RESEARCH ARTICLE

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

Ciprian B. Anea,[1] Matthew Lyon,[2] Itia A. Lee,[1] Joyce N. Gonzales,[1,3] Amidat Adeyemi,[1] Greer Falls,[4] Abdullah Kutlar,[5] and Julia E. Brittain[1]*

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.

Am. J. Hematol. 91:173–178, 2016.

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 fatality. 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

Additional Supporting Information may be found in the online version of this article.

[1]Vascular Biology Center, Georgia Regents University, Augusta, Georgia; [2]Department of Emergency Medicine, Georgia Regents University, Augusta, Georgia; [3]Department of Medicine, Division of Pulmonary/Critical Care, Georgia Regents University, Augusta, GA; [4]Department of Pathology, Georgia Regents University, Augusta, Georgia; [5]Department of Medicine, Division of Hematology/Oncology, Augusta, Georgia

Conflict of interest: Nothing to report

***Correspondence to:** Julia E. Brittain, Vascular Biology Center, CB3210B, Georgia Regents University, Augusta, GA 30912. E-mail: jbrittain@gru.edu

Contract grant sponsor: NIH; Contract grant numbers: NHLBI-7R21HL102635; MCGRI0043.
Received for publication: 26 June 2015; **Revised:** 16 October 2015; **Accepted:** 19 October 2015
Am. J. Hematol. 91:173–178, 2016.
Published online: 22 October 2015 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.24224

cleaves vWF. Without this cleavage vWF forms ultra large multimers that can be released in the bloodstream and promote platelet activation [17]. The deposition of these ultra large vWF multimers on the endothelium also plays a role in platelet adhesion and occlusion in the microvasculature. The results of this occlusion can be devastating to most end organs [18]. At present, the best treatment for TTP is plasma exchange [19] to remove factors such as complement—which promotes clearance of the ADAMTS-13 enzyme [16]. It is worthy to note that hemolysis may inactivate ADAMTS-13 due to elevation in unconjugated bilirubin [20], or free heme which may activate complement [21]. Plasma exchange may deplete these hemolytic moieties as well as circulating vWF that binds to activates platelets or deposits on endothelial cells. Alternatively, platelet activation itself, via the release of thrombospondin-1, is also a potent inhibitor the ADAMTS-13 enzyme [22] consequently the ultra large multimers of vWF have been detected in patients with SCD [22,23].

A key feature of pulmonary pathology at autopsy in patients with SCD is the profound pulmonary artery remodeling and plexiform lesion [24]. Although the mechanism through which these restrictive and proliferative pathologies occur, they are associated with a rise in pulmonary artery pressure and symptoms of pulmonary hypertension. The incidence of pulmonary hypertension in SCD is thought to be increasing due to the increasing lifespan of patients [25]. Recurrent lung injury from ACS or other sources is believed to set the stage for this potentially fatal condition.

In this autopsy study, we examined pulmonary pathology of patients with fatal cases of ACS in order to determine if platelet sequestration/thrombi are present in patients with ACS. We also set out to determine the factors that may contribute to such thrombi formation. To this end, we interrogated pulmonary histopathology and overall clinical presentation in the perimortem. We also examined the pulmonary artery remodeling and plexiform lesion associated with the development of pulmonary hypertension.

Materials and Methods

Autopsy case series. A study of pulmonary histology in patients with SCD and ACS was conducted by forming a case series comprised of 20 autopsies conducted at Georgia Regents Medical Center from 1997 to 2013. According to the medical record or autopsy report, 10 cases had ACS listed concurrent with or as primary cause of death. As a negative control a series of 10 additional cases were sampled whose cause of death was listed as something other than ACS. The cause of death, genotype, and age of this cohort (Patient# 11–20) are listed in Supporting Information Table I. No pediatric cases were included in either group. Age range for all cases was 18–63 years old. Paraffin-embedded lung samples were procured from all cases. At minimum, slides from each case were prepared from middle and inferior lobe of the right lung and the anterior section of the superior lobe and inferior lobe of the left lung. Seven of the ACS cases were women. In the negative control cohort, four cases were women. Two cases had S/β^0Thalassemia (S/β^0Thal) listed as genotype in the ACS positive group, one case was listed as SC in the negative control group the rest were SS genotype. In 8 of the 10 cases, including all platelet positive cases, pain preceded ACS. All ACS cases had thrombi or microthrombi noted in the autopsy report. Platelet counts from the autopsy study were abstracted from the patient medical record, and from 17 of the 20 cases, medications at steady state, transfusion (exchange or simple) during current intervention, and therapies known to affect platelet activation (count of function we abstracted as well. A summary of the therapies and medications for each case is found in Supporting Information Table II. This study was declared as nonhuman subject's research by the IRB at Georgia Regents University according to DHHS 45 CFR 46.

