Bacterial infections in hospitalised severely malnourished children in Durban, South Africa†

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**Background:** Severe acute malnutrition (SAM) results in alterations of host defence mechanisms, which leads to increased susceptibility to infections. This study describes culture-confirmed bacterial infections in a cohort of HIV-negative severely malnourished children and compares it with a previously described cohort of HIV-positive children.

**Methods:** A retrospective chart review was conducted of all HIV-negative children with SAM admitted to King Edward Hospital, Durban between January 1, 2015 and December 31, 2015. All positive bacterial cultures obtained within 2 days of admission (admission infections) and during 2 to 30 days of admissions (hospital acquired infections) were documented. A cohort of HIV-positive children with SAM was prospectively recruited between July 2012 and February 2015 at the same facility for the MATCH study.

**Results:** A total of 101 patients were eligible for the study, 53% were HIV unexposed; 73% of the total 250 cultures obtained were during admission. *Escherichia coli* (E. coli) contributed 26% of all positive cultures on admission. Significant differences were noted in laboratory variables between HIV-negative vs. HIV-positive children admitted with SAM. Extended-spectrum beta-lactamase (ESBL) producers in HIV-positive patients constituted 40% of all Gram-negative isolates vs. 24% in HIV-negative patients.

**Conclusion:** Gram-negative organisms remain an area of concern in both HIV-positive and HIV-negative patients with SAM with resistant organisms more prevalent in HIV-positive patients. Prevention of mother-to-child transmission of HIV reduces prevalence and incidence of HIV, which has been shown to contribute to the burden of bacterial infections in malnourished children.

**Keywords:** bacterial infections, HIV, severe acute malnutrition

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Introduction

Severe malnutrition is defined as weight for height z-score <−3SD, bilateral oedema, or mid-upper arm circumference (MUAC) < 11.0 cm (if > 65 cm in length). The World Health Organization (WHO) also defines severe acute malnutrition (SAM) by a very low weight for height (below −3z scores of the median WHO growth standards), by visible severe wasting, or by the presence of nutritional oedema. Marasmus involves inadequate intake of protein and calories, representing the end result of starvation. It occurs in the first year of life due to lack of breast-feeding and use of dilute animal milk. Poverty and famine, ignorance and poor maternal nutrition are among the major contributory factors.

Pre-school aged children in developing countries are often at risk of malnutrition because of their dependency on others for food, increased protein and energy requirements, and an immature immune system causing greater susceptibility to infections. Severe malnutrition affects 1–2% of pre-school children, mainly in the developing countries.

The ability of the malnourished child to handle infections is lower. Common infections are those caused by *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli* and *Haemophilus influenzae*. Malnutrition and infections are interrelated.

Gram-negative organisms, especially non-typhoidal Salmonella species, were the predominant cause of bacteraemia in severely malnourished children, supporting early results from Uganda and recent studies from Kenya, Malawi and Ethiopia. Although there was no difference in the types of bacterial organisms by human immunodeficiency virus (HIV) status, blood specimens from severely immuno-suppressed children were more likely to grow *Salmonella enteritidis*. The mechanism for this is not very clear and may include the difficulty in clearing Salmonella infections from infected macrophages and a weak immune system; the human immunodeficiency virus may predispose the host to infection with *Salmonella enteritidis* and this, in turn, promotes the production of human immunodeficiency virus (HIV) in the macrophages of the gastrointestinal tract mucosal cells, thus completing a vicious cycle.

In a meta-analysis study done in Kenya a total of 21 977 children were included. HIV prevalence was around 40%. Bacteraemia levels ranged from 3% to 30% (mean 17%). Mortality varied greatly from 18% to 47.4% among SAM cases. Mortality from bacteraemia was 17%, but doubled with SAM, HIV or tuberculous, and was multiplied by five with bacterial resistance. There is also substantial evidence that Gram-negative bacteria, *Escherichia coli*, non-typhi Salmonella, and other enterobacteria-aceae, represent about 60% (range 58%–77%) and Gram-positive...
bacteria, mainly Streptococci and Staphylococci, about 40% of blood culture isolates.\textsuperscript{12}

King Edward Hospital is situated in Durban, KwaZulu-Natal province, South Africa and offers regional and tertiary care. The aim of the study was to determine the bacterial infections isolated from HIV-negative children aged between 6 and 60 months with SAM admitted to King Edward Hospital between January and December 2015 during the first 30 days following admission.

