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ACS in children with sickle cell anaemia in Uganda: prevalence, presentation and aetiology

Odong Ochaya,[1] Heather Hume,[1] Sam Bugeza,[2] Freddie Bwanga,[3] Rosemary Byanyima,[2] Harriet Kisembo[2] and James K. Tumwine[1]

[1]Department of Paediatrics and Child Health, School of Medicine, College of Health Sciences (CHS), Makerere University, [2]Department of Radiology, School of Medicine, College of Health Sciences, Makerere University, and [3]Department of Medical Microbiology, School of Biomedical Sciences, College of Health Sciences, Makerere University, Kampala, Uganda

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Correspondence: Odong Ochaya, Makerere University College of Health Sciences, Paediatrics and Child Health, Kampala, Uganda.
E-mail: phillodong@gmail.com

Summary

ACS (ACS) is a serious complication of sickle cell anaemia (SCA). We set out to describe the burden, presentation and organisms associated with ACS amongst children with SCA attending Mulago Hospital, Kampala, Uganda. In a cross-sectional study, 256 children with SCA and fever attending Mulago Hospital were recruited. Chest X-rays, blood cultures, complete blood count and sputum induction were performed. Sputum samples were investigated by Ziehl-Nielsen staining, culture and DNA polymerase chain reaction (PCR) for *Chlamydia pneumoniae*. Of the 256 children, 22·7% had ACS. Clinical and laboratory findings were not significantly different between children with ACS and those without, besides cough and abnormal signs on auscultation. Among the 83 sputum cultures *Streptococcus pneumoniae* (12%) and *Moraxella* spp (8%), were the commonest. Of the 59 sputa examined with DNA PCR, 59·3% were positive for *Chlamydia pneumoniae*. *Mycobacterium tuberculosis* was isolated in 6/83 sputa. These results show that one in 5 SCA febrile children had ACS. There were no clinical and laboratory characteristics of ACS, but cough and abnormalities on auscultation were associated with ACS. The high prevalence of *Chlamydia pneumoniae* in children with ACS in this setting warrants the addition of macrolides to treatment, and *M. tuberculosis* should be differential in sub-Saharan children with ACS.

Keywords: ACS, Africa, Uganda, infections.

The prevalence of the sickle cell gene or sickle cell trait (SCT) in sub-Saharan Africa is estimated to be between 3–30% with the homozygous state estimated at 1–3% in some populations. In Uganda, recent estimates place SCT at 13·3% and the prevalence of sickle cell disease (SCD) at 0·7% (Ndeezi *et al*, 2016). Hence, the World Health Organization (WHO) has declared SCA as a public health priority (World Health Organisation Regional Office for Africa 2010).

Acute pulmonary complications occur frequently in patients with sickle cell anaemia (SCA). Acute chest syndrome (ACS) is such a complication and is similar to pneumonia in presentation (Bainbridge *et al*, 1985). ACS is defined as a new infiltrate on the chest X-ray (CXR) in a child with SCA in the presence of fever and other respiratory findings including cough, chest pain, tachypnoea / dyspnoea or hypoxia. (Charache *et al*, 1979) The need for a CXR therefore makes the diagnosis of ACS difficult in low income settings where X-rays are difficult to obtain. It has been suggested that a clinician's diagnosis of ACS without a CXR is likely to be unreliable (Morris *et al*, 1999).

ACS occurs more often in children with haemoglobin (Hb) SS and severe African haplotypes, which are more prevalent in sub-Saharan Africa compared to other SCA types (Ojwang *et al*, 1987; Serjeant, 1989). ACS contributes significantly to the morbidity, hospitalisation and death of children with SCA, and higher incidences of ACS are reported in children aged between 1 and 4 years than all other age groups (Akinyanju, 1989; Castro *et al*, 1994; Platt *et al*, 1994; Vichinsky *et al*, 2000; Makani *et al*, 2007). Despite the contribution of ACS to morbidity and mortality in persons with SCD, there is little published work on the prevalence, aetiology and clinical characteristics in sub-Saharan Africa (Castro *et al*, 1994; Morris *et al*, 1999) and none from Uganda. The role of infections in the aetiology of ACS, especially in children under 5 years of age, has been highlighted in some low-to-middle income countries (Castro *et al*, 1994; Vichinsky *et al*, 2000). Little has been reported

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about the aetiology or organisms associated with ACS in Uganda.

