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Molecular testing for respiratory pathogens in sickle cell disease adult patients presenting with febrile acute chest syndrome[☆]

Apport de la biologie moléculaire dans la détection des pathogènes respiratoires chez les patients drépanocytaires adultes présentant un syndrome thoracique aigu

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Résumé

Introduction. – Lors d'un épisode pulmonaire aigu fébrile chez un sujet drépanocytaire, il est parfois difficile de différencier un syndrome thoracique aigu (STA) d'une pneumopathie infectieuse (ressemblance sémiologique, absence fréquente de documentation microbiologique). Notre objectif était d'évaluer l'apport de la PCR pour détecter des pathogènes respiratoires, en association avec les examens microbiologiques standards, dans la documentation microbiologique des STA fébriles des patients drépanocytaires adultes.

Matériels et méthodes. – Étude prospective, monocentrique et observationnelle réalisée de février 2015 à avril 2016. Des échantillons sanguins, respiratoires et urinaires ont été prélevés systématiquement avant et après antibiothérapie à des fins de cultures, antigénuries, sérologies et PCR pour détecter des pathogènes respiratoires.

Résultats. – Un pathogène a été détecté dans 12 des 61 STA fébriles (19,7 %) : quatre virus (6,6 %) (Rhinovirus; Influenza A/B), sept bactéries (11,4 %) (*S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *L. pneumophila*, *M. pneumoniae*), une co-infection (1,6 %) (*S. aureus* et Influenza B). La PCR a détecté une seule infection à *L. pneumophila* (séro-groupe 2). Si la durée d'antibiothérapie était significativement plus courte (6,1 vs 7,8 jours, $p=0,045$) dans le groupe des STA sans documentation microbiologique, aucune autre différence n'a été mise en évidence entre le groupe des STA microbiologiquement documentés et de ceux non documentés.

Conclusion. – Chez les patients drépanocytaires adultes, l'utilisation de la PCR à la recherche de pathogènes respiratoires améliore modérément le diagnostic microbiologique des STA fébriles, dans lesquels la part infectieuse est secondaire.

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Mots clés : Biologie moléculaire ; Drépanocytose ; Pneumopathie aiguë communautaire

Abstract

Background. – Differentiating acute chest syndrome (ACS) from community-acquired pneumonia (CAP) is challenging in adults presenting with major sickle cell disease (SCD) (semiological similarity, rare microbiological documentation). We aimed to assess the usefulness of nucleic

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acid amplification test (NAAT) for respiratory pathogens, in combination with standard bacteriological investigations, in febrile ACS adult patients presenting with major SCD.

Methods. – We performed a prospective, monocentric, observational study of 61 SCD adults presenting with febrile ACS from February 2015 to April 2016. Systematic blood, urine, and respiratory specimens were collected, before antibiotic initiation, for culture, urinary antigen tests, serology, and NAAT for respiratory pathogens.

Results. – A pathogen was detected in 12 febrile ACS (19.7%): four viruses (6.6%) (Rhinovirus; Influenza A/B), seven bacteria (11.4%) (*S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *L. pneumophila*, *M. pneumoniae*), one mixed infection (1.6%) (*S. aureus* and Influenza B). NAAT only detected *L. pneumophila* in one case (serogroup 2). Apart from a significantly shorter antibiotic therapy duration (6.1 vs. 7.8 days, $P=0.045$), no difference was observed between undocumented and microbiologically-documented febrile ACS.

Conclusion. – Using NAAT for the detection of respiratory pathogens in adults presenting with SCD slightly improved the microbiological diagnostic of febrile ACS, although respiratory infections are not the main etiological factor.

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Keywords: Nucleic acid detection; Sickle cell disease; Acute community-acquired pneumonia

1. Introduction

Acute chest syndrome is one of the leading causes of hospitalization and death in adult patients presenting with sickle cell disease [1].

Distinguishing acute chest syndrome from community-acquired pneumonia is difficult because of semiological similarities and of the low rates of microbiological documentation in both acute chest syndrome and community-acquired pneumonia [2–4]. Functional asplenia in sickle cell disease predisposes patients to encapsulated bacterial infection, particularly to *Streptococcus pneumoniae* and such infections are a classical trigger of acute chest syndrome [5].

