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2021
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MORTALITY, RELAPSE, MORBIDITY AND GROWTH OF SEVERE ACUTELY MALNOURISHED CHILDREN IN THE SIX MONTHS FOLLOWING DISCHARGE

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of MSc in Child Health (Community Paediatrics)

Johannesburg, 2021

DECLARATION

I, Angelika Peczak declare that this Research Report is my own, unaided work. It is being submitted for the Degree of MSc in Child Health (Community Paediatrics) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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20 July 2021 in Johannesburg

ACKNOWLEDGEMENTS

This research report would not be complete without the support received from exceptional people.

Firstly, thank you to my supervisor, Prof Saloojee for the immense patience and assistance I received from the planning stages to the write up. Your calming guidance has breathed life into this report.

Secondly, to my partner, Phillip who has always been my number one cheerleader. I will forever appreciate the space you provided for me to complete this journey.

Thirdly, my daughter, Kaja. Thank you for being a constant reminder of the purpose of this research. I hope to give you the life that all children deserve.

Fourthly, to all my other family and friends that have been my sound boards to clear up my mind. Your support has been irreplaceable.

And finally, to little KS who was one of the first SAM patients I treated. You have been in my thoughts since you were lost to follow up. Thank you for being with me in spirit and an inspiration for this research. I hope that it will help other children with SAM flourish into powerful people.

PUBLICATIONS

This research report has not been published in any journal and is being presented in the submissible format.

I intend to submit the report to PLos ONE for publication. The submissible paper has been formatted to meet their submission guidance criteria (presented in Appendix D).

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COVER PAGE

Type of article: Research article

Study's contribution: The study provides evidence that discharging children with SAM only once anthropometrically recovered and continuing support through nutritional supplementation may improve and reduce relapse rates. It is the first of its kind from an upper-middle-income country. Similar studies emanating from less resourced settings have found varying rates of mortality and relapse, but few have offered the same length of post-discharge nutritional supplementation. The study follows new guidelines on reporting relapse to facilitate standardisation and comparability to other relapse studies.

Prior interactions: None

Academic editors: Diego G. Bassani, Faculty of Medicine and Dalla Lana School of Public Health, CANADA.

Opposed reviewers: None

Affiliations: Student of Department of Paediatrics and Child Health, University of the Witwatersrand

1. Title page

Long title

Clinical and growth outcomes of severely malnourished children following hospital discharge in a South African setting

Short title

Outcomes of severely malnourished children following hospital discharge

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2. Abstract

Background

Data on outcomes of children with severe acute malnutrition (SAM) following treatment are scarce with none described from any upper-middle-income country. This study established mortality, clinical outcomes and anthropometric recovery of children with SAM six months following hospital discharge.

Methods

A prospective cohort study was conducted in children aged 3-59 months enrolled on discharge from two hospitals in the Tshwane district of South Africa between April 2019 and January 2020. The primary outcome was mortality at six months. An analysis was undertaken of factors associated with persistent malnutrition.

Results

Forty-three children were enrolled with 86% of participants followed up to six months. Only a third of the participants had normal anthropometry at discharge – a quarter still had ongoing SAM. There were no deaths, although four children (9%) were re-hospitalised (including two for complicated SAM). Mean weight-for-length z-scores (WLZ) and wasting rates improved at one month but deteriorated by three months. At three months, six children (14%) either had ongoing or relapsed SAM – a SAM incidence rate of 20 per 1000 person-months. Almost half (46%) of children had persistent malnutrition, despite more than half of the participants still receiving nutritional supplements at the time.

Factors associated with persistent malnutrition included a low WLZ on admission (relative risk [RR] 3.3, 95% confidence interval [95%CI] 1.2-9.2), failing to meet WHO discharge criteria (RR 5.3, 95%CI 1.3-14.8) or having any illness by three months (RR 8.6, 95%CI 1.3-55.7).

Conclusion

Post-discharge mortality and morbidity was lower than in other less resourced settings. However, anthropometric recovery was poorer than expected. Modifying discharge criteria, optimising the use of available nutritional supplements and better integration with community-based health and social services may improve outcomes for children with SAM post-hospitalisation.

3. Introduction

Most programmes managing severe acute malnutrition (SAM) in children focus on initial recovery only, resulting in post-discharge outcomes, such as mortality, ongoing SAM, regression and relapse being poorly understood and underestimated.[1] Relapse rates post-discharge from treatment for wasting (SAM) has been identified as one of the top five global research priority questions to achieve scale-up of treatment of wasting.[2]

Studies conducted in Ethiopia, Malawi, Nigeria, Zimbabwe, Zambia, Bangladesh, and India have described mortality rates ranging between 0.8-10.3% from discharge to one year after either hospital or community-based management of SAM.[3-9] Factors associated with death included younger age (<12 months) and greater malnutrition severity at admission.[3-9] Mortality was highest among children living with HIV.[4,6]

A 2019 systematic review indicated that 0-37% of children treated for SAM relapsed with most cases occurring within six months of discharge from the treatment programme. Common risks for relapse included lower anthropometric scores at hospital admission and on discharge along with illness simultaneous to the time of relapse.[1]

Malnutrition remains a significant problem in South Africa, despite the country's status as an upper-middle-income country. SAM was a direct or contributory cause of a third of deaths in children under 5 years in 2013.[10] There has been a reduction in SAM in-patient mortality at South African hospitals from 19.3% in 2010 to 7.1% in 2019.[11] However, there is no national, district or institutional data on outcomes following discharge.

This study aimed to prospectively establish mortality and relapse rates, the frequency and type(s) of morbidities experienced, and anthropometric changes in children with SAM following hospital discharge. A secondary aim was to identify factors associated with poorer long-term growth, particularly those that might be amenable to change or that could assist in identifying children requiring more targeted support. It also explored the uptake and benefit of local SAM support services.

4. Methods

4.1 Study setting and design

This was a prospective cohort study of children aged 3-59 months with SAM following their discharge from a regional (Mamelodi) or district (Bronkhorstspruit) hospital in the Tshwane district of Gauteng, South Africa. The catchment area of these two hospitals

consists of urban and rural sub-districts, including various townships. Enrolment occurred from April 2019 to January 2020, with children followed up for six months post-discharge.

4.2 Study enrolment

All participants were hospitalised and had confirmed SAM on admission. SAM was defined as (a) a weight-for-length or -height z-score (WLZ) less than -3 on WHO growth standards, or (b) a mid-upper arm circumference (MUAC) of less than 11.5 cm, or (c) the presence of bilateral pedal oedema. For children less than 6 months old, a WLZ of less than -3 or the presence of bilateral pitting oedema was used for SAM diagnosis, the MUAC was not considered. Treatment was based on the South African guidelines for in-patient SAM care.[12]

Participants aged 3 to 60 months were identified by the researcher (AP) while being treated for SAM and children surviving to discharge were consecutively enrolled into the study. Study participants hospitalised more than once during the study period were only enrolled during the first admission. No exclusion criteria were applied. The primary outcome was mortality at six months post discharge. Children with chronic illnesses or disability such as cerebral palsy were included in the study. These children are especially vulnerable to SAM and although there may be other factors contributing to their malnourishment, they are important to follow up and determine their outcomes post-discharge.

Children were discharged from in-patient care when they met the national discharge criteria: return of appetite, pedal oedema resolved, clinically well and alert, all medical

complications resolved, and persistent and good weight gain for five consecutive days.[12] Discharge decisions were made by the treating clinicians.

Demographic, household and medical information was obtained through verbal primary caregiver interviews, while anthropometric (birth, admission and discharge) and clinical details were obtained from participants' case records and Road to Health booklets. A one-month first follow up date was set based on routine hospital practice. If a child was born preterm (<37 completed weeks of pregnancy) their corrected age was charted.

On discharge, all participants received a nutrition supplement in the form of a commercial infant formula or paediatric milk powder, ready to use therapeutic food (RUTF), children's porridge or a combination of these, as per hospital protocol. Supplementation was intended to be continued until the patient's weight returned to normal and was maintained for three consecutive months. Supplementation could be continued for more than three months at the treating dietitian's discretion.

Applications were submitted for all South African participants for participation in the South African Social Security Agency (SASSA) Zero Hunger project, a six-month programme enabling households with a malnourished child to receive monthly food parcels. All but one child was referred to the district ward based outreach team (WBOT) (community health care workers) for home visitations. The WBOT was expected to conduct at least one home visit and further follow up visits if necessary. Most children (60%) were referred to various social and health care specialists for added support. These included physiotherapists (67%), social workers (47%), occupational therapists (28%) and speech therapists (7%).

During the study period, monthly sub-district level meetings to reduce SAM mortality and improve the continuum of care through multi-disciplinary team work were ongoing. These were established in 2017 and discontinued in November 2019 when programme objectives of lower levels of SAM case fatality and appropriate communication networks were established. The meetings were led by the District Health Specialist Team and sub-district dietitians and aimed to audit SAM cases as well as integrate SAM care by creating relationships between relevant stakeholders (clinic, hospital, SASSA and WBOT staff). However, there was poor engagement by SASSA representatives and WBOT members.

4.3 Follow up procedure

Participants were expected to attend follow-up sessions at one and three months post-discharge when anthropometry (weight, length and MUAC) was undertaken, and information about any morbidities experienced and pertinent medical and social changes were attained. The follow up occurred either at the facility in which the child had been treated or at a down-referral clinic.

Participants were evaluated by the researcher or a dietitian. If a caregiver failed to attend a scheduled visit, she was contacted telephonically and all information, except anthropometry, collected. A third and final follow up session was conducted telephonically six months after discharge.

Complete follow-up at one and three month visits was defined by the physical presence of the participant and the follow-up deemed partial if only a telephonic interview was possible. Participants unreachable after three attempts within three weeks of the scheduled visit date were classified as ‘lost to follow-up’ for that visit. All participants were

contacted at six months regardless of whether they attended the one and three month follow-up visits.

