Early View

Original research article

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Endothelial dysfunction and hypercoagulability in severe sickle cell acute chest syndrome

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Take home message.

Severe sickle cell acute chest syndrome is associated with an activation of the pulmonary vascular endothelium and of coagulation, with higher levels of procoagulant microparticles in case of associated acute pulmonary hypertension.

ABSTRACT

Rationale: Acute pulmonary hypertension (PH) may develop during sickle-cell acute chest syndrome (ACS), and is associated with an increased mortality. Its mechanisms remain poorly known. The question was to assess if there is an endothelial dysfunction and a hypercoagulability in severe ACS, with and without acute PH.

Methods: In a prospective monocenter cohort follow-up study, all sickle-cell adult patients with ACS admitted to the intensive care unit underwent a trans-thoracic echography (TTE), and measurements of biomarkers of coagulation, endothelial activation, and platelet and erythrocyte activation. Acute PH was defined as a high echocardiographic probability of PH. The biological profiles of sickle-cell patients were analyzed at the time of ACS, contrasting the existence of acute PH, and compared with steady state and with non-sickle-cell controls (healthy subjects and community-acquired pneumonia (CAP)).

Results: Most patients (36 patients with 39 ACS episodes; 23 males; 27 years old) had thoracic pain, dyspnea and CT scan lung consolidation. Acute PH was diagnosed in 7 patients (19%). Erythrocyte and platelet-derived microparticles (MPs) and the pro-coagulant activity of MPs were higher in ACS patients with acute PH, as compared with their counterparts. As compared with healthy controls, ACS patients had higher levels of tissue factor, fibrin monomers, D-dimer, release of pro-coagulant microparticles, and erythrocyte and platelet-derived MPs. As compared with CAP patients, ACS patients had increased levels of fibrin monomers, and erythrocyte and platelet-derived MPs.

Conclusions: Severe ACS is characterized by endothelial dysfunction and hypercoagulability state, with a marked pro-coagulant profile in case of associated PH.

Sickle cell disease is one of the most common autosomal recessive genetic disorder affecting 300000 newborns in the world each year [1, 2]. Sickle cell disease is caused by a point mutation of the gene coding for the beta-globin chain of hemoglobin (Hb), substituting a glutamic acid by a valine, and resulting in the production of sickle hemoglobin (Hbs), that polymerizes when deoxygenated, resulting in red-cell sickling, chronic hemolytic anemia and vaso-occlusion [3]. This life-threatening disease presents very variable faces, and is associated with a reduction in life expectancy by approximately 30 years [1]. Among acute complications, the acute chest syndrome (ACS) is one of the leading causes of death [4]. This syndrome is characterized as the combination of chest pain, fever, leukocytosis and new radiological infiltrates [5]. It may result from several causes (lower respiratory tract infection, fatty embolism, pulmonary infarction) that lead to regional alveolar hypoventilation and HbS polymerization [4, 6]. The clinical expression of ACS is heterogeneous, ranging from a "simple form" requiring hospitalization in wards to acute respiratory distress syndrome (ARDS) [7]. Acute pulmonary hypertension (PH) is one of the markers of ACS severity [8]. The mechanisms of acute PH in ACS are incompletely understood and may be multiple [9–11]. However, the pulmonary vascular endothelium function and the coagulation pathway and have not been assessed in ACS. The question was to assess if there is an endothelial dysfunction and a hypercoagulability state in severe ACS, with and without acute PH.

MATERIAL AND METHODS

Patients and study design

Sickle-cell patients

A prospective monocenter cohort follow-up was conducted in the ICU of Tenon Hospital, Assistance Publique Hôpitaux de Paris, France, a referral center for SCD. All sickle-cell adult patients admitted to the ICU from November 2015 to May 2017 with a diagnosis of ACS were included. Pregnant or breastfeeding women, patients with a known chronic PH (mean pulmonary arterial pressure ≥ 25 mm Hg at rest measured by right cardiac catheterization) or a known systolic or diastolic heart failure, and those transfused in the 4 preceding months were excluded. The diagnosis of ACS was established in case of fever or chest pain in combination with a new radiological infiltrate of at least one lung segment[8]. Patients were followed-up until a medical visit at steady state, at least three months after the index ACS episode (from May 2016 to April 2018). Adapted from Ballas et al, the steady state was defined by a point in time where the patient was not experiencing an acute painful crisis or any changes due to therapy, at least 3 months after the acute chest syndrome with no history of an acute painful episode, of intercurrent illness such as infection and inflammation and no need for antibiotics during the previous 4 weeks, for at least four consecutive weeks after a previous painful crisis and no need for transfusion since the ACS episode [12]. When a patient had several episodes of ACS, only one visit at steady state was performed.

Control populations

Clinical and biological data of patients with severe community-acquired pneumonia (CAP), and healthy volunteers (medical students or members of the medical or laboratory staffs, without medical history) were used as control populations.

Characteristics at diagnosis, data recording and outcomes

The following data were collected on ICU admission, after patient-centered interview and medical charts analysis: demographics, SCD baseline characteristics, past medical history and comorbidities, chronic treatments, and ACS characteristics. Conventional laboratory tests included blood count, liver and kidney functions, fibrinogen, and lactate dehydrogenase (LDH). Specialized biology focused on the coagulation pathway, the pulmonary vascular endothelium function and the platelet and erythrocyte activation. The coagulation pathway was assessed on the measurement of *in vivo* thrombin generation marker (fibrin monomers), fibrinolysis debris (D-dimers) and a test for the release of pro-coagulant microparticles (Procoag-PPL) (See methods in the online supplement). The pulmonary vascular endothelium function was assessed on the dosage of tissue factor (See methods in the online supplement). Platelet and erythrocyte activation was assessed on the measurement of erythrocyte and platelet-derived microparticles, using a flow cytometry assay, as previously described [13].

