Pre-infection Systemic Inflammatory Markers And Risk of Hospitalization due to Pneumonia

Online Supplement

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Running head: Inflammatory markers and pneumonia

Methods (Online supplement)

Outcome measure

The primary outcome was CAP requiring hospitalization. All participants were asked to report all hospitalizations for ≥24 hours to the study personnel. Additionally, every 6 months, study personnel surveyed participants regarding recent hospitalization, often uncovering unreported additional events. Adjudication of CAP was performed by an adjudicator at each site and criteria to adjudicate CAP were established prospectively. Adjudicators were blinded to inflammatory marker levels. We used a combination of discharge summary, ICD-9 diagnoses, admission history and physical examination, and radiology reports for each hospitalization. Although previous studies have relied on each of these reporting strategies individually (1,2), this combined approach was chosen to reduce the likelihood of error in estimating CAP events. The diagnosis of pneumonia was based on combination of symptoms of fever, cough, and sputum production, rales and/or dullness on percussion during physical examination, and chest x-ray findings of consolidation or cavitation, with or without pleural effusion. In addition the following ICD-9 codes were used: 481 (pneumococcal pneumonia), 482.0 (pneumonia due to Klebsiella pneumoniae), 482.1 (pneumonia due to Pseudomonas), 482.2 (pneumonia due to Hemophilus influenzae), 482.3 (pneumonia due to Streptococcus), 482.4 (pneumonia due to Staphylococcus), 482.83 (pneumonia due to other gram negatives), 482.9 (bacterial pneumonia, unspecified), 483.0 (pneumonia due to Mycoplasma pneumoniae), 486 (pneumonia, organism unspecified), and 507.0 (aspiration pneumonia). Once a diagnosis of pneumonia was established, the adjudicator also determined the primary reason for hospitalization. To exclude nosocomial pneumonia events, pneumonia was

considered to be 'community-acquired' only if it was the primary reason for hospitalization. Results of bacteriology for etiology of CAP were available in fewer than 5% of CAP cases.

Inflammatory markers

Blood samples were obtained in the morning (mean time 0925 hours). After processing, the specimens were aliquoted into cryovials, frozen at -70ºC, and shipped to the Core Laboratory. Citrated plasma was used for analysis of TNF and IL-6 assays, and special coagulation (SCAT-I) plasma for CRP (3). Plasma TNF and IL-6 levels were measured in duplicate by ELISA kits (R&D Systems, Minneapolis, MN) using HS600 Quantikine and HSTA50 kits, respectively. Serum levels of CRP were also measured in duplicate by ELISA based on purified protein and polyclonal anti-CRP antibodies (Calbiochem, EMD Biosciences Inc, Darmstadt, Germany). The CRP assay was standardized according to the World Health Organization First International Reference Standard. TNF, IL-6, and CRP data were missing in 6.6%, 5.3%, and 2.3% participants, respectively.

Statistical analysis

Kaplan Meier survival curves and failure plots were plotted to ascertain relationship between tertiles of inflammatory markers and CAP events (Figure 1). However, the assumptions of the Cox proportional hazards model were not met. Therefore, the logistic regression model was used for multivariable analysis. Each inflammatory marker was entered individually, and those markers that were associated with CAP susceptibility were entered concurrently in the model. Covariates for the logistic regression model

were chosen a priori, based on previous studies and biological possibility of confounding the association. They included age, race, gender, site, smoking status, serum creatinine, FEV_1, use of oral steroids, and a history of congestive heart failure, coronary heart disease, and diabetes. Nutritional markers and lean body mass were not significant in the univariate analysis. A second logistic regression model was constructed to ascertain role of body mass index and lean body mass. These variables were not added to the original model to avoid overfitting the model. Model fit was ascertained by the Hosmer-Lemeshow test with $P>0.05$. All analysis was performed using SAS 9.0 (SAS Institute, Cary, NC)

Results (Online supplement)

A second logistic regression model was constructed with body mass index and lean body mass. Race and site were excluded from this model. The association between highest tertile of TNF and IL-6 and susceptibility to hospitalization for CAP remained significant (OR=1.7 and 2, respectively) [Table 5] and estimates were similar to those reported in table 3.