At follow-up visits, weight was measured to the nearest 100 g, using an electronic scale, length/height to the nearest 0.5 cm using a measuring board and MUAC to the nearest 1 mm, using WHO/UNICEF colour-coded tapes. A child was considered to have no acute malnutrition (NAM) if they had a WZL ≥ -2 and MUAC ≥ 12.5 cm, moderate acute malnutrition (MAM) if they had a WZL between -3 and -2 and/or a MUAC of 11.5-12.5 cm and SAM if the WZL was ≤-3 and MUAC ≤ 11.5 cm.[13] Participants were regarded as relapsed, when there was a recurrence of SAM (as defined at study onset).[2] Persistent malnutrition was defined as the presence of MAM or SAM in a child who previously had SAM.

4.4 Sample size estimation

The study intended to enrol 50 participants, allowing for detection of 10% mortality with a 95% confidence interval of 3-22%. A 10% mortality rate at six months following discharge was predicted based on the 0.8-10.3% mortality range identified in previous studies.[3-9] The actual sample size was, however, limited by fewer than anticipated children with SAM being admitted during the study period.

4.5 Data analysis

Data was entered into Excel 2010 (Microsoft, Seattle, USA), cleaned and exported to STATISTICA 13.5 (Tibco, California, USA) for analysis. World Health Organization Anthro software v3.2.2 (WHO, Geneva, Switzerland) was used to calculate the WLZ. Differences in proportions were compared by the chi-squared test or Fisher exact test when cell

numbers were <5. Strength of association was determined by calculating relative risks (RR) and related 95% confidence intervals (95% CIs), using IBM SPSS Statistics version 27.0 (SPSS Inc, Chicago, USA). A p-value <0.05 was considered statistically significant.

4.6 Ethical considerations

The study was approved by the University of Witwatersrand's Human Research Ethics Committee (clearance certificate number: M180812) and the National Health Research Database (GP_201812_016). Signed consent for participation was obtained from the primary caregivers of participating children.

5. Results

Forty-three children were enrolled, 32 at the regional and 11 at the district hospital. Follow up was 100% at one month (28% partial follow up), 89% at three months (33% partial follow up) and 86% of participants' caregivers were interviewed at six months.

5.1 Baseline characteristics

Participants' baseline characteristics are shown in Table 1. Their median (IQR) age was 11 (8, 20) months with 11% aged between three and six months. More were female (63%). All were black African, and 86% were South African nationals. All but one of the participant's primary caregiver was their biological mother. Two-thirds came from a single headed household. Most caregivers had no fixed monthly income (77%) and were recipients of a government social grant (79%). Half the children lived in informal houses constructed of corrugated iron. Water sources varied with 42% of participants reporting

an indoor tap, 21% an outside tap and 37% using a communal tank or tap. Two-thirds of homes had electricity and one-half had a fridge.

Table 1: Baseline characteristics of participants discharged from SAM in-patient treatment (n=43)

Characteristic	Frequency	Percent
Female	27	63
Caregiver nationality		
South African	37	86
Mozambican	2	5
Zimbabwean	4	9
Primary caregiver		
Mother	42	98
Grandmother	1	2
Secondary caregiver		
Father	18	42
Grandmother	9	21
Grandfather	1	2
Aunt	1	2
None	14	33
Social support		
Child support grant	31	72
Care dependency grant	1	2
Zero Hunger Project	1	2
None	10	23

5.2 Medical history

Forty percent of children had been previously admitted to hospital, most often for neonatal complications (65%), diarrhoea (12%) and pneumonia (6%). Only one child had a previous SAM admission. Three participants had a long-term disability (cerebral palsy). These children were included as SAM based on their anthropometry which was analysed using the same WHO growth standards as the other children. None of the children had any contractures preventing adequate length measurements. There was high HIV exposure - 19 of the caregivers (44%) were HIV positive, all were on antiretroviral treatment. Five children (12%) were HIV positive and were initiated or continued on

antiretroviral therapy. Co-morbidities during the admission included diarrhoea (35%), pneumonia (16%) and tuberculosis (7%).

5.3 Admission status

At admission, one-half of the participants (49%) presented with a WLZ below -3, 40% had a MUAC less than 11.5 cm and 35% had bilateral pedal oedema. Ten children (23%) presented with a low WLZ and MUAC and three (7%) presented with a low MUAC and oedema. No participant had both a low WZL and oedema, nor did any have all three SAM criteria.

5.4 Discharge status

Since discharge was based on clinical criteria and not the extent of anthropometric recovery, only a third of the participants had normal anthropometry (no acute malnutrition [NAM]) at discharge. Forty-two percent of the children were discharged with MAM and one-quarter had ongoing SAM. Of the MAM group, 33% had a low WLZ, 39% a low MUAC and 28% had both. In the SAM group, 36% qualified based on WLZ alone, 9% on a low MUAC and over half (55%) had both.

5.5 Mortality and morbidity

No participant died during the six month follow up period, three children relapsed (recovered then regressed to SAM) for an incidence rate of 20 per 1000 person-months. Three children never recovered from SAM. Thus, six children (14%) either had ongoing or relapse SAM. Two of the children were readmitted for complicated SAM and the other

four were managed as outpatients. One of the three relapsed children was less than six months old at enrolment and of the three ongoing SAM, two had cerebral palsy.

During the six months, over half (23/43 [53%]) of the participants were ill; (10/43, [23%]) had a serious acute illness (such as pneumonia, diarrhoea and tuberculosis). Four children were hospitalised, all four for pneumonia (two had concurrent TB). Other common morbidities by three months included a cold or fever (13%) and diarrhoea (11%). One child was newly diagnosed with HIV and five were exposed to TB during the follow up period.

Of the three children with cerebral palsy, two had ongoing SAM until three months. One child was discharged as NAM and remained NAM at one month follow up with no anthropometry taken at three months (partial follow up). All three were still receiving nutritional supplements at six months. None of the three experienced any illness at one month. Of the two children who had ongoing SAM, one had experienced a diarrhoeal episode by three months and the other had a cold or fever. The former was also treated for pneumonia by six months.

Of the five children living with HIV, three were discharged with SAM. Two recovered to MAM by one month with no anthropometry taken at three months. One had ongoing SAM until three months. One child was discharged as MAM and recovered to NAM by one month, and one was discharged NAM which continued until one month. None of the five children experienced any serious acute illness during the follow up period. One child who acquired HIV in the six months was discharged as a MAM and remained MAM until three months. She had not experienced any serious acute illness until six month follow up

where she was noted to have been hospitalised for SAM and TB treatment, and was initiated on HIV treatment.

5.6 Anthropometric recovery

Fig 1 depicts participants' anthropometric status at discharge and at one and three months follow-up. In 14 children with normal discharge anthropometry (NAM) there was one regression to MAM at one month, but this corrected by three months.

In the MAM at discharge group, 8/13 (62%) recovered to NAM by one month, with five regressing to MAM and two to SAM thereafter.

Of the six children discharged with SAM, three still had SAM at three months, two had MAM and one recovered. In summary, by three months, just over half the participants (54%) had NAM, 29% had MAM and 17% had ongoing SAM.

Fig 1. Anthropometric status at discharge related to one and three month follow-up status

Fig 2 shows the WLZ of 24 individual children (with available data) at admission, discharge, and one and three months following discharge. In all participants, WLZ increased from admission to discharge. There was a continued increase in WLZ for most participants for the first month. However, almost half of the participants (46%) had a decrease in their WLZ between one and three months.

Fig 2. Weight for length/height z-scores of individual children at admission, discharge, and one and three months following discharge (n=24)

Weight gain following discharge was calculated at one and three months. At one month, the median (IQR) weight gain since discharge was 2.9 (0.9, 4.3) g/kg/day, with a reduction to 1.6 (0.2, 2.6) g/kg/day between one to three months. The average gain was 1.4 (0.4, 2.0) g/kg/day over the three month period.

Table 2 shows the anthropometric status of children at the different follow up periods. Over three months, the WAZ, HAZ and MUAC improved. Stunting was present in 72% of the participants at discharge with 42% severely stunted; 63% of the children were still stunted at three months. The mean WLZ and wasting rate improved at one month but deteriorated by three months. At discharge only 42% of participants had a normal MUAC (>12.5cm) which improved to 77% and 79% at 1 and 3 months, respectively.

Table 2: Anthropometry at discharge, one month and three month follow up

	Admission n=varied*		Discharge n=43		One month n=31		Three months n=24	
	Mean (SD)	**Proportion** n (%)	**Mean (SD)**	**Proportion** n (%)	**Mean (SD)**	**Proportion** n (%)	**Mean (SD)**	**Proportion** n (%)
Weight for age z-score (WAZ)	-3.97 (1.11)		-2.95 (1.30)		-2.46 (1.38)		-2.38 (1.98)	
Moderately underweight		2 (7)		16 (37)		12 (39)		6 (25)
Severely underweight		25 (89)		18 (42)		9 (29)		8 (33)
Height/ length for age z-score (HAZ)	-2.53 (1.53)		-2.68 (1.53)		-2.66 (1.54)		-2.28 (1.55)	
Moderately stunted		11 (26)		13 (30)		10 (32)		6(25)
Severely stunted		17 (40)		18 (42)		13 (42)		9 (38)
Weight for length z-score (WLZ)	-3.44 (0.98)		-1.99 (1.20)		-1.33 (1.47)		-1.63 (1.42)	
Moderately wasted		5 (18)		13 (30)		6 (19)		6 (25)
Severely wasted		22 (79)		8 (19)		4 (13)		4 (17)
Mid-upper arm circumference (MUAC) [Median (IQR)]	11.5 (11.0-12.9)		12.3 (11.8-12.8)		13.0 (12.5-14.5)		13.6 (12.6-14.2)	
Moderately wasted		6 (14)		16 (37)		5 (16)		5 (21)
Severely wasted		22 (51)		9 (21)		2 (7)		0 (0)

*varied sample size. Admission WAZ & WLZ n=28 due to fifteen participants with oedema. HAZ and MUAC n=43.

5.7 Persistent malnutrition

Participants who fully recovered anthropometrically at three months (n=13, 54%) were compared to those who were still moderately or severely malnourished (n=11, 46%). Persistent malnutrition (MAM or SAM) was significantly more likely to be present in children admitted as severely wasted (9/12 (75%) vs. 2/12 (17%), RR 3.3, (95% CI 1.2-9.2), p=0.01) and less likely if admitted with pedal oedema (0/8 (0%) vs. 11/16 (69%), RR 0.31 (95%CI 0.15-0.65), p=0.002). Participants discharged with ongoing SAM were

trending to be more likely to remain malnourished (5/6 (83%) vs. 6/18 (33%), RR 4.0, (95%CI 0.65-24.7), p=0.06) while those discharged as NAM were significantly less likely to be malnourished at three months (0/6 (0%) vs. 11/18 (61%), RR 0.36 (95%CI 0.22-0.69), p=0.02).