CT angiography of the chest findings included the presence of alveolar consolidation, ground glass opacity, pleural effusion, pulmonary embolism (proximal, segmental or sub-segmental), as well as the pulmonary artery to ascending aorta diameter ratio (PA:A) and the right to left ventricular diameter ratio (RV:LV). A TTE was performed at inclusion by the same experimented cardiologist (VL), blinded to the results of the specialized biology measurement (See Methods in the online supplement). The echocardiographic probability of acute PH was classified as low, intermediate or high, on the basis on the echocardiographic signs (peak

tricuspid regurgitation velocity (PTRV) and other echo PH signs) used to assess the probability of chronic PH [14] (Table E1 and E2). As echocardiographic evaluation has a low positive predictive value for pulmonary hypertension in sickle cell disease [15] and to be confident in the diagnosis of acute PH, acute PH was defined when there was a high echocardiographic probability of PH.

The use of mechanical ventilation, blood products transfusion and antimicrobial therapy, as well as the ICU and hospital lengths of stay and corresponding vital status were recorded. When possible, patients had a follow-up medical visit at steady state, at least three months after the index ACS episode, with repeated specialized biology and TTE.

Ethic consideration

The institutional review board for human studies approved the experiments for the sickle cell patients and for healthy and pneumonia controls (reference number 15020 and number 16143, Comité de protection des personnes Ile de France V) and all the subjects provided written informed consent. After inclusion, all data regarding the clinical status, main outcomes, biological and radiological features were recorded in an anonymous database registered to the National Commission on Informatics and Liberty (n°1852665).

Statistical analysis

Quantitative data were expressed as median (interquartile range) (IQR, presented as first quartile - third quartile). Qualitative data were expressed as number of occurrence, n (%). In patients with several episodes of ACS, each episode was considered as individual episode, but the patients characteristics were considered once. Patients were separated in two groups, according to their echocardiographic profiles, as follows: low-to-intermediate probability and

high probability of PH. Acute PH was defined when there was a high echocardiographic probability of PH. Groups were compared using the non-parametric Mann-Whitney U test or the Kruskal Wallis test with Dunn's multiple comparisons test for continuous variables, and Fisher's exact test for categorical variables. Within-group comparisons were performed using the Wilcoxon matched-pair signed rank test.

Differences were considered significant when *P* value was less than 0.05. Analyses were performed using GraphPad Prism v8.0 (La Jolla, CA, USA).

RESULTS

Clinical characteristics of patients on ICU admission

During the study period, 82 SCD patients (90 episodes) were admitted to the ICU for the management of ACS. Of those latter, 36 patients (39 ACS episodes) were included (**Figure 1**). Most patients (64% males, 27, IQR 22-31 years) had homozygous sickle cell disease and had already experienced an episode of ACS. The most frequent chronic complications were biliary lithiasis and retinopathy. Two-thirds of the patients were treated with hydroxyurea (**Table 1**). The time from ACS symptoms onset to ICU admission was 3, IQR 1-7 days. The majority of patients presented with thoracic pain and dyspnea, but only one-third of them had fever. All patients needed oxygen therapy, at a median level of 4, IQR 2-5 L/min (**Table 2**). Baseline characteristics of the control populations are detailed in **Table E3**.

Biology on ICU admission (Table 2)

Leukocytosis and hyperfibrinogenemia were frequent and associated with a marked hemolysis. There was no thrombopenia but the platelet count remained normal. As compared with healthy controls, specialized biomarkers were suggestive of a hypercoagulability (increase in the levels of fibrin monomers and D-dimers, and in the release of pro-coagulant

microparticles, as assessed by a shorter time of anticoagulation in the Procoag-PPL test), as well as of an activated pulmonary vascular endothelium (increase in the level of tissue factor) in ACS patients. Erythrocyte and platelet-derived microparticles were also higher in ACS patients than in healthy controls (**Figure 2**). As compared with severe CAP controls, specialized biomarkers were also highly suggestive of a hypercoagulability state (increase in the levels of fibrin monomers) and of platelet and erythrocyte activation (increase in the levels of erythrocyte and platelet-derived microparticles) in ACS patients (**Figure 2**).

Radiology on ICU admission

All patients except one underwent a chest CT-scan. Lung consolidation was the most frequent pattern (92% of cases), and lesions predominated in inferior lobes in all cases. CT pulmonary angiography was performed in 37 patients, demonstrating an acute pulmonary embolism (PE) in 2 patients (one segmental and one sub-segmental), one of whom had golden sputum and an associated acute PH. A leg doppler ultrasound was realized in the 2 patients with PE, excluding deep vein thrombosis. The pulmonary artery to ascending aorta diameter ratio was enlarged in 42% of cases, but the right ventricle was not dilated. The injection quality was sub-optimal in 19 exams, not allowing the search for segmental and sub-segmental PE in those latter exams. The **Figure 3** shows typical CT-scans in 4 included patients.

Echocardiography on ICU admission

A TTE was performed in 34 patients. There was no evidence of left or right ventricular dysfunction, and no right ventricle dilation. A high probability of acute PH was diagnosed in 7 patients, on the following criteria: PTRV ≥ 3.4 m/sec (n=2), PTRV ranging between 2.8 and 3.4 m/sec in association with right ventricular outflow Doppler acceleration time <105 msec (n=4),

or with early diastolic pulmonary regurgitation velocity >2.2 m/sec ($n=1$). Intermediate and low probabilities of PH were documented in 13 patients and 14 patients, respectively (**Table E4**). Patients were separated in two groups, according to their echocardiographic profiles, as follows: low-to-intermediate probability and high probability of PH which was considered as acute PH (**Table 3**).

Management and outcomes (Table 4)

All patients received antibiotics during their ICU stay. Bacterial and viral lower respiratory tract infection was diagnosed in 8 patients (21%) and 3 patients (8%), respectively. Curative anticoagulation was initiated in the 2 patients diagnosed with PE. One-third of the patients received at least one blood product transfusion during their ICU stay.

