruited in the Medical Respiratory Intensive Care Unit, Surgery Trauma Intensive Care Unit, or Neuroscience Intensive Care Unit. This study met criteria for Waiver of Consent per the IRB. Inclusion criteria were subjects requiring mechanical ventilation who were intubated within 24 hours of study enrollment and were 18 years old or older. Patient demographics are depicted in Table 1. Subjects receiving inhaled nitric oxide were excluded. EBC samples, Clinical Pulmonary Infection Scores (CPIS), chest radiograph scores (CXRAY), and confirmation of pneumonia, sepsis, or patient death were obtained daily for the first five days of admission to the ICU unless the patient was discharged from the ICU. EBC biomarkers were correlated with CPIS, CXRAY, development of pneumonia, development of sepsis, or death. Collection and analysis methods are detailed below.

Exhaled Breath Condensate Collection –

EBC was collected at −80°C over 20 minutes using the RTubeVent (Respiratory Research, Inc., Austin, TX, USA) inline with mechanical ventilator. The RTubeVent was placed on the proximal end of the expiratory limb of the ventilator to the patient as previously described(18, 32). Collection was taken at the time of study enrollment and then at the same hour of the day as when initial collection occurred, 24 hours later, for each subject. Minute ventilation and ventilatory pattern were not controlled during collection and varied between patients according to their ventilator settings and breathing pattern. Sampling was monitored closely and terminated and restarted in the case of coughing or introduction of sputum into sample. After collection, samples were immediately aliquoted into 0.5 mL cryovials and stored at −80°C.

Exhaled Breath Condensate Cytokine Analysis –

Prior to analysis, EBC samples were thawed on ice. EBC samples were homogenized by repeated cryovial tube inversion, and subsequently analyzed using the MesoScale Discovery (MSD, LLC, Rockville, MD, USA) Human Proinflammatory Panel 1 10-plex kit. The 10-plex analyzed for the following cytokines: IFN-γ, IL-10, IL-6, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8, and TNF-α. Samples, standards and appropriate controls were analyzed using an MSD Quickplex SQ 120 instrument as previously described(33). Sample cytokine concentrations were calculated according to a standard curve for each individual cytokine.

Clinical Pulmonary Infection Score –

The CPIS is a validated scoring system used by our facility that combines the status of tracheal secretions, chest radiograph, body temperature, white blood cell count, oxygenation, and tracheal secretion microbiology for a numerical score of 0-12, with higher numbers correlating with worsening disease severity (Table 2)(13). Tracheal secretion score increases based on their presence and volume; chest radiograph score increases based on infiltrate presence and severity; body temperature score increases based on the presence of a fever or hypothermia; white blood cell count score increases with abnormally decreased or increased counts, suggesting impaired or escalated immune response; oxygenation score increases based upon increased oxygen requirements (indicated by a PaO2/FiO2 ratio < 240) and a subjective diagnosis of ARDS; and tracheal secretion microbiology score increases based upon the presence and number of different pathogens detected in tracheal secretions. CPIS was calculated daily for study subjects and correlated with daily EBC cytokines.

Chest Radiograph Scoring System –

This study used a modified scoring system for evaluating chest radiographs that was developed at our center and preferred by our clinical team (CXRAY, Table 3). This system combines the status of visual disease progressions from previous chest radiographs, infiltrates, and pleural effusions for a numerical score between 0-6, with higher numbers correlating with worsening disease severity. CXRAY was calculated daily for study subjects and correlated with daily EBC cytokines.

Clinical Outcomes –

Subjects were screened daily for sepsis, pneumonia, or death. Sepsis and pneumonia were confirmed by a combination of physician-guided diagnoses and clinical scoring systems entered into the chart. Pneumonia diagnosis was based upon intensivist clinical diagnosis and not specific criteria. These outcomes were correlated with daily EBC cytokines.