Children who did not meet the WHO criteria of either a MUAC of ≥12.5 or a WLZ of >-2 at discharge were more likely to be malnourished at three months (8/8 (100%) vs. 3/16 (19%), RR 5.3 (95%CI 1.9-14.8), p<0.001).

If participants were discharged with a WLZ -2.0 or higher, none (0%) (p<0.001) were malnourished at three months. Persistent malnutrition rates were 8% (p=0.006) for a WLZ <-2.1, 15% (p=0.003) for WLZ <-2.2 and 21% (p=0.011) if below a WLZ of -2.3. If the respective WLZ was combined with a discharge MUAC >12.0 cm, 0% (p<0.001) of children were malnourished at three months with a WLZ of <-2.0 or <- 2.1, 11% (p=0.013) for a WLZ <-2.2 and 22% (p=0.89) with a WLZ <-2.3.

A serious acute illness within three months was another factor associated with persistent malnutrition (4/4 (100%) vs 7/20 (35%), no RR estimate, p=0.03) as was having any illness within three months (9/10 (90%) vs 2/14 (14%), RR 8.6 (95%CI 1.3-55.7), p=0.001).

At one month, WBOT's visited seven children, all of whom were subsequently malnourished at three months, while of 17 children not visited only 4 (24%) were subsequently malnourished (p<0.001). Receiving any nutritional supplements at one or three months was not associated with a reduced or higher risk of persistent malnutrition.

No statistical significant difference was identified for any demographic or household factor. Neither the presence of any coexisting illness in the child or caregiver, nor other supportive care measures provided to the child proved to be a significant risk or benefit. Participant HIV exposure or infection was also not related to persistent malnutrition.

5.8 Nutritional supplements

Children were offered either an age-appropriate commercial infant formula, porridge, ready-to-use therapeutic food (RUTF), or a combination of these. Selection of supplements was based on the availability of supplements at the hospitals or clinics and on the clinical judgement of the treating dietitian.

All children were provided with nutritional supplements at discharge. Almost three-quarters remained supplement recipients at one month. This decreased to 58% by three months and to 38% by six months (Table 3). Provision of commercial infant formula was pervasive throughout the six month follow-up period, while less than half of the participants received RUTF at discharge and only about a third at one and three months. The study monitored the types of supplements given but not the amount.

Table 3: Provision of nutritional supplements based on discharge anthropometric status and type of supplement

Anthropometric category (at discharge)	Discharge		One month		Three months		Six months	
	no.	%	no.	%	no.	%	no.	%
No acute malnutrition	(n=14)		(n=14)		(n=14)		(n=13)	
Any	14	100	9	64	6	43	5	38
Commercial formula	13	93	9	64	4	29	4	31
Porridge	2	14	3	21	5	36	5	38
Ready-to-use therapeutic food	5	36	2	14	2	14	0	0
Moderate acute malnutrition	(n=18)		(n=18)		(n=16)		(n=17)	
Any	18	100	13	72	10	63	6	35
Commercial formula	18	100	11	61	7	44	6	35
Porridge	5	28	6	33	7	44	3	18
Ready-to-use therapeutic food	11	61	5	28	4	25	0	0
Severe acute malnutrition	(n=11)		(n=11)		(n=8)		(n=7)	
Any	11	100	9	82	6	75	3	43
Commercial formula	11	100	8	73	4	50	2	29
Porridge	2	18	5	45	5	63	3	43
Ready-to-use therapeutic food	4	36	3	27	1	13	1	14
Any form of malnutrition (total)	(n=43)		(n=43)		(n=38)		(n=37)	
Any	43	100	31	72	22	58	14	38
Commercial formula	41	95	28	65	15	39	12	32
Porridge	9	21	14	33	17	45	11	30
Ready-to-use therapeutic food	20	47	10	23	7	18	1	3

5.9 Access to services

Less than one-half of children ever received a WBOT home visit, and only 16% of participants were beneficiaries of the Zero Hunger programme during the six month follow-up period (Table 4).

Table 4: Access to support services at one, three and six months

	One month	Three months	Six months
Zero Hunger programme	1/37 (3%)	4/33 (12%)	5/32 (16%)
Ward based outreach team home visit (one or more)	11/42 (26%)	16/37 (43%)	13/36 (36%)

6. Discussion

This is the first report from an upper-middle-income country on the survival, recovery and relapse of children admitted and treated for SAM, six months after hospital discharge. Most gratifying was the finding that none of the participants that were successfully followed up died. Rates of ongoing SAM and relapse rates were lower than reported in other low and middle income country settings.[1,6,8,13-15] Nevertheless, almost half of the cohort remained malnourished (MAM or SAM) three months following discharge. Risk factors for adverse outcomes were similar to those identified in other low resourced settings, such as low WLZ on admission or discharge[1,6,14] and having an illness during the recovery period.[1,8]

6.1 Mortality

Studies reporting post-discharge mortality of children with SAM are limited and mainly emanate from low income settings. Mortality rates at 3-6 months of 2.7% to 8.7% have been described.[3,5,7,8] A systematic review reported mortality rates between 0.6% and 10.4%, 6-24 months after discharge from community-based therapeutic feeding centres following nutritional rehabilitation for uncomplicated SAM.[16] To our best knowledge, only one study, from Pakistan,[13] has previously reported a zero six-month mortality. Various factors could have contributed to this positive outcome including (i) the presence of a district-based SAM intervention programme, (ii) the availability of nutritional supplements post-discharge (which has been shown to be protective against relapse and improve nutritional recovery)[1,6,7] (iii) accessible follow-up services at the clinic or hospital, and (iv)

participant involvement in a study - more intensive follow-up with ready access to health care advice through mobile phones has proven benefit.[17]

6.2 Relapse

Relapse data across studies are largely not comparable because of differences in discharge criteria, diverse post-discharge treatment protocols including supplementary food provision, variable follow-up periods, inconsistent definitions of relapse, and differing reporting of relapse as a point prevalence, cumulative prevalence, and incident rate.[1,2] The study's relapse rate of six children (14%) with either ongoing or recurring SAM is on the lower end of reported incidence data. A 2019 systematic review indicated that 0-37% of children treated for SAM relapsed with most cases occurring within six months of discharge from treatment programme.[1] Factors contributing to the study sites zero mortality, as described earlier, were probably as relevant to this outcome.

6.3 Morbidity and illness

The fact that more than half the children experienced an illness during the study period is indicative of their ongoing underlying immunosuppressed status despite recovering from SAM, as well as their ongoing exposure to social and environmental risk factors that may have led to their developing SAM originally. Participants illness profile (mostly fever, cough and diarrhoea) was similar to that reported in studies from Bangladesh,[8] and Malawi,[18] albeit with lower incidence rates. The latter study compared home-based RUTF to standard care in children with SAM, and found significantly fewer deaths, lower relapse rates and lower incidence of cough, diarrhoea among RUTF recipients at the two-month

follow up.[18] The provision of nutritional supplements to our study participants may have assisted similarly.

A critically important study finding is that children who experienced an illness by three months were significantly more likely to remain malnourished. This underscores the importance of offering specific disease prevention and health promotion advice to the caregivers of these children on discharge and at follow-up, even in better-resourced settings.

6.4 Anthropometric recovery

Despite the laudable mortality and relapse rates, the anthropometric recovery of children with SAM was disappointing, with just over half the participants achieving a normal WLZ by three months. The critical regression period for growth was from one to three months, with almost half the participants demonstrating a reversal in mean WLZ (a -0.3 z-score reduction), and daily weight gain halving. During in-patient treatment for SAM a weight gain less than 5 g/kg/day is considered suboptimal.[19] No comparable standards exist for the post-discharge period, but the gains achieved by our cohort are clearly suboptimal.

The unsatisfactory anthropometric outcome may mainly be attributed to a combination of inappropriate discharge criteria, concomitant new infections, inadequate or inappropriate nutritional supplementation and dysfunctional access to support services.

6.5 Discharge criteria inappropriate

The current WHO recommendations are to transfer children from inpatient to outpatient care when their medical complications are resolved, have a good appetite and are

clinically well and alert. Furthermore children can be discharged from SAM treatment altogether when they are anthropometrically recovered (WLZ ≥-2 or MUAC ≥12.5cm and no oedema for at least two weeks) and to admit and discharge children into SAM treatment on the same anthropometric criteria for example if MUAC was used to identify SAM then it should be used to confirm nutritional recovery.[2,19] Adhering to the WHO guidelines has been shown to be protective against relapse.[1] However, by following the WHO guidelines for transferring children out of inpatient care as the two study hospitals did based on clinical rather than anthropometrical recovery, our findings suggest negative consequences. This resulted in over two-thirds of children still being malnourished at hospital discharge, with a higher risk of remaining malnourished at three months. This situation is pervasive in resource poor settings. In a recent study conducted in Zambia and Zimbabwe almost half of children had ongoing SAM at the time of discharge.[6]

Although longer hospital stays to allow for achievement of improved anthropometric recovery may be an obvious recommendation, this approach may itself have negative consequences such as increased risk of nosocomial infection and longer hospital stay costs. A more cost-effective approach would be to provide better nutritional and social support following hospital discharge. Our findings suggest that, in a setting where nutritional support for moderate malnutrition is provided after hospitalisation, children can be discharged from in-patient care once a WLZ of ≥-2.1, or a WLZ of ≥-2.2 and MUAC of ≥12.0 cm, is achieved; if a persistent malnutrition (MAM or SAM) risk of 8% or 11%, respectively, is considered acceptable at three months.