The course was favorable in most patient with a decrease in the level of supplemental oxygen at 48h (Table 3). The ICU length of stay was 4, IQR 3-5 days. One death occurred in a 34-year-old man with SS sickle cell disease with an initial intermediate probability of PH.

Comparisons of patients according to the presence of acute PH

Altogether, seven patients had a high echocardiographic probability of acute PH. As compared with their counterparts at baseline, they were more likely Caribbeans, had less frequently SS disease and less past medical history of ACS (**Table E5**). At the time of ACS, they required higher level of supplemental oxygen despite less frequent alveolar CT scan consolidation; they had more frequent lower limb pain and conventional biology was remarkable by higher platelet count and a lower level of total bilirubin level (**Table E6**). The activity of procoagulant MPS (35, IQR 29-41 sec vs. 50, IQR 38-56 sec; $p=0.016$) and the levels of erythrocyte-derived (7834, IQR 4401-13963 MPs/ μ L plasma vs. 3414, IQR 1397-5606 MPs/ μ L plasma; $p=0.018$) and

platelet-derived MPs (44686, IQR 16171-53138 MPs/ μ L plasma vs. 13956, IQR 9332-24076 MPs/ μ L plasma; $p=0.012$) were higher in ACS patients with high echocardiographic probability of PH, as compared with their counterparts (**Figure 4**). However, the levels of tissue factor, fibrin monomers and D-dimer were similar (**Figure 4**). The outcomes of patients are detailed in **Table E7**, contrasting low-to-intermediate and high-probability of PH.

Evaluation at steady state

Twenty-eight sickle-cell patients (78%) had a follow-up visit at steady state, 4.5 months (IQR 3-6) after the index episode of ACS. All had returned to their baseline clinical condition. A significant reduction of PTRV from 2.8 m/sec (IQR 2.6-3.0) to 2.5 m/sec (IQR 2.1-2.6) ($p=0.003$) was noted in the 19 patients who had an available measure of PTRV both during ACS and at steady state (**Figure E1 and Table E8**).

A significant reduction of tissue factor and D-dimer was observed at the time of steady state, as compared with the time of ACS (**Figure E2**). Fibrin monomers, PPL activity and erythrocyte and platelet-derived microparticles did not significantly decrease at steady state, as compared with the time of ACS (**Figure E2**). Those latter biomarkers remained significantly different from those of healthy controls at steady state (**Figure E3**). Erythrocyte MPs remained higher at steady state after an episode of ACS with acute PH, as compared with an episode of ACS with no PH (**Figure E4**).

DISCUSSION

In this pilot study, we investigated the pro-coagulant activation of the endothelium, the activation of coagulation pathway and the place of erythrocyte and platelet-derived microparticles in a cohort of SCD patients with ACS admitted to the ICU. Our findings were the following: (1) the endothelium was activated during ACS, and there was an hypercoagulability state, as compared with healthy and severe pneumonia controls (2) erythrocyte and platelet-derived microparticles and the procoagulant activity of microparticles were particularly increased in case of acute PH (3) at steady state, the levels of tissue factor and D-dimer significantly decreased, as compared with the time of ACS, but the procoagulant activity of microparticles and erythrocyte and platelet-derived microparticles levels remained increased, despite the significant reduction of PTRV in most patients.

ACS is a common acute complication of sickle cell disease [16] and the leading cause of death [17, 18]. A moderate increase in systolic pulmonary arterial pressure (PAPs), assessed indirectly by a PTRV ≥ 2.5 m / sec on TTE, has been shown to be a major independent risk factor for mortality [19]. Moreover, chronic PH is known to be a frequent and severe complication of SCD, as it has been shown to occur in 6 to 10% of patients, and to be associated with an overall survival of 60% at 5 years [15, 20]. A study comparing PTRV at steady state and at the time of vaso-occlusive crisis in 26 sickle cell patients showed a significant increase in PTRV from 2.4 ± 0.07 m / sec to 2.9 ± 0.07 m / sec, and a resolution at distance from the crisis, regardless of the intensity of hemolysis [21]. In a series of 70 episodes of severe ACS, the PTRV at admission was greater than 3 m / sec in 31% of the cases [8]. Of note, the patients treated

with mechanical ventilation (n=5) and those who died (n=4) were all characterized by a PTRV greater than 3 m / sec [8]. Another study has suggested that acute pulmonary vascular dysfunction and acute PH were more common during ARDS associated with SCD than during ARDS from other causes [22]. In our study, we used the echocardiographic probability of PH, based on PTRV measure and associated echocardiographic signs of PH, as PTRV measure alone is known to lack of precision for estimating the pulmonary arterial pressures in sickle cell disease [15]. Altogether, 7/34 patients (21%) were classified as having a high probability of acute PH. This prevalence is in the low range, as compared with other series [8]. This discrepancy might be related to the more strict definition of acute PH we used, as we combined PTRV with other echo PH signs, while the others used PTRV alone, but also to an overall lower severity of ACS in our series, as compared with others [8, 18].