\textbf{Methods}

The King Edward VIII (KEH) Hospital Paediatric Resuscitation Unit (PRU) admission record book between January 2015 and December 2015 was reviewed. All HIV-negative children aged between 6 and 60 months who were classified as SAM were screened for the study. Weight for height less than 3sd and mid-upper arm circumference less than 3sd were looked at to confirm patients that were included in the study. Age, HIV status and date of admission was confirmed and recorded.

Blood (1-3 ml) taken using a standard aseptic technique was inoculated into blood culture bottles. In the laboratory BacT (BioMerieux, France) a fully automated continuous monitoring blood culture system was used. When a blood culture is detected as positive, Gram staining is performed on broth for presumptive identification. The blood culture medium is then sub-cultured onto agar plates to obtain bacteria colonies which are identified using biochemical testing. Induced sputum samples were obtained using in-and-out sterile catheterisation or aseptic suprapubic aspiration. Lumbar puncture was done depending on whether the sample was taken within two days of admission or after two days, respectively.

The HIV status of patients was determined by reviewing the discharge forms and/or NHLS Laboratory Online results system (NHLS-TrakCare). An HIV-negative result was based on: negative HIV PCR or ELISA result, documented Road To Health Card HIV negative result and, if there was clinical suspicion, mother’s Voluntary Testing and Counselling for Human Immunodeficiency Virus (VCT) on admission to KEH PRU.

Full blood count, C-reactive protein, albumin levels, potassium level and INR were recorded. If multiple samples were taken, those done with cultures were recorded.

Hospital numbers and names of eligible patients were used to check culture results from the microbiology laboratory at King Edward Hospital and the South African National Health Laboratory Service Online record system. Cultures were from sputum, cerebrospinal fluid, urine and blood specimens and were done during the first 30 days of admission. Organisms isolated and sensitivity were recorded if available. Positive cultures were classified into community acquired and hospital acquired depending on whether the sample was taken within two days of admission or after two days, respectively.

When counting the number of patients eligible for study one patient was allocated to a positive culture despite the number of organisms grown; however, all the organisms isolated were described. All cultures were reviewed by the clinical team, in conjunction with the microbiologist and paediatric infectious diseases specialist to determine clinically significant infections versus contamination.

Comparison was also made with HIV-positive patients admitted to the same hospital with the same management protocols to determine any differences in demographic data, laboratory parameters, organisms and resistance profiles. These patients were looked at in the MATCH Study, which enrolled 82 HIV-positive children aged between 1 month and 10.6 years prospectively between July 2012 and February 2015.

Management of patients was undertaken according to WHO Guidelines Management of Severe Acute Malnutrition in Infants and Children.\textsuperscript{13} Infections on the ward were managed according to the local protocol, which dictates benzylpenicillin and gentamycin as first-line empirical antibiotics for unwell children. Second-line antibiotics are piperacillin-tazobactam and amikacin, with use of carbapenems as third line. Quinolones are used if indicated by culture results.

\textbf{Statistical analysis}

Descriptive analysis of all pathogens isolated within 30 days of each patient’s admission to hospital was completed and divided into admission and hospital acquired samples. If a sample grew multiple pathogens that patient was analysed as though he/she had a single positive culture.

Variables explored in univariate analysis included age, sex, length of stay and the laboratory parameters, particularly white cell count, platelet count, albumin, potassium, INR and C-reactive protein.

Demographic data including means of age, length of stay and the means of laboratory parameters were compared with the HIV-positive group using unpaired t-test. All tests were two tailed and a \( p \)-value of less than 0.05 was considered to be significant.

The outcome of patients was also noted in terms of survival during the study review period.

Analysis was performed using SSPS\textsuperscript{®} Version 20 (IBM Corp, Armonk, NY, USA) and no imputations were made for missing variables.

\textbf{Ethics approval}

The study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (Reference Number BE020/16) and King Edward Hospital. Confidentiality of patient details was maintained.

\textbf{Results}

\textbf{Patient characteristics}

A total of 101 patients were eligible of whom 51 were female. The mean age on the day of admission was 14.8 months (SD 8.7) with 47 patients (47%) HIV exposed and the remaining HIV unexposed. In total, 54 patients had a confirmed negative HIV test (51 HIV PCR and 3 HIV ELISA) and for the remainder HIV status was based on a negative maternal HIV rapid test.