In this study we determined the prevalence, clinical presentation and microorganisms associated with ACS amongst children with SCA attending the Mulago National Referral Hospital in Kampala, Uganda.

Methods

Study design and setting

This descriptive cross-sectional study was carried out among children in the Sickle Cell Clinic (SCC) and the Acute Care Unit at Mulago hospital between January and April 2013. Mulago Hospital is Uganda's National Referral and Teaching Hospital with a bed capacity of 1500. The Department of Paediatrics and Child Health has 7 wards and runs specialised outpatient clinics, such as the SCC.

At the time of the study, the SCC had over 9000 confirmed SCA patients registered - over 7000 had been registered between 2000 and 2013 of whom half actively attended the clinic at least once a year. The SCC runs from Monday to Friday and it has a day treatment centre where emergency and other initial treatment is provided.

The acute care unit (ACU) is the emergency general paediatric medical admission ward where critically ill children are admitted for initial care, after which they are transferred to the other wards.

Inclusion criteria

We included children who had confirmed SCA status by Hb electrophoresis or equivalent Hb separation analysis, for example iso-electric focusing (IEF). They had to be aged 6 months to 12 years and presenting with fever. All children had to have informed caregiver or parental consent. Children aged 8–12 years provided assent according to the Uganda National Council for Science and Technology (2014).

Exclusion criteria

Children who did not have a CXR for any reason were excluded from the study.

Sample size estimation

The sample size of 262 for the prevalence was calculated using the Kish Leslie formula (Kish, 1965) assuming a 21·8% prevalence of ACS in a Jamaican study (Wierenga *et al*, 2001) with a desired precision of 5%.

Study procedure

Patients who fulfilled the inclusion criteria were recruited by sequential sampling. Enrolment continued until the sample size was attained. SCA children aged 6 months to 12 years who presented to the SCC or ACU of Mulago Hospital were screened for fever (axillary temperature ≥37·5°C). After explanation of the study to the caregiver/parent/child, written consent from the caregiver/parent and assent from children aged 8–12 years was sought. All children who fulfilled the inclusion criteria were recruited after the consent process. These children were then reviewed, which included assessing vital signs and providing emergency treatment where necessary, taking a history and performing a physical examination. Blood was drawn before antibiotics were administered. CXR was done within 24 h after initial treatment, including antibiotics, was instituted.

Venous blood was drawn as follows: 1–2 ml in EDTA vacutainer bottles for complete blood count (CBC) and peripheral blood smears- including thick and thin films for microscopy for malaria parasites; and 1–3 ml in 100 ml BACTEC^{TM} Paediatric culture bottles (BD Kenya, Nairobi, Kenya). The blood in EDTA vacutainers were transported to the central haematology laboratory of Mulago Hospital, where the CBC was performed on a Celltac F, MEK-8222 automated haematological analyser (Nihon Kohden, Tokyo, Japan) within 30 min and a thin film made for staining. Blood cultures were transported at the end of the working day (4:00 pm) to the MBN clinical Laboratories, Kampala, where they were incubated for up to 7 days at 35°C. Blood cultures that showed bacterial growth were subcultured on Blood and Chocolate and MacConkey agars. Any growth was followed up and identified biochemically. Sensitivity was determined by disk diffusion techniques. The oxacillin disk screening test was used for susceptibility to β-lactams, with oxacillin zone sizes >20 mm considered sensitive to penicillin (Clinical and Laboratory Standards Institute 2007). Minimum inhibitory concentration (MIC) determination tests were not readily available.

After infiltrates on the CXR were identified at the point of care, sputum induction was done on these children using nebulised 3% saline solution as previously described (Nantanda *et al*, 2008). The sputum was collected after chest massage and suction from the oropharynx into sterile sputum container. These sputum samples were transported within 2 h to MBN laboratories, Kampala. A sputum gram stain, Ziehl Nielsen staining and culture and sensitivity were done on the day of receipt of sputum samples (same media and techniques as for blood culture above). All sputum samples were frozen in aliquots at −20°C degrees for 3 months until the remaining patient samples were attained. DNA was extracted in the laboratory, and 2 μl of the extracted DNA was added as DNA template for amplification. In the DNA amplification laboratory, the 463 base pair 16S rRNA gene of *Chlamydia pneumoniae* was amplified using the polymerase chain reaction (PCR) programme for *Chlamydia pneumoniae* (van Kuppeveld *et al*, 1994).