The mechanisms of acute PH during ACS are probably multiple. Indeed, ACS is associated with many pathological modifications such as worsening of hemolytic anemia, hypoxic vasoconstriction, high cardiac output which can temporarily increase pulmonary arterial pressure [21]. The increase in hemolysis can also be accompanied by a decrease in the bioavailability of NO by sequestration by free hemoglobin, and thus by acute endothelial dysfunction [23]. This acute endothelial dysfunction may be exacerbated by an increase in endothelin 1, a potent vasoconstrictor, which has been observed during vaso-occlusive crisis [24]. Another explanatory mechanism for acute PH during ACS may be acute microvascular or more proximal pulmonary vascular thrombosis [25, 26]. Acute pulmonary vascular thrombosis may be favored by fatty emboli, by the increase in the adhesion of red blood cells to the pathological endothelium, but also by a state of hypercoagulability favored by acute endothelial dysfunction, which possibly increases during ACS, with the intervention of ischemia-reperfusion lesions. The presence of pulmonary microvascular thrombi associated

with pulmonary arteriolar changes has been described in an autopsy series [27]. In our population, 2 patients with ACS developed acute PE, but only one had acute PH. None had associated deep-vein thrombosis, suggesting *in situ* thrombosis [26]. Noteworthy, lower limb pain was more often reported in the high probability group, as compared with the low-intermediate probability group, which may promote bone necrosis and ultimately pulmonary fat embolism. Interestingly, in our study, platelet count was higher in patients with acute PH. Among 121 ACS episodes, Dessap et al reported higher platelet counts in ACS episodes associated with pulmonary embolism than in ACS episodes without pulmonary embolism, and the authors discussed the direct relationship between increased platelet count and thrombosis [26]. Another study reported the association of thrombocytosis and elevated pulmonary artery pressures in sickle cell disease [28]. High platelet count could therefore participate to local thrombosis and to the onset of acute PH. The increase in erythrocyte and platelet-derived MPs and in the procoagulant activity of MPs could also be responsible of *in situ* microvascular thrombosis, and be one of the missing links to explain the pathophysiology of ACS, especially in case of acute PH. Circulating MPs are anucleated membrane fragments of 0.1 to 1 μm in diameter produced by damaged or activated cells [29, 30]. They contain surface proteins but also intracellular material such as ribonucleic acid (RNA). Several series have already shown increased rates of erythrocyte and platelet-derived-MPS in SCD patients, as compared with healthy controls [31–33], but the increase of these MPs during acute episodes compared to steady state is more controversial [34, 35]. Our study is the first to show an increase of these MPs in severe ACS, as compared with severe pneumonia, suggesting that these MPs should be more than a spectator in the pathophysiology of ACS. Moreover, it is the first to show a higher increase of erythrocyte- and platelet-derived MPs in case of acute PH. A series found that levels of erythrocyte-derived vesicles and intravascular haemolysis were

linked as suggested by the correlation between levels of vesicles and plasma Hb [36], what was consistent with a higher level of LDH in patients with acute PH and higher levels of MPs in our series. It has also been shown that erythrocyte-derived MPs promoted thrombin generation [13, 36]. Platelet-derived MPs have also been shown to be implicated in ACS, as it has been shown in an *in vivo* model of SCD that the activation of platelets produced platelet-derived MPs which activated neutrophils and other platelets to form large platelet–neutrophil aggregates that occlude pulmonary arterioles [30]. We therefore assume that erythrocyte- and platelet-derived MPs could promote acute PH in ACS, by favouring acute pulmonary endothelial dysfunction by an action on local NO and an activation of microvascular thrombosis, through the promotion of thrombin generation.

Our study has limitations. First, because of the necessary strict exclusion criteria like transfusion in the previous 4 months, only a small population was included. Moreover, given its design, this study showed an association but did not allow us to conclude on a causal link between increased MPs levels and acute PH in ACS. Given its prospective and real-life design, some exams were not performed in all patients, and the number of patients assessed at steady state was limited. Furthermore, as it was a routine care study and it was expected that we would not draw more blood from patients on inclusion than the usual amount, we have chosen to assay only certain markers, representative of endothelial dysfunction and activation of thrombosis. We were unable to assay certain markers of endothelial dysfunction such as adhesion molecules and von Willebrand factor which would have given a more complete characterization of endothelial dysfunction. Likewise, we were not able to characterize all the plasma microparticles and we focused on the erythrocyte and platelet microparticles which are the most relevant in sickle cell disease. Finally, the healthy control population and the

control CAP population were not matched for age and sex with the SCD population and the groups were too small to allow multivariate analysis. Nevertheless, these limits were reduced by the fact that the healthy controls had similar age and sex-ratio and the control CAP population had similar severity of lung disease as only one developed ARDS, and as most of these patients were treated with high level of oxygen like in the SCD population.

The activation of the endothelium with an increase in thrombosis, in the procoagulant activity of microparticles and in erythrocyte and platelet derived-MPs in ACS could be one of the missing links to explain the pathophysiology of ACS. This study is a pilot study which does not currently allow the results to be applied directly in clinical practice or in terms of prognosis or prediction. However, further and larger studies are needed to evaluate biomarkers assessing endothelial dysfunction and hypercoagulability as prognostic markers to prompt to more aggressive treatment or close monitoring of patients or as therapeutic targets in ACS.

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CONFLICT OF INTEREST STATEMENT

EMJ, HM, PVD and GG have nothing to disclose. GV reports grants and personal fees from Biomérieux, grants from Janssen, grants from SOS Oxygene, outside the submitted work. VL reports personal fees from AMOMED, outside the submitted work. Dr. Savale reports personal fees and non-financial support from Actelion, personal fees and non-financial support from MSD, grants, personal fees and non-financial support from GSK, outside the submitted work. Dr. Fartoukh reports grants from BioMerieux, personal fees from Pfizer, outside the submitted work.