Statistical Analysis –

We considered 10 cytokines in the statistical analyses. We restricted analyses to those analytes with mean replication Pearson correlation $r^2 > 0.25$ ($|r| > 0.5$). The mean replication was assessed as the average of the replication between the initial cytokine measure and all replicate measures taken after the initial collection of the breath condensate. Of the ten analytes, six (IFN-γ, IL-1β, IL-2, IL-6, IL-13, and TNF-α) passed this cutoff. We then defined outliers as observations more than 1.5 times the interquartile range from either the first or third quartile. Finally, before analysis, cytokines were log-transformed and scaled to the standard deviation. The five outcomes considered were death, CPIS, CXRAY, diagnosis of pneumonia, and diagnosis of sepsis. CPIS and CXRAY were converted to binary indicators (CPIS ≥ 6; CXRAY ≥ 4). For CPIS and CXRAY, our primary modeling approach used generalized estimating equations to utilize all observations while accounting for the correlation between measurements taken from the same individual. We compared associations from this model to those obtained using just the initial observation for each person. For all other outcomes, we only used the initial measurement from each individual

as there was no variation in the outcome within each individual. A full description of statistical methods and modeling is described in the supplement.

Results:

Association between EBC IL-2 and CXRAY score

There were 49 participants in the study and 115-129 observations for each cytokine (Table 4). A description of the overall study group is given in Table 1. We observed associations between IL-2 and CXRAY. A standard deviation increase in log-transformed IL-2 was associated with a substantial increase in the odds of observing a CXRAY score ≥ 4 (OR = 1.68; CI = 1.09 – 2.60; P = 0.02). IL-13 had associations of similar direction and magnitude with both CXRAY though with P-values above the 0.05 cutoff (OR = 1.62; CI = 0.96 – 2.74; P = 0.07) and CPIS (OR = 1.51; CI = 0.96 – 2.39; P = 0.08) (Figure 1, Table 5).

As a sensitivity analysis we evaluated both the correlation between the log odds ratio for the GEE model and a model based solely on the initial observation (i.e. the same model used for the other outcomes). The models had good agreement as assessed using Spearman correlations as the distribution of log odds ratios is not likely to be normal (Spearman r = 0.78). The GEE model was attenuated slightly to the null (22% smaller log odds ratio) but higher precision, 67% reduction in 95% confidence interval of log odds ratio. The substantial increase in precision of the GEE model further justified our use of this modeling approach.

EBC TNF-α and IL-1β are associated with pneumonia and sepsis, respectively

The outcomes Pneumonia, Sepsis, and Death did not alter over the course of study participant observation so were not suitable for a GEE model. Our primary approach was to model associations using the first measurement of each of the cytokines as described in the Methods. Secondarily, we also modeled associations using only those taken on first diagnosis of Pneumonia or Sepsis. The correlation between the associations for these two approaches was very high (log odds ratio Pearson $r^2 = 0.88$) with slightly smaller standard errors for the primary approach. Given the strong correlation between the two approaches taken to analyzing Pneumonia, Sepsis, and Death, differences between them is likely due to random chance, the slightly smaller sample size in the second approach (as not everyone had samples from the day of diagnosis analyzed), and slight sensitivities of the associations to date of sample collection. Using the primary approach we observed associations between TNF-α and Pneumonia (OR = 3.20; CI = 1.19 – 8.65; P = 0.02) as well as between IL-1β and Sepsis (OR = 0.33; CI = 0.11 – 0.98; P = 0.045) (Figure 2, Table 6).

Using the secondary approach where only measurements taken on the first day of diagnosis were included we still observed associations between TNF-α and Pneumonia indicating an increased risk of Pneumonia with increasing TNF-α concentrations in EBC exhaled breath condensate (OR = 2.93; CI = 1.02 – 8.21; P = 0.04) (Table 7). However, the association between IL-1β and Sepsis weakened considerably (OR = 0.43; CI = 0.13 – 1.42). We also observed an association between IL-2 and Sepsis in this approach (OR = 0.35; CI = 0.12 –

1.00; P = 0.05) which was actually similar in direction and magnitude to the association seen between IL-2 and Sepsis in the primary approach (OR = 0.05; CI = 0.23 – 1.11).

