Phospholipase A\textsubscript{2} Levels in Acute Chest Syndrome of Sickle Cell Disease

By Lori A. Styles, Casper G. Schalkwijk, Anton J. Aarman, Elliott P. Vichinsky, Bertram H. Lubin, and Frans A. Kuypers

Acute chest syndrome (ACS) is associated with significant morbidity and is the leading cause of death in patients with sickle cell disease (SCD). Recent reports suggest that bone marrow fat embolism can be detected in many cases of severe ACS. Secretory phospholipase A\textsubscript{2} (sPLA\textsubscript{2}) is an important inflammatory mediator and liberates free fatty acids, which are felt to be responsible for the acute lung injury of the fat embolism syndrome. We measured sPLA\textsubscript{2} levels in 35 SCD patients during 20 admissions for ACS, 10 admissions for vaso-occlusive crisis, and during 12 clinic visits when patients were at the steady state. Eleven non-SCD patients with pneumonia were also evaluated. To determine if there was a relationship between sPLA\textsubscript{2} and the severity of ACS we correlated sPLA\textsubscript{2} levels with the clinical course of the patient. In comparison with normal controls (mean = 3.1 ± 1.1 ng/mL), the non-SCD patients with pneumonia (mean = 68.6 ± 82.9 ng/mL) and all three SCD patient groups had an elevation of sPLA\textsubscript{2} (steady state mean = 10.0 ± 8.4 ng/mL; vaso-occlusive crisis mean = 23.7 ± 40.5 ng/mL; ACS mean = 336 ± 209 ng/mL). In patients with ACS sPLA\textsubscript{2} levels were 100-fold greater than normal control values, 35 times greater than values in SCD patients at baseline, and five times greater than non-SCD patients with pneumonia. The degree of sPLA\textsubscript{2} elevation in ACS correlated with three different measures of clinical severity and, in patients followed sequentially, the rise in sPLA\textsubscript{2} coincided with the onset of ACS. The dramatic elevation of sPLA\textsubscript{2} in patients with ACS but not in patients with vaso-occlusive crisis or non-SCD patients with pneumonia and the correlation between levels of sPLA\textsubscript{2} and clinical severity suggest a role for sPLA\textsubscript{2} in the diagnosis and, perhaps, in the pathophysiology of patients with ACS.

© 1996 by The American Society of Hematology.

A CUTE CHEST SYNDROME (ACS) is the second most common cause of hospitalization and the leading cause of death in sickle cell disease (SCD).\textsuperscript{1,2} A majority of SCD patients will experience at least one episode of ACS\textsuperscript{3,5} and repeated episodes can lead to chronic lung disease.\textsuperscript{4,6} Despite its substantial morbidity and mortality, little is known about the etiology of ACS. Although generally attributed to infection and inflammation, \textsuperscript{10-11} recent evidence suggests that pulmonary fat embolism is a cause of many cases of severe ACS.\textsuperscript{12,13} In patients who do not have SCD, pulmonary fat embolism classically presents as a syndrome with pulmonary disease with hypoxia, mental status changes, and a fall in platelets or hemoglobin.\textsuperscript{13,15} Prospective studies in trauma patients have revealed that although fat embolism occurs in the majority of trauma victims, the above constellation of symptoms occurs in about 10\% of these same patients.\textsuperscript{14,17} This suggests that the "fat embolism syndrome" represents only the most severe form of fat embolism and that pulmonary fat embolism is clinically unrecognized in most cases. The pathophysiology of pulmonary complications associated with fat embolism is incompletely understood, but experimental evidence suggests that the free fatty acids released by the hydrolysis of phospholipids in the embolized fat directly cause acute lung injury.\textsuperscript{15,14,18} Phospholipase A\textsubscript{2} (PLA\textsubscript{2}) is an enzyme that cleaves phospholipids at the sn-2 position generating free fatty acids and lyso-phospholipids. When arachidonic acid is the fatty acid product, a variety of inflammatory mediators including thromboxanes, leukotrienes, and prostaglandins are generated. In addition to free fatty acids these mediators have been implicated in acute lung injury.\textsuperscript{19-22} Secretory PLA\textsubscript{2} (sPLA\textsubscript{2}) is found in low concentration in normal plasma,\textsuperscript{23} however, its levels are increased in response to inflammation.\textsuperscript{24,27} In the acute respiratory distress syndrome (ARDS) sPLA\textsubscript{2} levels are very high.\textsuperscript{26-30} We postulated that sPLA\textsubscript{2} may be important in the pathophysiology of ACS and have measured this enzyme in serum or plasma from SCD patients in steady state, during vaso-occlusive crisis (VOC), and during ACS.