The use of nucleic acid amplification tests (NAAT) for respiratory pathogen detection has improved the microbiological diagnostic yield in community-acquired pneumonia [6]. The sensitivity of NAATs is superior to the traditional procedures. Furthermore, the widespread use of pneumococcus and influenza vaccinations in sickle cell disease patients may have changed the distribution of pulmonary pathogens [1].

However, before generalizing the use of NAAT in routine practice, two studies conducted on sickle cell disease adults presenting with acute chest syndrome reported discrepant results concerning the microbiological detection rates in respiratory samples [2,3]. Maître et al. did not report any pathogen in 107 episodes of acute chest syndrome, but the bronchoscopy sampling was carried out after antibiotic initiation [2]. Vichinsky et al. detected pathogens in 32% of acute chest syndrome episodes, mainly *Chlamydo-philum pneumoniae*, *Mycoplasma pneumoniae*, and respiratory viruses [3].

The aim of this study was to assess the usefulness of NAAT, in combination with standard bacteriological investigations, in the microbiological detection of respiratory pathogens in febrile acute chest syndrome in adults presenting with major sickle cell disease.

2. Methods

2.1. Patients and data collection

We conducted an observational prospective monocentric study from February 2015 to April 2016 in the sickle cell disease Reference Center of Henri Mondor University Hospital (Créteil, France). This center manages a cohort of around 3500 patients, with an annual rate of 1000 hospitalizations and 200 acute chest syndromes.

Patients were deemed eligible for enrolment if they:

- presented with acute chest syndrome with fever strictly above 38 °C;
- were aged 18 years or above;
- had hemoglobin of phenotype SS, SC, or S-beta-thalassemia on electrophoretic analysis of the hemoglobin chains. Informed consent was obtained from patients beforehand.

The diagnosis of acute chest syndrome was based on respiratory symptoms (chest pain, tachypnea, cough, wheezing) and/or abnormal chest sounds, and a new pulmonary infiltrate on chest X-rays. If radiological findings at admission were normal with clinical features of acute chest syndrome, a new chest X-rays or CT-scan was performed 48 hours after hospitalization.

Standardized and anonymized forms were used to collect data.

The study was approved by the ethics committee Ile-de-France V (January 2015) and registered at the French Agency for the Safety of Health Products (French acronym ANSM) (ID-RCB 2014-A01749-38).

2.2. Specimen collection and laboratory testing

Before introducing antibiotics, we collected high-quality sputum for Gram stain and culture, blood samples for culture,

and urines for *Legionella pneumophila* serogroup 1, and pneumococcal urinary antigen tests (BinaxNOW, Alere). Serological tests for *Mycoplasma pneumoniae* (ELISA-Medac®, Germany), *Chlamydomphila pneumoniae* (ELISA-plus Medac® for IgG; ELISA-Medac® for IgM, Germany), and *Legionella* spp. (ELISA-Zeus scientific®, and if positive IFI-Eurobio®) were performed at baseline and at Week 2. NAATs were performed on sputum, irrespective of sample quality, for the detection of *Legionella* spp. (Diagenode®, France), and on nasopharyngeal swab for Influenza A/B viruses, respiratory syncytial virus (RSV), metapneumovirus (hMPV), rhinovirus/enterovirus (Rhino&EV/CC), adenovirus/bocavirus (AD/hBoV), coronavirus/parainfluenza virus (HCoV/HPIV), *C. pneumoniae*, *M. pneumoniae*, and *Bordetella* spp. (respiratory multi-well system r-geneTM®, Argene/bioMérieux Marcy l'Étoile, France).

White blood cell count, hemoglobin level, lactate dehydrogenase, C-reactive protein, and procalcitonin (PCT) at admission and in the first 72 hours of hospitalization were measured.

2.3. Microbiological diagnostic criteria

Bacterial pneumonia, except for atypical bacterial agents, was defined by the detection of Gram-positive or Gram-negative bacteria in the sputum or in blood samples, in the absence of other sources of infection, or by a positive pneumococcal urinary antigen test.

Chlamydomphila pneumoniae pulmonary infection was defined by a positive serology (presence of IgM > 1.1 and kinetics of IgG titer of a primary infection; or absence of IgM but increased IgG titer by a factor of at least four more than two weeks after the onset of infection; or IgG > 512 AU/mL at admission with a positive PCR, consistent with reinfection), or a positive PCR on nasopharyngeal swab only if associated with a positive serology [7]. The serology for *C. pneumoniae* was matched with a serology for *Chlamydia trachomatis* to check the absence of cross-reactivity.

