STIMULUS REPORT

blood advances



# IL-6 levels are dramatically high in the sputum from children with sickle cell disease during acute chest syndrome

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**Key Points**

- Sputum interleukin-6 (IL-6) level is high during acute chest syndrome (ACS) in pediatric sickle cell disease, supporting anti–IL-6 trials.
- Sputum IL-8, CCL2, and CCL3 levels are also high during ACS, possibly contributing to recruitment of inflammatory cells in the lungs.

## Introduction

Sickle cell disease (SCD) is a severe hemoglobin (Hb) disorder characterized by hemolytic anemia, recurrent painful vasoocclusive events, and ischemia/reperfusion-driven inflammation.[1] Acute chest syndrome (ACS), a common and potentially life-threatening form of acute lung injury in SCD, is classically defined as fever and/or respiratory symptoms, accompanied by a new pulmonary infiltrate on chest radiograph.[2] Although ACS is considered a leading cause of morbidity and premature death in SCD patients, underlying pathophysiological mechanisms remain incompletely understood, and therapeutic options are therefore limited.[3] Multiple factors may contribute to the development of ACS, including viral or bacterial infection, hypoventilation secondary to pain during vasoocclusive crisis (VOC), fat embolism, and thromboembolism, but in most cases, especially in severe forms with acute respiratory failure, pathogenesis remains unclear.[4,5] A role of inflammation induced by ischemia/reperfusion and hemolysis has been strongly suggested,[1,6] which may be mediated by activation of lung endothelium,[6,7] as well as innate immune cells, including neutrophils, platelets, and monocytes.[8-10] However, levels of proinflammatory cytokines and chemokines in the lungs during ACS have not yet been studied. Interestingly, golden sputum, a hallmark of ACS defined by the yellowish coloration of the sputum during ACS, was shown to be related to an intense exudative process rather than to the presence of bilirubin, but its origin remains unknown.[11]

The objective of our study was to investigate the levels of the main proinflammatory cytokines and chemokines in the sputum from children with SCD during ACS, compared with non-ACS sputum and plasma levels.

## Methods

### Study design

We performed a prospective observational study between 2017 and 2019 in a pediatric French university hospital SCD reference center. Eligibility criteria were SCD of any type, including Hb SS, SC, $S/\beta^0$, and $S/\beta^+$, and age $\geq$1 but $<$18 years. Exclusion criteria were other disease resulting in increased systemic or pulmonary inflammation (eg, inflammatory disease, asthma) and anti-inflammatory treatment. We recruited 42 SCD patients and collected sputum samples during ACS (n = 12) or during non-ACS respiratory events in the setting of VOC (ie, respiratory symptoms such as cough, tachypnea, and hypoventilation without a new pulmonary infiltrate on chest radiograph; n = 6) and blood samples during ACS (n = 12), during VOC (n = 12), at steady state (n = 12), and from controls recruited among unaffected siblings (Hb AA) of SCD children (n = 9). All patients with ACS were hospitalized in the pediatric intensive care unit (PICU) and were treated with early noninvasive ventilation, according to the local protocol.[12] Patients with VOC were hospitalized either in the pediatric department or in the PICU. Inclusion and sample collection were performed during the first 3 days of hospitalization or during

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**Table 1. Main clinical and biological characteristics of patients with SCD and controls**

| | Controls (n = 9) | Steady state (n = 12) | VOC (n = 12) | ACS (n = 12) | Non-ACS (n = 6) |
|---|---|---|---|---|---|
| Age, y | 12.4 (7.9-15.6) | 10.1 (7.9-14.2) | 11.9 (9.6-14.5) | 9.7 (7.2-11.0) | 8.6 (6.1-11.5) |
| Female sex, % | 67 | 58 | 33 | 33 | 33 |
| SCD type (Hb SS/S$\beta^0$/SC), n | — | 10/1/1 | 10/1/1 | 12/0/0 | 6/0/0 |
| Hydroxyurea, % | — | 42 | 75 | 33 | 50 |
| Transfusion program, % | — | 42 | 0 | 8 | 0 |
| Hb level, g/dL | 11.7 (11.3-12.1) | 8.8 (8.2-10.3) | 8.3 (7.6-8.8) | 7.1 (6.0-7.8) | 7.6 (7.0-7.8) |
| White blood cells, $\times 10^9$/L | 4.7 (3.8-5.1) | 7.2 (5.6-8.7) | 10.4 (8.2-12.5) | 21.1 (16.0-23.1) | 12.6 (10.6-22.3) |
| Neutrophils, $\times 10^9$/L | 2.5 (1.7-3.1) | 2.8 (2.5-3.5) | 5.3 (3.8-8.5) | 14.0 (9.5-16.3) | 7.7 (4.9-14.9) |
| Platelets, $\times 10^9$/L | 283 (233-304) | 371 (272-395) | 306 (169-378) | 215 (175-352) | 430 (330-495) |
| CRP level, mg/L | — | — | 22 (15-42) | 149 (134-187) | 41 (21-62) |
| Interval between first symptoms and sample collection, d | — | — | 1 (1-2) | 2.5 (2-3) | 2 (1-2) |
| Golden sputum, % | — | — | — | 100 | 0 |
| Mechanical ventilation (noninvasive/invasive), % | — | — | 0 | 100 (75/25) | 0 |
| Duration of hospital stay, d | — | — | 6 (6-8) | 13 (10-16) | 6 (4-8) |
| Death, % | — | — | 0 | 0 | 0 |

