Lower respiratory tract infection with *Staphylococcus aureus* in sickle-cell adult patients with severe acute chest syndrome - the STAPHACS Study

Acute chest syndrome (ACS) is the most common acute pulmonary complication of sickle cell disease (SCD). It may progress to a life-threatening event requiring the use of mechanical ventilation, with a mortality rate ranging from 3% to 50% when acute respiratory distress syndrome develops. Lung infection may account for 30% of the aetiologies of ACS. The causal relationship between *Staphylococcus aureus* (S. aureus) and ACS has been described for many years. However, this microorganism has been identified in only 4% of cases in the largest series published to date. To our knowledge, there is no specific study focusing on acute lower respiratory tract infection (LRTI) associated with *S. aureus* in SCD adult patients with ACS, in the pneumococcal vaccine era. The objectives of this pilot study were to describe *S. aureus* LRTI in SCD patients with severe ACS, in terms of prevalence, clinical and laboratory findings and outcomes.

We conducted a retrospective observational study from April 2015 to December 2017 in SCD patients with ACS admitted to the intensive care unit (ICU) of Tenon Hospital, Paris, France, a tertiary university hospital and referral center for SCD. The definition of ACS combined fever or chest pain and a new pulmonary infiltrate of at least one segment on thoracic imaging, ACS was considered to be associated with *S. aureus* (alone or associated with another microorganism) when respiratory tract samples or blood cultures yielded *S. aureus*, in the absence of any identifiable clinical source other than the lung. Patients with ACS associated with *S. aureus* (*S. aureus* group) were compared to patients with ACS in whom another microorganism was identified or in whom no microbiological documentation was obtained despite a comprehensive microbiological workup (control group).

The workup included i) respiratory tract samples (sputum, tracheal aspirate [TA], or bronchoalveolar lavage [BAL]) with Gram staining and quantitative culture for bacterial microorganisms; ii) blood cultures; iii) urinary antigen testing for *Streptococcus pneumoniae* and *Legionella pneumophila*. Additionally, a respiratory multiplex polymerase chain reaction (mPCR) test (FilmArrayTM Respiratory Panel system) has been available in our unit since 2016.

This study was conducted in accordance with the French law, and was approved by the Ethical Review Board of the Société de Pneumologie de Langue Française (CEPRO 2019-021).

During the study period, 119 episodes of ACS were recorded in 114 patients. Forty-two episodes (40 patients) were excluded from the analysis because of incomplete microbiological investigation (Figure 1). Overall, *S. aureus* was identified in 29 of 119 episodes (24%), including respiratory tract samples cultures in 28 episodes, and blood culture in one episode (Table 1). Bacterial and viral co-infections were respectively diagnosed in four (14 %) and three (10%) episodes in the *S. aureus* group. The overall distribution of the ACS episodes associated with *S. aureus* was sporadic throughout the year (Online Supplementary Figure S1).

| Microbiological investigations, n (%) | S. aureus ACS group, N=29 | Control ACS group, N=48 | P     |
|-------------------------------------|---------------------------|-------------------------|-------|
| Spumum                            | 26 (90)                   | 47 (98)                 | 0.15* |
| Tracheal aspirate                  | 1 (3)                     | 7 (15)                  | 0.25* |
| Broncho-alveolar lavage            | 1 (3)                     | 3 (6)                   | 0.98* |
| Blood culture                      | 28 (97)                   | 47 (98)                 | 0.98* |
| *Streptococcus pneumoniae* urinary antigen test† | 24 (83) | 47 (98) | 0.03 |
| *Legionella pneumophila* urinary antigen test† | 26 (90) | 47 (98) | 0.15* |
| *Chlamydia pneumoniae* serology    | 7 (24)                    | 8 (17)                  | 0.42  |
| *Mycoplasma pneumoniae* serology   | 8 (28)                    | 9 (19)                  | 0.37* |
| Nasopharyngeal swab (multiplex PCR)† | 19 (66) | 31 (65) | 0.93 |
| Parvovirus B19 serology            | 11 (38)                   | 14 (29)                 | 0.43  |
| **Microbial Identification, n (%)** |                           |                         |       |
| Respiratory tract                  |                           |                         |       |
| MSSA£                              | 27 (93)                   | N/A                     |       |
| MRSA£                              | 2 (7)                     | N/A                     |       |
| Other bacterial microorganism      | 4 (14)§                   | 10 (21)#                | 0.44  |
| Respiratory virus†                 | 3 (10)§                   | 5 (10)†                 | 0.99* |
| Blood                              | 1 (3)                     | 3 (7)‡                  | 0.99* |