Discussion:

Early detection and treatment improves outcomes in sepsis and pneumonia(4, 6, 8, 9). However, non-invasive, quantitative metrics for detection of these diseases are lacking which causes most decisions for early treatment to be based upon subjective patient assessment(1–5, 8, 12, 13). Due to the heterogeneous nature of these disorders and a lack of specific diagnostics, most first-line treatments involve broad-spectrum antibiotics which can lead to microbial resistance(4, 9, 12). Identifying and implementing optimal treatment methods for patients with sepsis and pneumonia remains a challenge for healthcare providers.

This challenge is even greater when caring for CIMV. These patients are more vulnerable to acquire pneumonia and sepsis due to their weakened health state and presence of artificial airways and other indwelling support devices. Moreover, the presence of an artificial airway impairs their ability to participate in the patient assessment and inform their healthcare providers of potential symptoms of illness. Conventional methods for diagnosing pneumonia such as tracheal aspiration and bronchoalveolar lavage are invasive and some patients may be too unstable to safely undergo bronchoscopy(14, 18). Collection of EBC from these patients has been validated and standardized and may provide a superior method for disease detection(14, 17, 18).

This study found that several cytokines that were measurable in EBC collected during mechanical ventilation were associated with critical illness and disease severity. Specifically, associations were found between EBC cytokines and subjective chest radiograph scores, and clinical diagnoses of pneumonia and sepsis. These biomarkers may provide an objective and quantitative indicator of disease severity. The association of TNF-α with pneumonia suggests that this cytokine may be indicative of this outcome and could have a mechanistic role in this disease – though disease mechanisms would need future studies to be properly evaluated. TNF-α mediates inflammation, and a previous study of bacterial pneumonia found significantly elevated levels of TNF-α in acute pneumonia compared to convalescent pneumonia(34). These cytokines could provide therapeutic targets for these disorders.

Some of the results are confounding. For instance, since chest radiograph scoring is a component of the CPIS, it is somewhat surprising that CPIS did not also correlate with IL-2. The CXRAY score used is more comprehensive than that used in the CPIS, which may explain this observation. Alternatively, it is possible that the mechanism by which IL-2 is increased in EBC is linked to processes that more significantly alter chest radiography (such as hydrostatic pressure changes). The lack of differences in other pro-inflammatory cytokines in our subjects was unexpected. Since all subjects evaluated were CIMV, we speculate that the study may have been limited by not having enough representation across the spectrum of disease severity; for example, more significant differences may be observed if less ill subjects were included. Also, the associations we observed between IL-1β and IL-2 with sepsis are opposite with the hypothesis and reports in previous literature. We observed a decrease in these cytokines associated with sepsis, while previous reports have found IL-1β

levels to be increased in patients in septic shock compared to healthy controls(25), and IL-2 levels have been found to increase in parallel with disease severity of sepsis in patients(35). This may be due to differing criteria for sepsis between institutions, but is confounding nonetheless and should be interpreted with caution. The observation that patient death was not correlated with an increase in pro-inflammatory cytokines was also suprising; however, most death in intensive care units is not ultimately due to pulmonary inflammation and therefore EBC cytokines may not be as useful for predicting acute risk for death.

Beyond confounding results, this study had several limitations. The primary limitation of the study is the small sample size which limits the power for statistical inference. Despite this we were able to observe multiple associations, and differing approaches to modeling the association showed high concordance indicating stability of the associations. Future studies should include larger populations to better resolve associations. The study population included a wide variety of patient demographics with heterogeneous pathophysiology treated in three different ICUs. Pneumonia and sepsis encompass a variety of disease mechanisms with hundreds of potential causes all of which may lead to a different inflammatory phenotype. However, the findings from this study may be useful in identifying biomarkers of worsening disease that would benefit from detection regardless of causality. Future studies in more focused study populations are warranted and these results may help frame those studies. Also, this study included subjects from only one center. This narrows the potential patient population to those from the served region of this healthcare center. Multi-center studies are warranted to confirm the usefulness of these findings for other regions and facilities. The findings of the association of cytokine TNF-α with pneumonia (both viral and bacterial) should be confirmed in a larger study in non-ventilated patients both with initial signs of disease and convalescent patients after therapy(33). Finally, EBC collected in this study was not fractionated by end-tidal carbon dioxide signal or breathing pattern and therefore represented the entire area of ventilated lung rather than specific compartments such as alveolar or bronchial. Due to this, we cannot speculate as to the anatomic origin of the EBC biomarkers we evaluated(19, 20, 22).