Data are median (interquartile range [IQR]) unless otherwise indicated.
CRP, C-reactive protein.

a routine consultation for patients in steady state and controls. Sputum was obtained during chest physiotherapy or by endotracheal suctioning for intubated patients. Blood was collected in ethylenediamine tetraacetic acid, and plasma was obtained by centrifugation (10 minutes, 3500 *g*, 4°C). Sputum and blood were stored at −80°C. Levels of the main proinflammatory cytokines and chemokines tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, CCL2, and CCL3 in sputum and plasma were measured with a multiplex assay (Bio-Plex Pro Human Cytokine 27-Plex Assay; Bio-Rad), following the manufacturer's instructions. Several clinical and biological data were obtained from the medical files of all patients (Table 1). Informed consent was obtained from parents or legal guardians of all children. The study was approved by a medical ethics committee (GR-Ex/CPP-DC2016-2618/CNIL-MR01).

### Statistical analysis

Data are expressed as median (IQR). Differences between groups were assessed with Mann-Whitney *U* test or 1-way analysis of variance with post hoc test, as appropriate. Paired Student *t* test was used to compare differences between patients for whom IL-6 was measured both in sputum and plasma, collected concomitantly (n = 5). Spearman's rank correlation was used for correlation analyses. Statistical significance threshold was set at a *P* value of .05.

## Results and discussion

### IL-6 levels are dramatically high in golden sputum from SCD patients during ACS

Median (IQR) plasma IL-6 level was not significantly increased during ACS (49 [37-94] pg/mL) compared with during VOC (54 [52-72] pg/mL), at steady state (36 [27-50] pg/mL), and in controls (36 [28-43] pg/mL; Figure 1A). By contrast, median IL-6 level in sputum was dramatically elevated during ACS (3320 [1233-6459] pg/mL) compared with non-ACS patients (13 [11-19] pg/mL; $P = .0009$; Figure 1B). For the 5 patients who had concomitant sputum and plasma collections during ACS, median IL-6 level was >150-fold higher in sputum (6892 [6314-7114] pg/mL) than in plasma (42 [35-48] pg/mL; $P = .0009$; Figure 1C). No correlation was found between sputum IL-6 level and C-reactive protein level during ACS ($r = -0.035$; $P = .9$). These results suggest that massive production of IL-6 in the lungs by activated endothelial cells or other inflammatory cells may be involved in ACS pathophysiology, by inducing local inflammation independently of systemic inflammation. Of note, the 5 patients with the highest sputum IL-6 levels (>6000 pg/mL) had the most severe ACS forms, with 2 patients requiring invasive mechanical ventilation for respiratory failure, 1 requiring high oxygen level under noninvasive ventilation, and 2 experiencing multiorgan failure. Therefore, sputum IL-6 level might be a marker of severity in ACS. Because our hospital is a reference center for SCD, patients admitted to the PICU are frequently transferred from other hospitals for particularly severe ACS, which may have contributed to the dramatically high levels of IL-6 measured in the sputum of our participants. Therefore, future multicenter studies are needed to confirm whether sputum IL-6 level is positively correlated with ACS severity.

Increases in bronchoalveolar IL-6, IL-1β, and TNF-α levels have been previously reported in transgenic sickle cell mice exposed to prolonged hypoxia.[13] However, in our patients, no significant increases in the levels of IL-1β and TNF-α were observed in plasma (Figure 1D,F) or sputum (Figure 1E,G) during ACS, suggesting a predominant role of IL-6 in ACS pathophysiology. Sputum IL-6 level is a well-known biomarker of respiratory inflammation, which was recently found increased at steady state in 139 SCD children (mean, 43 ± 4 pg/mL) compared with 123 healthy age-matched controls (mean, 20 ± 4 pg/mL).[14] Importantly, a positive correlation was reported between sputum IL-6 level and number of ACS episodes, supporting our hypothesis of an involvement of IL-6 in ACS pathophysiology.