Platelet and vWF staining in human lung tissue. Standard immuno-histochemical techniques were used to detect platelet thrombi (anti-CD41 antibody, Abcam, ab63983) and vWf (Abcam, ab6994) in preserved lung samples. Hematoxylin and Eosin (H&E) staining was also conducted. Each interrogation of platelets was conducted in specimens from at least four separate lung blocks corresponding to different areas of the lung as indicated above. Staining for vWF was interpreted by a hematopathologist and scored by blinded observers.

Pulmonary artery remodeling and plexiform lesion scoring. Pulmonary artery remodeling and plexiform lesions were visualized from preserved lung blocks using H&E staining. The pathology blinded scoring schema and types of plexiform lesions included in the analysis are described in the legend for Table II, and illustrated in Supporting Information Fig. 2.

Statistical analysis. To compare the difference in patient age between the groups with platelet thrombi and those without, we used Moods Median Test to estimate P value. All correlations between continuous correlations were analyzed via Spearman's regression. Pathology score correlations between pulmonary arterial remodeling and age/ACS severity were assessed using Kendall's regression and calculation of τ to avoid error introduced by "tied" values in the discrete dataset. The drop in platelet count during ACS was evaluated using Wilcoxon's matched pairs signed rank test. Fisher's Exact Test was used to explore the relationship between hydroxyurea use and pulmonary artery remodeling. Data are presented as median with interquartile range (IQR). For all studies $P < 0.05$ is considered significant.

Results

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: [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