Interestingly, all eight children who presented with oedema at admission were anthropometrically normal by three months, confirming the different clinical and

anthropometric course of this form of SAM. This finding has been described in other settings.[4,6]

6.6 Nutritional supplements

A distinguishing feature of the study, compared to previous studies, was the high rates of nutrition supplement provision for participants post-discharge with more than one-half of children still receiving it at three months. However, the poor improvements in anthropometric status raises concern about the effectiveness of the intervention. There was modest provision of RUTF compared to commercial formula. Greater use of this product rather than commercial formula could support better growth.[20]

A recent systematic review found the use of nutritional supplements with high-quality protein and adequate micronutrient content for three months improved anthropometric growth for children with MAM, with food products having more benefit than nutritional counselling and/or micronutrient supplementation.[21] This was particularly the case if supplementary food was of suitable quality and provided for an adequate period. Further research is warranted on what constitutes effective post-discharge supplementation. Measured outcomes should be functional extending beyond anthropometric proxies.

6.7 Support services

A disappointing finding was the limited reach of the WBOT and Zero Hunger interventions. Their failure to engage in the district's SAM intervention project was recognised by the project team who lamented their inability to extract accountability.[22] All seven children who received a WBOT home visit by one month were still malnourished at three months

suggesting that the WBOT's activities had minimal influence on growth outcomes. Nevertheless, most caregivers (72%) were receiving a child support grant worth about $US30 (ZAR450) - a useful child survival and thriving intervention.[1] A single child who relapsed twice within six months was a non-South African national excluding him/her from benefiting from either a child support grant or the Zero Hunger intervention.

6.8 Strengths and limitations

The study's greatest strength is its prospective nature and duration of follow-up. It is the first such among malnourished children in South Africa and also in any upper-middle-income country setting. Another strength is the satisfactory follow-up rate – 86% is particularly good for a setting such as where the study was done, reducing bias associated with 'lost to follow up' which may undercount deaths. The study's location in a district with an active SAM intervention project also allowed for assessment of components of that project. Few studies have explored anthropometric recovery in children with SAM supplemented for a long period.

The study has several limitations. The greatest is the small sample size. This was the consequence of lower than predicted SAM hospitalisations during the dedicated study period. The zero mortality and low relapse rate prevented planned analysis of contributory risk factors for these outcomes. The primary researcher being the dietitian responsible for the care of some participants could have biased outcomes but this was considered unlikely, as no extraordinary measures were instituted by her. We did not collect data on several potential risk factors for persistent malnutrition such as home food security, health seeking behaviour and adherence to nutritional supplementation. Another potential limitation relates to seasonal variations in the risk of malnutrition. Although enrolment was

spread over 10 months and included both winter (hunger) and summer (harvest) seasons, this could have influenced study outcomes. Lastly, the study did not collect data on the children's feeding practices after discharge including breastfeeding and dietary diversity. These practices could have had an effect on the anthropometric recovery of the children.

The study findings cannot be easily generalised to other South African settings since few districts are implementing SAM projects similar to that undertaken in the study district. Nevertheless, project interventions such as nutritional supplements, WBOTs and social support programmes are ubiquitous in the country, although variably implemented.

7. Conclusions and implications for policy and practice

The study has provided evidence that discharging children only once nutritionally recovered and continuing nutritional support through supplementation may improve survival and reduce relapse rates. This accentuates the case for a more holistic perspective on SAM management. Post-discharge management is not just about food - this is necessary but not sufficient on its own. SAM programmes must work more closely with other child health services to form a more clearly defined and seamless functional continuum of integrated care. This is a tough ask and demands special effort to develop a collaborative work ethic, clear communication lines and common programme goals. Although post-discharge care is vital, identifying SAM children in the community and providing sufficient support early must remain an important part of the care process.

Despite its limitations, the study highlights the potential of several interventions to improve post-discharge outcomes for children with SAM. Many children were still severely malnourished at discharge and this was associated with a poorer outcome. The response should not simply be to prolong the hospital stay to meet WHO discharge criteria. Instead, children could be risk stratified so that, for example, those with a lower admission WLZ or weaker home and community support structures are discharged later. We have also proposed a possible modification to the WHO discharge criteria for adoption in the district.

However, an even better response would be to strengthen home based care. Addressing comorbidities and integrating primary health care services allowing for timely referrals for treating morbidities could aid in lower relapse rates. Allowing children to be discharged to their closest facility may improve follow up rates and access to health care, thereby reducing relapse.[1,8,14]

Identification of preventable risk factors for SAM relapse requires further research involving considerably larger sample sizes than the current study. Better understanding of the contextual factors that constrain the management of SAM at service points and at home are needed. Future research will also need to unravel exactly which post-discharge interventions are most effective and cost-effective for these vulnerable children, including exploring interventions addressing underlying risks for malnutrition such as household food security, caregiver well-being, and sub-optimal home environments.

A. Acknowledgments

Our sincere thanks to all dietitians, doctors and nurses at Mamelodi and Bronkhorstspruit Hospitals and dietitians at Tshwane sub-districts 5 and 7 clinics for their invaluable

support and contribution during patient enrolment and data collection. Dr Marshè Maharaj is recognised for offering supervision and guidance given during data collection.

B. Author contributions

AP and HS conceived and designed the study, analysed the data and wrote the manuscript: AP collected the data. Both authors edited the manuscript and approved the final version.

The study was undertaken in partial fulfilment of an MSc in Child Health at the University of Witwatersrand, Johannesburg by AP.

The study was self-funded.

8. References

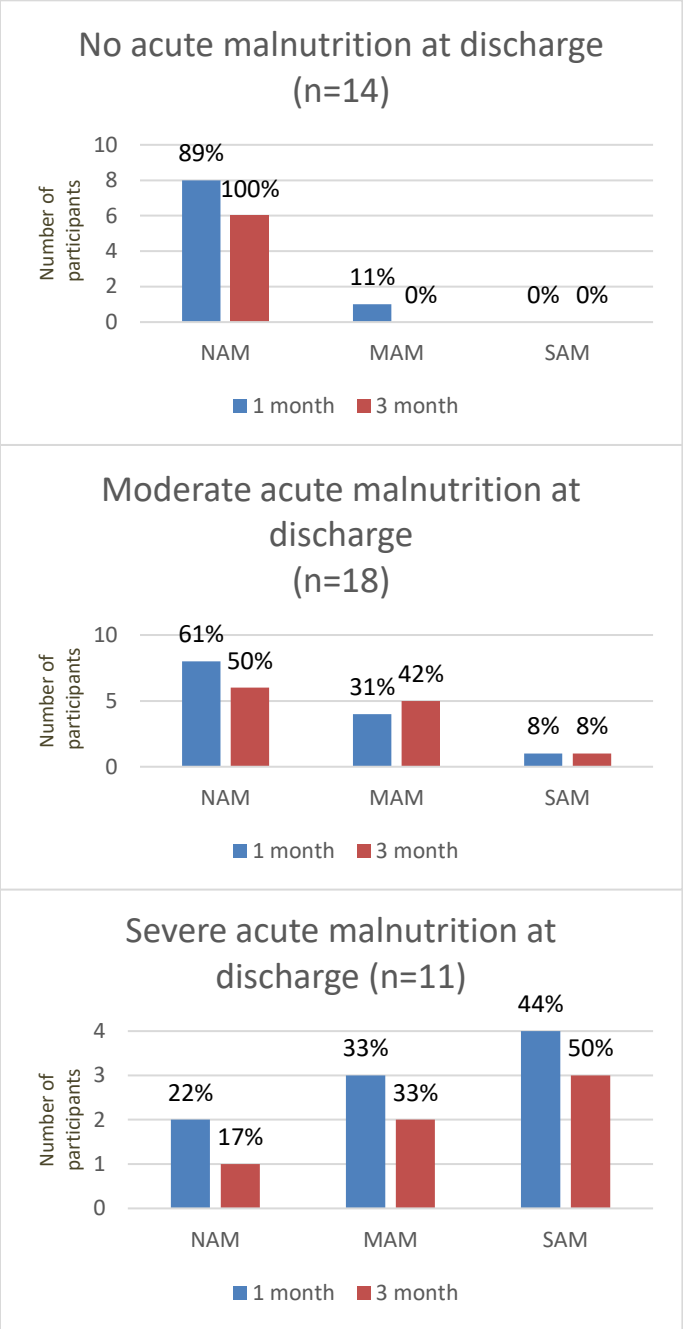
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List of figures



Abbreviations: NAM= No acute malnutrition, MAM= Moderate acute malnutrition, SAM= Severe Acute Malnutrition

Figure 1: Anthropometric status at discharge related to one and three month follow-up status.