Mycoplasma pneumoniae pulmonary infection was defined by a positive serology (presence of IgM or IgA > 10 and IgG > 64 AU/mL and kinetics of IgG titer of a primary infection; or absence of IgM and IgA but increased IgG titer by a factor of at least four two weeks or more after infection onset, consistent with reinfection), or by a positive PCR on nasopharyngeal swab [8].

Legionella spp. pulmonary infection was defined by a positive serology (indirect immunofluorescence with presence of IgM and IgG > 256 AU/mL at admission; or positive IgM with an increase in IgG titer by a factor of at least four 15 days later, with a second titer > 128 AU/mL for *L. pneumophila* and > 256 AU/mL for other species), or by a positive PCR on sputum; or by a positive urinary antigen test for *L. pneumophila* serotype 1; or by a positive culture of sputum or blood [9–11].

Viral bronchopulmonary infection was determined if any of the respiratory viruses included in the multiplex PCR was detected on a nasopharyngeal swab.

2.4. Treatment protocol

All sickle cell disease patients were treated according to the French guidelines for the management of acute chest syndrome: intravenous rehydration, multimodal analgesia with controlled-release morphine if needed, anxiety treatment, preventive anticoagulation, nasal cannula for oxygen therapy, and respiratory kinesiotherapy (incentive spirometry) [1]. Patients received red blood cell transfusion if recommended [1]. Antimicrobials were chosen as per European guidelines for the management of community-acquired pneumonia and French guidelines for the management of acute chest syndrome [1,12].

2.5. Statistical analysis

Descriptive results are expressed as medians (range) and proportions. Comparisons according to microbiologically-documented or undocumented acute chest syndrome status were performed using Mann Whitney tests for quantitative variables (duration of antibiotic course and hospital stay) and Fisher's exact test for qualitative variables (treatment by hydroxyurea). All analyses were performed using Stata V14.1 software (StataCorp, College Station, TX, USA).

3. Results

3.1. Characteristics of patients and acute chest syndrome episodes

Sixty-one patients were included. Demographics and sickle cell disease characteristics are summarized in Table 1.

Characteristics of the acute chest syndrome episodes are presented in Table 1. The main symptoms associated with fever were chest pain ($n = 58$, 95%) and tachypnea ($n = 42$, 68.9%). A vaso-occlusive crisis preceded acute chest syndrome in 39 patients (63.9%) with a mean interval of two days. Routine blood work results of patients presenting with vaso-occlusive crisis were typical [13]. Patients with documented bacterial infection had elevated PCT [0.2–2.4 $\mu\text{g/L}$], except one patient with pneumococcal pneumonia (0.2 $\mu\text{g/L}$). With PCT threshold > 0.5 $\mu\text{g/L}$ for a bacterial infection, its sensitivity and specificity were 91% and 61%, respectively. PCT positive and negative predictive values were 38.5% and 96.2%, respectively. Overall, no other difference regarding the recorded variables was observed between microbiologically documented and undocumented febrile acute chest syndrome episodes.

3.2. Detection of pathogens in febrile acute chest syndrome

Various samples were collected for the analyses, e.g. blood to perform culture from 57 patients (93.4%), high-quality sputum specimens for culture from 41 patients (67.2%), sputum specimens for *Legionella* spp. PCR from 21 patients (51.2%), nasopharyngeal swabs for multiplex PCR from 54 patients (88.5%), and urine samples to perform *Legionella* and pneumococcal urinary antigen tests from 51 patients (83.6%). Blood samples for *C. pneumoniae* and *M. pneumoniae* serological

Table 1
 Characteristics of patients and febrile acute chest syndrome episodes.
 Caractéristiques cliniques des patients et des syndromes thoraciques aigus fébriles.

