Serum Biomarkers for Identifying Acute Chest Syndrome Among Patients Who Have Sickle Cell Disease and Present to the Emergency Department

James T. Naprawa, MD; Bema K. Bonsu, MBChB; Deborah G. Goodman, RN; and Mark A. Ranalli, MD

ABSTRACT. Objective. To compare the accuracy of biomarkers for identifying acute chest syndrome (ACS) in patients with sickle cell disease presenting to a pediatric emergency department (ED).

Methods. We conducted a 13-month-long (2002–2003) cohort study with nested case-control in patients with sickle cell disease presenting to the pediatric ED with vaso-occlusive crises or fever in which we compared levels of secretory phospholipase A2 (sPLA2), endothelin-1, interleukin-6 (IL-6), and peripheral white blood cell count (WBC) in cases that were complicated by ACS and in control subjects with uncomplicated illnesses. For diagnosis, a test was considered to be accurate when the area under its receiver operator characteristic curve (AUC) was >0.70. Laboratory tests with AUC values ≥0.70 were entered into a binary recursive partitioning model for diagnosis.

Results. For the period of study, samples from 72 visits were obtained from 51 patients who presented with vaso-occlusive crises (range: 1–4 visits per patient; 15 were enrolled more than once). ACS complicated 19 of 72 visits (26%, 95% confidence interval: 17%–38%). At an AUC value of 0.79, only the sPLA2 test was accurate for diagnosing ACS. AUC values for peripheral WBC, endothelin-1, and IL-6 were 0.68, 0.51, and 0.52, respectively. Binary recursive partitioning retained only sPLA2 at a cutoff of 13.7 ng/mL to be accurate for diagnosis. This cutoff had a sensitivity of 74% (14 of 19), a specificity of 87% (46 of 53), a positive likelihood ratio of 5.6, and a negative likelihood ratio of 0.18.

Conclusions. Secretory phospholipase A2 but not endothelin-1, IL-6, or WBC is an accurate test for identifying present or incipient ACS in young patients who present to the ED with sickle cell pain crisis.

ABBREVIATIONS. ACS, acute chest syndrome; SCD, sickle cell disease; VOC, vaso-occlusive crises; sPLA2, secretory phospholipase A2; IL, interleukin; ED, emergency department; WBC, white blood cell count; ROC, receiver operator characteristic; AUC, area under the curve; SaO2, oxygen saturation.

From the *Department of Medicine, Division of Emergency Medicine, and the ‡Division of Hematology-Oncology, Children’s Hospital, Columbus, Ohio.

Accepted for publication Mar 31, 2005.

doi:10.1542/peds.2004-2107

No conflict of interest declared.

Reprint requests to (J.T.N.) Children’s Hospital, Division of Emergency Medicine, 700 Children’s Dr, OH 43205. E-mail: naprawa@pediatrics.osu-state.edu

PEDIATRICS (ISSN 0031 4005). Copyright © 2005 by the American Academy of Pediatrics.
The clinical treatment of patients was unaffected by their decision to participate in the study and routinely included hydration and pain management. For patients who agreed to participate in the study, 3 mL of blood was obtained in tubes that contained calcium ethylenediaminetetraacetic acid. Plasma from the sample was stored in calcium ethylenediaminetetraacetic acid–containing tubes at a temperature of −80°C. At the end of the study, sPLA2, IL-6, and endothelin-1 levels for each sample were measured by an off-site laboratory (Cayman Chemical, Ann Arbor, MI). Samples were diluted 1:20 and 1:50 in EIA buffer and assayed in triplicate. An enzyme immunometric assay was used to measure the level of each biomarker in each sample. Samples for which test absorbencies fell outside the range of standard curves were assayed a second time at appropriately lower (1:5 and 1:10) or higher (1:100 and 1:200) dilutions. Assay results were not available until the end of the study and so were not used in the evaluation and treatment of participants.