 doi:10.1002/ajh.24224

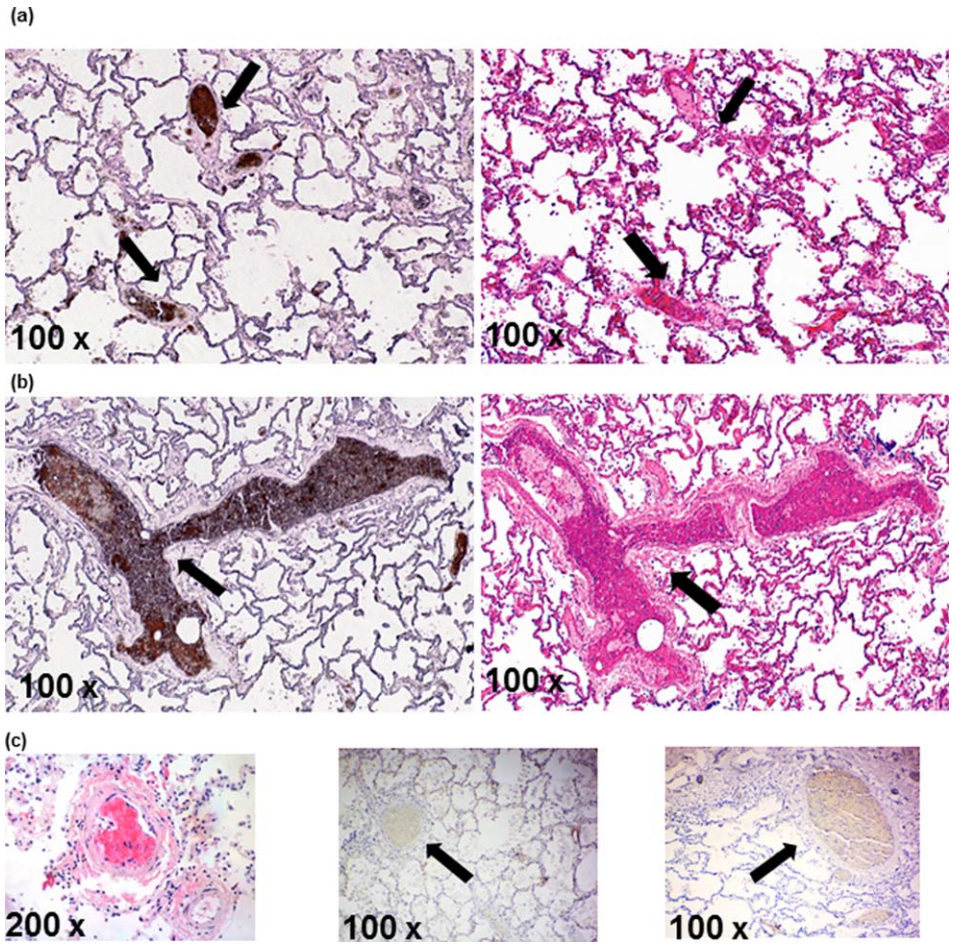

Figure 1. Pulmonary platelet occlusion in lungs from SCD patients with fatal ACS. (a) Intense positive anti-CD41 staining revealed platelet laden thrombi in small size arteries (left panel) with the corresponding serial section H&E staining (right panel). (b) Platelet occlusion in the large vessels of 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.

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 that those without platelet thrombi [median = 581 $\times$ 10^3 cells ml^{-1}, IQR =547,651 for patients with platelet thrombi versus 324 $\times$ 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. 2e).

Patient medication and therapy

We queried each patient's chart for medication or therapy that might associate 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 could find 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