Oedema was present at admission in 46 patients (46%). On admission the mean WCC was 17 \( \times 10^3 \) (SD: 5), platelet count was 401 \( \times 10^9 \) (SD:154) C-reactive protein was 27.7 mg/l (SD 84) and albumin level was 26 g/l (SD 8.7). The average length of stay in hospital was 14 days.

A total of 250 samples were collected, 73% of which on admission.
**Bacterial isolates**

A total of 93 blood cultures were collected; 81 were collected on admission. Of the 93 cultures collected, 13 were considered to be a pathogenic organism, 33 were contaminated (as determined by the microbiologist and treating clinician) and 48 had no growth. Gram-positive organisms constituted the majority of organisms grown from admission blood culture with *Staphylococcus aureus* having been isolated from 3 out of 10 positive blood cultures on admission.

A total of 77 urine cultures were collected, 19 of which were positive, 14 contaminated and 44 did not show any growth. Gram-negative organisms were the predominantly isolated organisms in both admission and hospital acquired urine samples with *E. coli* being the most frequently isolated organism.

Fifty-seven sputum samples were collected, of which 54% were collected between 2 and 30 days after admission. Only eight cerebrospinal fluid samples were collected, the majority (75%) being collected on admission. There were 15 stool samples, with 8 being collected on admission, and all were negative. One patient had *Haemophilus influenzae* isolated from both blood and CSF with cultures being taken on admission.

Some 55% of positive admission cultures were sensitive to first-line antibiotics whereas 43% of hospital acquired cultures were sensitive to second-line antibiotics.

Among the Gram-negative organisms, 24% were extended spectrum beta-lactamase (ESBL) producers. Of note was that 8 of the 12 ESBL producers were isolated from sputum with *Klebsiella pneumoniae* being the predominant organism isolated.

Table 1 shows cultures done on admission showing that Gram-negative organisms are predominant, in particular *E. coli*, which was cultured in 11 of 44 positive cultures. Gram-positive organisms were also noted with *Staphylococcus aureus* being the majority.

Table 2 shows hospital acquired organisms isolated during the period 2–30 days. Gram-negative organism are also the predominant organisms with *Klebsiella pneumoniae* being isolated in 35% of all the hospital acquired infections. It also shows that most of the organism acquired in the hospital are isolated from sputum.

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### Table 1: Admission cultures in HIV-negative patients with severe acute malnutrition (total = 183)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Blood</th>
<th>Urine</th>
<th>Sputum</th>
<th>Cerebrospinal fluid</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><em>Haemophilus influenza</em></td>
<td>1</td>
<td></td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td><em>Streptococcus group C</em></td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Streptococcus viridans</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

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Sex, age and length of stay were also compared with HIV-positive patients reviewed under the MATCH Study. Laboratory parameters were also looked at in comparison with HIV-positive patients. As shown in Table 3, significant differences were noted in age, length of hospital stay, albumin and platelet count of HIV-negative vs. HIV-positive children admitted with SAM.

Eight HIV-negative patients of the 101 admitted during the study period died within 30 days of admission. Three of the eight who died had positive cultures (two *E. coli* and one *Staphylococcus aureus*).

**Discussion**

Bacteraemia can be caused by both Gram-positive and Gram-negative bacteria. In this study 73% of cultures from South African HIV-negative SAM patients were done during the first two days of admission. Positive cultures were mainly Gram-negative organisms, of which *E. coli* was the predominant organism, which is different from a study done by Abraha et al in Ethiopia, which showed a predominance of Gram-positive organisms. Only two of the *E. coli* organisms were ESBL producers. *Acinetobacter baumannii*, which is traditionally a nosocomial organism, was also isolated in two sputum specimens collected on admission, showing its prevalence in communities. Gram-positive organisms contributed 30% of the total organisms cultured on admission of which *Staphylococcus aureus* was the predominant organism.

Hospital-acquired infections are predominantly caused by Gram-negative organisms, as shown in this study. *Klebsiella pneumoniae* and *Acinetobacter baumannii* were the main organisms isolated. Only 6% of the hospital-acquired organisms were Gram-positive, with *Streptococcus pneumoniae* and *Enterococcus faecium* being the only organisms isolated.