Initial interpretation of each CXR at the clinic was used to determine the presence, or not, of infiltrate and thus make decisions on the treatment and need for sputum induction.

The CXRs were later interpreted by two senior radiologists and a third opinion sought if there was any discordance in the interpretations of the two radiologists. The radiology reports were captured in a pre-set structured CXR form.

Study instruments and tools

A pretested structured questionnaire was used to collect socio-demographic characteristics, clinical history, physical examination findings, and laboratory and CXR results.

Chest radiographs

A hospital radiographer performed baseline postero-anterior (PA) or antero-posterior (AP) (in patients who were unable to stand) chest radiography within 24 h of admission. CXR was not repeated if a patient had undergone a chest radiograph within the previous 7 days.

Two radiologists, certified by the National Medical Association Board, each with more than a decade of experience, reviewed the hard copies of all radiographs using a standardized interpretation form. The interpretation form was developed by the authors (including 3 board-certified radiologists) based on the Fleischner Society's terms for thoracic imaging (Hansell *et al*, 2008), adding descriptive modifiers which we thought important in describing radiographs in ACS in SCD (e.g. alveolar infiltrates and cardiomegaly). The radiologists evaluated the radiographs independently. Films were assessed for quality and those that were technically inadequate were excluded. A third radiologist was involved to adjudicate whenever there were differences in interpretation, and differences were resolved by consensus. Radiologists were blinded to all clinical and laboratory data, and to the final diagnosis.

Patient management

The children received standard management according to the Uganda Clinical Guidelines, the Mulago National Referral Hospital Paediatric Department guidelines and the SCC protocols. Findings from the clinical examination and laboratory investigation results were shared with the attending team on duty. Parents/caregivers/children were informed about the laboratory findings. Children recruited from the SCC day care centre who qualified for admission to hospital were referred to the ACU for further management.

Variables

The following variables were captured in this study: social demographic characteristics, hospital admissions in the last 2 years, history of cough, chest pain, difficulty in breathing, wheeze, asthma, trauma/surgery in the past 1 month. We also included use of hydroxycarbamide, folic acid, penicillin prophylaxis, antimalarial prophylaxis and recent use of oral morphine. We also captured vaccination status with pneumococcal vaccine; the respiratory rate, presence of dyspnoea, dull percussion note, presence of rales; oxygen circulation, and the axillary temperature. Laboratory findings included: white blood cell (WBC) and platelet counts and Hb level, blood slide for malaria, blood culture and sensitivity, sputum alcohol- and acid-fast bacilli smear, gram stain and sputum bacterial culture and sensitivity.

Outcome measure

Presence of ACS defined as an infiltrate on the CXR in a febrile child with SCA and pulmonary features.

Data management

All data was recorded in pre-coded questionnaires which were checked at the end of the day for completeness. The data was entered, cleaned, edited and double entered into a statistical software package *Epidata Ver 3·1* (http://www.epidata.dk/download.php) before a back-up was performed. The data was exported to *STATA Ver 12* for analysis (StataCorp LP, College Station, TX, USA).

The demographic, clinical and laboratory variables were classified as either categorical or continuous. Categorical variables were reported as proportions and continuous variables as median and interquartile ranges for descriptive purposes where appropriate. Comparison of characteristics between those with ACS and those without, was determined by logistics regression. Factors with a *P*-value <0·2 on univariate analysis were subjected to multivariate analysis. On multivariate analysis, only factors with *P*-value <0·05 were deemed significant.

An elevated total WBC count was defined as $>17 \times 10^9$/l. In children less than 6 years old and $>15 \times 10^9$/l among children aged 6–12 years (Omoti, 2005). Elevated platelet counts were defined as $>500 \times 10^9$/l (Omoti, 2005). For haemoglobin concentration a cut-off of 50 g/l was chosen because this level is used for transfusion requirement in children at Mulago Hospital. Infiltrates on the CXR were defined by the presence of alveolar/consolidation pattern and/or interstitial pattern and/or pleural effusion/thickening. The level of agreement for CXR interpretation was analysed using Kappa statistics.

Quality control

The pre-coded questionnaire was pre-tested before the commencement of the study. Research assistants were trained on how to identify children who fulfilled the inclusion criteria and how to obtain written consent and assent. The laboratory research assistant was trained how to collect blood samples and a standard procedure for blood culture sample collection was followed according to standard operating procedures (SOPs). All questionnaires were checked for completeness and completed forms stored securely by the

investigator. Stickers were used for identification of patients and numbering of the enrolled patients. Blood slides for malaria were reviewed by a senior laboratory technologist.