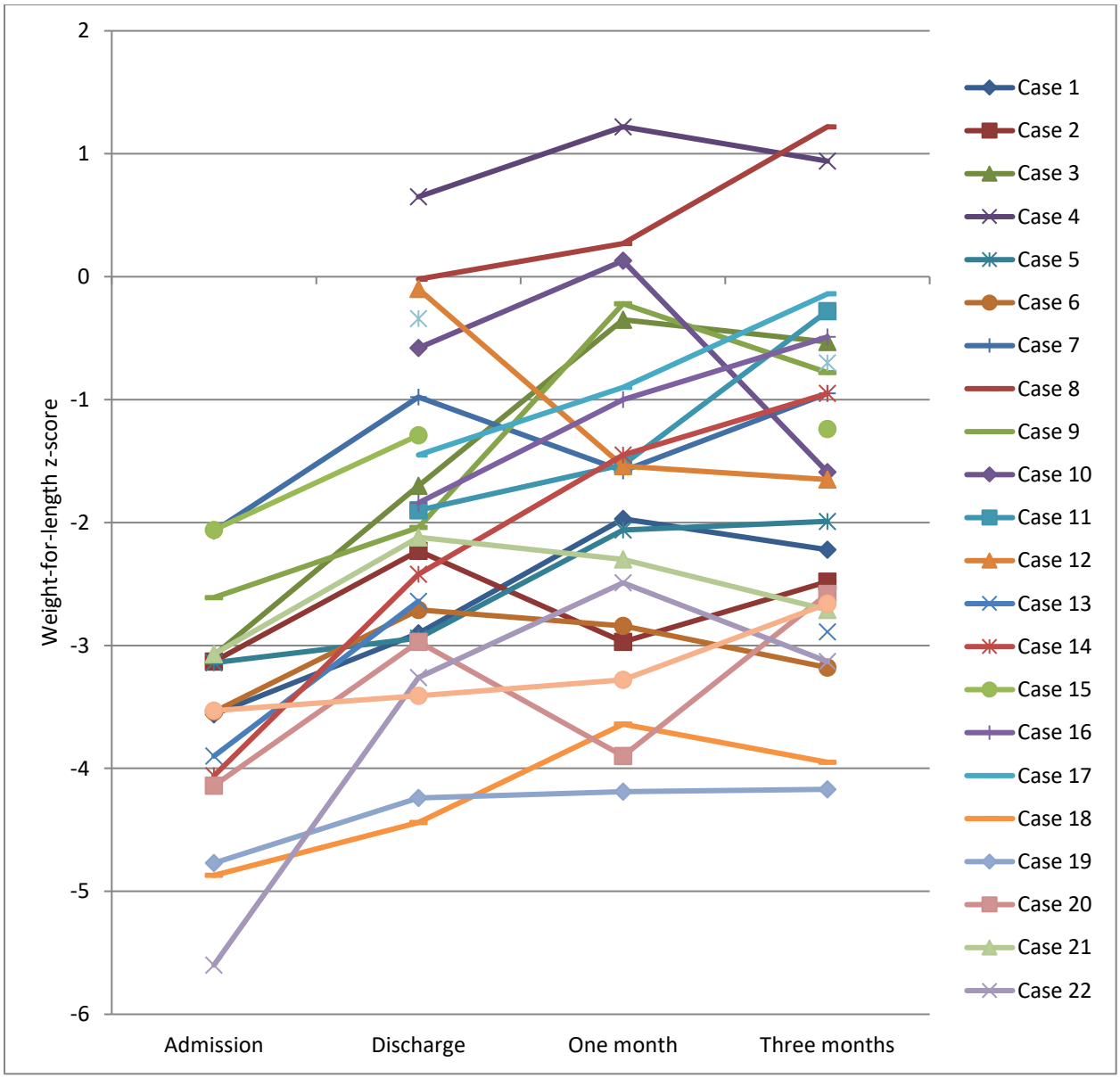


Figure 2: Weight for length/height z-scores of individual children at admission, discharge, and one and three months following discharge (n=24).

Appendices

A. Data Collection Questionnaires

Study participants Enrolment Form Baseline, Admission and Discharge Information			
Study Number:			
Enrolment date:	DD MM YY	Enrolment personnel:	
Case definition/ SAM diagnosis: 1 = W//L 2 = MUAC 3 = oedema	Answer:	Admission hospital 1= Mamelodi 2 = Bronkhorstspruit	Answer:
	Information needed	Options	Answer
Demographic Information			
1	Age	____ months	
2	Sex	1 = Male 2= Female	
3	Race	1= African 2= Caucasian 3= Indian 4= Coloured 9= Other: -	If 9: _____
4	Caregiver nationality	1= South African 2= Mozambican 3= Zimbabwean 9= Other: -	If 9: _____
Caregiver and family information			
5	Primary caregiver?	1= Mother 2= Father 3= Grandmother 4= Grandfather 5= Aunt 9= Other: -	If 9: _____
6	Secondary caregiver?	1= Mother 2= Father 3= Grandmother 4= Grandfather 5= Aunt 6= Not applicable 9= Other: -	If 9: _____
7	Does the child live in a single headed household?	1= Yes 2=No	
8	How many people does the child live with?	1= Adults: 2= Children:	1: _____ 2: _____
9	Monthly income of the caregiver(s)	Primary: R _____ Grants: _____ Secondary: R _____	
10	Home language	1= Afrikaans 2= English 3= Ndebele 4= Sepedi 5= Sotho 6= Swazi	

		7= Tsonga 8= Tswana 9= Venda 10= Xhosa 11= Zulu 99= Other: -	If 99: _____
11	Social aid	1= Child Support 2= Zero Hunger 3= Foster Care 4= Care Dependency 9= Other: -	If 9: _____
Housing Information			
12	Type of housing	1= Brick 2= Corrugated iron 9= Other: -	If 9: _____
13	Water source	1= Inside 2= Outside 3= Communal	
14	Electricity source	1= Electric 2= Gas 3= None	
15	Cooking facilities	1= Electric stove 2= Gas stove 3= Paraffin stove 4= Outside 9= Other:-	If 9: _____
16	Does the house have a fridge	1= Yes 2= No	
Participants medical history			
17	How many times has the child been admitted into hospital before?	Number of admissions: _____	
18	Admitted for?	1= Pneumonia 2= Diarrhoea 3= Tuberculosis 4= Burns 5= Poisoning 6= Injuries 7= Malaria 8= Measles 9= Not applicable 99= Other:	If 99: _____
19	Does the child have a disability?	1= Yes 2= No	
20	If yes, what disability do they have?	1= Cerebral palsy 2= Cleft palate 3= Trisomy 21 4= Not applicable 9= Other: -	If 9: _____
21	Has the child ever been treated for SAM before this?	1= Yes 2= No	
22	If yes, when were they treated?	Date 1: __/ __/_____ (dd/mm/yyyy) Date 2: __/ __/_____ (dd/mm/yyyy)	
23	Child HIV status	1= Positive 2= Negative 3= Exposed	
24	If positive, on treatment?	1= Yes 2= No 3= Not applicable	
25	Mothers HIV status?	1= Positive 2= Negative	
26	If positive, on treatment?	1= Yes 2= No 3= Not applicable	

27	Gestational age at birth?	_____ (in weeks)	
28	Mothers age at birth	_____ in years	
		Anthropometry	
29	Child's birth anthropometry?	Weight: _____(kg) Length: _____(cm) Head circumference: _____ (cm)	
30	Anthropometry on current admission?	Weight: _____kg Length: _____cm MUAC: _____cm W//A: _____ z-score L//A: _____z-score W//L: _____ z-score	
31	Anthropometry at current discharge?	Weight: _____kg Length: _____cm MUAC: _____cm W//A: _____ z-score L//A: _____z-score W//L: _____ z-score	
		Current admission and discharge information	
32	Discharge diagnosis?	1.Pneumonia 2.Diarrhoea 3.Tuberculosis 4.Burns 5.Poisoning 6.Injuries 7.Malaria 8.Measles 9.SAM 99.Other:	If 99: _____
33	Was there odema present at discharge?	1= Yes 2= No	
34	Nutritional supplements given on discharge	1= Pediasure 2= Nutrikids 3= Peptamen Jr 4= Children's porridge 5= Imunut 6= Starter formula 7= Follow on formula 8= None 9= Other	If 9: _____
35	Application to SASSA Zero Hunger project done?	1= Yes 2= No	
36	Referral to WBOT?	1= Yes 2= No	
37	Referral to specialists?	1= OT 2= Physio 3= Speech therapists 4= Social worker 5= No referrals 9= Other: -	If 9: _____
38	Next follow up date?	DD MM YY	

Study participants Follow Up Form One Month Follow Up			
Study Number:			
Date:	DDMMYY	Research personnel:	
	Information needed	Options	Answer

1	Survival status	1= Dead 2= Alive 3= Unknown	
2	Cause of death	1= Pneumonia 2= Diarrhoea 3= Tuberculosis 4= Burns 5= Poisoning 6= Injuries 7= Malaria 8= Measles 9= Meningitis 10= SAM 99= Other:	If 99: _____
3	Date of death	__/ __/_____ (dd/mm/yyyy)	
4	Has the child suffered from any condition since discharge?	1= Pneumonia 2= Diarrhoea 3= Tuberculosis 4= Malaria 5= Measles 6= Meningitis 7= SAM 9= Other:	If 9: _____
5	Since discharge has the child received in-patient treatment for SAM	1= Yes 2= No	
6	Since discharge has the child received out-patient treatment for SAM?	1= Yes 2= No	
7	Anthropometry	Weight: ____kg Length: ____cm MUAC:____cm W//A: _____ z-score L//A: _____z-score W//L: _____ z-score	
8	Social aid since discharge?	1= Child Support 2= Zero Hunger 3= Foster Care 4= Care Dependency 5= No aid given 9= Other: -	If 9: _____
9	Has WBOT conducted a home visit since discharge?	1= Yes 2= No	
10	What supplements are given at this follow up?	1= Pediasure 2= Nutrikids 3= Peptamen Jr 4= Children's porridge 5= Imunut 6= Starter formula 7= Follow on formula 8= None 9= Other: -	
11	Is the child HIV positive?	1= Yes 2= No	
12	If yes, is the child on HIV treatment?	1= Yes 2= No 3= Not applicable	
13	Has the child been exposed to TB?	1= Yes 2= No	
14	Has the caregivers contact details changed?	1= Yes 2= No	
15	Has the child moved to a new address?	1= Yes 2= No	

Study participants Follow Up Form

Three Month Follow Up			
Study Number:			
Date:	DD MM YY	Research personnel:	
	Information needed	Options	Answer
1	Survival status	1= Dead 2= Alive 3= Unknown	
2	Cause of death	1= Pneumonia 2= Diarrhoea 3= Tuberculosis 4= Burns 5= Poisoning 6= Injuries 7= Malaria 8= Measles 9= Meningitis 10= SAM 99= Other:	If 99: _____
3	Date of death	__/ __/_____ (dd/mm/yyyy)	
4	Has the child suffered from any condition since discharge?	1= Pneumonia 2= diarrhoea 3= Tuberculosis 4= Malaria 5= Measles 6= Meningitis 7= SAM 9= Other:	If 9: _____
5	Since discharge has the child received in-patient treatment for SAM	1= Yes 2= No	
6	Since discharge has the child received out-patient treatment for SAM?	1= Yes 2= No	
7	Anthropometry	Weight: ____kg Length: ____cm MUAC:____cm W//A: _____ z-score L//A: _____z-score W//L: _____ z-score	
8	Social aid since discharge?	1= Child Support 2= Zero Hunger 3= Foster Care 4= Care Dependency 5= No aid given 9= Other: -	If 9: _____
9	Has WBOT conducted a home visit since discharge?	1= Yes 2= No	
10	What supplements are given at this follow up?	1= Pediasure 2= Nutrikids 3= Peptamen Jr 4= Children's porridge 5= Imunut 6= Starter formula 7= Follow on formula 8= None 9= Other: -	
11	Is the child HIV positive?	1= Yes 2= No	
12	If yes, is the child on HIV treatment?	1= Yes 2= No 3= Not applicable	
13	Has the child been exposed to TB?	1= Yes 2= No	