MATERIALS AND METHODS

Patients. The sPLA\textsubscript{2} level was determined in 35 SCD patients. Patients ranged in age from 1 to 20 years (mean = 11 years). All patients had a diagnosis confirmed by standard electrophoresis and isoelectric focusing methods. There were 30 patients with hemoglobin SS, two with hemoglobin SC, and three with hemoglobin S-β thalassemia. Serum sPLA\textsubscript{2} levels were measured during 20 admissions for ACS and 10 admissions for VOC. Four patients were tested during more than one hospitalization. Eleven SCD patients had PLA\textsubscript{2} levels drawn during a comprehensive health care visit in the sickle cell clinic when there was no evidence of illness. There was no overlap in patients between the steady state and ACS groups. Secretory PLA\textsubscript{2} levels were also drawn in 19 normal controls and in 11 children without SCD who were admitted to the hospital with pneumonia.

Hospitalizations. Acute chest syndrome was defined as the development of a new infiltrate on chest radiography in combination with fever, respiratory symptoms, or chest pain. Patients admitted with ACS were treated following a standard protocol that included hydration at one and one-quarter times maintenance, parenteral cefuroxime and oral erythromycin, arterial blood gas monitoring, and daily complete blood counts. Tranfusion was used at the attending physician's discretion based on the patient's clinical course. Intravenous narcotics and nonsteroidal antiinflammatory medications were used to treat accompanying pain events.

Vaso-occlusive crisis was defined as an admission for pain, which required parenteral narcotics and indicated no cause for pain other than SCD. Patients admitted with VOC were treated following a

From the Department of Hematology/Oncology, Children's Hospital Oakland, and the Children's Hospital Oakland Research Institute, Oakland, CA; Centre for Biomembranes and Lipid Enzymology, Department of Lipid Biochemistry, University of Utrecht, Utrecht, The Netherlands.

Submitted April 25, 1995; accepted November 2, 1995.

Supported in part by National Institutes of Health Grants No. HL 20985, HL 27059, DK32094

Address reprint requests to Lori Styles, MD, Department of Hematology/Oncology, Children's Hospital Oakland, 747 52nd St, Oakland, CA 94609.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1996 by The American Society of Hematology.

0006-4971/96/8706-0021$3.00/0

Blood, Vol 87, No 6 (March 15), 1996: pp 2673-2678

2573
protocol that included intravenous hydration, not to exceed one and one-half times maintenance, intravenous narcotics, and nonsteroidal antiinflammatory drugs. If fever developed, patients were evaluated with chest radiography, blood and urine cultures, and intravenous cefuroxime was started.

For the non-SCD patients admitted with pneumonia, diagnosis was established with a chest radiograph 

**Clinical and laboratory assessment.** Clinical and laboratory data were collected on all hospitalized SCD patients including history of preceding or accompanying pain, PaO₂ on room air arterial blood gas, transfusion history, and the presence or absence of fever. Arterial blood gas measurements were determined using an AVL 995 (AVL Scientific Corp, Roswell, GA). Alveolar-arterial oxygen gradient was calculated from room air arterial blood gas values according to the following formula: 

\[ (A - a) \text{PO}_2 = (713 \times \text{FiO}_2) - (\text{PaCO}_2 \times 1.2) - \text{PaO}_2 \]

All sPLA₂ levels were measured using the method described below. Fifteen SCD patients had two or more sPLA₂ level determinations during a single hospital admission. In the patients that were followed with sequential sPLA₂ levels from before the onset of ACS through convalescence, the sample with the highest sPLA₂ value was used in the calculation of statistical significance.