Finally, all the patients had at least one respiratory tract sample, except 1 patient in the *Staphylococcus aureus* (S. aureus) group in whom *S. aureus* was identified in blood culture.

#Urinary antigen testing for *Streptococcus pneumoniae* and *Legionella pneumophila* was the BinaxNOW kits (Alere, Jouy en Josas, France). £MSSA: Methicillin-sensitive *S. aureus*; MRSA: Methicillin-resistant *S. aureus*. °*Streptococcus pneumoniae* (n=3), *Klebsiella pneumoniae* (n=1), *Mycoplasma pneumoniae* (n=1). †*Chlamydia pneumoniae* (n=2), *Streptococcus pneumoniae* (n=2), *Legionella pneumophila* (n=1). The workup included i) respiratory tract samples (sputum, tracheal aspirate [TA], or bronchoalveolar lavage [BAL]) with Gram staining and quantitative culture for bacterial microorganisms; ii) blood cultures; iii) urinary antigen testing for *Streptococcus pneumoniae* and *Legionella pneumophila*. Additionally, a respiratory multiplex polymerase chain reaction (mPCR) test (FilmArrayTM Respiratory Panel system) has been available in our unit since 2016.

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Letters to the Editor

specifically, *S. aureus* was identified during the flu season in 24% of episodes (Online Supplementary Table S1), a rate that did not differ from that of the other episodes of ACS (35%; \( P=0.3 \)).

The baseline characteristics were similar between the two groups, except the presence of a more frequent prior history of *S. aureus* infections in the *S. aureus* group, as compared with the control group (28% vs. 6%; \( P=0.01 \)) (Online Supplementary Table S1). Despite similar rates of influenza and pneumococcal vaccination, the pneumococcal vaccination strategies differed (13-valent pneumococcal vaccine only: none in the *S. aureus* group vs. 17% in the control group; \( P=0.02 \)).

The characteristics, management and outcomes of ACS were also similar between the two groups. Post-hospital outcomes marginally differed, in terms of number of and time to hospital readmission for vaso-occlusive crisis or ACS (Online Supplementary Figure S2).

Among the 29 ACS episodes with *S. aureus*, 21 isolates (72%) were sent to the National Staphylococcus Reference Center for genotyping analysis. *S. aureus* strains were genetically diverse, covering the four accessory gene regulator groups and assigned to 12 clonal complexes (CC) (Table 2). At least one toxin gene was found in 13 isolates (62%); two methicillin-sensitive (MSSA) isolates (10%) had a gene coding for panton-valentine leukocidin (PVL).

This study underlines the importance and the clonal diversity of *S. aureus* during severe episodes of ACS. Although unrelated to the influenza epidemic, ACS associated with *S. aureus* appeared inversely related to the pneumococcal vaccination strategy, raising the question of how the pneumococcal vaccination may affect the nasopharyngeal colonization of those patients. A history of *S. aureus* infection was associated with the subsequent development of a documented ACS episode with *S. aureus*.