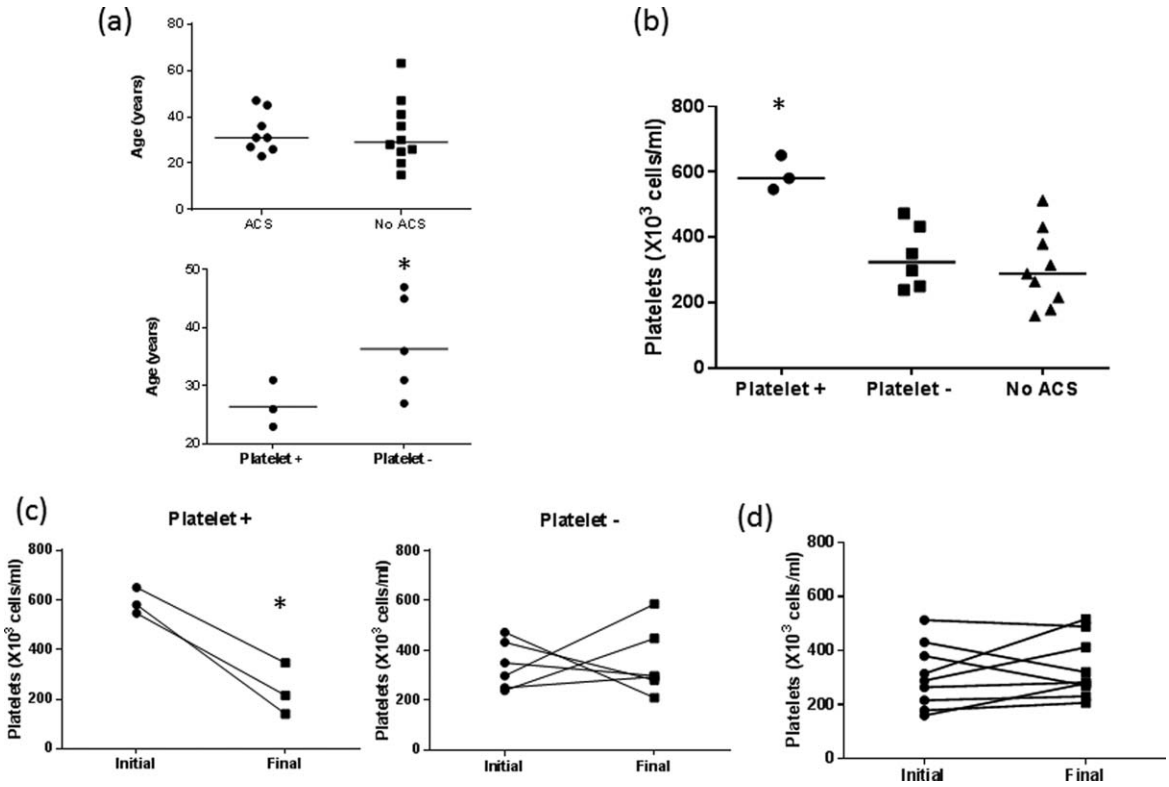

Figure 2. Age and platelet count correlate in patients with pulmonary platelet thrombi. Age associates with pulmonary platelet thrombi. Top panel—There is no significant difference in age between the group with ACS and the group without. Bottom panel—Patients with pulmonary platelet thrombi in ACS are significantly younger than those patients without these thrombi. Median 26.00 versus 36.00 years [IQR: 23,31 and 23,31]. *$P = 0.03$. (b) Initial platelet count is higher in those patients with pulmonary platelet thrombi. There is no difference between initial platelet count amongst the platelet negative group with ACS, and those without ACS. (c) Left panel: Platelet count drops in patients with pulmonary platelet thrombi detected at autopsy. Median initial value: 581 $\times$ 10^3 cells ml^{-1} [IQR: 547 $\times$ 10^3,651 $\times$ 10^3]. Median final value: 215 $\times$ 10^3 cells ml^{-1} [IQR: 180 $\times$ 10^3, 346 $\times$ 10^3] $P = 0.023$. Right panel: There is no change in perimortem platelet count in those patients without pulmonary thrombi. $P = 0.046$. Median initial value: 581 $\times$ 10^3 cells ml^{-1} [IQR: 547 $\times$ 10^3, 651 $\times$ 10^3]. Median final value: 215 $\times$ 10^3 cells ml^{-1} [IQR: 180 $\times$ 10^3, 346 $\times$ 10^3] $P = 0.023$. (d) There was no net change in platelet count in those patients without ACS.

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 ACS examined one striking feature of pulmonary pathology in all 20 of these patients was the degree of restrictive pulmonary artery remodeling regardless of age. In fact, the representative pulmonary artery shown in Supporting Information Fig. 2a is derived from a 23-year-old patient. We also observed profound plexiform lesions in these patients, of both the thrombotic and proliferative nature. We then scored all 20 cases for both remodeling pathology (R-Score) and both types of plexiform lesion (see Table II for scoring schema and results). We then determined if there was a relationship between the frequency and severity of ACS (scoring schema and scores also in Table II) and the noted pulmonary artery remodeling. Surprisingly, we found no relationship ($\tau = -0.429$, $P = 0.623$). We also noted no relationship between hydroxyurea use and pulmonary remodeling score ($P = 0.6437$). However, when we examined age and pulmonary artery remodeling, we found a significant, but inverse relationship ($\tau = -0.489$, $P = 0.0276$), suggesting that those older patients had less pulmonary artery remodeling.

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. 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.

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 platelet activation. Platelet activation and platelet mediated inflammation increases during pain crisis, making them attractive therapeutic targets [29]. There have been several studies of anti-platelet agents in steady state SCD, mainly ADP-receptor antagonists [30,31]. The use of such agents are well tolerated and may mitigate pain. One agent, eptifibatide, has been administered during both steady state [32] and acute pain crisis [33] and appears to be well tolerated in both settings.

 doi:10.1002/ajh.24224

TABLE I. Endothelial Cell vWF (EvWF) Deposition is Increased in Amount and Present in Aggregates in Patients with ACS and Platelet Pulmonary Thrombi

Patient no.	Age	Genotype	EvWF	EvWF aggregates	Platelet thrombi
1	31	SS	4	3	Yes
2	23	SS	3	4	Yes
3	26	SS	4	3	Yes
4	27	SS	2	0	No
5	45	SS	1	0	No
6	36	SS	0	0	No
7	31	SS	2	1	No
8	47	SS	2	0	No
9	18	SβOThal	1	0	No
10	27	SβOThal	1	0	No
11*	26	SS	1	0	No
14*	25	SC	1	0	No