**Secretory PLA₂ activity and concentration.** Phospholipase A₂ activity was measured with 1-acyl-2-[14C]phosphoethanolamine, prepared as described by Van den Bosch et al., as substrate. Enzymatic activity was assayed by incubating 0.2 mmol/L radioactive substrate (specific radioactivity 3,000 dpm/nmol) in 0.2 mol/L Tris/HCL (pH 8.5). 10 mmol/L Ca²⁺ and 5 µL plasma in a final volume of 200 µL. After 30 minutes at 37°C, reactions were stopped by extracting the liberated [14C]-labeled fatty acid with an equal volume of 1 mol/L HClO₄ to each well and the absorbance was read at 490 nm in a microtiter plate reader (EAR 400; SLT-LabInstruments, Austria). Results were compared with those obtained with cultured medium from Hep G2 cells stimulated with human interleukin-6. The amount of sPLA₂ in this cultured medium was assessed by comparison with purified recombinant human sPLA₂ (kindly provided by Dr H.M. Verheij, Department of Enzymology and Protein Engineering, University of Utrecht, Utrecht, the Netherlands). The lower limit of detection was approximately 1 ng/mL and the inter-measurement variability on a single sample was up to 10% to 15%.

Secretory PLA₂ concentration as measured with ELISA in plasma was shown to have an excellent linear correlation with sPLA₂ activity (r² = 0.953), confirming that the sPLA₂ found in the plasma is in an active form (Fig 1). Virtually identical results were found when either plasma or serum was used. Hence, sPLA₂ concentration data was used to analyze the relationship between the presence of active sPLA₂ and ACS. Nineteen normal (hemoglobin AA) controls also had PLA₂ determination to confirm that assay values were in the expected range reported in other series.

**Statistical evaluation.** Statistical evaluation was performed using a nonparametric procedure for the four patient groups (ACS, VOC, SCD at steady state, and non-SCD with pneumonia) using the Kruskal-Wallis One Way Analysis of Variance on Ranks. Dunn’s Method was used to determine if individual group medians were significantly different.

**RESULTS**

Compared with values obtained from control patients, mean sPLA₂ concentrations were elevated in all three SCD patient groups studied (ACS, VOC, and steady state) and in the non-SCD group with pneumonia (Table 1). Steady state SCD patients had a mean sPLA₂ level of 10.0 ± 8.4 ng/mL (median = 9 ng/mL), which was three times higher than values in normal controls (mean = 3.1 ± 1.1 ng/mL, median = 3.1 ng/mL). Sickle cell disease patients with VOC had a similar threefold to fivefold elevation above normal controls. The sPLA₂ concentration of patients with VOC (mean = 23.7

![Graph](https://via.placeholder.com/150)

**Table 1. Secretory PLA₂ Levels in Sickle Cell Disease**

<table>
<thead>
<tr>
<th>Patient Groups</th>
<th>Mean (median) (ng/mL)</th>
<th>Range (ng/mL)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady state SCD (n = 11)</td>
<td>10.0 ± 8.4 (9)</td>
<td>1.1-28.5</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>VOC (n = 10)</td>
<td>23.7 ± 40.5 (8.7)</td>
<td>1.8-134.6</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>ACS (n = 20)</td>
<td>336 ± 209 (289)</td>
<td>12-725</td>
<td>—</td>
</tr>
<tr>
<td>Pneumonia (n = 11)</td>
<td>68.6 ± 82.9 (38)</td>
<td>6-267</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

* P values are for differences between ACS and the other patient groups using analysis of variance techniques (see the Materials and Methods section). All other comparisons are not significant.
Acute chest syndrome patients had a mean sPLA2 level of 336 ± 209 ng/mL (median = 289 ng/mL), which was 100 times greater than normal controls and 35 times greater than in samples from SCD patients in the steady state. Four of the ten patients with VOC had fever during hospitalization. Comparison of the febrile and afebrile groups showed no difference in sPLA2 concentration (P = .45).