Sputum is collected in symptomatic patients, hence all patients with positive cultures are treated because they are high-risk patients in view of immune-paresis from malnutrition. No organism was isolated from stool collected upon admission or during the hospital stay. As a cost–benefit measure it will be necessary to collect stool from symptomatic patients. Stool specimens also aid in deciding on use of antibiotics in those that would have grown an organism; for example in Shigella dysentery where the use of ciprofloxacin is indicated.
were Gram-positive. This study suggests a change in epidemiology from the predominant Gram-negative aetiologies in bacteraemia revealed that Gram-positive organisms, especially Staphylococcus species, were the predominant cause of bacteraemia. In a study done in Ethiopia and published in 2010 coagulase-negative Staphylococcus species and S. aureus were common amongst coagulase-negative ESBL producers being isolated in 50% of all the ESBL Gram-negative isolates. In this study done in 2015 at King Edward Hospital, regardless of their HIV serological status, this is in keeping with the current study, which has noted that there are significant differences in terms of length of stay in hospital and age of patients enrolled in the two studies. This study looked at patients between the ages of 6 and 60 months, whereas the MATCH Study did not have any restrictions in terms of age upon enrolment. Length of stay differs, presumably due to intercurrent and opportunistic infections in HIV-positive patients, which prolong hospital stay.

Laboratory parameters that also showed significant differences include platelet count and albumin, which were both noted to be lower in HIV-positive patients. Opportunistic infections play a significant role in HIV-positive patients, leading to reduced appetite and significant gastrointestinal losses secondary to diarrhea and malabsorption. Preventable diseases are still prevalent, with Haemophilus influenzae being isolated in 17% of the Gram-negative cultures done on admission. This also represented 15% of hospital-acquired Gram-negative organisms. Only one culture out of the six cerebrospinal fluid specimens grew Haemophilus influenzae. The patient who grew Haemophilus influenzae was a six-month-old girl who was not immunised due to social circumstances. Streptococcus pneumoniae was the second commonest Gram-positive organism cultured during admission and the commonest Gram-positive organism cultured during 2–30 days of admission. In this study serotypes were not performed in the Streptococcus cultures; hence it is unknown whether they were included in the vaccines. In comparison a study in Uganda found a very low proportion of H. influenzae infection in these children and this may be explained by the incorporation of Hib vaccine into their expanded programme on immunisation (EPI) from 2002. There is a need to be aggressive in terms of education of the public on immunisations and efforts in the expanded programme of immunisation in KwaZulu-Natal to reduce the incidence of vaccine-preventable diseases.

ESBL producers in HIV-positive patients constitute 40% of all Gram-negative isolates vs. 24% in HIV-negative patients. The role of HIV in frequency of Gram-negative infections and resistant organisms has been shown in the study. Measures that include prevention of mother-to-child transmission of HIV reduce frequency of infections and reduce length of hospital stay, thereby also reducing the frequency of resistant organisms. Prevention of HIV in women of child-bearing age also helps to reduce the incidence and prevalence of HIV.

Table 2: Hospital-acquired infections in HIV-negative patients with severe acute malnutrition (total = 67 done)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Blood</th>
<th>Urine</th>
<th>Sputum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>3</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>3</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>2</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Resistance to antibiotics is still a challenge, with 67% of all Gram-negative ESBL producers being isolated in sputum. Strict infection control measures, which include isolation of patients with positive sputum cultures, can help prevent spread of these resistant organisms. Klebsiella pneumoniae remains the main ESBL producer, being isolated in 50% of all the ESBL Gram-negative organisms as shown in this study.

In a study done in Ethiopia and published in 2010 coagulase-negative Staphylococcus species and S. aureus were common amongst Gram-positive bacteria whereas Enterobacteriaceae were the most common among Gram-negative isolates. It also revealed that Gram-positive organisms, especially Staphylococcus species, were the predominant cause of bacteraemia (68.6%) in severely malnourished children, consistent with other reports where they found 71% of their total isolates were Gram-positive. This study suggests a change in epidemiology from the predominant Gram-negative aetiologies in this study done in 2015 at King Edward Hospital.

Comparing HIV-negative SAM patients and HIV-positive children admitted to King Edward Hospital in Durban, South Africa it is noted that there are significant differences in terms of length of stay in hospital and age of patients enrolled in the two groups.