REFERENCES

1. Piel FB, Steinberg MH, Rees DC. Sickle Cell Disease. *N. Engl. J. Med.* 2017; 376: 1561–1573.
2. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions. *PLoS Med.* 2013; 10: e1001484.
3. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, Smith WR, Panepinto JA, Weatherall DJ, Costa FF, Vichinsky EP. Sickle cell disease. *Nat Rev Dis Primers* 2018; 4: 18010.
4. Gladwin MT, Vichinsky E. Pulmonary complications of sickle cell disease. *N. Engl. J. Med.* 2008; 359: 2254–2265.
5. Charache S, Scott JC, Charache P. “Acute chest syndrome” in adults with sickle cell anemia. Microbiology, treatment, and prevention. *Arch. Intern. Med.* 1979; 139: 67–69.
6. Vichinsky EP, Neumayr LD, Earles AN, Williams R, Lennette ET, Dean D, Nickerson B, Orringer E, McKie V, Bellevue R, Daeschner C, Mancini EA. 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.
7. Medoff BD, Shepard J-AO, Smith RN, Kratz A. Case records of the Massachusetts General Hospital. Case 17-2005. A 22-year-old woman with back and leg pain and respiratory failure. *N. Engl. J. Med.* 2005; 352: 2425–2434.
8. Mekontso Dessap A, Leon R, Habibi A, Nzouakou R, Roudot-Thoraval F, Adnot S, Godeau B, Galacteros F, Brun-Buisson C, Brochard L, Maitre B. Pulmonary hypertension and cor pulmonale during severe acute chest syndrome in sickle cell disease. *Am. J. Respir. Crit. Care Med.* 2008; 177: 646–653.
9. Hebbel RP, Osarogiagbon R, Kaul D. The endothelial biology of sickle cell disease: inflammation and a chronic vasculopathy. *Microcirculation* 2004; 11: 129–151.
10. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. *Blood* 2016; 127: 801–809.
11. Bennewitz MF, Jimenez MA, Vats R, Tutuncuoglu E, Jonassaint J, Kato GJ, Gladwin MT, Sundd P. Lung vaso-occlusion in sickle cell disease mediated by arteriolar neutrophil-platelet microemboli. *JCI Insight* 2017; 2: e89761.
12. Ballas SK. More definitions in sickle cell disease: steady state v base line data. *Am J Hematol* 2012; 87: 338.

13. Gerotziafas GT, Van Dreden P, Chaari M, Galea V, Khaterchi A, Lionnet F, Stankovic-Stojanovic K, Blanc-Brude O, Woodhams B, Maier-Redelsperger M, Girot R, Hatmi M, Elalamy I. The acceleration of the propagation phase of thrombin generation in patients with steady-state sickle cell disease is associated with circulating erythrocyte-derived microparticles. *Thromb. Haemost.* 2012; 107: 1044–1052.
14. Galiè N, Humbert M, Vachiery J-L, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur. Respir. J.* 2015; 46: 903–975.
15. Parent F, Bachir D, Inamo J, Lionnet F, Driss F, Loko G, Habibi A, Bennani S, Savale L, Adnot S, Maitre B, Yaïci A, Hajji L, O’Callaghan DS, Clerson P, Girot R, Galacteros F, Simonneau G. A hemodynamic study of pulmonary hypertension in sickle cell disease. *N. Engl. J. Med.* 2011; 365: 44–53.
16. Castro O, Brambilla DJ, Thorington B, Reindorf CA, Scott RB, Gillette P, Vera JC, Levy PS. The acute chest syndrome in sickle cell disease: incidence and risk factors. The Cooperative Study of Sickle Cell Disease. *Blood* 1994; 84: 643–649.
17. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N. Engl. J. Med.* 1994; 330: 1639–1644.
18. Cecchini J, Lionnet F, Djibré M, Parrot A, Stojanovic KS, Girot R, Fartoukh M. Outcomes of adult patients with sickle cell disease admitted to the ICU: a case series*. *Crit. Care Med.* 2014; 42: 1629–1639.
19. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, Minter K, Brown B, Coles WA, Nichols JS, Ernst I, Hunter LA, Blackwelder WC, Schechter AN, Rodgers GP, Castro O, Ognibene FP. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N. Engl. J. Med.* 2004; 350: 886–895.
20. Savale L, Habibi A, Lionnet F, Maitre B, Cottin V, Jais X, Chaouat A, Artaud-Macari E, Canuet M, Prevot G, Chantalat-Auger C, Montani D, Sitbon O, Galacteros F, Simonneau G, Parent F, Bartolucci P, Humbert M. Clinical phenotypes and outcomes of precapillary pulmonary hypertension of sickle cell disease. *Eur. Respir. J.* 2019; 54.
21. Machado RF, Mack AK, Martyr S, Barnett C, Macarthur P, Sachdev V, Ernst I, Hunter LA, Coles WA, Nichols JP, Kato GJ, Gladwin MT. Severity of pulmonary hypertension during vaso-occlusive pain crisis and exercise in patients with sickle cell disease. *Br. J. Haematol.* 2007; 136: 319–325.