In the 15 SCD patients followed with serial sPLA2 measurements, sPLA2 levels seemed to parallel their clinical course. Seven patients with VOC were followed with sequential sPLA2 levels and four of these went on to develop ACS. In all four of these patients, sPLA2 levels rose abruptly with the development of ACS and then decreased as the patient clinically improved (Fig 3). In the three patients who did not develop ACS, sPLA2 levels remained low. The remaining eight patients were admitted with a diagnosis of ACS. Sequential evaluation of sPLA2 concentration in these patients documented that sPLA2 levels were highest with the onset of ACS and declined as the patient recovered.

Secretory PLA2 levels were highest in patients with clinically more severe lung disease as assessed by arterial blood gas results and the need for transfusion. Arterial blood gas measurements in room air were performed on 15 ACS patients. Comparisons of ACS patients with and without significant hypoxia (PaO2 <70 and ≥70 mm Hg) and with and without increased alveolar-arterial O2 gradients (>30 and ≤30 mm Hg) revealed an excellent correlation between elevated sPLA2 clinical severity (Fig 4).

Secretory PLA2 concentration was also compared in the transfused versus untransfused patient groups. One patient from the transfused group was removed from the analysis because he was transfused secondary to aplastic crisis and not due to pulmonary disease. Also, one severely alloimmunized patient was removed from the analysis because, despite

Fig 2. sPLA2 levels in different SCD patient groups. Comparison of mean sPLA2 levels in three different groups of SCD patients and in non-SCD patients with pneumonia. ACS, n = 20; VOC, vaso-occlusive crisis, n = 10; steady state SCD patients at the time of routine comprehensive health care visit, n = 11; non-SCD pneumonia patients, n = 11.

Fig 3. Sequential sPLA2 levels in four SCD patients admitted with VOC. Four patients admitted with VOC were followed with sequential sPLA2 levels and went on to develop ACS. Their levels are shown here. Three patients admitted with VOC and monitored in time did not develop ACS, and sPLA2 levels remained low. Hospital day 0 is the day the diagnosis of ACS was made. Hospital days before and after the day ACS was diagnosed are designated by negative or positive numbers, respectively.

Fig 4. Correlation of sPLA2 levels with measures of severity. Comparison of sPLA2 levels in ACS patients with and without hypoxia (PaO2 ≤70 and >70 mm Hg, respectively), with and without increased alveolar-arterial O2 gradient [(A – a)O2 >30 and ≤30 mm Hg, respectively] and in those who did (+) and did not (−) need transfusion (TXN). Error bars indicate ± 1 SD. N = 15 for all three comparisons. PaO2, partial pressure of oxygen in arterial blood.
severe hypoxia, he could not be transfused secondary due to a lack of compatible blood. Secretory PLA$_2$ levels were significantly higher in the group needing transfusion, suggesting a relationship between sPLA$_2$ concentration and clinical severity (Fig 4).

**DISCUSSION**

Elevated levels of sPLA$_2$ have been reported in ARDS, sepsis, multi-organ dysfunction, and arthritis. Secretory PLA$_2$ is felt to be an important mediator of inflammation in these conditions as it can hydrolyze arachidonic acid from the sn-2 position of phospholipids providing the essential substrate for a number of eicosanoids. Secretory PLA$_2$ is upregulated in response to proinflammatory cytokines such as tumor necrosis factor and interleukin-1. In animal models, PLA$_2$ administered intravenously or instilled intratracheally, produces diffuse ARDS-like changes including diffuse alveolar edema and an inflammatory cell influx. This same lung injury can be prevented by pretreatment with inhibitors of PLA$_2$. In humans, sPLA$_2$ is increased in patients with ARDS and has been shown to correlate with outcome, severity of lung injury, and alveolar-arterial oxygen gradient.

We found dramatically elevated levels of sPLA$_2$ in SCD patients with ACS. Similar elevations were not seen in SCD patients with VOC alone and the presence or absence of fever with pain crisis did not alter this result. Despite the fact that most of these ACS patients were not seriously ill, their sPLA$_2$ levels were similar to that found in critically ill patients with ARDS and sepsis. Additionally, sPLA$_2$ levels in the ACS group were nearly five times greater than levels in non-SCD patients with pneumonia and suggest that sPLA$_2$ elevation is not just a secondary marker for lung damage.