Chest radiographs were obtained at the discretion of the attending ED physician. At our institution, chest radiographs are performed only when warranted by the clinical examination. Because of the cumulative risk from repeated exposure to ionizing radiation, radiographs are generally performed for chest pain, fever, hypoxia, difficulty breathing, and auscultatory findings that suggest pulmonary air space disease. We assumed, however, that all cases of ACS missed or incipient at the initial clinical encounter would progress in severity, necessitating a return visit to the ED or clinic within 7 days of discharge with symptoms that would prompt the ordering of a chest radiograph. Thus, if ACS was not diagnosed at the ED visit or within 7 days of discharge, we assumed that it was not present at enrollment or was clinically indiscernible. We gave a designation of overt ACS to patients who had a new pulmonary infiltrate(s) at the time of enrollment in the ED. Likewise, a designation of occult or incipient ACS was given to patients when ACS was potentially missed by the clinical examination or absent at the first ED encounter but reported during a 7-day interval after discharge from the ED, ie, during hospitalization or at a follow-up visit to the ED or hematology clinic.

The medical chart of each participant was reviewed for data concerning the chief complaint, respiratory symptoms, fever, peripheral blood white blood cell (WBC) count, chest radiograph report, admission to hospital, hospital course, duration of hospitalization, discharge diagnosis, and complications after enrollment to the study. The investigator who abstracted clinical data was blinded to the results of serum biomarkers. The diagnostic performance of sPLA2, IL-6, endothelin-1, and the peripheral WBC for ACS was estimated by calculating the area under the receiver operator characteristic (ROC) curves (AUCs) of these tests. The ROC curve plots sensitivity versus 1 − specificity (likelihood ratio) for each predicted value of a test. The AUC determines the diagnostic value of a given test. An AUC value of 0.5 indicates that the test has no discriminatory ability, whereas an AUC value of 1.0 indicates perfect diagnostic capability. We adopted a nominal AUC value of 0.7 to be the lowest acceptable threshold for stating that a test had discriminatory value. Tests with an AUC value >0.7 were entered into a binary recursive partitioning analysis. This tree-based analytic tool uses a nonparametric algorithm that balances the cost/complexity of adding successive binary splits of the data against improvements in discriminatory performance to optimize diagnostic efficiency. The process creates an overly complex tree that tends to over-fit the observed data. For increasing the parsimony, internal validity, and likely fit of the model to new settings, a cross-validation process is used to prune unstable branches of the tree. Sensitivity, specificity, and positive and negative likelihood ratios are calculated for the resultant tree. We considered each assay/visit to be independent for the purpose of estimating these indices. Last, for participants who enrolled on >1 occasion, we determined whether levels of diagnostic tests differed for visits with dissimilar outcomes.

Data were analyzed with the Stata statistical package (version 8.2; Stata Corp, College Station, TX) and for recursive partitioning with the RPART routine of the R statistical program (R Foundation for Statistical Computing, Vienna, Austria; www.R-project.org). The institutional review board of the hospital approved the study.

RESULTS

Blood samples were collected from 52 children who presented to the ED on 81 occasions (1–4 visits per child; 15 enrolled more than once). Samples were collected in the ED except for 1 obtained in the outpatient hematology clinic. Five samples were excluded because they were extraneous samples obtained from hospitalized participants who already had a study specimen collected in the ED. Four additional samples were excluded because they were mislabeled. Mislabeled blood samples were not re-drawn, and information for the corresponding visits were excluded from data analysis.

After these 9 visits were removed, 72 visits by 51 patients were retained in the data set for analysis. The median age at retained visits was 12.1 years (interquartile range [IQR]: 6.9–16.6 years; 1 participant at 26.7 years was the only patient who was older than 20 years). Boys constituted 63% (32) of participants. Fever (temperature in triage >38°C) was documented in triage at 10 visits. Arms and legs (30 patients >1 location) were the most common sites of bone pain. Chest radiographs were performed at all visits for fever and at 33 (49%) of 62 visits for VOC at which fever was absent. All visits for fever, and 34 of 62 visits for VOC without fever resulted in admission to the hospital.

