ORIGINAL ARTICLE 10.1111/j.1469-0691.2012.03771.x

Carriage of encapsulated bacteria in Gabonese children with sickle cell anaemia

F. Schaumburg[1,2], B. Biallas[2,3], E. Ngoune Feugap[2,3], A. S. Alabi[2,3], B. Mordmüller[2,3], P. G. Kremsner[2,3], M. P. Grobusch[2,3,4], B. Lell[2,3], M. van der Linden[5], G. Peters[1] and A. A. Adegnika[2,3,6]

1) *Institute of Medical Microbiology, University Hospital Münster, Münster, Germany,* 2) *Medical Research Unit, Albert Schweitzer Hospital Lambaréné, Lambaréné, Gabon,* 3) *Institute of Tropical Medicine, University Hospital Tübingen, Tübingen, Germany,* 4) *Division of Internal Medicine, Centre for Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands,* 5) *National Reference Centre for Streptococci, University Hospital RWTH Aachen, Aachen, Germany, and* 6) *Leiden University Medical Centre, Leiden, the Netherlands*

Abstract

Sickle cell anaemia (SCA) is a haemoglobin disorder that alters the deformability of erythrocytes through abnormal polymerization of haemoglobin. Children with SCA have an increased risk of infections with encapsulated bacteria. To guide the antibiotic prophylaxis and vaccinations in children with SCA in Gabon, we characterized *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* from children with and without SCA. We performed a cross-sectional study and compared nasal and pharyngeal *S. pneumoniae*, *Staph. aureus* and *H. influenzae* isolates from SCA children (n = 73) with comparators matched for age, residence and sex (n = 143) in a matched-comparison analysis. The resistance pattern and capsular type were identified for each isolate. The total carriage rate for *S. pneumoniae*, *Staph. aureus* and *H. influenzae* was 13.8%, 46.7% and 12.5%, respectively, and did not differ between groups (p >0.05). The mean number of days under antibiotic treatment in the past year was higher in children with SCA than in controls (penicillin: 70.1 vs 0.1 days, p 0.00002). The total non-susceptibility rate was 30% for oral and parenteral (meningitis) penicillin in *S. pneumoniae*, resistance rates were 1.6% for oxacillin in *Staph. aureus* and 14.8% for ampicillin in *H. influenzae*. Susceptibility to antibiotic agents and distribution of capsular types did not differ significantly between both groups. In conclusion, carriage and resistance rates are similar in children with and without SCA. Our data provide the basis to guide empiric therapy of invasive diseases caused by *S. pneumoniae*, *Staph. aureus* and *H. influenza* in children in Gabon.

Keywords: Antibiotic prophylaxis, *Haemophilus influenzae*, matched-pair analysis, sickle cell anaemia, *Staphylococcus aureus*, *Streptococcus pneumoniae*

Original Submission: 6 October 2011; **Revised Submission:** 8 January 2012; **Accepted:** 9 January 2012

Editor: E. Bottieau

Clin Microbiol Infect

Corresponding author: F. Schaumburg, Institute of Medical Microbiology, University Hospital Münster, Domagkstrasse 10, 48149 Münster, Germany

E-mail: frieder.schaumburg@ukmuenster.de

Introduction

Sickle cell disease is a monogenic disorder of haemoglobin and affects 0.74% of all newborns in sub-Saharan Africa each year [1]. One nucleotide mutation of the β-globulin gene causes an alteration of the amino acid sequence resulting in an abnormal polymerization of haemoglobin (Hb) under certain conditions such as deoxygenation. Heterozygous children are asymptomatic carriers of this point mutation and are protected against malaria [2]. Homozygous carriers develop sickle cell anaemia (SCA), which is characterized by the HbSS phenotype. Clinical syndromes associated with SCA are vaso-occlusive crisis, acute chest syndrome, aplastic crisis, cerebrovascular accident and haemolytic anaemia [1]. Hypoperfusion of the spleen leads to functional hyposplenism and may culminate in 'autosplenectomy' as the result of recurrent infarction and haemorrhages [3]. The clearance of pathogens and the stimulation of the immune system are