Characteristics	All patients (N=61)	No microbiological documentation (N=49)	With micro- biological documentation (N=12)	P-values
Sex				
Male (%)	29 (47)	23 (47)	6 (50)	1.000
Female (%)	32 (52)	26 (53)	6 (50)	
Mean age (years) (standard deviation)	31.2 (± 9.28)	31.4 (± 9.91)	30.3 (± 6.34)	0.716
Type of sickle cell disease				
SS (%)	55 (90)	44 (90)	11(92)	0.748
SC (%)	3 (5)	2 (4)	1(8)	
S-beta-thal (%)	3 (5)	3 (6)	0	
Treatment				
Hydroxyurea (%)	24 (39)	16 (33)	8 (67)	0.047
Chronic transfusion therapy (%)	9 (15)	6 (12)	3 (25)	0.361
Bloodletting (%)	4 (7)	2 (4)	2 (17)	0.170
Vaccinations				
Seasonal flu vaccination (%)	38 (74)	28 (57)	10 (83)	0.250
<i>Haemophilus influenzae</i> type B vaccination (%)	55 (90)	43 (88)	12 (100)	0.572
Pneumococcal vaccination (%)	50 (82)	40 (82)	10 (83)	1
Characteristics of acute chest syndrome episodes				
Fever > 38 °C (%)	61(100)	49(100)	12(100)	
Cough (%)	28(46)	19(39)	9(75)	0.049
Chest pain (%)	58(95)	46(94)	12(100)	1
Expectoration (%)	21(34)	12(25)	9(75)	0.002
Tachypnea (%)	42(69)	31(63)	11(92)	0.083
Oxygen saturation (median %) [range]	96[96–98]	97[96–98]	95[94–98]	0.086
Baseline laboratory values (median) [range]				
Leucocytes (Giga/l)	1.52[1.11–1.95]	1.54[1.11–2.02]	.435[1.105–1.585]	0.284
Hemoglobin (g/dL)	8.3[7.3; 9.1]	8.3[7.3–8.9]	8.2[6.8–10.6]	0.554
CRP (mg/L)	104.2[35.1–156.0]	96.7[32.7–136.0]	06.50[59.0–164.8]	0.231
LDH (IU/L)	559[396–728]	553[392–725]	698[510; 941]	0.132
PCT (µg/L)	0.4[0.2–1.0]	0.4[0.2–1]	0.6[0.2–2.4]	0.549
Samples obtained for microbiological detection				
Blood cultures (%)	57 (93)	45 (74)	12 (100)	–
Sputum for culture (%)	41 (67)	32 (65)	9 (75)	–
Urinary antigen tests for <i>Streptococcus pneumoniae</i> and <i>Legionella pneumophila</i> (%)	51 (84)	39 (80)	12 (100)	–
PCR for respiratory virus and <i>Chlamydomphila pneumoniae</i> and <i>Mycoplasma pneumoniae</i> (%)	56 (92)	44 (90)	12 (100)	–
PCR for <i>Legionella pneumophila</i> (%)	21 (34)	15 (31)	6 (50)	–
<i>Chlamydomphila pneumoniae</i> and <i>Mycoplasma pneumoniae</i> serology on Day 0 (%)	47 (77)	38 (78)	9 (75)	–
<i>Chlamydomphila pneumoniae</i> and <i>Mycoplasma pneumoniae</i> serology >Day 15 (%)	19 (31)	14 (29)	5 (42)	–
<i>Legionella pneumophila</i> serology on Day 0 (%)	42 (69)	33 (67)	9 (75)	–
<i>Legionella pneumophila</i> serology >Day 15 (%)	18 (29)	14 (29)	4 (33)	–
Duration of antibiotic therapy (mean days)	6.4 (± 3.89)	6.1 (± 3.96)	7.8 (± 3.38)	0.045
Duration of hospitalization (mean days)	8.6 (± 4.10)	8.4 (± 3.79)	9.2 (± 5.33)	0.813

tests were collected from 45 patients (77%) at admission and 19 patients (31%) 15 days later. *Legionella* spp. serology was performed for 42/61 patients (68.8%) at admission and 18/61 patients (29.5%) 15 days later. All patients had at least three samples collected for microbiological detection. These results are detailed in Table 1.

Microbiological characteristics of febrile acute chest syndrome are summarized in Table 2. We detected the following pathogens in 12 patients (19.7%): bacteria in seven patients (11.4%) including methicillin-susceptible *Staphylococcus aureus* (n = 2), *Streptococcus pneumoniae* (n = 2), *Klebsiella pneumoniae* (n = 1), *Legionella pneumophila* (n = 1), and *Mycobacterium*

Table 2

Microbiological characteristics of the 61 febrile acute chest syndrome episodes.
 Caractéristiques microbiologiques des 61 épisodes de syndrome thoracique aigu fébrile.