MBN laboratory ran tests for blood and sputum cultures and sensitivity. Internal quality control at the MBN laboratory was ensured by the following: having qualified personnel, using standard calibrated and serviced equipment, inclusion of positive and negative controls on periodic basis, SOPs were followed and compliance with Good Clinical and Laboratory Practices. The MBN laboratory is currently undergoing ISO 15189 accreditation. External Quality in the MBN laboratory is done periodically by Limbach laboratories in Germany, Uganda Virus Research Institute and the National TB Reference Laboratory.

Ethical considerations

Permission to carry out the study was obtained from the Department of Paediatrics and Child Health of Makerere University, College of Health Sciences, the School of Medicine Research and Ethics Committee (SOMREC) and the Uganda National Council of Science and Technology. Written informed consent was sought from the caregiver/parent of eligible children, and assent from children 8–12 years.

Results

Study profile

A total of 270 children with SCA and fever were screened. Two hundred and sixty-three (263/270) fulfilled the inclusion criteria and were thus enrolled into the study. Seven children were excluded from analysis because they did not have the outcome variable because their CXRs were not available for radiological interpretation.

Baseline characteristics

Over one-half of the study participants – 156 (59·3%) – were male; the median age was 3·8 years [interquartile range (IQR): 1·9–6·3].

Prevalence of acute chest syndrome

The prevalence of ACS among children with SCA and fever in this study was 22·7% (58/256). The prevalence of ACS among the females was 18·5% (19/103) while among the males it was 25·5% (39/153). Prevalence of ACS was 22·8% (42/184) among children in the 0·5–5 years age group, and 22·2% (16/72) in the age group 6–12 years age group.

Demographic, symptoms and physical characteristics

As shown in Table I, cough was a common symptom in these children, especially among those with ACS. Difficulty in breathing, limb pain and history of prior pneumonia was present in the smaller proportion of patients with ACS.

High grade fever, low SpO_2 levels (<92%) and un-palpable spleen were seen in a small proportion of patients with ACS when compared with children without ACS (Table II). Overall, only 29/256 (11·3%) had abnormal chest auscultatory findings, of which the majority 17/29 (58·6%) were among

Table I. Comparison of findings in the history of children with and without acute chest syndrome.

Characteristic	Acute chest syndrome $N = 58$	No acute chest syndrome $N = 198$	*P*-value
Cough, *n* (%)			
Absent	6 (10·3)	79 (39·9)	<0·001
Present	52 (89·7)	119 (60·1)	
Difficulty in breathing, *n* (%)			
Present	15 (25·9)	35 (17·7)	0·177
Absent	43 (74·1)	163 (82·3	
Limb pain, *n* (%)			
Present	11 (19·0)	50 (25·3)	0·325
Absent	47 (81·0)	148 (74·7)	
Prior pneumonia, *n* (%)			
Present	15 (25·9)	26 (13·1)	0·026
Absent	43 (74·1)	172 (86·9)	
Daily folate, *n* (%)			
Adherent	36 (62·1)	140 (70·7)	0·220
Non-adherent	22 (37·1)	58 (29·3)	
Malaria prophylaxis, *n* (%)			
Present	36 (62·1)	133 (67·2)	0·474
Absent	22(37·9)	65 (32·8)	

Table II. Clinical findings in the physical examinations of children with and without acute chest syndrome.

Characteristic	Acute chest syndrome $N = 58$	No acute chest syndrome $N = 198$	*P*-value
Temperature °C, *n* (%)			
37·5–38·9	45 (77·6)	160 (80·8)	0·597
39·0–40·4	13 (22·4)	38 (19·2)	
Median temperature, °C, *n* (IQR)	38·3 (37·9–38·9)	38·1 (37·8–38·8)	0·194
Spleen			
Palpable, *n* (%)	6 (10·3)	29 (14·7)	0·404
Not palpable, *n* (%)	52 (89·7)	169 (85·3)	
Respiratory rate			
Tachypnoea, *n* (%)	40 (69·0)	91 (46·0)	0·002
No tachypnoea, *n* (%)	18 (31·3)	107 (54·0)	
Chest auscultation			
Normal, *n* (%)	41 (70·7)	186 (93·9)	<0·001
Abnormal, *n* (%)	17 (29·3)	12 (6·1)	
SPO_2 ($N = 242$)			
>92%, *n* (%)	41 (74·6)	143 (76·5)	0·770
<92%, *n* (%)	14 (25·4)	44 (23·5)	

IQR, interquartile range; SPO_2, blood oxygen saturation.