ACS complicated 19 (26%) of 72 visits (15 patients). ACS was diagnosed in the ED at 13 visits and within a 7-day interval after such an encounter at 6 visits (during hospitalization at 4 visits and on return to the ED after discharge to home at 2 visits). Table 1 shows demographic and clinical features, as well as results of selected biomarkers at 19 visits that were assigned a diagnosis of ACS. For the remaining 53 visits in 36 patients who did not receive a diagnosis of ACS, ACS was excluded on the basis of the clinical examination without the aid of chest radiographs at 26 (49%) visits. There were no differences in age and gender distributions of patients with and without ACS.

At an AUC value of 0.79 (unchanged after adjusting for intrapatient correlation), only the sPLA2 test exceeded the nominal AUC threshold value of 0.7 (Fig 1). AUC values for other tests were 0.68 for WBC, 0.51 for endothelin-1, and 0.52 for IL-6. The median sPLA2 level among children with ACS was 29.7 ng/mL (IQR: 6.5–53.7 ng/mL). This varied significantly from a median of 5.9 ng/mL (IQR: 1.9–9.2 ng/mL) noted in children without ACS (P = .0002). When entered into the recursive partitioning model, only the sPLA2 test at a level of 13.7 ng/mL was chosen to be optimal for diagnosis with sensitivity of 74% (14 of 19), specificity of 87% (46 of 53), positive likelihood ratio of 5.6, and negative likelihood ratio of 0.18. Peripheral blood WBC, endothelin-1, and IL-6 were not retained for diagnosis after 10-fold cross-validation.

For 53 visits that were not assigned a diagnosis of ACS, pain was the presenting complaint at 49 visits and fever at 4 visits. When pain was the presenting complaint, it was localized to the chest at 13 of 49 visits. Chest radiographs that were performed to
evaluate chest pain at these 13 visits showed no new pulmonary infiltrates. Nine of 13 visits for chest pain resulted in hospitalization for pain management. All were discharged without complications. For the remaining 36 visits with pain localized to other sites, 13 patients were hospitalized for pain management and none developed ACS. Patients of all 4 non-ACS visits for fever had negative chest radiographs in the ED and did not develop ACS on admission or after discharge to home.

Of the 53 visits that were not assigned a diagnosis of ACS, the sPLA2 level was >13.7 ng/mL during 7 visits. The chief complaint in 2 of these visits was chest pain (1 also had a cough). Fever and dyspnea were the chief complaints in a third visit. Fever alone was the chief complaint in a fourth. Each one of these 4 patients had a normal chest radiograph in the ED. The remaining 3 had pain crises alone (no chest pain). None of these patients had a chest radiograph, and none went on to develop overt ACS.

Thirteen of 19 visits that were designated as ACS were established at the initial ED encounter by chest radiographs that revealed a new pulmonary infiltrate. Six (32%) remaining cases in 5 patients (patients 3, 4, 5, 8, and 9; Table 2) were not diagnosed at the initial ED visit but established within 7 days of this encounter—after admission to the hospital or discharge from the ED—and designated occult or

