Use of tuberculin skin test for assessment of immune recovery among previously malnourished children in Ethiopia

Paluku Bahwere1,2*, Philip James1,3, Alemseged Abdissa4, Yesufe Getu5, Yilak Getnet5, Kate Sadler1 and Tsinuel Girma6

Abstract

Objective: To compare levels of immunity in children recovering from severe acute malnutrition (cases) against those of community controls (controls).

Results: At baseline children recovering from severe acute malnutrition had lower, mid upper arm circumference (122 mm for cases and 135 mm for controls; \( p < 0.001 \)), weight-for-height Z-score (\(-1.0\) for cases and \(-0.5\) for controls; \( p < 0.001 \)), weight-for-age Z-score (\(-2.8\) for cases and \(-1.1\) for controls; \( p < 0.001 \)) and height/length-for-age Z-score (\(-3.6\) for cases and \(-1.4\) for controls; \( p < 0.001 \)), than controls. Age and gender matched community controls. At baseline, prevalence of a positive tuberculin skin test, assessed by cutaneous delayed-type hypersensitivity reaction skin test, was very low in both cases (3/93 = 3.2%) and controls (2/94 = 2.1%) and did not significantly increase at 6 months follow up (6/86 = 7.0% in cases and 3/84 = 3.4% in controls). The incidences of common childhood morbidities, namely fever, diarrhoea and cough, were 1.7–1.8 times higher among cases than controls. In conclusion, these results show that tuberculin skin test does not enable any conclusive statements regarding the immune status of patients following treatment for severe acute malnutrition. The increased incidence of infection in cases compared to controls suggests persistence of lower resistance to infection even after anthropometric recovery is achieved.

Keywords: Severe acute malnutrition, Immunity, Delayed-type hypersensitivity response, Tuberculin skin test, Morbidity, Tuberculin purified protein derivative, Community-based management of acute malnutrition

Introduction

Globally, 5.8 million children younger than 5 years died in 2015 and millions more are permanently disabled by the physical and mental effects of a poor dietary intake in the earliest months of life [1]. Approximately 2% of children living in low income countries suffer from severe acute malnutrition (SAM) [2]. SAM is believed to account for at least 4.4% of the global under-five death burden and 6.0% of disability-adjusted life-years lost [1]. Despite the tremendous progress of recent years, Ethiopia remains one of the countries with the highest burden of SAM [3]. The rapid scale-up of the community-based management of acute malnutrition (CMAM) approach in many high burden countries has enabled better treatment coverage and has contributed to saving lives of millions of children with SAM [4–11]. However, several studies have reported that children who recovered from SAM remain at high risk of infections and death several months after exiting treatment programmes [12–19]. One hypothesis is that the anthropometric and clinical discharge criteria that are used in treatment programmes may have weak
correlation with optimal recovery of body functions including immunity.

In Ethiopia, children with SAM are discharged from CMAM as recovered when they have fulfilled the following criteria: weight gain of 20%, mid upper-arm circumference (MUAC) > 11.0 cm, resolved oedema and clinically stable for 2 consecutive weeks [20]. Here we describe a study conducted in Jimma Zone, Eastern Ethiopia, using a tuberculin purified protein derivative (PPD) skin test to verify if children who reached these discharge criteria had also recovered their immune response. This paper presents the results and discusses the utility of the test for this purpose.

Main text

Methods

This research was a sub-study of a larger 12 months prospective cohort study conducted from September 2013 to January 2015. The main study aimed to describe the mortality, morbidity and nutritional status profiles of children aged 6–59 months discharged as recovered from CMAM for SAM treatment. It enrolled 215 post-SAM cases (‘cases’) discharged as recovered from CMAM sites of Jimma area and 215 non-wasted community controls (‘controls’) matched from the same village with the cases by age, sex, mother’s education and roof material. The controls were apparently healthy children with no history of treatment for acute malnutrition. Cases and controls were followed up concurrently at their homes each month, assessing nutritional status (weight, height, MUAC, bilateral pitting oedema) and reported morbidity of the past 2 weeks (history of fever, diarrhoea or cough) and vital status (alive or dead).

The extent of immune system recovery was assessed using the difference in incidence of infectious diseases between cases and controls and by a cutaneous delayed-type hypersensitivity reaction skin test (DTHR) at enrolment and at the 6-month follow up. The DTHR was assessed using the Tuberculin skin test (TST) [21]. TST was chosen because of its practicality for community-based surveys, its commercial availability, and its wide usage in DTHR response and immune diagnosis of tuberculosis infection [22–24]. We used Tuberculin PPD RT23 (Statens Serum Institute, Copenhagen, Denmark). The tuberculin PPD was kept refrigerated and transported to the field on ice and avoiding light exposure. This maintained the temperature of the vials between 2 and 8 °C throughout. The DTHR tests were performed by trained research nurses. 0.1 mL (2 Tuberculin Units/0.1 mL) of antigen was injected into the intradermal space on the volar surface of the forearm, using a 27-gauge needle with the bevel up and at an angle of 5–15°. Adherence to the injection technique was confirmed by the apparition of a small wheal of 6–10 mm as the antigen was injected into the dermis and by the absence of bleeding. Reading and classification of the DTHR response was based on the diameter of the induration measured 48–72 h after the antigen injection [25]. A TST was regarded as positive if the transversal diameter of skin induration was ≥ 10 mm. Children were classified as anergic if there was no induration.

