Response of C-Reactive Protein and Serum Amyloid A to Influenza A Infection in Older Adults

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Influenza epidemics are associated with significant morbidity and mortality in the elderly, with a substantial proportion of deaths due to cardiovascular events. Elevations of acute-phase proteins have been associated with an increased risk of atherosclerotic events. Therefore, serum amyloid A (SAA) and C-reactive protein (CRP) were measured during influenza illness and 4 weeks later in 7 young persons, 15 elderly outpatients, and 36 hospitalized adults. Striking elevations were seen in mean acute SAA and CRP levels in all groups, but hospitalized patients had the highest levels (SAA, 503 vs. 310 µg/mL [P = .006]; CRP, 120 vs. 34 µg/mL [P < .001]). The presence of dyspnea, wheezing, and fever was also associated with high CRP levels. Influenza infection is associated with significant elevations of SAA and CRP levels in elderly patients, especially those who require hospitalization. It is possible that direct effects of CRP may exacerbate preexisting atherosclerotic lesions and may help explain cardiovascular events associated with acute influenza.

Patients and Methods

Patients. Patients with possible influenza A were recruited in Rochester, NY, between November and April during the winters of 1996–1998. Elderly adults and the staff members of a senior day care center were invited to participate in a prospective surveillance for respiratory illnesses. Elderly adults who attended day care were physically frail and were eligible for nursing home care, by New York State standards. Most of the day care staff members were healthy young women. During the winter of 1998, patients >65 years old or patients with chronic heart or lung disease admitted to the hospital with acute cardiopulmonary illness, exclusive of angina and myocardial infarction (MI), also were recruited. Study nurses evaluated patients in the prospective surveillance when they experienced >3 of the following symptoms: nasal congestion, sore throat, new or increased cough, wheezing, sputum production, or respiratory difficulty. One or 2 symptoms plus fever, abnormal chest examination results, or illness that required a physician visit also warranted an evaluation. Patients with known inflammatory conditions or metastatic malignancies were excluded. Evaluations consisted of a medical history and physical examination, SaO2 level measurement, nasopharyngeal swab sampling for virus culture, and acute and convalescent serum samples (4–6 weeks) for laboratory analysis. Bacterial sputum samples were obtained for culture from patients when possible.

Laboratory methods. Specimens for viral culture were transported to the laboratory on ice, were inoculated onto Rhesus monkey kidney cells, and were examined for cytopathic effect for 10 days. Hemadsorption was done on day 10. Influenza A infection was confirmed with influenza A–specific monoclonal antibodies...
(Baxter Healthcare). Acute and convalescent serum samples were tested for influenza-specific IgG to whole virus antigen by EIA. Influenza infection was defined as a positive viral culture or a ≥4-fold increase in influenza titers. SAA and CRP levels were measured within the first week of symptoms and 4–6 weeks later in the prospective surveillance groups. Admission and convalescent specimens were measured in hospitalized patients. SAA levels were determined by using the Cytoscreen immunoassay kit (Biosource International): the range of values was 10–600 μg/mL. CRP levels were measured by use of a human CRP ELISA kit (AngioMax; Angiopharm) with a minimum detectable value of 0.15 μg/mL. Normal SAA and CRP levels are considered to be ≤10 μg/mL.

**Statistical methods.** The Student’s t test, with a Welch correction factor, as appropriate, was used to compare means. The χ² analysis was used to compare proportions. For analysis of variance (ANOVA) and multiple regression, data were analyzed on both the linear and the log scales. A 1-way ANOVA with the Tukey-Kramer test was done to compare the clinical variables of the 3 study groups. Regression analysis was done with stepwise model selection of variables affecting acute CRP levels. The following variables were used in the regression analysis: culture status, age, sex, chronic obstructive pulmonary disease (COPD), Katz functional score, dyspnea, number of days with symptoms, wheezing, temperature, respiratory rate, SaO₂ level, hospitalization, use of steroids or antibiotics, and pneumonia.

### Results

Ninety staff members and 214 elderly attendees of a senior day care center participated in the prospective surveillance. Influenza A infections were documented in 7 (7.8%) of 90 staff members and in 16 (7.5%) of 214 elderly patients. One hundred twenty-nine patients admitted to the hospital with acute respiratory illnesses were recruited. Forty-eight (37%) were diagnosed with influenza A, and 37 of these patients had serum samples available. One elderly day care attendee and 1 hospitalized patient were excluded because of underlying rheumatoid arthritis. Documented bacterial infections were rare. None of the staff members or nonhospitalized elderly patients had sputum or blood samples cultured. Among the 36 hospitalized patients with influenza, 15 (42%) had sputum samples cultured, of which 10 were considered to be of good quality. Three specimens grew *Streptococcus pneumoniae*, 1 of which was also culture positive for influenza virus. Blood samples obtained from 23 patients (64%) had negative culture results. The 3 hospitalized patients who had *S. pneumoniae* isolated in their sputum were excluded from the SAA and CRP analyses because of the potential confounding effects of bacterial infection.

Thus, 55 patients with influenza A infections were analyzed for SAA and CRP levels (table 1): 28 (51%) had illnesses confirmed by positive culture results, whereas 27 were seropositive only. Influenza isolates in the community for each of the 3 seasons were identified as H3N2 viruses. Illnesses were relatively mild in the prospective groups, with the hospitalization of only 1 elderly day care attendee. As expected, illnesses were significantly more severe in the hospitalized patients, as evidenced by hypoxia, the presence of pneumonia, and the use of steroids. The average duration of symptoms before evaluation was 3.6 days, with no significant difference among groups.