Table III. Comparison of laboratory results of children with and without acute chest syndrome.

Characteristic	Acute chest syndrome $N = 58$	No acute chest syndrome $N = 198$	P-value
WBC count, $\times 10^9$/l			
Elevated*, n (%)	49 (84·5)	167 (84·3)	**0·979**
Not elevated, n (%)	9 (15·5)	31 (15·7)	
Median WBC count, $\times 10^9$/l (IQR)	24·9 (18·1–29·8)		0·536
Hb level ($N = 254$)			
>50 g/l, n (%)	43 (74·1)	153 (78·1)	0·213
<50 g/l, n (%)	15 (25·9)	43 (21·9)	
Median Hb, g/l (IQR)	65 (48–75)		
Platelet count			
$\geq 500 \times 10^9$/l, n (%)	22 (37·9)	58 (29·3)	0·257
$<500 \times 10^9$/l, n (%)	36 (62·1)	140 (70·7)	
Median platelet count, $\times 10^9$/l (IQR)	440·5 (360·0–538·5)	387 (293–501)	0·044
Median MPV, fl	5·3 (4·5–8·5)	4·9 (4·2–7·8)	0·204

Hb, haemoglobin; IQR, interquartile range; MPV, mean platelet volume; WBC, white blood cell.
*Defined as WBC count $\geq 17 \times 10^9$/l in children 0·5–5 years of age and $\geq 15 \times 10^9$/l among children aged 6–12 years.

those with ACS. The remainder 12/29 were among those without ACS. This difference was statistically significant.

Laboratory results

Table III shows the laboratory findings of children with and without ACS. The WBC counts, haemoglobin concentration and platelet counts were not significantly elevated among children with ACS. However, the median WBC count was higher than the cut-offs (15×10^9/l among 6–12 years and 17×10^9/l among under 6 years) in all age groups among children with ACS.

Radiological characteristics among children with acute chest syndrome

The two primary radiologists reported that CXRs showed infiltrates in 85/256 patients, with an overall agreement of 78·9% and a Cohen's Kappa statistics for inter-rater agreement of 0·4. However, the reviewers agreed on only 31/85 as having an infiltrate (as defined in the methods). The remaining 54 were then reviewed by a third radiologist who identified 27/54 as having an infiltrate giving a total of 58 (31 + 27) CXRs confirmed as having a chest infiltrate.

Microbiology results

Table IV summarises the organisms isolated from participants' sputum and blood samples. *Chlamydia pneumoniae* was the commonest organism isolated in the population, positive in 35/59 (59·3%), however it occurred almost equally among children with ACS compared to those without ACS – 19/35 (54·3%) and 16/35 (45·7%) respectively.

All the sputum ($N = 83$) and blood ($N = 263$) cultures samples collected and run provided valid results. The blood cultures were positive in 24/256 (9·3%) of cases. Positivity rates between children with and without ACS were not significantly different: 19/198 (9·9) and 4/58 (6·9%). Twenty (7·2%) grew pathogenic organisms and 5 (1·9%) grew contaminants in the blood cultures. Among children with ACS with gram positive bacteria, *Staphylococcus aureus 2* and *Streptococcus pneumonia 1* were the most frequent.

Sputum positivity rate was 20/83 (24·1%), and *Streptococcus pneumoniae* (8/83) was the most frequent bacterial isolate in sputum culture.

The sensitivity patterns varied. Of the 256 blood cultures only 11 (4·3%) grew *Staphylococcus aureus*, the most frequently isolated organism in blood, and all were susceptible to gentamicin and cloxacillin, while sensitivity to ciprofloxacin (9/11), vancomycin (8/11) and chloramphenicol (7/11) was also significant.

In the sputum, *Streptococcus pneumoniae* was the most frequently isolated organism (8/83) and it was mainly susceptible to the commonly used antibiotics, erythromycin (8/8) and chloramphenicol (7/8), but also to clindamycin (7/8). *Moraxella spp* (7/83), was the second frequently isolated organism and it was mainly susceptible to ciprofloxacin (7/7), gentamicin (6/7), chloramphenicol (5/7) and erythromycin (5/7).