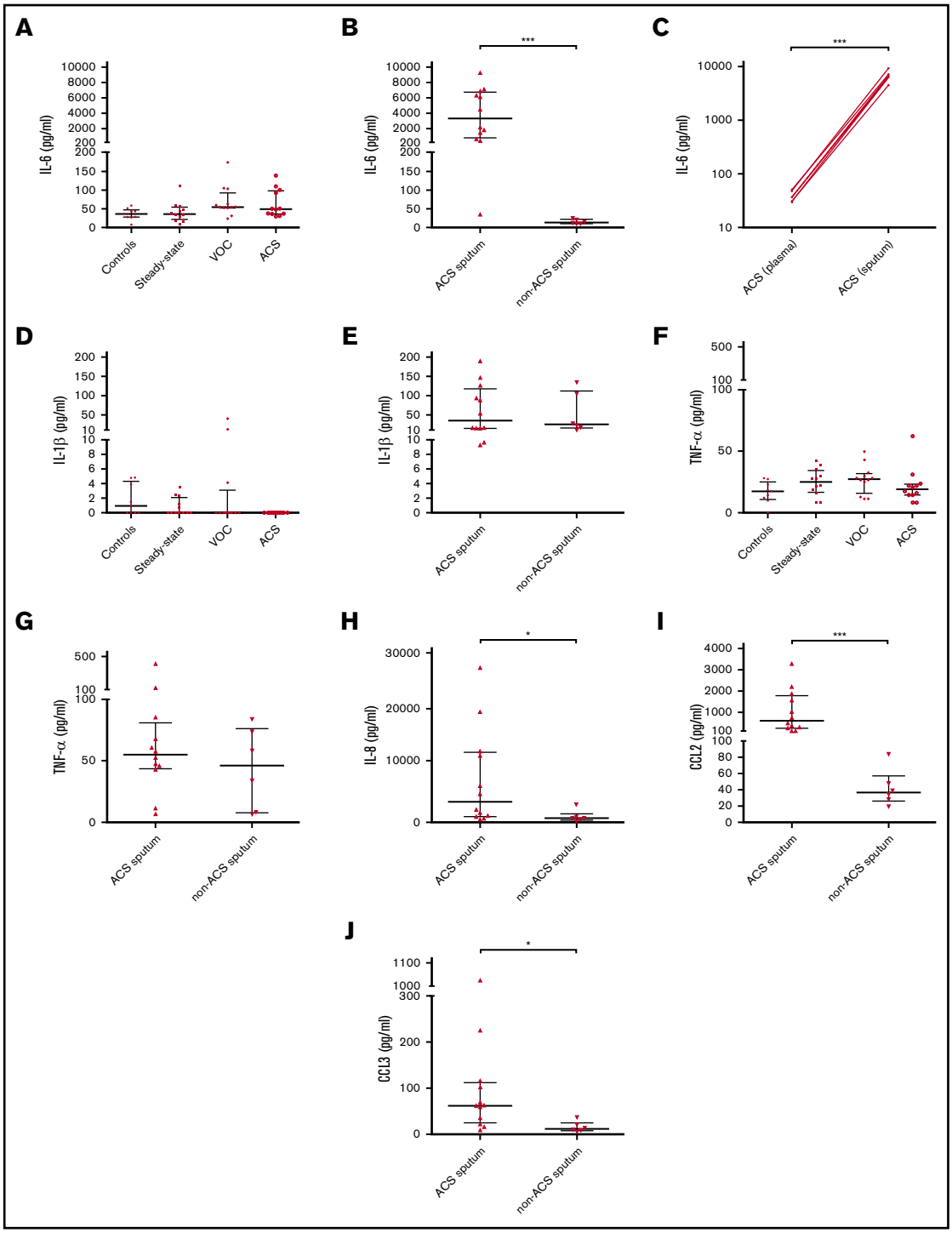


**Figure 1. Plasma and sputum levels of the main proinflammatory cytokines and chemokines in children with SCD.** (A) Plasma level of IL-6 in 9 healthy controls and 36 SCD patients, at steady state (n = 12), during VOC (n = 12), and during ACS (n = 12). Median (IQR) plasma IL-6 level was not significantly higher during ACS (49 [37-94] pg/mL) compared with during VOC (54 [52-72] pg/mL), at steady state (36 [27-50] pg/mL), and in controls (36 [28-43] pg/mL). (B) By contrast, median (IQR) IL-6 level in sputum was dramatically elevated (3320 [1233-6459] pg/mL) in SCD patients during ACS (n = 12) compared with non-ACS (n = 6) respiratory events (13 [11-19] pg/mL; $P = .0009$). (C) For the 5 patients who had concomitant sputum and plasma collections during ACS, median (IQR) IL-6 level was >150-fold higher in sputum (6892 [6314-7114] pg/mL) than in plasma (42 [35-48] pg/mL; $P = .0009$). (D,F) Median plasma IL-1β and TNF-α levels were not increased during ACS compared with during VOC, at steady state, and in controls. (E,G) Median levels of IL-1β and TNF-α in sputum were not significantly increased during ACS compared with non-ACS respiratory events. (H-J) Median (IQR) sputum chemokine levels were higher during ACS compared with non-ACS respiratory events for IL-8 (3632 [1121-11 976] pg/mL vs 774 [575-948] pg/mL; $P = .044$) (H), CCL2 (591 [263-1624] pg/mL vs 37 [30-46] pg/mL; $P = .0009$) (I), and CCL3 (62 [32-105] pg/mL vs 12 [9-19] pg/mL; $P = .01$) (J). $^*P < .05$, $^{***}P < .001$.

Il-6 is a pleiotropic proinflammatory cytokine involved in the induction of acute-phase responses, which is produced by immune (eg, monocytes, macrophages, neutrophils) and nonimmune (eg, endothelial cells, epithelial cells, fibroblasts) cells in response to infectious or noninfectious lung injury.[15] The elevated sputum IL-6 levels in our study are quite unlikely to be attributable to viral or bacterial infection, because screening for respiratory viruses (human respiratory syncytial viruses, seasonal coronaviruses, parainfluenza and influenza viruses, metapneumovirus and rhinovirus/enterovirus) and atypical bacteria (*Chlamydia pneumoniae* and *Mycoplasma pneumonia*) was negative for all patients, as were blood cultures and bacteriological examination of sputum. Moreover, reported levels of IL-6 in endotracheal fluid from non-SCD children with severe pneumonia requiring mechanical ventilation are much lower than those observed in our patients.[16]