Conclusion:

EBC can be safely collected from critically ill, mechanically-ventilated patients and many cytokines can be measured in this biofluid. EBC IL-2 concentrations are associated with CXRAY severity scores after adjustment for age and race, suggesting that the processes leading to worsening CXRAY scores may be IL-2-mediated and that EBC IL-2 may be a useful biomarker for disease severity. EBC TNF-α levels are associated with pneumonia and may be useful biomarkers for detection and management of this prevalent disease. We propose that measurement of EBC cytokines in CIMV patients is feasible and could facilitate future research to hopefully address the large segment of CIMV patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Conflict of Interest/Funding Disclosure:

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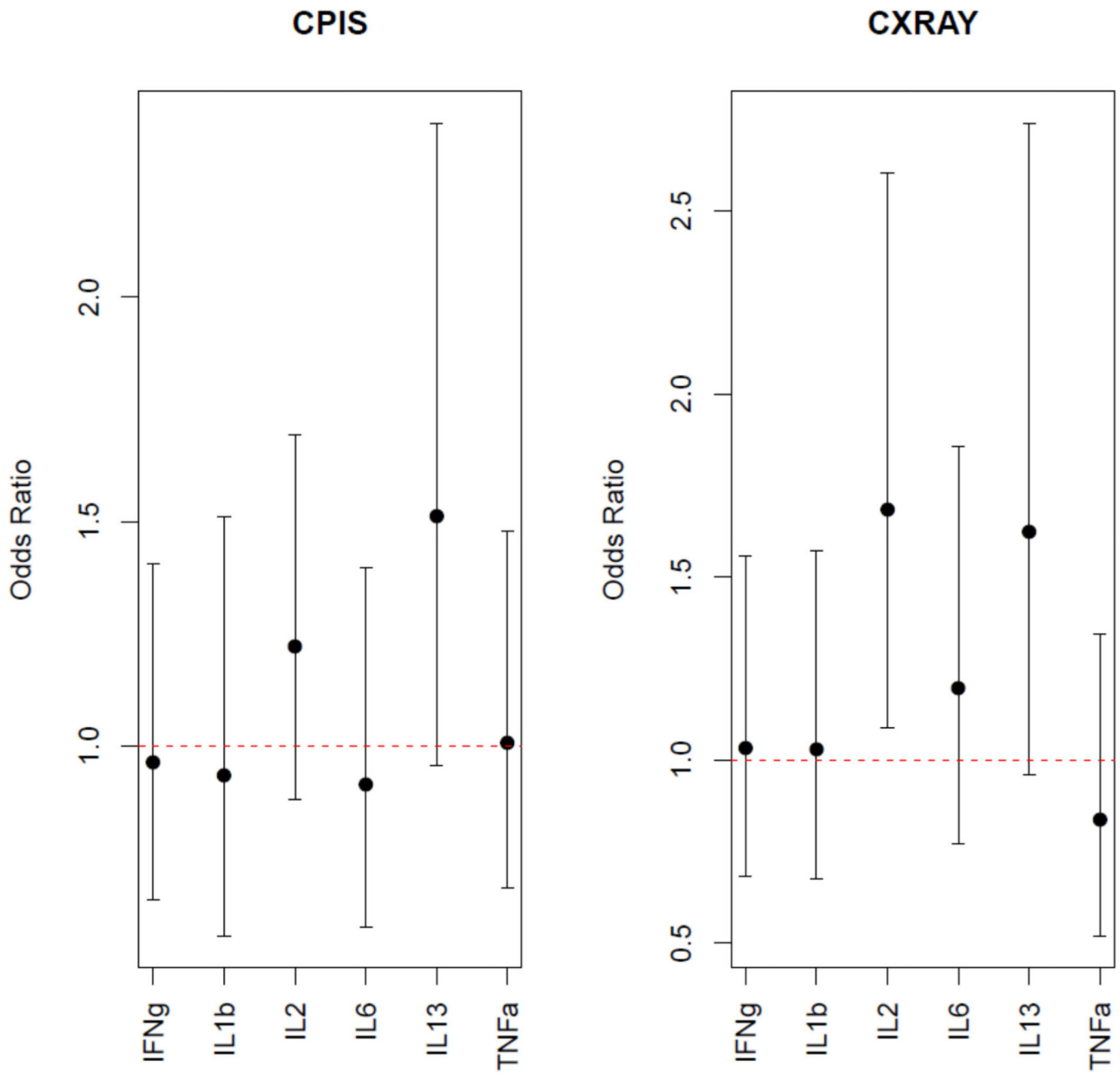