In our study, one quarter of the episodes of ACS were associated with *S. aureus*, although this microorganism is infrequently involved in community-acquired LRTI1 and in ACS.1 Current guidelines advise using antibiotics targeting *S. pneumoniae* and intracellular bacteria.3 In our series, *S. pneumoniae* was identified in only four episodes of ACS (accounting for 5% of episodes with a complete microbiological investigation) including two co-infections with *S. aureus*. This low proportion of *S. pneumoniae* is probably due to the good pneumococcal vaccination coverage in our cohort. One hypothesis explaining the increase in the prevalence of *S. aureus* could be a decrease in the carriage of *S. pneumoniae* related to the extensive vaccination in this fragile population. The effectiveness of pneumococcal vaccination has led to the decrease in invasive pneumococcal infection in SCD patients, and may have changed the prevalence of *S. aureus* infection in this population.6 An inverse relationship between the oropharyngeal carriage of *S. pneumoniae* and *S. aureus*

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**Figure 1. Selection of the episodes of acute chest syndrome.** The microbiological investigation was incomplete in 42 episodes, including 26 episodes (25 patients) with no respiratory tract samples, 14 episodes (14 patients) with no blood culture, and 16 episodes (14 patients) with no pneumococcal and legionella urinary antigen testing. Altogether, 77 episodes (in 74 patients) with a complete microbiological investigation were analyzed, including 29 episodes (24%) associated with *Staphylococcus aureus* (*S. aureus*) in 28 patients (1 patient had two episodes of acute chest syndrome [ACS] associated with *S. aureus*), and 48 episodes (in 49 patients) associated either with another microorganism or with no microorganism.
has already been suggested, and could be related to an inhibitory effect of \textit{S. pneumoniae} on \textit{S. aureus} via the production of hydrogen peroxide.\textsuperscript{7} These findings raise the question whether oropharyngeal carriage may be associated with a risk for developing ACS with \textit{S. aureus}. In the general population, an increase in the prevalence of \textit{S. aureus} carriage over a prolonged period has been suggested after pneumococcal vaccination.\textsuperscript{3} Moreover, \textit{S. aureus} infection might be more common in patients who are colonized.\textsuperscript{10} Some series have highlighted the risk of \textit{S. aureus} oropharyngeal carriage and the risk of \textit{S. aureus} infection in the general population\textsuperscript{9} as well as in the critical care setting.\textsuperscript{10} A sickle-cell pediatric series suggested that \textit{S. aureus} colonization was also associated with a subsequent risk of \textit{S. aureus} infection, without specifying the site of infection.\textsuperscript{11} In our study, a history of \textit{S. aureus} infection was associated with the subsequent occurrence of ACS associated with this microorganism. This finding may also suggest a chronic carriage of \textit{S. aureus} in this population, and the subsequent risk for developing another \textit{S. aureus} infection.

\textit{S. aureus} has been implicated in influenza LRTI.\textsuperscript{12} In our study, 24\% of the ACS episodes associated with \textit{S. aureus} occurred during the flu season, a rate that did not differ from that of the other ACS episodes. The viral co-infection rate was 10\%, but influenza was identified in only one episode, despite a broad search using mPCR (66\% of patients with \textit{S. aureus} documentation). In addition, the ACS episodes associated with \textit{S. aureus} were not preponderant during the flu season.

We identified two MRSA, accounting for 3\% of the \textit{S. aureus} strains, a rate higher than that usually reported in community-acquired pneumonia,\textsuperscript{3} but lower than the 16\% rate in a recent series concerning community acquired pneumonia.\textsuperscript{13} SCD patients are regularly hospitalized and exposed to repeat antimicrobial therapies, which could partly explain this proportion. Nevertheless, a pediatric series suggested a similar rate of MRSA oropharyngeal carriage between SCD and non-SCD populations.\textsuperscript{14}

Despite a higher initial clinical severity, the \textit{S. aureus} group had similar short-term outcomes to the control group, a finding that may be related to the fact that early antimicrobial treatments were administered to all patients, as recommended.\textsuperscript{3}

Genotypic analysis demonstrated a significant clonal diversity, with a 62\% rate of toxin genes, similarly to colonizing and pathogenic \textit{S. aureus} strains.\textsuperscript{15} While the PVL strains may be involved in necrotizing community-acquired pneumonia,\textsuperscript{13} the two ACS episodes were not associated with necrotizing pulmonary lesions, and had favorable outcomes.