22. Cecchini J, Boissier F, Gibelin A, de Prost N, Razazi K, Carteaux G, Galacteros F, Maitre B, Brun-Buisson C, Mekontso Dessap A. Pulmonary Vascular Dysfunction and Cor Pulmonale During Acute Respiratory Distress Syndrome in Sicklers. *Shock* 2016; 46: 358–364.
23. Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO, Schechter AN, Gladwin MT. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat. Med.* 2002; 8: 1383–1389.
24. Ergul S, Brunson CY, Hutchinson J, Tawfik A, Kutlar A, Webb RC, Ergul A. Vasoactive factors in sickle cell disease: in vitro evidence for endothelin-1-mediated vasoconstriction. *Am. J. Hematol.* 2004; 76: 245–251.
25. Bhalla M, Abboud MR, McCloud TC, Shepard JA, Munden MM, Jackson SM, Beaty JR, Laver JH. Acute chest syndrome in sickle cell disease: CT evidence of microvascular occlusion. *Radiology* 1993; 187: 45–49.
26. Mekontso Dessap A, Deux J-F, Abidi N, Lavenu-Bombled C, Melica G, Renaud B, Godeau B, Adnot S, Brochard L, Brun-Buisson C, Galacteros F, Rahmouni A, Habibi A, Maitre B. Pulmonary artery thrombosis during acute chest syndrome in sickle cell disease. *Am. J. Respir. Crit. Care Med.* 2011; 184: 1022–1029.
27. Anea CB, Lyon M, Lee IA, Gonzales JN, Adeyemi A, Falls G, Kutlar A, Brittain JE. Pulmonary platelet thrombi and vascular pathology in acute chest syndrome in patients with sickle cell disease. *Am. J. Hematol.* 2016; 91: 173–178.
28. Pashankar FD, Carbonella J, Bazzi-Asaad A, Friedman A. Prevalence and risk factors of elevated pulmonary artery pressures in children with sickle cell disease. *Pediatrics* 2008; 121: 777–782.
29. Hebbel RP, Key NS. Microparticles in sickle cell anaemia: promise and pitfalls. *Br. J. Haematol.* 2016; 174: 16–29.
30. Vats R, Brzoska T, Bennewitz MF, Jimenez MA, Pradhan-Sundt T, Tutuncuoglu E, Jonassaint J, Gutierrez E, Watkins SC, Shiva S, Scott MJ, Morelli AE, Neal MD, Kato GJ, Gladwin MT, Sundt P. Platelet Extracellular Vesicles Drive Inflammasome-IL-1 β -Dependent Lung Injury in Sickle Cell Disease. *Am. J. Respir. Crit. Care Med.* 2020; 201: 33–46.
31. Shet AS, Aras O, Gupta K, Hass MJ, Rausch DJ, Saba N, Koopmeiners L, Key NS, Hebbel RP. Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. *Blood* 2003; 102: 2678–2683.
32. van Beers EJ, Schaap MCL, Berckmans RJ, Nieuwland R, Sturk A, van Doormaal FF, Meijers JCM, Biemond BJ, CURAMA study group. Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. *Haematologica* 2009; 94: 1513–1519.

33. van Tits LJ, van Heerde WL, Landburg PP, Boderie MJ, Muskiet F a. J, Jacobs N, Duits AJ, Schnog JB. Plasma annexin A5 and microparticle phosphatidylserine levels are elevated in sickle cell disease and increase further during painful crisis. *Biochem. Biophys. Res. Commun.* 2009; 390: 161–164.
34. Kasar M, Boğa C, Yeral M, Asma S, Kozanoglu I, Ozdogu H. Clinical significance of circulating blood and endothelial cell microparticles in sickle-cell disease. *J. Thromb. Thrombolysis* 2014; 38: 167–175.
35. Tantawy AAG, Adly AAM, Ismail EAR, Habeeb NM, Farouk A. Circulating platelet and erythrocyte microparticles in young children and adolescents with sickle cell disease: Relation to cardiovascular complications. *Platelets* 2013; 24: 605–614.
36. Westerman M, Pizzey A, Hirschman J, Cerino M, Weil-Weiner Y, Ramotar P, Eze A, Lawrie A, Purdy G, Mackie I, Porter J. Microvesicles in haemoglobinopathies offer insights into mechanisms of hypercoagulability, haemolysis and the effects of therapy. *Br. J. Haematol.* 2008; 142: 126–135.

FIGURE LEGENDS

Figure 1. Flow chart of the study

ACS: acute chest syndrome; ICU: intensive care unit; TTE: transthoracic echocardiography

Figure 2. Biomarkers of endothelial dysfunction and hypercoagulability state in sickle cell patients with acute chest syndrome, as compared with controls

A. Levels of tissue factor in ACS patients (n=38), and in controls (healthy subjects, n=10, and patients with severe community-acquired pneumonia, n=13)

B. Levels of fibrin monomers in ACS patients (n=38), and in controls (healthy subjects, n=10, and patients with severe community-acquired pneumonia, n=13)

C. Levels of D-dimer in ACS patients (n=38), and in controls (healthy subjects, n=10, and patients with severe community-acquired pneumonia, n=13)

D. Levels of PPL clotting time in ACS patients (n=38), and in controls (healthy subjects, n=10, and patients with severe community-acquired pneumonia, n=13)

E. Levels of erythrocyte-derived microparticles in ACS patients (n=38), and in controls (healthy subjects, n=10, and patients with severe community-acquired pneumonia, n=13)

F. Levels of platelet-derived microparticles in ACS patients (n=38), and in controls (healthy subjects, n=10, and patients with severe community-acquired pneumonia, n=13)

Comparisons were made using Kruskal Wallis test with Dunn's multiple comparisons test. *P-value < 0.05; ***P-value < 0.0001; ****P-value < 0.00001 vs. ACS patients

ACS : acute chest syndrome ; MP, microparticles

Figure 3. Typical high-resolution computed tomography (HRCT) of the chest of patients with severe acute chest syndrome included in the study

- A. Axial HRCT image of the middle lobe, lingula and lower lobes, in a 19-year-old male showing alveolar consolidation with air bronchogram and ground glass opacities in the lower lobes.
- B. Axial HRCT image of the middle lobe, lingula and lower lobes, in a 26-year-old female showing alveolar consolidation with air bronchogram in the lower lobes.
- C. Axial HRCT image of the middle lobe, lingula and lower lobes, in a 19-year-old male showing alveolar consolidation and ground glass opacities in the lower lobes.
- D. Axial contrast-enhanced HRCT image of the upper lobes in a 18-year-old female showing a segmental pulmonary embolism in the right upper pulmonary artery and alveolar consolidation in the upper lobes.