As in ARDS, sPLA$_2$ concentration in patients with ACS correlated with several measures of clinical severity. The correlation between sPLA$_2$ and arterial-alveolar gradient is particularly relevant as Emre et al reported this to be the strongest predictor of clinical severity in ACS. In the ACS patients followed sequentially, the increase in sPLA$_2$ coincided with the onset of ACS and levels declined as the patient improved. In total, these results suggest that there is a relationship between sPLA$_2$ and ACS.

The detection of fat embolism and elevated levels of sPLA$_2$ in ACS suggests a causal relationship between free fatty acids, fatty acid-derived lipid mediators, and ACS. Since vaso-occlusive crisis can result in the intravascular release of bone marrow fat and lead to pulmonary fat embolism, this may trigger the upregulation of sPLA$_2$ and generate more free fatty acids, either systemically or locally in the lung. While the pulmonary toxicity of increased free fatty acids in an in vitro setting, as well as in animal models, is well established, documenting the toxic effects of free fatty acids in vivo has been more difficult. A recent report, however, indicates that both palmitic and oleic acid levels as well as total free fatty acids are elevated in ACS but not in vaso-occlusive crisis and further supports the above hypothesis. Bronchoalveolar lavage (BAL) has recently been demonstrated to be a safe and effective means to detect pulmonary fat embolism. The ACS patients in this study did not undergo BAL but correlating sPLA$_2$ levels with the results of BAL in the future, could also help document a relationship between sPLA$_2$ and fat embolism. Implicating sPLA$_2$ in the pathophysiology of ACS and fat embolism has clinical importance in that sPLA$_2$ may be useful as an early marker for ACS. In the vaso-occlusive crisis patients followed sequentially, sPLA$_2$ levels seemed to increase in the 2 to 3 days before ACS. These data are preliminary, however, and sequential sPLA$_2$ levels on a large number of SCD patients admitted with pain will be necessary to further evaluate sPLA$_2$ usefulness as a predictor for ACS. If sPLA$_2$ proves accurate in predicting ACS, therapies such as transfusion or sPLA$_2$ inhibitors could be considered earlier in disease. Because of the association of sPLA$_2$ with rheumatoid arthritis and sepsis, there is already considerable interest in the development of PLA$_2$ inhibitors.

Our study also demonstrated elevated sPLA$_2$ levels in SCD patients at baseline compared with normal controls. If bone marrow fat embolism is a stimulus for sPLA$_2$, increased baseline sPLA$_2$ levels may reflect ongoing or intermittent leakage of marrow fat intravascularly. Alternatively, higher levels of sPLA$_2$ at baseline may reflect a disturbed balance of inflammatory mediators. In support of this latter hypothesis, reports in SCD patients have documented elevations of endotoxin, tumor necrosis factor and interleukin-1, all known to upregulate sPLA$_2$. Other studies have also reported that SCD patients often have altered levels of lipid mediators related to the action of sPLA$_2$, including prostaglandins, leukotrienes, and thromboxanes.

In summary, we documented dramatically elevated levels of sPLA$_2$ in association with ACS. The rise in sPLA$_2$ coincided with the onset of ACS and the degree of sPLA$_2$ elevation correlated with several measures of clinical severity. In addition to documenting high levels of sPLA$_2$ in ACS, we found that SCD patients have elevated levels at baseline compared with normal controls. The significance of this is unknown at present but may reflect an altered regulation of the inflammatory system. Secretory PLA$_2$ may be useful in identifying patients at risk for ACS and provide justification for the evaluation of additional therapies to treat this complication.

**ACKNOWLEDGMENT**

We are indebted to Jolene Edwards for editorial assistance.

**REFERENCES**

5. DeCuelaer K, McMullen KW, Maude GH, Keatinge R, Ser-
PHOSPHOLIPASE A2 IN ACUTE CHEST SYNDROME


gradient in acute chest syndrome of sickle cell disease. J Pediatr 123:272, 1993
Phospholipase A2 levels in acute chest syndrome of sickle cell disease


Updated information and services can be found at: http://www.bloodjournal.org/content/87/6/2573.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml