Pulmonary platelet thrombi and vascular pathology in acute chest syndrome in patients with sickle cell disease

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A growing body of evidence suggests a role for platelets in sickle cell disease (SCD). Despite the proinflammatory, occlusive nature of platelets, a role for platelets in acute chest syndrome (ACS), however, remains understudied. To provide evidence and potentially describe contributory factors for a putative link between ACS and platelets, we performed an autopsy study of 20 SCD cases—10 of whom died from ACS and 10 whose deaths were not ACS-related. Pulmonary histopathology and case history were collected. We discovered that disseminated pulmonary platelet thrombi were present in 3 out of 10 of cases with ACS, but none of the matched cases without ACS. Those cases with detected thrombi were associated with significant deposition of endothelial vWF and detection of large vWF aggregates adhered to endothelium. Potential clinical risk factors were younger age and higher platelet count at presentation. However, we also noted a sharp and significant decline in platelet count prior to death in each case with platelet thrombi in the lungs. In this study, neither hydroxyurea use nor perimortem transfusion was associated with platelet thrombi. Surprisingly, in all cases, there was profound pulmonary artery remodeling with both thrombotic and proliferative pulmonary plexiform lesions. The severity of remodeling was not associated with a severe history of ACS, or hydroxyurea use, but was inversely correlated with age. We thus provide evidence of undocumented presence of platelet thrombi in cases of fatal ACS and describe clinical correlates. We also provide novel correlates of pulmonary remodeling in SCD.

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Introduction

Sickle cell disease (SCD) is a genetic disease triggered by a point mutation in the β-globin chain of hemoglobin resulting in a glutamic acid in the sixth position of the β-chain instead of valine (Hbs). This illness is an autosomal recessive disorder affecting ~100,000 people in the US alone [1]. There are an estimated 300,000 births per year worldwide (WHO).

One of the leading causes of death in patients is acute chest syndrome (ACS) [2]. The pulmonary manifestations of ACS can appear suddenly, and often progress rapidly to mortality. There are multiple identified etiologies associated with the development of ACS including infection, fat or pulmonary embolism, or opiate intoxication [3]. In most cases, the cause cannot be attributed to a single agent, and if so it is likely determined authoritatively only at autopsy.

One potential commonality is that an acute pain event usually precedes the onset of ACS [3]. Although clearly much remains to be learned, acute pain events are one of the better characterized aspects of SCD. In most cases, there is an increase in inflammatory markers and indicators of endothelial dysfunction [4,5]. Platelet activation increases during pain events, as do platelet-derived markers of inflammation [6]. In fact, platelets are emerging as potentially pivotal contributors to the overall inflammatory state of patients [7]. Hemolysis is a defined activator of platelets [8–10], as is certain bacterial infections [11]. Inflammatory factors from the α-granules of platelets such as CD40L and thrombospondin circulate at higher levels in patients with SCD. These levels increase further as patients enter acute events [12,13].

Changes in platelet count are also associated with acute clinical events, including ACS [14]. Patients with SCD, even at steady state typically have higher platelet counts than those without the illness [15]. However, platelet count typically drops during acute events [14,15], and in some cases thrombocytopenia can occur in ACS [2]. This drop in platelet count is usually attributed to platelet adhesion and sequestration in the vasculature. Although platelet activation increases during the acute events, the mechanism through which this sequestration may occur is also understudied. Nonetheless, the magnitude of the decrease in platelet count is predictive of neurological outcome in ACS [2], so there is clearly merit in exploring the role of platelets during this life-threatening event in patients with SCD.

In illnesses where there is demonstrable platelet sequestration in the vasculature, such as thrombotic thrombocytopenic purpura (TTP), the etiology of platelet activation and sequestration is known [16]. In most cases of TTP, there is a profound inhibition of ADAMTS13—an enzyme that...
Platelet thrombi in the lungs of patients with SCD and ACS at autopsy

Paraffin embedded lung samples from 10 patients with ACS and 10 who died without ACS were analyzed for the presence of platelets. Profound arterial platelet thrombi were detected in 3 out of the 10 fatal cases of ACS. None were noted in the cohort of cases where ACS was not the cause of death. We noted that arterial vessels of all sizes ranging from medium arteries, arterioles, and part of the capillary system had platelet thrombi or were completely occluded by platelets (representative data shown Fig. 1a,b). All platelet positive cases had a genotype of SS. In two of the three cases, there was significant evidence with echocardiography of profound right heart strain. One of those patients exhibited McConnel’s sign (a finding at echocardiography describes a distinct regional pattern of right ventricular dysfunction, with akinesia of the mid free wall, but normal motion at the apex) but no evidence of pulmonary embolism was detected post-mortem [28]. In two of the three cases with platelet thrombi, none of the established causes of ACS [2] were mentioned in the medical record or autopsy report, whilst for one platelet positive case, lipid-laden macrophages in the lung were noted in the autopsy record. The presence of these cells could suggest a fat embolism concurrent with or as causal with the fatal ACS. All platelet positive cases presented with pain as the primary symptom. The platelet occlusion was detected in the middle and inferior lobes of the lung. Identical interrogation of lung tissue was conducted on cases where ACS was not listed as cause of death. No pulmonary platelet thrombi were detected. We did note pulmonary vessel occlusion from erythrocytes (RBCs) in those patients with ACS without platelet thrombi. In Fig. 1c, we highlight the appearance of such an occlusion (left) and also note that in these patients without platelet thrombi, these RBC occlusions fail to stain positive with the anti-CD41 antibody (Fig. 1c middle and left panel).

Clinical variables associated with pulmonary platelet thrombi in ACS

We then sought to examine variables which could account for the accumulation of platelets in the lung during ACS. Although our study includes cases from across adulthood in SCD, there was no difference in median age of the group with ACS and the group without (P = 0.48) (Fig. 2a, top panel). However, one of the most obvious differences between the ACS groups was patient age. The median age for the platelet positive group was 26.0 years [IQR: 23.31], whereas in the platelet negative group the median was 36.0 years [IQR: 23.31] (P = 0.03) (Fig. 2a, bottom panel) suggesting that younger age may be a risk factor for pulmonary platelet occlusions. We next examined platelet counts during the acute and fatal episodes of chest syndrome presented here, and in those from patients with SCD who died from other causes. We were able to abstract at least two platelet counts from 9 out of the 10 total cases—and all three cases with pulmonary platelet thrombi—during the ACS episode leading to their death. Peri-mortem platelet counts were available for 9 out of 10 cases in the negative control group as well. The median time between the two platelet count determinations for each ACS
case was 9.3 hr [IQR = 8.4, 14.2] and 14.2 hr [IQR = 11.4, 16.6] for those without ACS. For those medical records which contained more than two platelet counts, the two most proximal to death were used.

First, we noted that during this acute event those patients with pulmonary platelet thrombi had higher initial platelet counts than those without platelet thrombi [median 581 × 10^3 cells ml^-1, IQR =547,651 for patients with platelet thrombi versus 324 × 10^3 cells ml^-1, IQR = 239,324 for those without (P = 0.023)] (Fig. 2b). Second, we observed a significant drop in platelet count in those patients with pulmonary platelet thrombi (Fig. 2c, right panel, P = 0.01), one of the patients became thrombocytopenic. The group without pulmonary platelet thrombi, however, demonstrated no consistent elevation or drop in platelet count (Fig. 2d, left panel). There was no consistent change in platelet count in the negative control group (Fig. 2c).