14	Has the caregivers contact details changed?	1= Yes 2= No	
15	Has the child moved to a new address?	1= Yes 2= No	

Study participants Follow Up Form 6 months phone call			
Study Number:			
Date:	DD MM YY	Research personnel:	
	Information needed	Options	Answer
1	Survival status	1= Dead 2= Alive 3= Unknown	
2	Cause of death	1= Pneumonia 2= Diarrhoea 3= Tuberculosis 4= Burns 5= Poisoning 6= Injuries 7= Malaria 8= Measles 9= Meningitis 10= SAM 99= Other:	If 99: _____
3	Date of death	__/ __/_____ (dd/mm/yyyy)	
4	Has the child suffered from any condition since discharge?	1= Pneumonia 2= diarrhoea 3= Tuberculosis 4= Malaria 5= Measles 6= Meningitis 7= SAM 9= Other:	
5	Since discharge has the child received in-patient treatment for SAM	1= Yes 2= No	
6	Since discharge has the child received out-patient treatment for SAM?	1= Yes 2= No	
7	Social aid since discharge?	1= Child Support 2= Zero Hunger 3= Foster Care 4= Care Dependency 5= No aid given 9= Other: -	If 9: _____
8	Has WBOT conducted a home visit since discharge?	1= Yes 2= No	
9	What supplements is the child still receiving?	1= Pediasure 2= Nutrikids 3= Peptamen Jr 4= Children's porridge 5= Imunut 6= Starter formula 7= Follow on formula 8= None 9= Other:-	

10	Is the child HIV positive?	1= Yes 2= No	
11	If yes, is the child on treatment?	1= Yes 2= No 3= Not applicable	
12	Has the child been exposed to anyone with TB?	1= Yes 2= No	

B. Study protocol

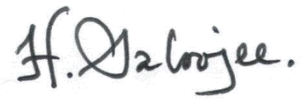
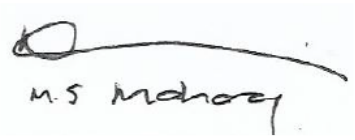
CANDIDATE'S SURNAME: Peczak		FIRST NAME/S: Angelika Barbara	STUDENT NUMBER: 607630
CURRENT QUALIFICATIONS: B.Dietetics			
TEL:	CELL:0720783160	E-MAIL: angepeczak@gmail.com	FAX:
DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: MSc Child Health			
PART-TIME OR FULL-TIME: Part time			
FIRST REGISTERED FOR THIS DEGREE:	TERM : 1 (2018)	YEAR: 1	
DEPARTMENT: Paediatrics and Child Health			
TITLE OF PROPOSED RESEARCH: Mortality, relapse, morbidity and growth of severe acutely malnourished children in the six months following discharge			
CANDIDATE'S SIGNATURE:		DATE: 11 July 2018	
SUPERVISOR 1 (NAME & SURNAME): Haroon Saloojee		80% Supervision	
SUPERVISOR'S QUALIFICATIONS: MBBCh, MSC, FCPaed (SA)			
SUPERVISOR'S DEPARTMENT: Division of Community Paediatrics			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: 011 717 2282; Haroon.Saloojee@wits.ac.za			
SUPERVISOR 2 (NAME & SURNAME): Dr Marshee Maharaj		20% Supervision	
SUPERVISOR'S QUALIFICATIONS: MBchB(UP), MMED(UP), FCPaed (SA)			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: 0834813777 marshemaharaj@gmail.com			

<table>
<tr><td colspan="3"><u>SYNOPSIS OF RESEARCH</u>:
Severe acute malnutrition (SAM) is a serious and common condition in children under 5 years of age in South Africa. There is limited local research done on outcomes after discharge from in-patient treatment. International data indicated high ongoing mortality and relapse rates – Local information may assist in identifying gaps in the care of these children post-discharge and improving overall survival of children with SAM. This study aims to determine the incidence and causes of death, relapse and morbidity experienced and the role of demographic, nutritional, social support and health related factors on outcomes of children with SAM in the six months following discharge. Children discharged from Bronkhorstspruit and Mamelodi Hospital will be followed up at one, three and six months following discharge. Medical, anthropometric and social information will be collected.</td></tr>
<tr><td colspan="2">ETHICS PENDING: Y

ETHICS APPROVED: N

(circle appropriate symbol)*

*Please note human ethics clearance/waiver certificate or animal ethics certificate must be in the student’s name</td><td>IF Y SUPPLY ETHICS

CLEARANCE No:</td></tr>
<tr><td colspan="3">As supervisor, I confirm that I have read the protocol which has been submitted for assessment.</td></tr>
<tr><td>SIGNATURE OF SUPERVISOR/S:</td><td></td><td></td></tr>
<tr><td>SIGNATURE PG OFFICE STAFF

……..</td><td>REGISTERED

YES….. NO…..</td><td>STAMP</td></tr>
</table>

1. Title

Mortality, relapse, morbidity and growth of severe acutely malnourished children in the six months following discharge

2. Introduction and Justification

Severe Acute Malnutrition (SAM) is a global issue and has become a priority for many countries to alleviate.[1] The lack of adequate nutrients and contributing morbidities can cause wasting, seen in children with SAM. Wasting causes both lower muscle mass and depleted fat stores which weakens the immune system allowing for infections to threaten lives, and causes developmental delay that impends on cognitive and physical growth.[1,2] The 2015 South African vital registration (VR) statistics show that although child mortality has decreased in the past decade, 32 000 children died before reaching the age of five, of whom 1400 (4.6%) were, according to the death certificates, due primarily to malnutrition.[3]

In 2017, in Tshwane sub-districts 5, 6 and 7, 68 children were admitted for SAM in the two hospitals in the area, with 11 in-patient deaths, a high 16.2% in-hospital mortality. Limited information is known on the outcomes of the children that were discharged subsequently.

Research is often conducted about the effectiveness of in-patient treatment of children with SAM that shows that the quality of care and adherence to the WHO guidelines can have positive outcomes.[4-7] There is however limited literature about outcomes and the

continuity of care following discharge. Studies conducted in Malawi, Bangladesh, Pakistan and India aimed to determine the mortality, relapse, morbidity and growth of children who have been discharged from either in-patient or community based treatment of SAM have indicated the importance of the post-discharge follow up period.[8-12]

A study in Malawi that followed children for 8 months after admission showed that 42% of the children died, with just over half of the deaths (56%) occurring during the inpatient treatment. The study showed that key risk factors for mortality included younger age and greater severity of malnourishment. Children with non-oedematous SAM and those with an underlying HIV infection and with a disability such as cerebral palsy were at higher risk of dying.[8]

In Bangladesh children treated for SAM at a day care clinic (community care) and followed up for six months found that in the three month post-discharge morbidities such as diarrhoea, fever and cough were common, with a decrease in diarrhoea in the subsequent three months. Relapse was seen in 17.8% of these children and 2.8% died within the 6 month period. While wasting improved at the 6 months follow up, severe stunting remained.[9]

Another study in Bangladesh of SAM children with pneumonia, found that 3 months later, 8.7% of the children had died with most deaths occurring within one month of discharge. Post-discharge death was experienced in those that were younger, were more malnourished at admission, were discharged early and had previously been admitted for pneumonia. Symptoms of respiratory and gastrointestinal infection were noted prior to death in some of these children.[10]

A 2018 Pakistani study of children who had been discharged from an outpatient treatment programme found that 7.4% of these children relapsed within 6 months of discharge, with a mean relapse time of 73 days.[11] This result is similar to a study of children discharged from a community based treatment program of SAM in India, where relapse rates of 9.1% and 2.9% were noted at 3 and 6 months respectively.[12] The anthropometry of the Pakistani children improved over six month follow-up period with more than 50% of children that began the study with moderate acute malnutrition (MAM) resolving.[11]

HIV infection is not discussed in detail in the studies done in Asia, however it is a variable that does need to be investigated in Africa, an HIV-prevalent continent. A recent meta-analysis done in Ethiopia found that HIV and Tuberculosis comorbidity increase the case fatality ratio of children with SAM.[13] Interestingly a further study in Malawi found that HIV-infected children with SAM did have higher mortality rates, however their nutritional recovery in a four month follow up were similar to the HIV-uninfected children.[14]

The studies discussed have shown the importance of post-discharge follow up in preventing further mortality and relapse in SAM children, as well as determining the morbidities they experience and the growth they achieve. The research proposed would be the first of its kind in the South African context. It aims to determine the health and growth outcomes the children discharged from in-patient care of SAM. If mortality and relapse rates are found to be as high reported as elsewhere, it can aid in enforcing the importance of follow up by both health care professionals and community based health care workers. This research will help towards achieving the vision of the global strategy of helping children survive SAM as well as the conditions associated with it, help them thrive by detection of problems that could cause poor outcomes and ensure that these

children grow and develop in a way that they can help transform their lives and reach their full potential.[15]

3. Study objectives

The objectives of this study are:

Primary objective

- To determine the mortality rate of children with severe acute malnutrition (SAM) during the six months following discharge from hospital.

Secondary objectives

- To evaluate growth of children with SAM one and three months following discharge from hospital.
- To describe in children with SAM at the one, three and six months following discharge from hospital :
    - Their relapse rate to SAM
    - The frequency and type(s) of morbidity experienced
    - Causes of death in children who demised
    - The nutritional, social and other interventions accessed
- To identify demographic, socio-economic, anthropometric and health related factors associated with mortality and/or relapse, six months following discharge from hospital

4. Methods

4.1 Design

The study will consist of a prospective cohort follow up for 6 months after hospital discharge, or until death or disappearance of study participants.

4.2 Site of the study

The study will be conducted in Tshwane sub-districts 5, 6 and 7 which comprise the eastern side of Tshwane including Mamelodi, Cullinan, and Bronkhorstspruit and surrounding areas. The sub-districts have one regional hospital, Mamelodi Hospital and one district hospital, Bronkhorstspruit Hospital in the area that treat all of the SAM in-patients in the catchment area. Referrals of these children are made from various clinics in the surround areas. Data will be collected from the children admitted and discharged from these two hospitals.