Elevations in mean CRP and SAA levels occurred during acute illness in all 3 groups. The differences between acute and convalescent specimens were highly significant (P < .001) for both SAA and CRP levels in the day care elderly patients and hospitalized adults. In all groups, there was a broad range of responses (figure 1). Thirty-one (56%) of 55 patients had acute SAA levels ≥600 μg/mL, whereas only 5 (9%) of 58 had levels ≤10 μg/mL. Nineteen patients (35%) had CRP levels ≥100 μg/mL, whereas only 10% had levels ≤10 μg/mL. SAA and CRP levels correlated well (r = .65). At convalescence, SAA levels returned to normal in 24 (44%) of 55 patients and were markedly diminished (<70 μg/mL) in 21 patients (38%). CRP levels returned to ≤10 μg/mL in 36 (65%) of 55 patients. Three patients had persistently high SAA (≥600 μg/mL) and CRP (163, 447, and 447 μg/mL, respectively) levels at 4 weeks. Although there were no clinically distinguishing features to their illnesses, these 3 patients all had underlying COPD. The 29 influenza cases with positive culture results were clinically indistinguishable from the cases that were seropositive only. In addition, the patient characteristics of the 2 groups were the same. The mean CRP levels were higher in the culture-positive group, compared with the culture-negative group, but these differences were not significantly higher.

### Table 1. Patient and illness characteristics and acute- and convalescent-phase serum amyloid A (SAA) and C-reactive protein (CRP) levels in 55 patients with influenza A infections.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Staff members (n = 7)</th>
<th>Elderly day care attendees (n = 15)</th>
<th>Hospitalized adults (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD protein level, μg/mL</td>
<td></td>
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<td></td>
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<tr>
<td>SAA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acute phase</td>
<td>205 ± 276</td>
<td>378 ± 263</td>
<td>501 ± 163</td>
</tr>
<tr>
<td>Convalescent phase</td>
<td>19 ± 24</td>
<td>45 ± 85</td>
<td>87 ± 175</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute phase</td>
<td>19 ± 25</td>
<td>40 ± 30</td>
<td>123 ± 113</td>
</tr>
<tr>
<td>Convalescent phase</td>
<td>4 ± 6</td>
<td>7 ± 6</td>
<td>42 ± 109</td>
</tr>
</tbody>
</table>

**NOTE.** Normal SAA and CRP levels are ≤10 μg/mL. CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease.  

a. P < .01, staff members vs. elderly day care attendees vs. hospitalized adults.  
b. P < .01, staff members vs. hospitalized adults.  
c. P < .01, hospitalized adults vs. staff members and elderly day care attendees.  
d. P < .05, staff members vs. elderly day care attendees vs. hospitalized adults.  
e. P < .01, hospitalized adults vs. elderly day care attendees.
Figure 1. Acute (○) and convalescent (□) levels of serum amyloid A (SAA; A) and C-reactive protein (CRP; B) for individual patients in each of the 3 study groups. Normal levels for both SAA and CRP are <10 μg/mL. Conv, convalescent.

statistically significant (acute level, 124 vs. 67 μg/mL; convalescent level, 48 vs. 8 μg/mL).

In stepwise regression analysis of variables that affect acute CRP, hospitalization was the most significant, with a parameter estimate of 1.9 ($P = .0009$). The mean acute CRP level in the 34 hospitalized patients was significantly higher than that in the 21 nonhospitalized patients (120 vs. 34 μg/mL, $P < .001$).

The presence of wheezing ($P = .02$), dyspnea ($P = .03$), and temperature ($P = .04$) also had significant impact in the model.

Because the presence or absence of COPD was a potential confounder, these 2 groups were analyzed separately also. Acute CRP levels were significantly higher in persons with COPD than in those without (149 vs. 57 μg/mL, $P = .01$). Patients with COPD were more likely to be in the hospitalized group...
older patients’ inflammatory response to infection with specific pathogens. In a recent report, older age correlated with prolonged circulation of inflammatory cytokines after pneumococcal pneumonia [10]. Age-related immune dysregulation is associated with a shift from a Th1 to a Th2 type cellular response, with increased production of pro-inflammatory cytokines, such as tumor necrosis factor, interleukin (IL)–1, and IL-6, which stimulates hepatic synthesis of CRP [11, 12]. Although the interaction between cytokines and immune cells is complex in the elderly, the net impact of aging may result in a less specific immune response with a greater and more harmful inflammatory reaction.

There is evidence that infection, particularly respiratory infection, is linked with ischemic events. Several case-control studies have shown that patients with acute MI and cerebrovascular accidents had a higher incidence of infection in the preceding month than did age- and sex-matched control subjects [2, 13, 14]. In a large study of 1922 patients with MI, the rate of acute respiratory tract infection in the 10 days before the MI was 2.7 times greater than that in 7649 matched control subjects [2]. Chronically elevated levels of acute-phase reactants, such as CRP and SAA, have been epidemiologically correlated with an increased risk of acute coronary and cerebrovascular thrombosis [3, 4, 15, 16]. Although the link between acute infection and the risk of thrombotic events is highly exploratory in nature, some interesting questions may be raised. CRP may directly exacerbate vascular inflammation by activating complement and inducing inflammatory cytokines from Th2 cells [3, 17]. In addition, CRP induces tissue factor expression by monocytes, thereby increasing procoagulant activity [18]. The effect of abrupt transient increases in CRP and SAA levels during acute infection on preexisting atherosclerotic lesions and the subsequent risk for vascular events is unknown.

In summary, influenza A infection in adults is associated with significant increases in the acute-phase reactants SAA and CRP. The magnitude of the response appears to be greatest in older patients with severe illness that requires hospitalization. In view of evidence that CRP may directly contribute to acute vascular events, the magnitude of the response may be important, and further study is warranted.

Acknowledgments

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References