Figure 4. Biomarkers of endothelial dysfunction and hypercoagulability state in sickle cell patients in acute chest syndrome, contrasting low-to-intermediate and high probability of acute pulmonary hypertension

- A. Levels of tissue factor in patients with low-to-intermediate probability of PH (n=27), as compared with high probability of PH (n=7)
- B. Levels of fibrin monomers in patients with low-to-intermediate probability of PH (n=27), as compared with high probability of PH (n=7)
- C. Levels of D-dimer in patients with low-to-intermediate probability of PH (n=27), as compared with high probability of PH (n=7)

- D. Levels of PPL clotting time in patients with low-to-intermediate probability of PH (n=27), as compared with high probability of PH (n=7)
- E. Levels of erythrocyte-derived microparticles in patients with low-to-intermediate probability of PH (n=27), as compared with high probability of PH (n=7)
- F. Levels of platelet-derived microparticles in patients with low-to-intermediate probability of PH (n=27), as compared with high probability of PH (n=7)

Comparisons were made using Mann-Whitney U test

*P-value < 0.05; **P-value <0.001

MP : microparticles; PPL: pro-coagulant microparticles

Table 1. Baseline characteristics of the patients included in the study (n=36)

Demographics	
Age, years	27 (22-31)
Gender, M/F (ratio)	23/13 (1.8)
BMI, kg/m ²	21.5 (19.6-24.1)
Smoker or ex-smoker	7 (20)
Ethnicity	
Sub-Saharan Africa	27 (75)
Caribbean	6 (17)
Other	3 (8)
Sickle cell disease characteristics	
Genotype	
SS	34 (94)
SC	1 (3)
S beta-thalassemia	1 (3)
Baseline hemoglobin, g/dL	8.8 (8.0-9.5)
History of acute complications	
Vaso-occlusive crisis	36 (100)
Acute chest syndrome	31 (86)
Health-care consumption in the preceding year	
Emergency room	2 (1-5)

Hospitalization in conventional ward	2 (1-4)
Hospitalization in ICU	0 (0-1)
Chronic complications	
Proteinuria	9 (25)
Chronic renal insufficiency	0 (0)
Retinopathy	18 (50)
Biliary lithiasis	23 (64)
Cholecystectomy	20 (56)
Splenectomy	3 (8)
Bone complications	13 (36)
Leg ulcers	1 (3)
Chronic treatments	
Hydroxyurea	24 (67)
Chronic exchange transfusions	0 (0)

Values are expressed as median (25-75 percentiles) or as number (%).

BMI: body mass index; Bone complications refer to osteonecrosis, arthrosis and osteomyelitis.

ICU: intensive care unit; PH: pulmonary hypertension

Table 2. Characteristics of the acute chest syndromes (n=39)

<i>Variables</i>	<i>Available data</i>	<i>All population (n=39)</i>
<i>Clinics</i>		
Time from symptoms onset to ICU referral, days	39	3 (1-7)
Thoracic pain	39	34 (87)
Cough	39	17 (44)
Dyspnea	39	29 (74)
Golden sputum	39	14 (36)
Fever	39	12 (31)
Limb's pain	39	26 (67)
Systolic blood pressure, mmHg	39	122 (117-135)
Diastolic blood pressure, mmHg	39	72 (67-84)
Heart rate, min ⁻¹	39	104 (92-117)
Temperature, °C	39	37.2 (36.9-38.1)
Respiratory rate, min ⁻¹	28	26 (21-30)
Crepitant	39	24 (62)
Tubal breath	39	7 (18)
<i>Biology</i>		
Hb, g/dL	39	7.5 (6.3-8.5)
Platelets, mm ⁻³	39	270 (200-391)
Leucocytes, mm ⁻³	39	17.1 (14.5-20.6)

Neutrophils, mm⁻³	39	12.6 (10.0-15.2)
Reticulocytes, mm⁻³	39	272 (180-335)
Total Bilirubin, micromol/L	39	51 (36-69)
LDH, IU/L	39	542 (414-882)
Fibrinogen, g/L	33	4.4 (3.8-5.7)

Microbiological documentation

MSSA	39	6 (15)
Streptococcus pneumoniae	39	2 (5)
Respiratory virus	39	3 (8)

HRCT data

Alveolar consolidation	38	35 (92)
Ground glass opacity	38	11 (29)
Pleural effusion	38	7 (18)
Pulmonary embolism	38	2 (5)
PA:A ratio >1	38	16 (42)
RV:LV ratio > 1	38	0 (0)

Values are expressed as median (25-75 percentiles) or number (%).

Hb: hemoglobin; ICU: intensive care unit; LDH: Lactate dehydrogenase; LVEF: left ventricular ejection fraction; MSSA: methicillin-sensitive Staphylococcus aureus; PA:A ratio: pulmonary artery to ascending aorta diameter ratio; PH: pulmonary hypertension; RV:LV ratio: right ventricular to left ventricular diameter ratio; TAPSE: tricuspid annular plane systolic excursion; HRCT: High Resolution Computed Tomography

Table 3. Echocardiographic parameters, contrasting low-to-intermediate and high-probability of acute pulmonary hypertension

<i>Echocardiographic parameters</i>	<i>Low-to-intermediate probability of PH, n=27</i>	<i>High probability of PH, n=7</i>	<i>P</i>
Heart rate, beat/minute	107 (86-113)	96 (83-117)	0.90
Left atrial surface, cm ²	20 (18-24)	22 (20-26)	0.33
Right atrial surface, cm ²	15 (13-17)	17 (14-20)	0.23
End-diastolic ventricular area ratio	0.56 (0.52-0.63)	0.64 (0.58-0.71)	0.09
E wave at the mitral level, cm/s	94 (79-103)	106 (84-120)	0.3
A wave at the mitral level, cm/s	68 (57-83)	68 (49-95)	0.88
E /A ratio	1.4 (1.1-1.7)	1.4 (1.3-1.9)	0.64
Lateral Ea wave, cm/s	17 (15-20)	16 (15-24)	0.78
E/Ea ratio	5.3 (4.2-6.9)	5.2 (4.7-7.0)	0.83
LV EF, %	63 (60-67)	57 (55-65)	0.15
Cardiac index, L/mn/m ²	3.2 (2.9-3.6)	3.2 (2.8-4.1)	0.97
TAPSE, cm	25 (21-29)	25 (22-25)	0.63
Tricuspid annular peak systolic	17 (15-19)	19 (14-21)	0.47

velocity (s'), cm/s

Peak tricuspid regurgitation

2.6 (2.5-2.8)

3.1 (3.0-3.4)

0.0001

velocity, m/sec

Right ventricular outflow

113 (94-125)

91 (81-109)

0.04

Doppler acceleration time, sec

Inferior cava diameter, mm

11 (8-17)

16 (14-18)

0.31

Systolic pulmonary artery pressure, mm Hg

31 (29-37)

44 (39-51)

0.0003

Values are expressed as median (25-75 percentiles) or number (%).