Characteristics	Patients N=61 (%)	Blood culture	Sputum culture	Urinary antigen test	PCR	Serology Day 0	Serology Day 15
No pathogen detection	49 (80.3)	–	–	–	–	–	–
Bacterial infections	7 (11.4)	0	5	1	1	1	1
<i>Staphylococcus aureus</i>	2 (3.3)	0	3	–	–	–	–
<i>Streptococcus pneumoniae</i>	2 (3.3)	0	1	1	–	–	–
<i>Legionella pneumophila</i>	1 (1.6)	0	0	0	1	0	0
<i>Klebsiella pneumoniae</i>	1 (1.6)	0	1	–	–	–	–
<i>Mycoplasma pneumoniae</i>	1 (1.6)	–	0	–	0	1	1
<i>Haemophilus influenzae</i>	0	0	0	–	–	–	–
<i>Chlamydomphila pneumoniae</i>	0	–	0	–	0	0	0
<i>Salmonella</i> spp.	0	0	0	–	–	–	–
Viral infections	4 (6.6)	–	–	–	4	–	–
Influenza virus A	1 (1.6)	–	–	–	1	–	–
Influenza virus B	1 (1.6)	–	–	–	1	–	–
Rhinovirus	2 (3.3)	–	–	–	2	–	–
Respiratory syncytial virus	0	–	–	–	0	–	–
Parainfluenza virus 1, 2, 3	0	–	–	–	0	–	–
Coronavirus	0	–	–	–	0	–	–
Metapneumovirus	0	–	–	–	0	–	–
Bocavirus	0	–	–	–	0	–	–
Adenovirus	0	–	–	–	0	–	–
Enterovirus	0	–	–	–	0	–	–
Co-infections ^a							
Influenza B and MRSA	1 (1.6)	–	1	–	1	–	–

MRSA: methicillin-resistant *Staphylococcus aureus*.

^a Co-infection included in bacterial and viral infections.

plasma pneumoniae ($n = 1$); viruses in four (6.6%) including Rhinovirus ($n = 2$), and Influenza virus ($n = 2$) (one type A and one type B); and one co-infection with methicillin-resistant *Staphylococcus aureus* and Influenza virus B (1.6%). The multiplex PCR enabled the detection of five viral pathogens (influenza viruses and rhinoviruses, but no respiratory syncytial virus). In one patient, serogroup 2 *L. pneumophila* was detected by specific PCR on sputum. All PCR assays for *C. pneumoniae* and *M. pneumoniae* were negative.

3.3. Treatment and outcomes

The in-hospital course and administered treatments are summarized in Table 3. Twenty-three patients (37.7%) were admitted to the intensive care unit but none died. The mean hospital stay was 8.6 [2–22] days, up to 12.1 [7–22] days for bacterial pneumonia and 9.5 [9,10] days for atypical pneumonia. No statistically significant difference was observed in hospital stay between microbiologically-documented and undocumented acute chest syndrome (Table 1).

All patients received oxygen therapy and four (6.5%) required mechanical ventilation. A red blood cell transfusion was administered to 14 patients (22.9%): for symptomatic anaemia with Hb < 6 g/dL ($n = 2$), unfavorable acute chest syndrome outcome after three days of hospitalization ($n = 2$), splenic sequestration ($n = 1$), pregnancy ($n = 1$), and chronic transfusion therapy ($n = 8$).

An empirical antibiotic course was introduced in 52 patients (85.2%) and was later adapted according to the bacterial detection results in nine patients. The mean time from initial

Table 3

Characteristics of the in-hospital course and administered treatments.

Caractéristiques de l'évolution hospitalière et des traitements reçus par les patients.