Nine of the 11 isolated *Streptococcus pneumoniae* were resistant to oxacillin but their susceptibility to penicillin by minimum inhibitory concentration (MIC) tests could not be ascertained because MIC E-test strips were not readily available. Chloramphenicol, which is commonly used in our setting, had a good coverage.

The blood smear for malaria was positive in 17/256 (6·6%) cases, none of which had ACS.

Discussion

In this cross-sectional study, we determined the prevalence, and described the clinical presentation and infections associated with ACS amongst children with SCA who attend Mulago National Referral Hospital.

Table IV. Organisms isolated from sputum and blood.

	Sputum $N = 83$				Blood $N = 256$			
	No ACS† ($n = 47$)		ACS* ($n = 36$)		No ACS* ($n = 198$)		ACS* ($n = 58$)	
Organism	Freq	%‡	Freq	%‡	Freq	%‡	Freq	%‡
Staphylococcus aureus	1	0	0	0	9	3·5	2	0·8
Streptococcus pneumoniae	6	7·2	2	2·4	2	0·8	1	0·4
Streptococcus viridans	0	0	0	0	1	0·4	0	0
Group D *Salmonella*	0	0	0	0	2	1·2	1	0·4
Salmonella Typhi	0	0	0	0	1	0·4	0	0
Group A *Streptococcus*	2	2·4	0	0	0	0	0	0
Klebsiella pneumoniae	1	2·4	1	1·2	0	0	0	0
Moraxella Spp	3	3·6	4	4·8	0	0	0	0
Contaminants§	NA	NA	NA	NA	5	2·0	0	0
Present AAFBs (ZN stain)	5	6·0	1	1·2	NA	NA	NA	NA
Positive *Chlamydia pneumoniae* DNA PCR (N-59)	19	32·2	16	27·1	NA	NA	NA	NA
Positive blood slides for malaria	NA	NA	NA	NA	0	0	18	6·9

AAFBs, alcohol- and acid-fast bacilli smear; ACS, acute chest syndrome; Freq, frequency; NA, not applicable; PCR, polymerase chain reaction; ZN, Ziehl-Neelsen.

*ACS defined by infiltrate on chest X-ray.

†No ACS- These include 2 groups of children; those with clinical signs but no radiological signs of ACS and those with fever but no other clinical signs and no radiological features of ACS.

‡Calculated as percentage of overall number of samples cultured.

§Contaminants in blood culture included Coagulase-negative *Staphylococcus* ($n = 1$), *Micrococcus* spp ($n = 2$) and *Bacillus* spp ($n = 2$).

Prevalence of acute chest syndrome

The prevalence of ACS among children with SCA presenting with fever in this study was 22·7%, which is comparable to figures from Jamaica (Wierenga *et al*, 2001), Oman (Jaiyesimi & Kasem, 2007), and Saudi Arabia (Hawasawi *et al*, 1998). This rate, however, differs from a recent report of 6% by Nansseu *et al* (2015) from Cameroon partly because of differences in target groups or even differences in haplotypes between Uganda and Cameroon. The Saudi prevalence was not different from the current study, possibly because African haplotypes of sickle cell gene prevail in the Saudi western region (Alabdulaali, 2007). In our study, the prevalence of ACS was similar in both age groups. This is surprising, as Castro *et al* (1994) reported that the highest incidence of ACS among children in North America was in the 2–4 years age group. In France, ACS was mainly observed in children under 5 years (Neonato *et al*, 2000). This difference could be explained by differences in the methods between the studies. Most of the studies obtained the prevalence of ACS among children admitted with SCA, but in the current study we estimated the prevalence among febrile children with SCA.

Demographics and other findings

In this study there was no significant difference between children with ACS and those without ACS when age, gender, history of difficulty breathing and limb pains were analysed. This is somewhat different from what Vichinsky *et al* (1997) found: Their patients with abnormal lung results were more likely to have a productive cough, shortness of breath/difficulty breathing and wheezing. In the current study 1 in 5 of the children with ACS had limb pains, which is comparable to that reported by Vichinsky *et al* (1997).

Even though children with ACS were more likely to have a cough, with a frequency of 89·6% in this study, a history of cough was also present in 60·1% of children without ACS. A history of cough is therefore an important finding but can still be found in those without ACS. These results are similar to those of other reports (Vichinsky *et al*, 1997; Taylor *et al*, 2004; Alabdulaali, 2007), but contrary to the findings of these studies, our univariate analysis showed that a history of prior episodes of pneumonia was significantly more common among children with ACS than those without. The reason for this difference is unclear.