Lung sections were stained for human vWF. Any increase in intensity over background staining in the endothelial layer was considered as positive. Intensity of staining was scored from 0 to 4, with 0 indicating essentially no vWF staining on the endothelium and 4 indicating an intense, concentrated stain in those cells. Aggregated EvWF was detected by areas of discrete, high-intensity staining on the lung endothelium. EvWF aggregates were scored from 0 to 4 with 0 indicating no aggregates noted by the observers, and 4 indicating multiple aggregates per vessel and multiple vessel involvement. In the cohort with no acute chest diagnosis, modest vWF staining was detected in the patients with pneumonitis due to aspiration (11*), and there was modest detection in the SC patient with Varicella and splenic sequestration (14*). No endothelial vWF staining was detected otherwise in this cohort without ACS. For representative staining please see supplemental Figure 1.

In fact, eptifibatide may reduce platelet mediated inflammation at steady state [32], as does prasaquel [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 ADAMTS-13 via indirect activation of platelets and release of TSP-1 and/or direct inhibition of the enzyme. Either way, multiple lines of evidence are converging. In fact, the acute clinical event most closely associated with inhibition of ADAMTS-13 in SCD is a strong history of ACS [34]. Thus, investigation into the platelet/hemolysis/vWF interaction in ACS clearly merits further study. The key mediator of progression to or severity of ACS may be the platelet. In instances of death unrelated to ACS, our data suggest little role for platelet occlusion or vWF.

Consistent with a role for vWF in platelet thrombi during ACS, it is notable that in the presence of platelet rich thrombi, we describe increased vWF deposition and aggregated vWF in the lungs suggesting a common etiology underlying platelet occlusion. However, this study cannot establish the definitive link between ACS and vWf composition in these patients, but can provide the basis for additional study. Further prospective inquiry in ACS should prove beneficial in verifying or refuting the role of ADAMTS-13, hemolysis, and vWF in ACS. Determining the activity of ADAMTS-13 during ACS, though out of reach of this study, is an essential step in validating our results.

However, even though the cultures for all patients with platelet thrombi were negative for bacteria, gram negative infection—such as *Streptococcus pneumonia*—could potentiate platelet activation via the TLR-4 receptor on the surface of the platelet [11]. Such infection could promote the adhesion of platelets to any site at which an adhesive substrate is exposed in the vasculature. In this mechanism we see a confluence of vWF deposition, coupled with infection that could create a significant and negative impact on lung function.

TABLE II. Pulmonary Remodeling and Plexiform Lesions in ACS and SCD

Patient no.	Age	Genotype	R_score	Plexiform lesion	Acute chest history
1	31	SS	2	2P	3
2	23	SS	4	4P/1T	4
3	26	SS	3	2T	2
4	27	SS	4	3P	4
5	45	SS	3	4P/2T	1
6	36	SS	2	2P	2
7	31	SS	3	3T	4
8	47	SS	4	3P/1T	1
9	18	SβOThal	4	3P	4
10	27	SβOThal	2	2T	Unavailable
11	26	SS	4	3P/1T	3
12	28	SS	2	1P/1T	4
13	41	SS	2	2P	1
14	25	SC	3	3P/1T	Unavailable
15	30	SS	2	2P	2
16	63	SS	1	1P	2
17	47	SS	1	1T	3
18	18	SS	4	4P/2T	Unavailable
19	36	SS	3	3P/1T	3
20	20	SS	3	3P/2T	4

Pulmonary artery remodeling **(R-Score)** accounts for both intimal hyperplasia and medial hypertrophy. A score of 4 indicates multiple arteries exhibiting both pathologies as shown in Fig. 4a. A score of 0 would indicate no pulmonary artery remodeling noted. **Plexiform lesions** were classified in two types: proliferative (P) and thrombotic (T). Proliferative lesions included angiomatoid lesions as shown in Supporting Information Fig. 4c, as well as lesion fed by a muscular artery, and subpleural plexiform lesions as described in Ref. 1. Thrombotic lesions included the recanalized thrombus in which thick bands of connective tissue with endothelial cells. partially occlude the arterial lumen as described in Ref. 2 and the colanderlike lesion shown in Fig. 4b. Both types of lesions were present in some cases and were scored separately. A score of 4 for either lesion indicates widespread and severe lesions. Scores shown are the integer value from two blinded observers. ACS History/Severity was a pooled score of frequency of ACS in the past year and severity of ACS (IE: Ventilator Support) and was abstracted from the patient's medical chart. Representative lesions are shown in Supporting Information Fig. 2.