PH: pulmonary hypertension; LVEF: left ventricular ejection; TAPSE: tricuspid annular plane systolic excursion

Table 4. Treatments and outcomes of the acute chest syndromes (n=39)

	<i>Available data</i>	<i>All population (n=39)</i>
Treatments		
Supplemental oxygen at admission, L.min-1	39	4 (2-5)
- 1-5L.min-1		29 (74)
- > 5L.min-1		10 (26)
Mechanical ventilation	39	1 (3)
Antimicrobial therapy	39	39 (100)
Blood product transfusion	39	14 (36)
Outcomes		
ICU length of stay, days	39	4 (3-5)
Hospital length of stay, days	39	10 (7-13)
Supplemental oxygen at 48h from admission, L.min-1	36	
- 0 L.min-1		3 (8)
- 1-5L.min-1		32 (89)
- > 5L.min-1		1 (3)
Death *	39	1 (3)

Values are expressed as median (25-75 percentiles) or number and frequency.

ICU: intensive care unit

*The patient who died was a non-smoker 34-year-old man, normal-weighted (BMI 18.4 kg/m²), with a history of multiple vaso-occlusive crises and ACS, bone complications and

cholecystectomy. He came first at hospital for isolated fever, but rapidly developed a meningeal syndrome, signs of ACS (fever, chest pain and dyspnea with chest X-ray opacities) with 6L/min need of oxygen, and was referred to the ICU. Large basal consolidations were evidenced on CT scan, without pulmonary embolism. A *Streptococcus pneumoniae* was identified in the cerebrospinal fluid, but respiratory tract samples remained sterile. Laboratory findings included a severe central bicytopenia (Hb 4.4 g/dL, reticulocytes 20,300/mm³, platelets 91,000/mm³) and high inflammation (leucocytes 35,600/mm³). The patient had an intermediate probability of PH at initial TTE. Initial levels of erythrocyte and platelet-derived MPs were low (1398 and 1601 MPs/ μ L plasma, respectively). Despite antibiotics and transfusion, the patient deteriorated and developed confusion, acute respiratory failure and acute renal failure requiring intubation and dialysis. The signs evolved towards a severe and fatal acute respiratory distress syndrome (ARDS) 6 days after ICU admission.

Figure E1. Changes in peak tricuspid regurgitation velocity at the time of the acute chest syndrome and at baseline
 A significant reduction of PTRV from 2.8 m/sec (IQR 2.6-3.0) during ACS to 2.5 m/sec (IQR 2.1-2.6) at steady state ($p=0.003$) was noted in the 19 patients for whom data were available.
 ACS: acute chest syndrome

Figure E2. Biomarkers of endothelial dysfunction and hypercoagulability state in sickle cell patients in acute chest syndrome, as compared with steady state

- A. Levels of tissue factor in patients during ACS, as compared with steady state (n=14)
 - B. Levels of fibrin monomers in patients during ACS, as compared with steady state (n=14)
 - C. Levels of D-dimer in patients during ACS, as compared with steady state (n=14)
 - D. Levels of PPL clotting time in patients during ACS, as compared with steady state (n=14)
 - E. Levels of erythrocyte-derived microparticles in patients during ACS, as compared with steady state (n=14)
 - F. Levels of platelet-derived microparticles in patients during ACS, as compared with steady state (n=14)
 - G. Levels of platelet-derived microparticles in patients during ACS, as compared with steady state (n=14)
- Comparisons were made using Wilcoxon matched-pair signed rank test
 *P-value < 0.05; ***P-value < 0.0001
 ACS : acute chest syndrome ; MP : microparticles; PPL: pro-coagulant microparticles

Figure E3. Biomarkers of endothelial dysfunction and hypercoagulability state in sickle cell patients at steady state as compared with healthy controls

- A. Levels of tissue factor in patients at steady state (n=14) as compared with healthy controls (n=10)
- B. Levels of fibrin monomers in patients at steady state (n=14) as compared with healthy controls (n=10)
- C. Levels of D-dimer in patients at steady state (n=14) as compared with healthy controls (n=10)
- D. Levels of PPL clotting time in patients at steady state (n=14) as compared with healthy controls (n=10)
- E. Levels of erythrocyte-derived microparticles in patients at steady state (n=14) as compared with healthy controls (n=10)
- F. Levels of platelet-derived microparticles in patients at steady state (n=14) as compared with healthy controls (n=10)
- G. Levels of platelet-derived microparticles in patients at steady state (n=14) as compared with healthy controls (n=10)

Comparisons were made using Mann-Whitney U test
*P-value < 0.05; **P-value < 0.001; ***P value < 0.0001

Figure E4. Levels of microparticles at steady state, contrasting low-to-intermediate and high probability of acute pulmonary hypertension at the time of the acute chest syndrome

A. Levels of erythrocyte-derived microparticles at steady state in patients with low-to-intermediate probability of PH (n=15) during ACS, as compared with patients with high probability of PH (n=3) during ACS

B. Levels of platelet-derived microparticles at steady state in patients with low-to-intermediate probability of PH (n=15) during ACS, as compared with patients with high probability of PH (n=3) during ACS
Comparisons were made using Mann-Whitney U test

**P-value <0.01

ACS : acute chest syndrome ; MP : microparticles; PH : pulmonary hypertension