### Sputum levels of IL-8, CCL2, and CCL3 chemokines are high during ACS

Median (IQR) sputum chemokine levels were higher during ACS compared with non-ACS respiratory events for IL-8 (3632 [1121-11 976] pg/mL vs 774 [575-948] pg/mL; $P = .044$; Figure 1H), CCL2 (591 [263-1624] pg/mL vs 37 [30-46] pg/mL; $P = .0009$; Figure 1I), and CCL3 (62 [32-105] pg/mL vs 12 [9-19] pg/mL; $P = .01$; Figure 1J). These high chemokine levels could induce pulmonary recruitment of innate immune cells, including neutrophils and monocytes, which in turn may produce inflammatory mediators, thus contributing to a vicious cycle of local inflammation during ACS. Indeed, in sickle cell mice exposed to prolonged hypoxia, increased neutrophil count was observed in bronchoalveolar lavage together with increased inflammatory mediators and neutrophil lung infiltrates.[13] Also, in SCD patients during ACS, positron emission tomography with $^{18}$F-fluorodeoxyglucose revealed increased lung uptake, which was consistent with lung neutrophilic inflammation.[17]

Here, we report for the first time markedly increased levels of IL-6, IL-8, CCL2, and CCL3 in the golden sputum from SCD children during ACS. Whether IL-6 should be considered a marker for or full actor in ACS requires further investigation. However, recent reports of dramatic improvement after tocilizumab in a child and an adult patient with SCD and ACS related to SARS-CoV-2 suggest that IL-6 may play an essential role in ACS.[18,19] Clinical trials with immunomodulatory agents targeting IL-6 in patients with severe ACS should be considered.

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## Authorship

Contribution: S.A., R.R.-B., T.T.M., and O.H. designed the study; S.A., M.d.M., M.T., J.B., J.-M.T., V.B., F.M., and C.H. were responsible for patient recruitment and management; S.A., R.R.-B., and T.T.M. performed the experiments; S.A., M.d.M., T.T.M., and O.H. analyzed all experiments and drafted the manuscript; and all authors reviewed the manuscript.

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