The 188 participants who reported a respiratory tract infection up to 2 weeks prior to the blood draw could confound the association between inflammatory markers and increased risk of CAP. We constructed a logistic regression model after excluding these participants and re-estimated risk of CAP. Again, the risk estimates remained similar.

References (Online supplement)

E1. Jokinen, C., Heiskanen, L., Juvonen, H., Kallinen, S., Karkola, K., Korppi, M., Kurki, S., Ronnberg, P. R., Seppa, A., Soimakallio, S. and et al. Incidence of community-acquired pneumonia in the population of four municipalities in eastern Finland. *Am J Epidemiol* 1993;137:977-988.

E2. Guevara, R. E., Butler, J. C., Marston, B. J., Plouffe, J. F., File, T. M., Jr., Breiman, R. F. Accuracy of ICD-9-CM codes in detecting community-acquired pneumococcal pneumonia for incidence and vaccine efficacy studies. *Am J Epidemiol* 1999;149:282-289.

E3. Macy, E. M., Hayes, T. E., Tracy, R. P. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clin Chem* 1997;43:52-58.

Tables (Online supplement)

Table E1. Adjusted risk* of susceptibility to Community-acquired Pneumonia for inflammatory markers after adjusting for nutritional markers

Variable	**Adjusted odds ratio with 95% CI**
TNF tertile III vs tertile I	1.8 (1.1-2.9)
TNF tertile II vs tertile I	1.5 (0.9-2.5)
IL-6 tertile III vs tertile I	1.7 (1.04-2.8)
IL-6 tertile II vs tertile I	1.3 (0.8-2.1)
Severity of lung disease	
Severe ($FEV_1 \leq 50\%$)	3.3 (2.1-5.2)
Moderate ($65 > FEV_1 > 50$)	1.6 (0.8-3.1)
Mild ($80 > FEV_1 \geq 65$)	1.3 (0.8-2.2)
History of coronary artery disease	1.2 (0.8-1.7)
History of congestive heart failure	1.4 (0.7-3)
Serum creatinine>1.5 mg/dl	1.2 (0.6-2.5)
History of diabetes	1.3 (0.8-2)
Smoking status (ever smoked vs never smoked)	1.4 (0.97-2.1)
Oral steroid use	1.4 (0.6-3.4)
Age (per year increase)	1.0 (0.9-1.1)
Gender (males vs females)	0.7 (0.4-1.4)
Body mass index (per kg/m^2 increase)	0.99 (0.93-1.04)
Lean body mass (per kg increase)	1 (0.99-1.01)

*Adjusted for age, gender, lean body mass, body mass index, congestive heart failure, coronary artery disease, smoking status, diabetes, renal failure, oral steroid use, and FEV_1. Only 2516 participants and 139 cases of community acquired pneumonia included in the adjusted model due to missing data for exposure variables and covariates. Both inflammatory markers were entered in the logistic regression model concurrently

Table E2. Adjusted risk* of susceptibility to Community-acquired Pneumonia for inflammatory markers after excluding participants reporting respiratory tract infection prior to the blood draw

Variable	**Adjusted odds ratio with 95% CI**
TNF tertile III vs tertile I	1.7 (1.01-2.8)
TNF tertile II vs tertile I	1.3 (0.8-2.2)
IL-6 tertile III vs tertile I	1.7 (1.1-2.9)
IL-6 tertile II vs tertile I	1.3 (0.8-2.2)
Severity of lung disease	
Severe ($FEV_1 \leq 50\%$)	3.1 (1.9-5)
Moderate ($65 > FEV_1 > 50$)	1.5 (0.7-3.2)
Mild ($80 > FEV_1 \geq 65$)	1.3 (0.8-2.2)

*Adjusted for age, race, site, gender, congestive heart failure, coronary artery disease, smoking status, diabetes, renal failure, oral steroid use, and FEV_1. Only 2511 participants and 127 cases of community acquired pneumonia included in the adjusted model due to missing data for exposure variables and covariates. Both inflammatory markers were entered in the logistic regression model concurrently