Table 3: Baseline characteristics and laboratory results comparing HIV-negative SAM patients vs. HIV-positive patients

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>HIV negative mean (SD), n = 101</th>
<th>HIV positive (match study) mean (SD), n = 82</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, males</td>
<td>50 (49.5%)</td>
<td>44 (53.6%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Age, months</td>
<td>14.8 (8.7)</td>
<td>24.15 (28)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Length of stay, days</td>
<td>14.1 (0.7)</td>
<td>20 (2.8)</td>
<td>0.0002</td>
</tr>
<tr>
<td>White cell count ×10^9</td>
<td>17 (5.0)</td>
<td>13.95 (6.84)</td>
<td>0.10</td>
</tr>
<tr>
<td>Platelet count ×10^9</td>
<td>401 (154)</td>
<td>330 (217)</td>
<td>0.02</td>
</tr>
<tr>
<td>C-reactive protein mg/l</td>
<td>27.7 (84)</td>
<td>40.4 (48.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>Albumin g/l</td>
<td>26 (8.7)</td>
<td>23.4 (7.4)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Change of antibiotics is done by the clinician who looks at the clinical condition, septic markers and any positive cultures. However, empirically the patient is changed from first-line (penicillin and gentamycin) to second-line antibiotics (pipercillin-tazobactam) and if not responding, the patient receives third-line antibiotics (carbapenems).

Preventable diseases are still prevalent, with Haemophilus influenzae being isolated in 17% of the Gram-negative cultures done on admission. This also represented 15% of hospital-acquired Gram-negative organisms. Only one culture out of the six cerebrospinal fluid specimens grew Haemophilus influenzae. The patient who grew Haemophilus influenzae was a six-month-old girl who was not immunised due to social circumstances. Streptococcus pneumoniae was the second commonest Gram-positive organism cultured during admission and the commonest Gram-positive organism cultured during 2–30 days of admission. In this study serotypes were not performed in the Streptococcus cultures; hence it is unknown whether they were included in the vaccines. In comparison a study in Uganda found a very low proportion of H. influenzae infection in these children and this may be explained by the incorporation of Hib vaccine into their expanded programme on immunisation (EPI) from 2002. There is a need to be aggressive in terms of education of the public on immunisations and efforts in the expanded programme of immunisation in KwaZulu-Natal to reduce the incidence of vaccine-preventable diseases.

ESBL producers in HIV-positive patients constitute 40% of all Gram-negative isolates vs. 24% in HIV-negative patients. The role of HIV in frequency of Gram-negative infections and resistant organisms has been shown in the study. Measures that include prevention of mother-to-child transmission of HIV reduce frequency of infections and reduce length of hospital stay, thereby also reducing the frequency of resistant organisms. Prevention of HIV in women of child-bearing age also helps to reduce the incidence and prevalence of HIV.

Ninety-one children were recruited for a study in Kenya. Of these, 60 had marasmus, 20 kwashiorkor and 11 marasmic kwashiorkor. HIV serology was positive in 43% of study subjects. There were 30 bacterial isolates from 26 subjects. Ten bacterial isolates were Gram-positive and 20 Gram-negative. Isolation rates did not vary by HIV serological status. Twenty-one of the 30 isolates were from blood culture. Gram-negative agents were responsible for most of the severe bacterial infections in children admitted to the Kenyatta National Hospital, regardless of their HIV serological status. This is in keeping with the current study, which has shown predominantly Gram-negative organisms.

Amongst the HIV-negative patients in the study group 8% died during the 30 days of admission. These patients were managed strictly according to World Health Organization Guidelines for the Management of Severe Acute Malnutrition, which explains the lower mortality. This is compared with another study where, among the children with bacteraemia, mortality was higher (43.5% vs. 20.5%) in the HIV positive than the HIV negative; OR 3.0 (95% CI 1.01–8.6). For the children without bacteraemia, the test of equality of survival distributions by HIV status was not significant (p = 0.07).
Limitations of the study include the fact that it was done with a small population in South Africa, which might not be a true representation in sub-Saharan Africa. In addition, the study looked at all patients meeting the WHO definition of severe acute malnutrition, thereby including cardiac patients (7 out of 101) and others with genetic problems.

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