**Patient medication and therapy**

We queried each patient’s chart for medication or therapy that might association with the presence of absence of platelet pulmonary thrombi. No specific anti-platelet drugs (triflusal, eptifibatide, abciximab, ticagrelor, clopidogrel, prasugrel, ticlopidine, cilostazol, tirofiban) were administered during steady state or listed in the patient chart during hospitalization. We also found no occurrence of aspirin or nonsteroidal anti-inflammatory drugs. Therefore, we focused on those drugs with a known effect on platelet count instead of function. We did not find any relationship between steady state hydroxyurea use (P = 0.891) or administration of heparin prior to death (P = 0.76), although we were unable to find a negative antibody screen for heparin-induced thrombocytopenia in either of the patient’s charts. We also believed that any anti-coagulants or fibrinolytics may have an effect on platelet thrombi. One patient with platelet thrombi differential diagnosis was strongly suggestive of a pulmonary embolism. The treatment included fibrinolytic therapy, which failed to ameliorate symptoms. A patient without platelet pulmonary thrombi was also administered tPA suggesting fibrinolytics were unlikely to induce or ameliorate these thrombi. We also found no consistent relationship between anti-coagulants and platelet thrombi. Virtually the entire cohort was transfused. Only one patient was treated with exchange transfusion, however. These data are summarized in Supporting Information Table II. Because these patients were treated at the same institution, even though these events occurred over the span of some years, critical care and life support measures were remarkably similar in all cases. Because of the matching of years between the non-ACS cohort and ACS cohort any variations however, would be controlled. Standard therapies such as antibiotics, hydration, and pain medication were administered across both cohorts and were not considered as contributory to platelet accumulation in the lungs.

**Detection of increased vWF deposition in the lungs of patients with pulmonary platelet thrombi**

With the accumulation of platelet thrombi and the declining platelet counts, and no clear role of medication, we next examined if we could identify a difference in vWF deposition on the endothelium (EvWF) between groups. Among those patients with no platelet pulmonary

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**Figure 1.** Pulmonary platelet occlusion in lungs from SCD patients with fatal ACS. (a) Intense positive anti-CD41 staining revealed platelet laden thrombi in small size arteries (left panel) with the corresponding serial section H&E staining (right panel). (b) Platelet occlusion in the large vessels of the lung (left panel); H&E serial section staining to the right. (c) Occlusion of vessels by RBCs representative images of lungs without pulmonary platelet occlusion, but occluded with RBCs. Left panel: Representative H&E image. Middle and right panel: Representative images of platelet negative lungs and negative CD41 staining. Faint brown stain is from RBCs (At arrows). Images in middle and right panel are briefly counter-stained with hematoxylin to highlight lung structure. Data of three cases with platelet occlusion shown in (a) and (b). Images in (c) are from three independent cases with ACS. n = 20 total cases analyzed.
thrombi, we detected a clear, though modest endothelial staining pattern for vWF. In one case without platelet thrombi there was no vWF detected on the endothelium. In an analysis using blinded observers we found that in the cases with platelet rich thrombi, there was increased endothelial vWF deposition versus those cases without. We also detected intensely staining discrete aggregates of vWF present in the vessel. The intensely staining vWF aggregates were not present in those cases without platelets. Scored data are summarized in Table I. Representative images of the stained tissue are found in Supporting Information Fig. 2.

**Pulmonary artery remodeling and plexiform lesion in SCD**

In addition to the prominent pulmonary platelet thrombi in 3 out of 10 of the cases with SCD who succumbed to ACS. These occlusions were associated with younger age, higher platelet counts in ACS coupled with a significant drop in platelet count as ACS worsened. We also noted an increase in quantity and nature of endothelial vWF deposition. Although it is one of the most common insults to the lungs in SCD, we found no relationship between a significant history and severity of ACS and pulmonary artery remodeling. We noted that the degree of pulmonary artery remodeling appears to decline with age. Taken together these data suggest a novel etiology of ACS that involves profound pulmonary platelet thrombi, but may preclude ACS as a significant contributor to pulmonary insult in SCD.