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.

Another possible predictor of pulmonary platelet thrombi was relatively young age which was also associated with higher platelet counts at steady state. Although all patients were adults there was a decade difference in median age between the groups. Thus the patient in late adolescence to mid-20s might be predisposed to platelet thrombi and sequestration. We suggest, however, that platelet count is likely not sufficient to identify patients with this underlying etiology. It is likely that combination of factors, including age, platelet count, vWF modifiers, and the potentially other as yet unknown contributing factors coalesce to create this profound sequestration of platelets in the pulmonary arterial circulation. Nevertheless, these data are thus indicative of a novel pathological role of platelets in ACS and open new avenues for study and treatment.

One patient with platelet thrombi was administered heparin prior to death. There was also one patient with ACS who received heparin therapy. Heparin-induced thrombocytopenia (HIT) is closely associated with thrombosis and pulmonary embolism [35]. There is also a pronounced drop in platelet count. In this study, the platelet measurements were within hours [35], not days of each other. Monitoring for HIT typically begins 1–2 days after administration of heparin.

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 restrictive 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.

Acknowledgments

The authors acknowledge the exemplary support staff, providers, and patients at the Georgia Regents Comprehensive Sickle Cell Program. They also recognize the families of the deceased patients. They also thank the MCG Dean's Scholar program for support of A.A. and the GRU Department of Biostatistics.