Figure 1:

Association between cytokines assessed in EBC and the clinical outcomes of CPIS and CXRAY using a generalized estimating equations model. An odds ratio greater than 1 is indicative of a positive association between the cytokine and the outcome; an odds ratio equal to 1 indicates that the cytokine and the outcome are independent of one another; an odds ratio of less than 1 is indicative of an inverse association between the cytokine and the outcome. CPIS = clinical pulmonary infection score; CXRAY = chest radiograph score.

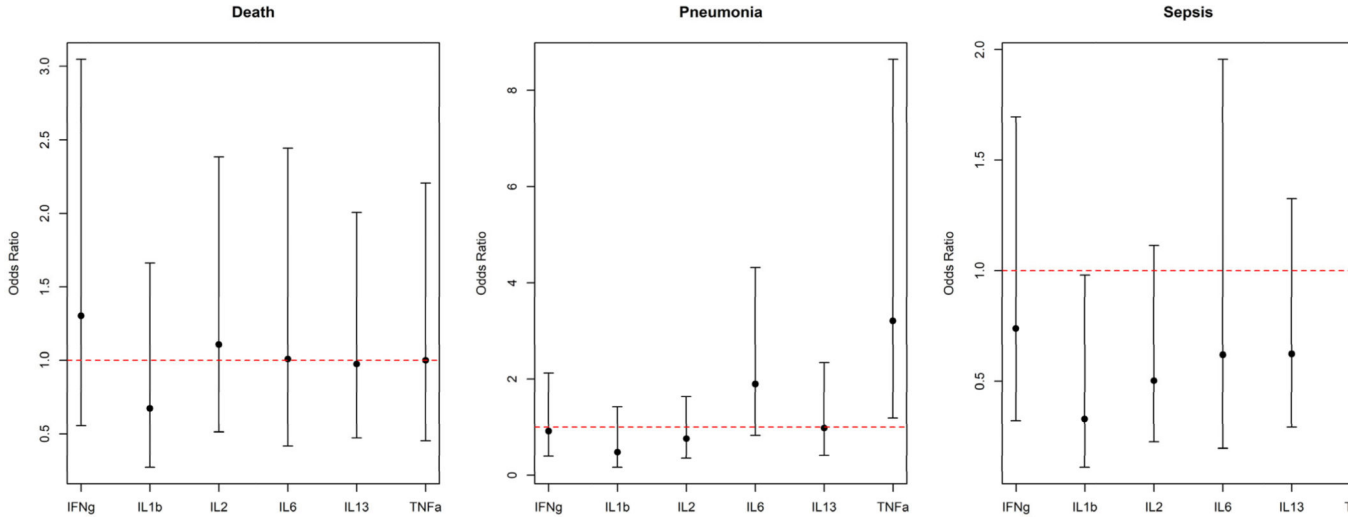

Figure 2:

Associations between cytokines assessed in EBC and the outcomes of Death, Pneumonia, and Sepsis. An odds ratio greater than 1 is indicative of a positive association between the cytokine and the outcome; an odds ratio equal to 1 indicates that the cytokine and the outcome are independent of one another; an odds ratio of less than 1 is indicative of an inverse association between the cytokine and the outcome.