Characteristics	N=61 (61%)
<i>Department of admission, number (%)</i>	
Emergency room	22 (36.1)
Intensive care unit	23 (37.7)
Internal medicine	16 (26.2)
Oxygen therapy	61 (100)
Mechanical ventilation, number (%)	4 (6.5)
Red blood cell transfusion, number (%)	14 (22.9)
Antibiotic therapy, number (%)	52 (85.2)
<i>Types of antimicrobials, number (%)</i>	
Amoxicillin ± clavulanic acid	39 (43)
Cefotaxime	18 (20)
Spiramycin	22 (24)
Telithromycin	8 (9)
Other antibiotic treatments ^a	4 (4)
Oseltamivir	5 (8.2)

^a Levofloxacin ($n = 1$); piperacillin-tazobactam ($n = 1$); oxacillin ($n = 1$); linezolid ($n = 1$).

empirical therapy to adapted specific therapy was 3.1 [2–7] days. Overall, prescribed antibiotics were amoxicillin ($n = 30$), amoxicillin-clavulanic acid ($n = 9$), cefotaxime ($n = 18$), spiramycin ($n = 22$), telithromycin ($n = 8$), levofloxacin ($n = 1$), piperacillin-tazobactam ($n = 1$), oxacillin ($n = 1$), and linezolid ($n = 1$). Some were prescribed as part of a combination therapy.

The mean duration of the whole antibiotic therapy was 6.4 [1–19] days. A statistically significant difference in the antibiotic therapy duration was observed between

microbiologically-documented (7.8 days) and undocumented (6.1 days) acute chest syndromes ($P = 0.045$). We did not observe more infections in patients on hydroxyurea than in those without it (Table 1).

All patients with influenza virus infections were treated with oseltamivir.

Nine patients did not receive any antibiotic therapy as they presented few clinical signs, moderate inflammatory features (temperature < 38.5 °C, CRP < 50 mg/L, PCT < 0.5 µg/L), and no pathogen was detected among them.

Overall, except for a significant shorter duration of antibiotic therapy in patients with undocumented acute chest syndrome, no other difference regarding recorded variables was observed between microbiologically documented and undocumented febrile acute chest syndromes.

4. Discussion

Our use of NAAT for the detection of respiratory pathogens, combined with standard bacteriological investigations, slightly increased the yield of microbiological diagnosis in sickle cell disease adults presenting with febrile acute chest syndrome, especially for viral detection. For bacterial pathogens, only one case of serotype 2 *Legionella pneumophila* was detected by NAAT. No significant difference was observed between microbiologically-documented and undocumented febrile acute chest syndromes apart from the statistically significant shorter duration of antibiotic therapy in patients in whom no pathogen was detected.

The use of NAAT for atypical bacterial and viral respiratory pathogens has considerably been evaluated over the past years, and has increased the microbiological diagnostic yield [6,14]. It should enable early de-escalation of empirical broad-spectrum antimicrobials to prevent the emergence of bacterial resistance, potential side effects, and drug interactions. By using NAAT, albeit performant, we detected fewer atypical microorganisms than previously reported in studies using traditional methods [3].

We detected few respiratory viruses despite them being the leading pathogens in respiratory tract infections [6,14] and despite our study being conducted during the winter epidemics. No respiratory syncytial virus infection was diagnosed, despite an ongoing outbreak in France during the study period [15]. The microbiological diagnosis of patients who tested positive for influenza virus infection (thus switched to specific oseltamivir treatment) could have been achieved by flu-targeted specific PCR, which is less expensive. If viruses play a role in sickle cell disease children presenting with acute chest syndrome, [5] they are less frequently observed in adults. The overall vaccination coverage in our cohort was higher than that of other targeted French populations (elderly people, immunocompromised patients, or respiratory insufficiency) [16–18]. Less than 5% of influenza infections were detected; this could be due to the high flu vaccine coverage (60%). We detected only two pneumococcal pneumonia cases (3.3%), which is less than in community-acquired pneumonia usually reported in immunocompetent adults [7,14]. This could be related to the conjugate

anti-pneumococcal vaccine effectiveness previously administered in 85% of our patients.

As bacterial clearance is fast for both *Legionella* and *M. pneumoniae*, there is no asymptomatic respiratory carriage of these pathogens [19,20]. Their detection suggests an acute respiratory tract infection but does not differentiate between upper and lower respiratory tract infection, hence the need to interpret PCR results according to the clinical findings [19,20]. We documented one pneumonia caused by serogroup 2 *L. pneumophila* by PCR. Conversely, respiratory carriage of *C. pneumoniae* is common and can last several months [21]; therefore, the microbiological criteria of *C. pneumoniae* infection is both a positive PCR and serology [7]. This latter diagnostic definition was not taken into consideration in some previous studies where positive PCR for *C. pneumophila* was considered sufficient to confirm the diagnosis [3,22]. This discrepancy may have underestimated the incidence of *C. pneumoniae* respiratory tract infections in our cohort compared with previous ones [3,22].