References

1. Brousseau DC, Panepinto JA, Nimmer M, Hoffmann RG. The number of people with sickle-cell disease in the United States: National and state estimates. Am J Hematol 2010;85:77–78.
2. Vichinsky EP, Neumayr LD, Earles AN, et al. Causes and outcomes of the acute chest syndrome in sickle cell disease. National Acute Chest Syndrome Study Group. N Engl J Med 2000;342:1855–1865.
3. Vichinsky EP, Styles LA, Colangelo LH, et al. Acute chest syndrome in sickle cell disease: Clinical presentation and course. Cooperative Study of Sickle Cell Disease. Blood 1997;89:1787–1792.
4. Ballas SK, Files B, Luchtman-Jones L, et al. Secretory phospholipase A2 levels in patients with sickle cell disease and acute chest syndrome. Hemoglobin 2006;30:165–170.
5. Styles LA, Schalkwijk CG, Aarsman AJ, et al. Phospholipase A2 levels in acute chest syndrome of sickle cell disease. Blood 1996;87:2573–2578.
6. Tomer A, Harker LA, Kasey S, Eckman JR. Thrombogenesis in sickle cell disease. J Lab Clin Med 2001;137:398–407.
7. Davila J, Manwani D, Vasovic L, et al. A novel inflammatory role for platelets in sickle cell disease. Platelets 2015;26:726–729.
8. Ataga KI, Brittain JE, Desai P, et al. Association of coagulation activation with clinical complications in sickle cell disease. PLoS One 2012;7:e29786.
9. Helms CC, Marvel M, Zhao W, et al. Mechanisms of hemolysis-associated platelet activation. J Thromb Haemost 2013;11:2148–2154.
10. Villagra J, Shiva S, Hunter LA, et al. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. Blood 2007;110:2166–2172.
11. Berthet J, Damien P, Hamzeh-Cognasse H, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. Clin Immunol 2012;145:189–200.
12. Lee SP, Ataga KI, Orringer EP, et al. Biologically active CD40 ligand is elevated in sickle cell anemia: Potential role for platelet-mediated inflammation. Arterioscler Thromb Vasc Biol 2006;26:1626–1631.
13. Browne PV, Mosher DF, Steinberg MH, Hebbel RP. Disturbance of plasma and platelet thrombospondin levels in sickle cell disease. Am J Hematol 1996;51:296–301.
14. Shanley LA, Ebeling M, Titus MO. Changes in platelet count as a predictive tool in sickle cell acute vaso-occlusive crises: A pediatric study. Clin Pediatr (Phila) 2011;50:657–661.
15. reedman ML, Karpatkin S. Elevated platelet count and megathrombocyte number in sickle cell anemia. Blood 1975;46:579–582.
16. Tsai HM. Pathophysiology of thrombotic thrombocytopenic purpura. Int J Hematol 2010;91:1–19.
17. Lopez JA, Dong JF. Cleavage of von Willebrand factor by ADAMTS-13 on endothelial cells. Semin Hematol 2004;41:15–23.
18. Wu YP, van Breugel HH, Lankhof H, et al. Platelet adhesion to multimeric and dimeric von Willebrand factor and to collagen type III preincubated with von Willebrand factor. Arterioscler Thromb Vasc Biol 1996;16:611–620.
19. George JN. How I treat patients with thrombotic thrombocytopenic purpura: 2010. Blood 2010;116:4060–4069.
20. Lu RN, Yang S, Wu HM, Zheng XL. Unconjugated bilirubin inhibits proteolytic cleavage of von Willebrand factor by ADAMTS13 protease. J Thromb Haemost 2015;13:1064–1072.
21. Frimat M, Tabarin F, Dimitrov JD, et al. Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome. Blood 2013;122:282–292.
22. Novelli EM, Kato GJ, Ragni MV, et al. Plasma thrombospondin-1 is increased during acute sickle cell vaso-occlusive events and associated with acute chest syndrome, hydroxyurea therapy, and lower hemolytic rates. Am J Hematol 2012;87:326–330.
23. Chen J, Hobbs WE, Le J, et al. The rate of hemolysis in sickle cell disease correlates with the quantity of active von Willebrand factor in the plasma. Blood 2011;117:3680–3683.
24. Haque AK, Gokhale S, Rampy BA, et al. Pulmonary hypertension in sickle cell hemoglobinopathy: A clinicopathologic study of 20 cases. Hum Pathol 2002;33:1037–1043.
25. Gladwin MT, Kato GJ. Cardiopulmonary complications of sickle cell disease: Role of nitric oxide and hemolytic anemia. Hematol Am Soc Hematol Educ Prog 2005;51–57.
26. Stacher E, Graham BB, Hunt JM, et al. Modern age pathology of pulmonary arterial hypertension. Am J Respir Crit Care Med 2012;186:261–272.
27. Pietra GG, Edwards WD, Kay JM, et al. Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. Circulation 1989;80:1198–1206.
28. Sosland RP, Gupta K. Images in cardiovascular medicine: McConnell's Sign. Circulation 2008;118:e517–e518.
29. Beurling-Harbury C, Schade SG. Platelet activation during pain crisis in sickle cell anemia patients. Am J Hematol 1989;31:237–241.
30. Wun T, Soulieres D, Frelinger AL, et al. A double-blind, randomized, multicenter phase 2 study of prasugrel versus placebo in adult patients with sickle cell disease. J Hematol Oncol 2013;6:17.
31. Semple MJ, Al-Hasani SF, Kioy P, Savidge GF. A double-blind trial of ticlopidine in sickle cell disease. Thromb Haemost 1984;51:303–306.
32. Lee SP, Ataga KI, Zayed M, et al. Phase I study of eptifibatide in patients with sickle cell anaemia. Br J Haematol 2007;139:612–620.
33. Desai PC, Brittain JE, Jones SK, et al. A pilot study of eptifibatide for treatment of acute pain episodes in sickle cell disease. Thromb Res 2013;132:341–345.
34. Novelli EM, Kato GJ, Hildesheim ME, et al. Thrombospondin-1 inhibits ADAMTS13 activity in sickle cell disease. Haematologica 2013;98:e132–e134.
35. Arepally GM, Ortel TL. Clinical practice. Heparin-induced thrombocytopenia. N Engl J Med 2006;355:809–817.
36. Vij R, Machado RF. Pulmonary complications of hemoglobinopathies. Chest 2010;138:973–983.
37. Stein PD, Sabbah HN, Mandal AK. Augmentation of sickling process due to turbulent blood flow. J Appl Physiol 1976;40:60–66.
38. Platt OS. Sickle cell anemia as an inflammatory disease. J Clin Invest 2000;106:337–338.
39. Queiroz RF, Lima ES. Oxidative stress in sickle cell disease. Rev Bras Hematol Hemoter 2013;35:16–17.