Table 1:

Demographics for the 49 study participants. Positive CPIS indicates the number of participants with a CPIS score ≥ 6 while Positive CXRAY indicates the number of participants with a CXRAY score ≥ 4. CPIS = Clinical Pulmonary Infection Score; CXRAY = chest radiograph score. Positive results at any time during study enrollment were included.

	Mean	**SD**
Age	56.4	14.3
	N	%
Males	28	57.1
Females	21	42.9
White	26	53.1
Black	18	36.7
Unknown Race	5	10.2
Positive CPIS	22	44.9
Positive CXRAY	24	49.0
Pneumonia	13	26.5
Sepsis	9	18.4

Table 2:

Criteria for the Clinical Pulmonary Infection Score.

Clinical Pulmonary Infection Score (CPIS) Range 0–12	
Temperature (°C)	**Points**
> 36.5 and < 38.4	0
> 38.5 and < 38.9	1
< 36.4 or > 39	2
Oxygenation (PaO2/FiO2)	
> 240 or ARDS diagnosis	0
< 240 and no ARDS diagnosis	2
White Blood Cell Count (mm^3)	
> 4000 and < 11000	0
< 4000 or > 11000	1
< 4000 or > 11000 and bands > 500	2
Chest Radiograph	
No infiltrate	0
Diffuse infiltrate	1
Localized infiltrate	2
Tracheal Secretions	
Scant	0
Present	1
Present and purulent	2
Tracheal Aspirate Culture	
Single pathogen or no growth	0
Multiple pathogens	1
Multiple pathogens and + gram stain	2

Table 3:

Criteria for the Chest Radiograph Scoring System.

Chest Radiograph Scoring System (CXRAY) Range 0–6		
General Impression		**Points**
	First film/no change	0
	Worse	1
Pleural Effusion		
	No	0
	Yes	1
Infiltrate		
	No	0
	Diffuse	1
	Localized	2
Consolidation		
	No	0
	Yes	1
	Yes with cavitation	2

Table 4:

Geometric mean, median, and geometric standard deviation for cytokines.

Cytokine	Total obs	Geometric Mean (pg/mL)	Median (pg/mL)	Geometric SD
IFN-γ	128	1.73	1.84	1.48
IL-1β	115	0.58	0.48	2.31
IL-2	129	0.48	0.49	1.73
IL-6	121	0.23	0.11	4.85
IL-13	119	1.57	1.58	2.19
TNF-α	126	0.13	0.09	4.43

Table 5:

Associations between outcomes used in (GEE) model and cytokines. Beta = log odds ratio; CPIS = Clinical Pulmonary Infection Score; CXRAY = chest x-ray score; LCI = lower 95% confidence interval; OR = odds ratio; SE = standard error; UCI = upper 95% confidence interval.

Cytokine	Outcome	Beta	SE	P-value	OR	OR LCI	OR UCI
IL-2	CXRAY	0.52	0.22	0.02	1.68	1.09	2.60
IL-13	CXRAY	0.48	0.27	0.07	1.62	0.96	2.74
IL-13	CPIS	0.41	0.23	0.08	1.51	0.96	2.39
IL-2	CPIS	0.20	0.17	0.23	1.22	0.88	1.69
IL-6	CXRAY	0.18	0.22	0.42	1.20	0.77	1.86
TNF-α	CXRAY	−0.18	0.24	0.46	0.84	0.52	1.35
IL-6	CPIS	−0.09	0.22	0.68	0.91	0.60	1.40
IL-1β	CPIS	−0.07	0.24	0.78	0.93	0.58	1.51
IFN-γ	CPIS	−0.04	0.19	0.84	0.96	0.66	1.41
IFN-γ	CXRAY	0.03	0.21	0.89	1.03	0.68	1.56
IL-1β	CXRAY	0.03	0.22	0.89	1.03	0.67	1.57
TNF-α	CPIS	0.01	0.20	0.98	1.01	0.68	1.48

Table 6:

Associations between cytokines and outcomes used in linear model based on the first day of EBC collection for each cytokine. Beta = log odds ratio; LCI = lower 95% confidence interval; OR = odds ratio; SE = standard error; UCI = upper 95% confidence interval.