<table>
<thead>
<tr>
<th>Patient Identification</th>
<th>Visit No.</th>
<th>Presenting Complaint(s)</th>
<th>Age, Y</th>
<th>Gender</th>
<th>sPLA2, ng/mL</th>
<th>Peripheral WBC, 1000 Cells/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Abdomen and limb pain, cough</td>
<td>4.1</td>
<td>M</td>
<td>72.35</td>
<td>13.3</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Fever, cough</td>
<td>2.0</td>
<td>M</td>
<td>71.03</td>
<td>33.7</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Fever, headache, cough</td>
<td>10.0</td>
<td>M</td>
<td>14.89</td>
<td>15.6</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Fever, dyspnea</td>
<td>10.9</td>
<td>M</td>
<td>56.52</td>
<td>22.1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>Fever, left knee pain</td>
<td>11.6</td>
<td>F</td>
<td>56.52</td>
<td>17.8</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Back, shoulder, and left knee pain</td>
<td>9.9</td>
<td>M</td>
<td>53.39</td>
<td>14.3</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Chest pain, dyspnea, cough</td>
<td>18.9</td>
<td>M</td>
<td>45.17</td>
<td>24.0</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Fever, chest pain, back pain, dyspnea</td>
<td>17.0</td>
<td>M</td>
<td>31.42</td>
<td>11.1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Leg and arm pain</td>
<td>11.2</td>
<td>M</td>
<td>53.65</td>
<td>17.0</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>Chest and arm pain</td>
<td>11.2</td>
<td>M</td>
<td>31.12</td>
<td>12.9</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>Leg and chest pain</td>
<td>7.1</td>
<td>M</td>
<td>2.44</td>
<td>7.8</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>Chest and back pain</td>
<td>7.1</td>
<td>M</td>
<td>29.74</td>
<td>7.9</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>Chest, back, and extremity pain</td>
<td>15.3</td>
<td>M</td>
<td>22.9</td>
<td>8.7</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>Abdominal pain limb, cough</td>
<td>3.4</td>
<td>M</td>
<td>14.29</td>
<td>22.9</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>Fever, abdominal pain</td>
<td>3.4</td>
<td>F</td>
<td>5.81</td>
<td>23.6</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>Chest and back pain</td>
<td>16.2</td>
<td>M</td>
<td>6.50</td>
<td>23.4</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>Back and leg pain, generalized aches</td>
<td>8.7</td>
<td>F</td>
<td>2.75</td>
<td>17.9</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>Back and extremity pain</td>
<td>15.7</td>
<td>F</td>
<td>2.47</td>
<td>36.5</td>
</tr>
</tbody>
</table>

High sPLA2 levels (>13.7 ng/mL) are in boldface.

Occult or incipient ACS: ACS not diagnosed at the ED visit but diagnosed within the 7-day interval after this visit, ie, ¶ during hospitalization or # at a follow-up ED visit.

![Fig 1. Diagnosing ACS: area under the ROC curve for sPLA2 levels (AUC = 0.79).](http://pediatrics.aappublications.org/Downloaded from http://pediatrics.aappublications.org/)
Patient 3 presented with headache, cough, and fever to 102.8°F. He was hypoxic in the ED (oxygen saturation by pulse oximetry [\(Sa_O_2\)] was 74\% on room air) with diminished breath sounds in the left lung fields. A chest radiograph performed in the ED was normal. A follow-up chest radiograph obtained after hospitalization, 8 hours later, revealed air space disease of both middle lobes, consistent with ACS. His sPLA2 level obtained in the ED was high at 14.89 ng/mL. He received a blood transfusion for ACS.

Patient 4 received a diagnosis of ACS on 2 occasions. She presented on the first occasion with knee pain and fever of 103.4°F. She was admitted for pain and fever of 103.4°F. She was admitted for pain management and subsequently developed respiratory distress and hypoxia on room air (\[Sa_O_2\] 82\%), prompting a chest radiograph, which showed peribronchial thickening but no obvious infiltrates. On day 2, she developed bibasilar pulmonary infiltrates and pleural effusions and received a transfusion of packed red blood cells with rapid improvement in his condition.

Patient 5 was admitted to the hospital with back, shoulder, and knee pain. His sPLA2 level on admission was 53.39 ng/mL. On day 2 of admission, he developed chest and abdominal pain with fevers. Chest radiograph at this time revealed a new left lower lobe infiltrate. Hypoxia (\[Sa_O_2\] 86\%) and worsening anemia on day 3 prompted transfusion of packed red blood cells with rapid improvement in his condition.