Usual standard tests for microbiological detection have lower sensitivity and specificity than NAAT. Sputum culture has excellent specificity (up to 100%), but rather moderate sensitivity (60% to 85%), especially after the administration of antibiotics [23]. Blood cultures are even less sensitive in bacterial pneumonia (0% to 14%), except in severe pneumonia [24]. No blood culture was positive in our samples. Pneumococcal urinary antigen testing has moderate sensitivity (60% to 70%) and good specificity (90% to 97%) [25] and may remain positive six months after pneumococcal pneumonia or after pneumococcal vaccination [26,27]. One of our patients, who was diagnosed with pneumococcal pneumonia based on a positive urinary antigen test, had not reported pneumococcal infection in the previous year. *Legionella* urinary antigen test has sensitivity of 79.7% and specificity of 97% [28] and only detects serogroup 1 *L. pneumophila* ($> 90\%$ in France), [10]. An early negative test does not rule out the diagnosis and a positive test cannot be interpreted in case of repeated pneumonia.

Another explanation for the low pathogen detection rate could be the suboptimal quality of the swab sample. Successful NAAT requires a correct technique of sampling which could be difficult in a busy standard healthcare setting. The result of the swab test differs according to the sampling site, e.g. testing on nasal swabs is less sensitive than on nasopharyngeal swabs [29]. We observed one false negative *M. pneumoniae* PCR result, yet with a positive serology and consistent clinical findings of pneumonia. After excluding potential PCR inhibitors, we hypothesized a poor quality of the nasopharyngeal swab. Unfortunately, a control swab was not performed.

Guidelines for the use of antibiotics in acute chest syndrome are based on clinical experience and are oriented to minimize the risk of encapsulated bacterial infections in functional asplenic sickle cell disease patients [1,6] with antipneumococcal penicillin-based antibiotics. Our findings revealed that *S. aureus* was also one of the leading detected pathogens. In febrile acute chest syndrome in sickle cell disease adults, empirical antibiotics could be broadened to amoxicillin-clavulanic acid as first-line therapy, at least during the flu epidemics. Macrolides combined with beta-lactams should be kept for severe acute chest

syndrome or when *Legionella* spp. infection is suspected, as recommended [1,12,30].

This study has several limitations. First, we were not able to enroll every consecutive eligible patient. However, the high incidence of acute chest syndrome in our hospital allowed us to constitute a representative cohort of patients during the study period. Secondly, we only included febrile acute chest syndrome episodes at admission, which may have excluded initially afebrile infections such as viral infections, or sickle cell disease patients who are used to take acetaminophen for pain. Given that in previous studies around 90% of patients with acute chest syndrome were febrile at admission [2,3], it provided additional evidence that this clinical feature is associated with acute chest syndrome. Thirdly, we did not perform a comparative study. Finally, not all of the required specimens were obtained from patients who had chest pain and non-productive cough, which may have underestimated the incidence of some pathogens.

5. Conclusion

NAAT slightly improves the microbiological diagnosis of infections in sickle cell disease adults presenting with febrile acute chest syndrome, in whom pathogen detection is low. Beyond the technical limits of NAAT in standard clinical settings, these findings suggest the limited role of infections in the pathophysiology of acute chest syndrome. However, as antibiotics are largely prescribed to acute chest syndrome patients, the use of atypical bacterial and viral PCR could lead to early de-escalation of antimicrobials, particularly for macrolides. This should be considered in future prospective studies.

Authors' contribution

A.R. collected and analyzed the data, and drafted the article. G.M. supervised the study and reviewed the article. E.A. performed the biostatistics and reviewed the article. A.H., K.R., R.L., C.G., M.K., and P.B. collected the data and reviewed the article. J.W.D. and S.F. performed the microbiological tests and reviewed the article. S.G. supervised the study, analyzed the data, drafted and reviewed the article. G.M. and S.G. personally reviewed the data, understood the statistical methods employed for efficacy analysis, and confirmed an understanding of this analysis, that the methods are clearly described and that they are a fair way to report the results. They confirm that the study objectives and procedures are honestly disclosed. They also reviewed study execution data and confirmed that procedures were followed to an extent that convinces all authors that the results are valid and generalizable to a population similar to that enrolled in this study.

Disclosure of interest

The authors declare that they have no competing interest.

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