4.3 Study population and study sample

The study population are children admitted and treated for SAM at hospitals in the whole of Tshwane. The study sample will be children with SAM discharged from sub-district 5, 6 and 7 specifically from Mamelodi and Bronkhorstspruit hospitals.

4.4 Sampling

There will be no sampling done as all eligible children during the study period will be enrolled consecutively to achieve maximum recruitment of participants.

4.5 Study period

The study will be conducted from October 2018 until December 2019.

4.6 Definition of SAM

SAM is defined by their (i) weight for height being less than a -3 z-score on growth charts or (ii) mid-upper arm circumference (MUAC) of less than 11.5cm or (iii) the presence of bilateral pitting oedema.

4.7 Study inclusion and exclusion criteria

The study will include children between the ages of 3 months and 59 months who have undergone and survived in-patient treatment for SAM. This may be their first or a recurrence of SAM. Children with SAM secondary to conditions such as cerebral palsy, cleft palate and trisomy 21, or children being cared for in institutions such as children's homes, will be eligible for the study.

The study will exclude children admitted and treated for SAM who are below the age of 3 months and children who have care givers that plan on relocating from Tshwane sub-districts 5, 6 and 7 in the three months following discharge. Children younger than 3 months have been excluded as the Integrated Management of Childhood Illnesses (IMCI) do not have clear SAM diagnostic guidelines for children below the age of 6 months. It is also reported that the in-patient mortality is high of children less than 3 months. However the study does require a maximum amount of participants and although the diagnosis and survival is problematic the ones above 3 months that do survive discharge should be

included and their participation in the study could be beneficial to determine the outcomes of children less than 6 months old.

4.8 Sample size and selection of subjects

A point estimate of expected mortality for the sample has been set at 10%. The estimate is based on the findings of mortality rates ranging from 2.8-10.3% in similar studies based in Bangladesh and Malawi[8,9,12] Tshwane sub-districts 5, 6 and 7 do not document the deaths of previous SAM children during follow up sessions; however the amount of episodes of SAM relapse to Mamelodi or Bronkhorstspruit Hospital in 2017 period has been approximately 5 episodes of the 55 discharged patients from the facilities.

I aim to enrol 50 participants. Table 1 shows the lower and upper 95% confidence intervals based on an estimated sample size of 50 participants and varying mortality rates. Table 2 shows the 95% confidence intervals of a mortality rate estimate of 10% and varying sample sizes. The range of the lower and upper values of the 95% CI is 0.19 in a study of 50 participants with a point estimate of 10% mortality as seen in both tables which is acceptable. The larger the sample size, the narrower the 95% CI becomes as seen in Table 2.

Table 1: Lower and upper 95% Confidence interval (95% CI) values in a sample size of 50 participants (n=50) with varying point estimations of mortality.

Mortality rate	Lower confidence interval	Upper confidence interval
5%	0.01	0.17
10%	0.03	0.22
15%	0.07	0.29
20%	0.10	0.34
25%	0.14	0.40

Table 2: Lower and upper 95% Confidence interval (95% CI) values with an estimated mortality of 10%, for various sample sizes.

Sample size	Lower confidence interval	Upper confidence interval
25	0.01	0.26
50	0.03	0.22
75	0.04	0.18
100	0.05	0.18
125	0.05	0.16
150	0.05	0.16

4.9 Measuring tool or instrument

The measurement tool used to gather information about the participants includes:

(a) An enrolment form (Appendix A) where details about the children's biological, social and environmental determinants of health as well as their clinical information based on their birth, admission and discharge criteria will be collected. This will be collected from hospital records and Road to Health booklets (where necessary) as well as from information provided by the care givers.

Much of this information will be collected by a trained hospital dietitian during the in-patient care treatment and from the Nutritional Care Documents (NCR) that are used in Gauteng hospitals. An updated version of this NCR has been implemented in 2018 to standardize the tool as prescribed by regulations from the HPCSA. The document includes anthropometric, biochemical, clinical, dietary and medical history and general information about the patient. The measurement tool used in this research will be an adaptation of the anthropometrical, clinical, medical history and general information sections as these explore more details of the participants' social and living conditions as well as the anthropometry from admission to discharge.

(b) A study-follow up form (Appendix B) where details about any illness that has been treated or the occurrence of relapse or death will be noted. This will be at the one and three month follow up visits. Anthropometric measurements such as weight, length and MUAC will be redone and questions determining any placement of social aid as well as changes to family income and location will be included.

(c) A final visit form (Appendix C) to be completed at the six month follow up visit will include the survival status of the child with cause of death data (if applicable); whether children have relapsed to SAM and the types and frequencies of morbidities they have experience in the preceding three months.

4.10 Study Procedures

4.10.1 Enrolment procedure

The enrolment will involve different personnel at the two facilities. For the participants enrolled at Mamelodi Hospital the investigator will be notified of all SAM admissions by the dietetics staff. When potential participants are being prepared for discharge (within 2-3 days), a research assistant(s) (an assigned and trained dietitian) will provide the caregiver information about the study and enrol participants whose caregiver consent to participation. The investigator will be responsible for the enrolment of the participants from Bronkhorstspruit Hospital as she will be treating the children and will be aware of them from admission. She will enrol caregivers prior to discharge. Consent forms will be signed by the caregivers (Appendix D).

4.10.2 Enrolment data collection

Baseline clinical and anthropometric admission and discharge information as well as general caregiver and participant's personal, housing and medical history information (Appendix A) will be taken during enrolment and completed at discharge by the research assistant at Mamelodi Hospital and the researcher at Bronkhorstspruit Hospital. If any information is missing, the form will be completed during the first (one month) follow up session or via telephone or hospital records. The research personnel will fill out the participants and caregivers information on a participant particulars form and there will be no personal identifying information on the enrolment data collection form.

4.10.3 Follow up sessions

Physical follow up of the study participants will occur at one and three months post-discharge. The research follow up sessions will be conducted simultaneously to the routine clinical and nutritional check-ups of the participants at the facilities. The research investigator will schedule these dates, accommodated by the facility staff and will be present at all follow up sessions. Data collected (Appendix B) will include mortality, relapse or morbidity experienced, current anthropometry (weight, height and MUAC), details of social aid (SASSA child grant or placement into the Zero Hunger Programme), and whether any changes have occurred to the living conditions and contact details of the participants and caregivers. The caregivers will receive SMS reminders the day before the scheduled follow up dates. If a participant does not arrive at a follow up session the caregiver will be contacted via telephone to determine the reason and what the survival status of the child is.

4.10.4 Final follow up session

The final follow up will take place at 6 months discharge and will focus on the primary outcome of the study. These follow up sessions will be done telephonically with the caregivers. The caregivers will provide information to the research investigator or if necessary a trained research assistant/ translator about details of the participant's mortality relapse, morbidity and social aid during the 3 months after the last follow up visit (Addendum C).

4.11 Pilot Study

A pilot study of the enrolment form and the one and three month follow up forms will be conducted in September 2018 (or as soon as ethical clearance is obtained) using three SAM children that are being followed up after discharge at Bronkhorstspruit Hospital.

5. Data Analysis

All data will be entered into MS Excel 2010 (Microsoft, Seattle, USA) for data management and analysed using STATA 15.1 (Stata Corporation, College Station, Texas, USA). Mortality and relapse rates will be analysed using frequency and percentage of group data. The secondary outcome of growth in the children will be analysed with z-scores for weight for age, length for age and weight for length. Z-scores will be calculated using Emergency Nutrition Assessment (ENA) for SMART software, (version July 2015). The changes of these measures will be recorded from admission, discharge and follow up at one and three months through measures of central tendency and dispersion depending on the distribution of the data, as well as through t-tests (if normally distributed) when comparing anthropometry in those who died-/-relapsed with

those who survive or are relapse free. The type and frequencies of morbidity and causes of deaths will be analysed through proportions at group level. Analysis of the factors associated with the mortality and/or relapse of the children in the six months will be done through comparison of those who have died/ relapsed and those who have survived/ did not relapse. For continuous variables such as age or anthropometry, a t-test or Wilcoxon test will be performed depending on the distribution of the data. Categorical variables such as HIV status and nationality will be compared using chi-squared tests. A p-value of less than 0.05 will be considered statistically significant.

5.1 Variables

Table 3 below indicates the variables that will be compared in the three cohorts of the children who died and or experienced relapse and those that survived.

Table 3: List of variables that will be compared for mortality and relapse

	Variable
Demographic	Age
	Sex
	Race
	Nationality
	Primary caregiver
	Secondary caregiver
	Home language
Socioeconomic	Caregiver(s) salaries
	Type of housing
	Water source
	Electricity source
	Cooking facilities
	Social grants/ aid accessed
Anthropometric	Birth, admission, discharge, one and three month anthropometry (Weight, length and MUAC)
	Oedema present
Health related factors	Frequency of hospital admissions before current SAM episode

	Types of morbidity the child experience
	Disability
	Prematurity
	Comorbidity with SAM episode
	HIV status
	Referral to other health care workers
	Prescribed medications
	Prescribed nutritional supplements

6. Ethical clearance

The research proposal will be submitted to the University of Witwatersrand Human Research Ethics Committee (HREC) as well as the Gauteng Department of Health. Permission will be sought from the chief executive officers of Mamelodi and Bronkhorstspruit Hospital to enrol participants. Consent will be sought from the children's caregivers for study participation. Any identifying information about the participants such as names, hospital file numbers or caregiver information will be excluded from the data collection form and will be collected in a data sheet only seen by the researcher. A study number will be allocated to each participant and the researcher will have a separate study participant information form with the participant's information. All data collection forms will be kept in a locked drawer by the researcher. It is the researcher's responsibility to have the wellbeing of all participants as a priority and when necessary she well ensure that participants receive appropriate referrals and the treatment they need. The data collected in this study will be written into a dissertation for dissemination of information. It will be submitted for publication and presentations may be done at various conferences or facility days. The findings will be shared with the two facilities and presented to Tshwane sub-districts 5, 6 and 7 district staff.

7. Study Limitations

The study has several limitations. First, the sample size is small. The sample size is based on the admissions and discharges of the two facilities and the time available for enrolment. This may result in a type II error since factors associated with the mortality and relapse may not show statistical significance when comparing the cohorts of those who survived to those who died. This study however has the potential to be used as a pilot for future research of this nature with a larger sample size. The risk of attrition bias is also greater with a smaller sample size. To avoid a high rate of loss of follow up the investigator will send SMS reminders to the caregivers before the one and three month follow up visits as well as continue to update caregivers' information at each of the follow up sessions.