Patient 8 was discharged from the ED after presenting with leg and chest pain. No chest radiograph was obtained. His sPLA2 level at the first visit was low at 2.44 ng/mL and would not have permitted early diagnosis. He returned 5 days later with chest, leg, and back pain. A chest radiograph showed a left lower lobe lung infiltrate, and he was admitted to the hospital with ACS. His sPLA2 level at the follow-up visit was high at 53.65 ng/mL.

Last, patient 9 was discharged from the ED after presenting with leg and chest pain. No chest radiograph was obtained. His sPLA2 level at the first visit was low at 2.44 ng/mL and would not have permitted early diagnosis. He returned 5 days later with chest, leg, and back pain. A chest radiograph showed a left lower lobe lung infiltrate, and he was admitted to the hospital with ACS. His sPLA2 level at the follow-up visit was high at a value of 29.74 ng/mL.

Fifteen children were enrolled on 1 occasion for a total of 36 visits. Nine children (enrolled 22 times) had ACS diagnosed on 1 or more occasions at or within 7 days of an enrollment visit for a total of 13 ACS visits. Of 13 visits classified as ACS, 10 (77\%) were associated with high sPLA2 levels. In contrast, all (100\%) 9 visits that were not classified as ACS were associated with a low sPLA2 level (Table 2).

Finally, Table 3 lists sPLA2 levels of 6 remaining patients who were enrolled more than once (total
visits: 14) and were never assigned a diagnosis of ACS. Eleven (79%) of these 14 visits were associated with low sPLA2 levels.

**DISCUSSION**

ACS is the second leading cause of hospitalization in patients with SCD and accounts for up to 25% of deaths associated with this disease (mortality per event is 1%–5%). The term, although applied variably by different authors, is generally used to describe the combination of respiratory symptoms (cough, respiratory distress, and chest pain) and new pulmonary infiltrates on chest radiographs in patients with SCD. When present in SCD patients presenting with fever, it is clinically unsuspected in 61% of cases. The cause of most cases of ACS is unknown, although pulmonary emboli and infection are believed to play important roles. Children who develop ACS have longer hospital stays and frequently require admission to the ICU. ACS may also be associated with development of chronic lung disease and early death in patients with SCD. The cost to the health care system in addition to the emotional strain placed on the patients and their families is enormous.

In a recent study, 48% of all patients who had SCD and developed ACS were initially admitted to the hospital with a diagnosis other than ACS. Early identification of these children is desirable, as it would permit aggressive treatment to be instituted. The identification of high-risk patients would also facilitate research into novel therapies that could abort or ameliorate this syndrome. A promising laboratory test that may achieve these goals is sPLA2. This enzyme cleaves phospholipids to generate free fatty acids and lyso-phospholipids such as arachidonic acid. Arachidonic acid, in turn, is a substrate for a number of proinflammatory mediators such as thromboxanes and leukotrienes. sPLA2 is normally present at low levels in plasma. It increases modestly in patients who do not have SCD and have pneumonia but to a comparatively lower level than is found in children who have SCD and ACS. It is also increased in adult respiratory distress syndrome and has been shown to be a potent mediator of lung inflammation in animal models.

In the present study, we show that this enzyme is predictive of ACS in children who present to the ED with VOC or fever. These results confirm findings by Styles et al., in a smaller study, who reported a dramatic increase in the serum levels of this enzyme 2 to 3 days before clinical manifestations of ACS in 4 patients who were admitted to the hospital for VOC. In that study, sPLA2 levels remained low in 3 other SCD patients who were admitted for VOC and did not develop ACS. Our study adds to these findings by providing an optimal cutoff for sPLA2 of 13.7 ng/mL on the basis of binary recursive partitioning.