Our study has limitations inherent to all retrospective monocentric studies, and our results should be extrapolated with caution. The selection criteria may have underestimated the prevalence of \textit{S. aureus}, but also the prevalence of other microorganisms, in particular intracellular bacteria. In some patients, the vaccination program may have been just initiated before the ACS episode, precluding any formal conclusion about the relationship between \textit{S. aureus} ACS and the pneumococcal vaccination strategy. Last, the distinction between \textit{S. aureus} colonization and \textit{S. aureus} infection may have been difficult in some cases.

Whether the identification of \textit{S. aureus} is associated with colonization in ACS, and whether this colonization is associated with a subsequent risk for developing a new infection is uncertain. In order to answer this, it would be necessary to take sequential samples at baseline and during episodes of ACS. In this context, and due to the limitations of the current diagnostic tests, the value of quantitative PCR may help to distinguish between colonization and deep pulmonary infection. Moreover, decontamination of the \textit{S. aureus} carriage sites could be useful. Finally, antibiotic therapy targeting MSSA on admission of patients with ACS having a prior \textit{S. aureus} infection may be warranted.

\textbf{Table 2. Genotypic markers of \textit{Staphylococcus aureus}.}

| Variables, n (%) | Strains, n=21 |
|------------------|--------------|
| Toxin            | 13 (62)      |
| TSST-1           | 2 (10)       |
| Enterotoxins\textsuperscript{4} | 11 (52) |
| PVL              | 2 (10)       |
| Exfoliative toxin\textsuperscript{4} | 1 (5) |
| \textit{agr} allele |
| I                | 10 (48)      |
| II               | 7 (33)       |
| III              | 3 (14)       |
| IV               | 1 (5)        |
| Clonal Complex   |              |
| CC15 MSSA        | 5 (24)       |
| CC12 MSSA        | 2 (10)       |
| CC8 MSSA         | 2 (10)       |
| CC8 MRSA         | 2 (10)       |
| CC398 MSSA       | 2 (10)       |
| CC188 MSSA       | 1 (5)        |
| CC152 MSSA       | 1 (5)        |
| CC121 MSSA       | 1 (5)        |
| CC97 MSSA        | 1 (5)        |
| CC96 MSSA        | 1 (5)        |
| CC38 MSSA        | 1 (5)        |
| CC45 MSSA        | 1 (5)        |
| CC390 MSSA       | 1 (5)        |

\textsuperscript{TSST: toxic shock syndrome toxin; PVL: Panton-Valentine leucocidin; \textit{agr}: accessory gene regulator; MSSA: methicillin-resistant \textit{Staphylococcus aureus}; MRSA: methicillin-sensitive \textit{Staphylococcus aureus}; CC: clonal complexes; SEA (n=2); SEB (n=3); SEC (n=2); SEF (n=1); SEI (n=1); SED (n=1); SEJ (n=1); SEK (n=2); SEL (n=1); SEN (n=1); SEO (n=1); SEP (n=4); SEI (n=2); SEQ (n=1); SER (n=2); \textit{ETA} (n=1).}

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\textsuperscript{1}Correspondence: ALEXANDRE ELABBADI - alexandre.elabbadi@aphp.fr
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Contributions: MF and AE collected, analyzed, and interpreted the data; AE and MF drafted the manuscript; MF, AE, and GV contributed to the study conception and design; FV, AT, and CV performed investigation of S. aureus strains; MF, GV, FL, FV, CV, and AT critically revised the manuscript. All the authors read and approved the final manuscript.

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