In the present study, many of the variables, i.e., median axillary temperature, spleen size and oxygen saturation by pulse oximetry, were not significantly associated with ACS. Most children with ACS presented with fever, cough and tachypnoea, a significant number (70%) having normal findings on auscultation and oxygen saturation. This is in agreement with other studies (Morris *et al*, 1999; Taylor *et al*, 2004).

One in 10 children with SCA and fever were likely to have abnormal findings on auscultation, 2 in 3 of these were likely to have ACS. These findings are comparable to those of Vinchisky (Vichinsky *et al*, 1997) but differ from Morris' (Morris *et al*, 1999). Despite this association, there was a significant proportion of children without ACS (70·6%) that

had normal chest auscultation. It is therefore important to note that normal chest auscultation does not mean absence of ACS.

Laboratory findings

In this study the contribution of normoblasts (immature red blood cells) to the automated WBC count was not corrected for, however the median WBC count was 24·9 (18·0–29·8) $\times$ 10^9/l, which is similar to reports from Jamaica (Wierenga *et al*, 2001). These high WBC counts could partly be due to the contribution of normoblasts. In Omani children, Jaiyesimi and Kasem (2007) corrected for immature RBCs and reported a lower mean WBC of 15·37 $\pm$ 8·39 $\times$ 10^9/l. There was no significant difference in median WBC counts between the children with ACS and those without ACS in this study.

The median haemoglobin concentration among children with ACS was 65 (48–75) which is relatively lower than the steady state haemoglobin (75·4 $\pm$ 22·6 g/l) found among children with SCA in Benin state Nigeria (Omoti, 2005). In the present study the individual drop of haemoglobin was not measured, but this difference of 10 g/l drop from steady state haemoglobin reported in Benin Nigeria (Omoti, 2005) is similar to previous reports from North America (Vichinsky *et al*, 2000). In India however, a much lower mean haemoglobin level, 56 $\pm$ 12·4 g/l was reported (Samal, 1997). Comparing the median haemoglobin levels between febrile children with ACS and febrile children without ACS in this study did not show a significant difference.

The median platelet count for the participants was 399 (301–509) $\times$ 10^9/l, which is within the reported range of mean steady state platelet counts in Benin state, Nigeria (Omoti, 2005) and is similar to findings in Jamaica (Wierenga *et al*, 2001), though much higher than the mean platelet count reported from the Bahamas (Taylor *et al*, 2004). Similar to findings from North America (Vichinsky *et al*, 2000), the present study did not find a significant relationship between high platelet counts and ACS.

Microbiological findings

In the current study blood smears for malaria were positive in 6·9% of children, though none of the children with malaria had ACS. This is not different from a recently completed work (Nakibuuka, unpublished observations) which showed a malaria prevalence of 5% in same population.

In this study the rate of isolating pathogenic organisms from blood culture from febrile children with SCA was 7·2%, which is similar to rates reported by other studies: 6% in Jamaica (Wierenga *et al*, 2001), 8·3% in Madina (Hawasawi *et al*, 1998) and 10% in India (Samal, 1997). It is however lower than the 28·5% rate reported in the same target population at Mulago by Kizito *et al* (2007). Perhaps a much higher positivity rate was observed in the latter report because Kizito recruited children with axillary temperatures $\geq$38·0°C different from the $\geq$37·5°C cut-off in the current study. Sputum culture and sensitivity yielded a 25·3% growth for pathogenic bacteria in this study, which too is not different from findings in the North American National Acute Chest Syndrome study (Vichinsky *et al*, 2000).