Cytokine	outcome	beta	SE	P-value	OR	OR LCI	OR UCI
TNF-α	Pneumonia	1.16	0.51	0.02	3.20	1.19	8.65
IL-1β	Sepsis	−1.11	0.56	0.05	0.33	0.11	0.98
IL-2	Sepsis	−0.69	0.41	0.09	0.50	0.23	1.11
IL-6	Pneumonia	0.64	0.42	0.13	1.90	0.83	4.32
IL-1β	Pneumonia	−0.73	0.55	0.19	0.48	0.16	1.42
IL-13	Sepsis	−0.47	0.39	0.22	0.62	0.29	1.33
IL-1β	Death	−0.40	0.46	0.39	0.67	0.27	1.66
IL-6	Sepsis	−0.48	0.59	0.41	0.62	0.20	1.96
TNF-α	Sepsis	−0.42	0.55	0.45	0.66	0.22	1.93
IFN-γ	Sepsis	−0.30	0.42	0.47	0.74	0.32	1.69
IL-2	Pneumonia	−0.27	0.39	0.48	0.76	0.35	1.63
IFN-γ	Death	0.26	0.43	0.54	1.30	0.56	3.05
IL-2	Death	0.10	0.39	0.80	1.11	0.51	2.38
IFN-γ	Pneumonia	−0.08	0.43	0.84	0.92	0.40	2.13
IL-13	Death	−0.03	0.37	0.94	0.97	0.47	2.01
IL-13	Pneumonia	−0.02	0.44	0.96	0.98	0.41	2.34
IL-6	Death	0.01	0.45	0.98	1.01	0.42	2.44
TNF-α	Death	0.00	0.40	1.00	1.00	0.45	2.21

Table 7:

Associations between cytokines and outcomes used in linear model based on the first day of Pneumonia or Sepsis diagnosis. Beta = log odds ratio; LCI = lower 95% confidence interval; OR = odds ratio; SE = standard error; UCI = upper 95% confidence interval.

Cytokine	outcome	beta	se	P-value	OR	OR LCI	OR UCI
TNF-α	Pneumonia	1.07	0.53	0.04	2.93	1.04	8.21
IL-2	Sepsis	−1.05	0.54	0.05	0.35	0.12	1.00
IL-13	Sepsis	−0.78	0.49	0.12	0.46	0.17	1.21
IL-1β	Pneumonia	−0.81	0.56	0.14	0.44	0.15	1.31
IL-1β	Sepsis	−0.83	0.60	0.17	0.43	0.13	1.42
IL-6	Pneumonia	0.44	0.45	0.34	1.55	0.64	3.76
IL-2	Pneumonia	−0.33	0.38	0.38	0.72	0.34	1.51
IL-1β	Death	−0.43	0.51	0.40	0.65	0.24	1.77
IL-6	Sepsis	−0.43	0.59	0.46	0.65	0.21	2.06
IL-13	Pneumonia	−0.30	0.44	0.50	0.74	0.31	1.77
IL-13	Death	−0.30	0.45	0.50	0.74	0.31	1.78
IFN-γ	Sepsis	−0.26	0.44	0.56	0.77	0.33	1.83
IFN-γ	Death	0.26	0.46	0.57	1.29	0.53	3.17
TNF-α	Sepsis	−0.25	0.59	0.67	0.78	0.24	2.49
IL-6	Death	0.17	0.47	0.71	1.19	0.47	2.98
IFN-γ	Pneumonia	−0.16	0.44	0.72	0.85	0.36	2.01
TNF-α	Death	0.14	0.46	0.75	1.15	0.47	2.82
IL-2	Death	−0.02	0.40	0.96	0.98	0.45	2.15