In our study, sPLA2 levels >13.7 ng/mL would have permitted earlier identification of ACS in 5 separate cases that were not recognized clinically or by chest radiograph at the initial encounter (Table 2). One case was a child who was discharged to home and returned within 4 days with ACS. Four additional cases were in 3 children (1 who developed the complication twice) who were admitted for VOC or fever and received a diagnosis of ACS during hospitalization. None of these cases was recognized or established clinically and/or by chest radiograph as ACS at discharge from the ED. Routine ordering of sPLA2 in the ED and serial ordering of the test among hospitalized children with initially normal sPLA2 levels may assist in the early detection of occult or incipient ACS. It is possible that early transfusion of packed red blood cells in children who are identified to be at higher risk for ACS on the basis of high sPLA2 levels may abort progression to or attenuate ACS with the additional long-term benefit of decreasing the incidence of chronic lung disease.

Finally, our findings offer clues for defining the cause of ACS (the elucidation of which has proved to be difficult) and for treating patients. Styles et al. proposed that an increase in sPLA2 may indicate a more important role for fat emboli.
marrow of long bones during VOC) as opposed to infection or pulmonary infarction. It is possible that alternative therapies that specifically block the expression of this proinflammatory enzyme may abort progression to ACS in high-risk patients. A drug that has been reported to block the activity of sPLA2 is mepacrine (quinacrine).16,17 Although not tested in children with SCD, animal studies show that this drug attenuates the development of artificially induced acute lung injury.17 Formal investigation of such novel therapies is needed because of the hazards associated with blood transfusions. It does not seem likely that IL-6 and endothelin-1 inhibitors will be beneficial for ACS.

Our study had a number of limitations. Although useful for diagnosis, the sPLA2 assay is not offered widely. In our study, samples were sent to an off-site laboratory. The cost and delay associated with transport of blood specimens limited the utility of the test during the study. The assay, however, is commercially available with a turnaround time of ~3 hours. This makes it a potentially useful test for diagnosis and management. A second limitation is that samples were stored for variable periods of time depending on the time of enrollment. We do not know to what extent storage at low temperatures for varying periods of time may have altered the accuracy of assays; however, we found no relationship between the length of storage and the levels of sPLA2. Future studies are needed to evaluate the performance of sPLA2 levels reported at the point of care and within a time frame that is practical for the management of SCD.

Last, chest radiographs were ordered selectively because of the risk of radiation. This had the side benefit of allowing us to assess the complementary role of tests to the clinical examination in predicting ACS, an assessment that would have been obscured by routine ordering of radiographs. We assumed that cases of ACS that were missed at the initial encounter would progress in severity, necessitating a return visit to the hospital within 7 days of discharge. It is possible, however, that some cases of ACS that were not apparent at the initial visit failed to progress to more overt disease. If so, these cases would be missed by a strategy of selective chest radiographs. This raises the possibility that some participants who had high sPLA2 levels and were not assigned a diagnosis of ACS at the initial encounter may have had or developed clinically occult ACS that resolved spontaneously.

CONCLUSIONS

In this new setting, elevation of sPLA2 in serum identified children with ACS. At a value >13.7 ng/mL, this test was associated with an increased risk for ACS. This information may be useful for informing medical decisions, for promoting novel approaches to treatment, and for fostering research of alternative drugs and treatment approaches in high-risk patients.

ACKNOWLEDGMENT

The authors would like to thank Phil Holt, RN, and the rest of the nursing staff of Columbus Children’s Emergency Department for their help in the collection of samples.

REFERENCES

Serum Biomarkers for Identifying Acute Chest Syndrome Among Patients Who Have Sickle Cell Disease and Present to the Emergency Department
James T. Naprawa, Bema K. Bonsu, Deborah G. Goodman and Mark A. Ranalli

Pediatrics 2005;116:e420
DOI: 10.1542/peds.2004-2107 originally published online August 11, 2005;
Serum Biomarkers for Identifying Acute Chest Syndrome Among Patients Who Have Sickle Cell Disease and Present to the Emergency Department
James T. Naprawa, Bema K. Bonsu, Deborah G. Goodman and Mark A. Ranalli
Pediatrics 2005;116:e420
DOI: 10.1542/peds.2004-2107 originally published online August 11, 2005;

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/116/3/e420