A further limitation is that the researcher will be involved in the treatment of the children that will be discharged from Bronkhorstspruit Hospital and will aid in follow up sessions at Mamelodi Hospital. This could bias the results as the care giver and study participants may receive more attention and care than usual, allowing them to avoid the negative outcomes of mortality and relapse. His limitation is unavoidable.

8. Study Timeline

The study timetable is shown below (Figure 1)

Figure 1: Study timetable of the research process (2018 – 2020).

	2018	2019	2020

Activities	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Research Protocol																					
Protocol Assessment																					
Ethics Submission																					
Pilot study																					
Enrolment																					
Data Collection																					
Data Analysis																					
Writing of research report																					
Submission of research report																					

9. Funding

An application will be made to the Faculty of health sciences research fund to cover the partial cost of the study. An estimated budget is show in table 4.

Table 4: Estimated budget

Activity	Unit costs	Total cost
Printing consent forms	1 pages per participant 50 participants R0.50 per page	1 x 50 x R0.05 = R25.00
Printing data collection forms	5 pages per participant	5 x 50 x R0.50 = R125.00

	50 participants R0.50 per page	
Transport costs for participants	R20 per visit 2 visits per participant 50 participants	R20 x 2 x 50 = R2000.00
Transport costs for researcher (to Mamelodi Hospital)	Distance: 40km 2x per month = 80km/month R3.41/km	80km x R3.41 = R272.80 per month 13 months follow up R272.80 x 13 = R3546.40
SMS reminders of Follow up sessions	R0.80 per SMS 2 follow up reminders per participant 50 participants	R0.80 x 2 x 50 = R80.00
Telephone calls for 6 month follow up	10 minute telephone call per participant R1.23 charge per minute 50 participants	10 x R1.23 x 50 = R615.00
Research assistant for enrolment	1 research assistant at Mamelodi Hospital 1 hour per participant R50 per hour 40 participants	1 x 1 x R50 x 40 = R2000.00
Research assistant for conversation/ translation at 6 months	1 research assistant 10 minute telephone call per participant 50 participants R50 per hour	1 x 10/60 x 50 x R50 = R417.00
	Total budget	R8808.40

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Addendum D: Informed consent form

INFORMED CONSENT FORM

Study title

Mortality, relapse, morbidity and growth of severe acutely malnourished children in the six months following discharge

Who we are

Hello, I am Angelika Peczak and I am a Child and Maternal Health Masters student at the University of Witwatersrand.

What we are doing

I am conducting research on what happens to children who have been treated in hospital for severe acute malnutrition (SAM) in the six months after their discharge. This research will find out how many children survive, how they grow and what the common illnesses are that they experience during these six months and help find out what is affecting the health of these children.

Your participation

I am asking you whether you will allow your child to be part of this study. If you agree, you will be asked to answer questions about the child's medical history, living conditions and general information when they are discharged from hospital.

One and three months after discharge you and your child will be requested to attend follow up visits (as scheduled with the doctors at the hospital) in which I will ask you a few more questions about your child's condition, take his/her body measurements and ask for any updates on your address and telephone number. After 6 months of your child being discharged I will phone you and ask you some more questions about your child's health condition.

Please understand that **your participation is voluntary** and you are not being forced to take part in this study. The choice of whether to participate or not, is yours alone. If you choose not to take part, you will not be affected in any way whatsoever. If you agree to participate, you may stop participating in the research at any time and tell me that you don't want to go continue. If you do this there will also be no penalties and you will not be disadvantaged in any way.

Confidentiality

Any study records that identify you will be kept confidential to the extent possible by law. The records from your participation may be reviewed by people responsible for making sure that research is done properly, including members of the ethics committee at the Wits Ethics Committee (all of these people are required to keep your identity confidential)

Otherwise, records that identify you will be available only to people working on the study, unless you give permission for other people to see the records.

The information you provide will not be published unless you give your specific permission in writing at the end of this consent form. All identifying information will be kept in a locked file cabinet and will not be available to others. We will refer to you by a code number or pseudonym (another name) in any publication.

Risks/discomforts

At the present time, we do not see any risks in your participation. The risks associated with participation in this study are no greater than those encountered in daily life.

Benefits

There are no immediate benefits to you from participating in this study, except that we will be checking on your child's progress (or deterioration) for six months after discharge. If we pick up any problem, we will respond to it immediately.

This study will be extremely helpful to us in better understanding how we as health care workers can improve the services given to your child and other children that have had the same condition as yours.

If you would like to receive feedback on our study, we will record your phone number on a separate sheet of paper and can send you the results of the study when it is completed.

Who to contact if you have been harmed or have any concerns

This research has been approved by the WITS Ethics committee. If you any complaints about ethical aspects of the research or feel that you have been harmed in any way by participating in this study, please call the WITS Ethics committee on 011 717 1252

If you have concerns or questions about the research you may call the project leader, Angelika Peczak, on 0103450701.

CONSENT

I hereby agree to allow my child to participate in this research. I understand that they are participating freely and without being forced in any way to do so. I also understand that they can stop participating at any point should I not want to continue and that this decision will not in any way affect me negatively.

I understand that this is a research project whose purpose is not necessarily to benefit me personally in the immediate or short term.

I understand that my participation will remain confidential.

Signature of caregiver:_____ **Date**:_____

I hereby agree to my child's participation in the study.

C. Ethical clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Angelika Peczak

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180812

NAME: (Principal Investigator)	Miss Angelika Peczak
DEPARTMENT:	Community Paediatrics Bronkhorstspruit Hospital Mamelodi Hospital
PROJECT TITLE:	Mortality, relapse, morbidity and growth of severe acutely malnourished children in the six months following discharge
DATE CONSIDERED:	31/08/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Haroon Saloojee
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/12/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore be due in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 12/12/2018

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

D. Submission guidelines to PLOS

Style and Format

File format	Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected. LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information. We encourage you to present and discuss your findings concisely.
Font	Use a standard font size and any standard font, except for the font named "Symbol". To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout and spacing	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.

Reference style

PLOS uses “Vancouver” style, as outlined in the ICMJE sample references.

See reference formatting examples and additional instructions below.

Equations

We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable.

Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., “$a^2 + b^2 = c^2$”), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values.

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Nomenclature

Use correct and established nomenclature wherever possible.

Units of measurement	Use SI units. If you do not use these exclusively, provide the S parentheses after each value. Read more about SI units.
Drugs	Provide the Recommended International Non-Proprietary Nam
Species names	Write in italics (e.g., *Homo sapiens*). Write out in full the genu both in the title of the manuscript and at the first mention of an a paper. After first mention, the first letter of the genus name f the full species name may be used (e.g., *H. sapiens*).
Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the genetic nomenclature database (e.g., HGNC for human genes; recommend using this tool to check against previously approv is sometimes advisable to indicate the synonyms for the gene t appears in the text. Gene prefixes such as those used for oncog cellular localization should be shown in roman typeface (e.g., MYC).
Allergens	The systematic allergen nomenclature of the World Health Organization/In Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature S should be used for manuscripts that include the description or use of allerg manuscripts describing new allergens, the systematic name of the allergen approved by the WHO/IUIS Allergen Nomenclature Sub-Committee prior publication. Examples of the systematic allergen nomenclature can be foun at the WHO/IUIS Allergen Nomenclature site.

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Prior to submission, authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing and copyediting services.

Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like "scientific editing service" or "manuscript editing service."

Submissions are not copyedited before publication.

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Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* • Title page: List title, authors, and affiliations as first page of manuscript • Abstract • Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* • Materials and Methods • Results • Discussion • Conclusions (optional)
Ending section	*The following elements are required, in order:* • Acknowledgments • References • Supporting information captions (if applicable)
Other elements	• Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. • Tables are inserted immediately after the first paragraph in which they are cited. • Supporting information files are uploaded separately.

 Refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

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The compiled submission PDF includes low-resolution preview images of the figures after the reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

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Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

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Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled "current address." At a minimum, the address must include the author's current institution, city, and country.

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Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

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Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include its name in the manuscript byline. Do not add it to the author list in the submission system. You may include the full list of members in the Acknowledgments or in a supporting information file.

PubMed only indexes individual consortium or group author members listed in the article byline. If included, these individuals must qualify for authorship according to our criteria.

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Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

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PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

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Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

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The Abstract should:

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- Not exceed 300 words

Abstracts should not include:

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The introduction should:

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- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
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Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

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These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

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Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

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References

Any and all available works can be cited in the reference list. Acceptable sources include:

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References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI

Source	Format
	included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

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Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

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Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

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Read the guidelines for figures and requirements for reporting blot and gel results.

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Manuscripts submitted to *PLOS ONE* are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously reported in accordance with community standards and that the statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

- SAMPL guidelines, for general guidance on statistical reporting
- *PLOS ONE* guidelines, for clinical trials requirements
- *PLOS ONE* guidelines, for systematic review and meta-analysis requirements
- EQUATOR, for specific reporting guidelines for a range of other study types

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In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

- List the name and version of any software package used, alongside any relevant references
- Describe the technical details or procedures required to reproduce the analysis
- Provide the repository identifier for any code used in the analysis (See our code-sharing policy.)

Statistical reporting guidelines:

- Identify research design and independent variables as being between- or within-subjects
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    - If data were transformed include this information, with a reason for doing so and a description of the transformation performed
- Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed
- If relevant, describe how missing/excluded data were handled
- Define the threshold for significance (alpha)
- If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.
- For analyses of variance (ANOVAs), detail any post hoc tests that were performed
- Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so
- Describe all options for statistical procedures. For example, if t-tests were performed, state whether these were one- or two-tailed. Include details of the type of t-test conducted (e.g. one sample, within-/between-subjects).
- For step-wise multiple regression analyses:
    - Report the alpha level used
    - Discuss whether the variables were assessed for collinearity and interaction
    - Describe the variable selection process by which the final model was developed (e.g., forward-stepwise; best subset). See SAMPL guidelines.
- For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

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Results must be rigorously and appropriately reported, in keeping with community standards.

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- **Regression analyses.** Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.

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