reduced in hyposplenism or asplenism, so patients with SCA have a high risk of infections with encapsulated bacteria similar to the so-called overwhelming post-splenectomy infections [4,5]. Bacterial infections are most frequently caused by *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* and non-Typhi *Salmonella* species [6–8]. Both immunization against *S. pneumoniae* and *H. influenzae* type b and antibiotic prophylaxis are recommended to reduce the risk of fatal infections. However, permanent antibiotic prophylaxis or frequent antibiotic therapies may raise the risk for the selection of resistant strains and may therefore limit therapeutic options [4]. As *S. pneumoniae*, *Staph. aureus* and *H. influenzae* are part of the nasopharyngeal flora, infections with these pathogens are mostly endogenous [9,10]. Knowledge of the distribution of capsular types and resistance to antibiotics is necessary to guide optimal vaccination, antibiotic prophylaxis and treatment of these pathogens. As these data have not been described for Gabon, we collected nasal and pharyngeal swabs from children with SCA and a matched healthy group to compare the susceptibility pattern and capsular types of *S. pneumoniae*, *Staph. aureus* and *H. influenzae*.

Material and Methods

Ethical statement

Ethical approval was obtained from the regional review board (Comité d'Éthique Régional Indépendant de Lambaréné, Gabon, CERIL09/2010). The participating children and their legal representatives were informed about the study procedures and the legal representative or the full-age participant, respectively, gave written informed consent before enrolment.

Population

We performed a cross-sectional study on the carriage of *S. pneumoniae*, *Staph. aureus* and *H. influenzae* in children with and without SCA. Consecutive children with SCA attending the regular consultations at the Albert Schweitzer Hospital, Lambaréné, Gabon were included in the SCA group if (i) HbSS had been confirmed by electrophoresis and (ii) they had not received a blood transfusion in the past 3 months. Exclusion criteria for both groups were fever and signs of acute infection. Four children with SCA declined participation. For each SCA child two controls matched for age (± 2 years), residence and sex were enrolled, if they were haemoglobin A (HbA) carriers as confirmed by electrophoresis. If controls did not meet all three criteria we gave priority to age over residence and residence over sex.

Demographic data were recorded for each participant. All children underwent a physical examination to assess them for hepatomegaly, splenomegaly and enlarged lymph nodes by palpation. EDTA-anticoagulated capillary blood was drawn to test for the presence of HbS and HbA by electrophoresis. Giemsa-stained thick blood smears were prepared to check for *Plasmodium* sp. and filarial infections.

Haemoglobin electrophoresis

To lyse red blood cells, capillary blood was diluted 1:10 in lysis buffer (0.2 M Tris–HCl (pH 8.9), 5.1 mM EDTA, 14.6 mM boric acid, 0.154 M sodium chloride, 4% Ficoll-Plaque, 1% nonidet P-40) and separated in a 2% agarose gel. The electrophoresis was performed in electrophoresis buffer (84.2 mM Tris–HCl (pH 8.9), 2.05 mM EDTA and 51.8 mM boric acid).

Bacterial culture

Nasal and pharyngeal swabs were stored in Amies transport medium for a maximum of 3 h until they were subjected to microbiological culture and identification at the medical research unit in Lambaréné, Gabon. Swabs were streaked on Columbia blood agar plates supplemented with 5% sheep blood. An aztreonam disc (13 μg; Oxoid, Hants, UK) was placed on the agar and plates were incubated at 35°C for 18–24 h. Swabs were also plated on chocolate agar plates supplemented with an oleandomycin disc (15 μg; Oxoid) and incubated at 35°C and 5% CO_2 for 18–24 h.

Identification and susceptibility test

Cultures on Columbia blood agar plates were screened for two or three presumptive *S. pneumoniae* colonies, which were tested for optochin susceptibility. Isolates displaying a zone of inhibition >14 mm were tested for bile solubility [11]. Susceptibility was tested applying the broth microdilution method and breakpoints according to the Clinical Laboratory Standards Institute [12]. For penicillin, we report the non-susceptibility rates to oral penicillin (MIC >0.06 μg/mL) and parenteral penicillin (meningitis: MIC >0.06 μg/mL; non-meningitis: MIC >2 μg/mL).

Presumptive *Staph. aureus* colonies were identified by positive catalase reaction and latex agglutination test (Pastorex Staph-Plus; Bio-Rad Laboratories, Marnes-la-Coquette, France). Species confirmation and susceptibility tests were performed using a Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France).

Colonies of *Haemophilus* sp. were tested for a positive satellite phenomenon and were subjected to biochemical identification using API NH (bioMérieux). As biochemical identification systems do not reliably differentiate between *H. influenzae* and *H. haemolyticus*, we confirmed the species of *H. influenzae* isolates by 16s RNA gene sequencing [13,14]. Susceptibility was tested using the agar diffusion method [12].