**Discussion**

In summary, we detected a platelet mediated occlusion in 3 out of 10 patients with SCD who succumbed to ACS. These occlusions were associated with younger age, higher platelet counts in ACS coupled with a significant drop in platelet count as ACS worsened. We also noted an increase in quantity and nature of endothelial vWF deposition. Although it is one of the most common insults to the lungs in SCD, we found no relationship between a significant history and severity of ACS and pulmonary artery remodeling. We noted that the degree of pulmonary artery remodeling appears to decline with age.

Although clearly, due to the limited size of the study, the data and any conclusions must be interpreted with a degree of caution. However, an autopsy case study of 10 patients who died with ACS, and 20 in total is, to our knowledge, one of the bigger studies of its kind. As such, this work has produced some novel, potentially relevant findings. Our data suggest that, since most of the patients in the ACS group presented with pain as their initial symptom, the evolution of a pain crisis into ACS may, in some cases, be related to platelet and pulmonary artery remodeling.

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In fact, epifibatide may reduce platelet mediated inflammation at steady state [32], as does prasugrel [30]. There may, however, be some concern about administering an anti-platelet agent to a patient with ACS and declining platelet counts. However, platelet inhibition at steady state, or in the hemodynamically stable acute crisis, might be an important therapeutic addition to prevent progression to ACS.

In addition to potentially reducing inflammation, by blocking platelet mediated TSP-1 release and ADAMTS-13 inhibition [22,34] platelet inhibition therapy may also benefit progression to or therapy for ACS. Hemolysis, a known activator of platelets may therefore be a multi-hit on ACS, our data suggest little role for platelet occlusion or vWF.

Our data also suggest a role for platelet count as a possible predictor for those patients with ACS who have platelet thrombi in the lung. Initial platelet count was higher in those patients with the thrombi than in those without. Such clinical predictors are going to be essential in leveraging this new potential mechanism of ACS into a viable therapeutic option. It is notable that even though there was a precipitous drop in platelet count prior to death, though counts remained in the acceptable range for two of the patients. We observed no consistent change in platelet count in those patients without platelet thrombi in the lung, however.
Without a negative screen for antibodies that induce HIT, we cannot rule out that one of the cases of pulmonary platelet thrombi may have been HIT, or HIT related. However, the association between increased vWF deposition and vWF aggregate is suggestive of a common mechanism leading to pulmonary platelet thrombi.

Here, we also provide intriguing data that a history of frequent and severe ACS may not provide basis for pulmonary artery remodeling, and the subsequent development of pulmonary hypertension. Although repeated ACS would be predicted to ultimately injure the lung, we suggest that this ACS-mediated injury may manifest as fibrotic changes in the structural components of the lungs from infarct [36], rather than directly inducing pulmonary artery remodeling. We suspect the consistent insult from turbulent blood flow [37], increased inflammation [38], and oxidative stress [39] at steady state provides a richer environment for the development of pulmonary artery remodeling. Likewise, both the thrombotic and proliferative plexiform lesion likely have origins arising from a steady state, rather than acute pathology. However, we do interpret these data with caution as our case series is small, and the clinical data noting history of ACS was only available for up to 2 years prior to death.

Another aspect of our data that might dispute our steady state contribution to pulmonary artery remodeling was the clear relationship between age and this restrictive lesion. We found that older patients, paradoxically, had less remodeling and fewer plexiform lesions. This finding is not consistent with the notion of continual arterial exposure to damaging factors. In fact, a consistent exposure would predict an increase in pulmonary artery remodeling with age. However, the lack of pulmonary artery remodeling, regardless of etiology, may serve as a profound protective mechanism of longevity, as it would decrease right heart ventricular strain.

Conclusions

We present here a novel potential mechanism of ACS in patients with ACS—a massive sequestration of platelets in the pulmonary vasculature. These data suggest that anti-platelet therapy might have application in ACS and open new avenues of thought and targeting in this too often fatal consequence of SCD.

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