Blood culture isolated, in order of frequency, *Staphylococcus aureus* 11(4·2%), *Streptococcus pneumoniae* 3 (1·1%) and Group D Salmonella 3 (1·1%). The organisms identified from the sputum in order of occurrence was *Chlamyidia pneumoniae* 20/55 (36·3%), *Streptococcus pneumoniae* 3/83 (1·1%), *Moraxella* Spp 7/83 (8·4%), *Klebsiella pneumoniae* 2/83 (2·4%). These findings do not differ from the extensive the national ACS study in both sputum and blood isolated organisms (Vichinsky *et al*, 2000). A prior report from the same study setting showed *Staphylococcus aureus* 28/47 (60%) samples, *Haemophilus influenzae* 9/47 (19%) and *Staphylococcus epidermidis* 4/47 (9%) as the most common organisms isolated in blood cultures (Kizito *et al*, 2007). This study and the report by Kizito *et al* (2007) showed that *Staphylococcus aureus* bacteraemia contributes significantly to morbidity in children with SCA at Mulago Hospital. Unlike Kizito *et al* (2007) but in line with findings in Kilifi, Kenya (Williams *et al*, 2009) we found that Non-Typhi Salmonella contributed significantly to bacteraemia. In this current study, *Haemophilus influenza* was not isolated in blood cultures but it was the second most common in the same setting as reported by Kizito, 2007 between October 2001 and January 2002. This is possibly because *Haemophilus influenza* type b (Hib) conjugate vaccine was introduced for routine infant immunization programme in Uganda in June 2002.

In this study, *Chlamyidia pneumoniae* was isolated in 19/47 of sputum samples analysed from children without ACS (Table IV). There is no published data on the prevalence of positivity of *Chlamyidia pneumoniae* among children with SCA who have fever but with no chest symptoms or signs. The significance of this is finding needs further study.

As described above, *Mycobacterium tuberculosis* was isolated in 6/83 sputum samples in the current study, and was a significant contribution to morbidity among children with SCA at Mulago hospital.

In this study the overall sensitivity of the most frequent organisms, *Staphylococcus aureus* and *Streptococcus pneumoniae*, to drugs commonly used in our settings; chloramphenicol, ceftriaxone and gentamicin, was reassuring.

Radiological findings

The overall interpretation by specialist radiologists of CXRs in this study had a fair agreement (kappa = 0·4). However, there was significant disagreement especially with positive CXRs. This shows the difficulty in relying on CXRs to make a diagnosis of ACS, especially in low income countries where CXRs are not readily available in rural health facilities.

The most common finding was of interstitial pattern, with consolidation pattern with pleural effusions occurring in only

2/58 cases. These findings are not different from those described in India (Samal, 1997). Pleural effusion is not common in ACS and, if present, is usually small. Therefore, if a child with SCA presents with massive pleural effusion a thorough work-up on other causes would be necessary.

Strengths of the study

This study is the first to outline the clinical and radiological characteristics of children with ACS in Uganda and provides a baseline for other studies in trying to understand ACS in sub-Saharan Africa. The laboratory results, particularly the sputum characteristics, in this study do not differ from other studies on ACS and febrile children with SCA, but it does add more information on the organisms isolated from the sputum of children with SCA, fever and ACS. This study used a good quality laboratory with good quality assurance which would validate the results therein.

Limitations of the study

Some variables in the study were dependent on clinical history and therefore required recall by parents/caregiver/patient. There was a probable recall bias on symptoms related to previous illnesses, prophylaxis measures and past illnesses. This recall bias may have influenced results from the variables dependent on patient history.

We used disk diffusion techniques which are not directly used to measure the susceptibility of *Streptococcus pneumoniae* to cephalosporins and to penicillin. MIC techniques were not available in the laboratory at the time of the study, therefore full and reliable results on *Streptococcus pneumoniae* susceptibility are not available. This limits the study's ability to conclude on treatment and prophylaxis of a commonly isolated organism – *Streptococcus pneumoniae,* especially in relation to penicillins. A major limitation was that sputum induction tests were based on an initial interpretation of an infiltrate on CXR at the point of care. Given the subjective and inconsistent interpretation for CXRs, some of the children who had ACS according to the senior radiologist assessment did not have sputum results. This reduced the number of reportable sputum isolates in relation to patients with ACS. Due to limited funds sputa samples were not analysed for viral pathogens.

Conclusions

One in five children with SCA and fever had ACS. Even though there were no specific laboratory characteristics of ACS, cough and abnormal findings on chest auscultation were associated with ACS. Infections seem to contribute to ACS in this environment and *Mycobacterium tuberculosis* should be considered in sub-Saharan African children with ACS. The high prevalence of *Chlamydia pneumoniae* in children with ACS warrants and emphasises the addition of a macroclide to treatment regimens.

Author Contributions

OO, JKT and HH conceptualized and supervised the study. OO was the principal investigator of the study and recruited and followed up the study participants. FB supervised the laboratory work while SB, HK and RB carried out interpretation of the chest radiographs. OO and JKT analysed the data, assisted by Deogratious Sebuwufu; and wrote the initial manuscript. All the authors have read and revised the manuscript.

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