Morbidity was assessed by caretaker face-to-face interviews using a 2-week recall approach and a pre-tested standardised questionnaire. Caretakers were asked if the child had experienced fever, diarrhoea, or persistent cough during 2 weeks preceding the interview. All socio-demographic, nutrition, medical and household information captured by the questionnaire followed the same methodology described in James et al. [26].

In absence of literature data allowing precise calculation of sample size, a convenience sample size of 100 cases and 100 controls was used for this study. Proportions and means were compared using Chi squared tests and unpaired Student t test, respectively. We generated incidence rate ratios to compare morbidity incidence between the cases and controls.

Results

Characteristics at enrolment

Ninety-three cases and 95 controls were included in the present sub-study. The two groups did not differ for most of the parameters checked except for orphan status, age of carers, number of children below 5 years of age in the household, proportion of food secure households and nutrition status (Table 1). At enrolment the nutritional status of cases, indicated by MUAC, weight-for-height Z-score, weight-for-age Z-score and height/length-for age Z-score, were lower than that of the controls (Table 1).

Results of cell mediated immunity assessment

In both cases and controls, less than 5% of children had a positive tuberculin skin test (Table 2). The rate of positivity did not increase between baseline and 6 months after enrolment (Table 2). Four cases anergic at enrolment had a positive TST test at 6-months while one who had an induration of 9.5 mm at enrolment was anergic at 6 months follow up. For the majority of reactive children, the diameter of induration was larger at 6-months while one who had an induration of 9.5 mm at enrolment was anergic at 6 months follow up than at enrolment (Fig. 1).

Results on incidence of reported common morbidities

The incidences of fever [12.45 per 100-child-months in cases and 7.25 per 100-child-months in controls; incidence rate ratio (IRR): 1.85 (1.33–2.20); p < 0.001],
diarrhoea [11.90 per 100-child-months in cases and 6.70 per 100-child-months in controls; IRR: 1.77 (1.37–2.29); p < 0.001], and cough [12.42 per 100-child-months in cases and 6.94 per 100-child-months in controls; IRR: 1.79 (1.37–2.29); p < 0.001] were 1.7–1.8 times higher among cases than controls.

Table 1  Socio-demographic, medical, nutrition and household characteristics of participating children

| Characteristics | Post-SAM cases (n = 93) | Community controls (n = 95) | p value |
|-----------------|-------------------------|----------------------------|---------|
|                 | n (%)                   | Median (IQR)               | n (%)   | Median (IQR) |         |
| Socio-demographic characteristics | | | | | |
| Female          | 39 (41.9)               | 41 (43.2)                  | 0.865   |
| Age (months)    | 15 (6; 48)              | 14 (7; 48)                 | 0.309   |
| Medical history | | | | | |
| Ever immunised  | 77 (82.8)               | 80 (84.2)                  | 0.794   |
| Presence of BCG scar | 54 (58.1) | 65 (68.4) | 0.326 |
| Recent vitamin A supplementation | 86 (92.5) | 87 (91.6) | 0.821 |
| Recent deworming | 52 (55.9)       | 44 (46.3)                  | 0.188   |
| Utilises insecticide treated bed net | 47 (50.5) | 53 (55.8) | 0.767 |
| Nutrition status | | | | | |
| Mid upper arm circumference (mm) | 122 (118; 131) | 135 (130; 143) | < 0.001 |
| Weight-for-age Z-score<sup>a</sup> | − 2.8 (− 3.6; − 2.2) | − 1.1 (− 1.8; − 0.5) | < 0.001 |
| Height-for-age Z-score<sup>a</sup> | − 3.6 (− 4.6; − 2.6) | − 1.4 (− 2.4; − 0.7) | < 0.001 |
| Weight-for-height Z-score<sup>a</sup> | − 1.0 (− 1.9; − 0.4) | − 0.5 (− 1.1; 0.2) | < 0.001 |
| % severe stunting<sup>a</sup> | 60 (65.2) | 17 (18.1) | < 0.001 |
| Caregivers information | | | | | |
| Both parents alive | 79 (85.9) | 92 (96.8) | 0.004 |
| Mother as principal caregiver | 89 (95.7) | 93 (97.9) | 0.442 |
| Caregiver ever attended school | 17 (18.3) | 22 (23.2) | 0.410 |
| Principal caregiver age (years) | 26 (13; 60) | 25 (18; 55) | 0.034 |
| Caregiver MUAC (mm) | 222 (182; 297) | 229 (122; 267) | 0.074 |
| Infant and child feeding index<sup>b</sup> | | | | | |
| Lowest category | 2 (2.1) | 4 (4.2) |         |
| Middle category | 42 (45.2) | 28 (29.5) |         |
| Highest category | 49 (52.7) | 63 (66.3) |         |
| Household characteristics | | | | | |
| Male headed household | 88 (95.6) | 92 (96.8) | 0.668 |
| Household head attended school | 30 (32.3) | 34 (36.2) | 0.573 |
| Household size | 6 (2–22) | 5 (2–12) | 0.081 |
| Number children below 5 years | 2 (1–2) | 1 (1–2) | 0.020 |
| Food secure | 50 (56.2) | 77 (82.8) | 0.001 |
| WASH practices category | | | | | |
| Good WASH index<sup>c</sup> | 55 (59.1) | 58 (61.0) |         |
| Poor WASH index | 38 (40.9) | 37 (39.0) |         |
| Wealth quartiles distribution<sup>d</sup> | n = 88 | n = 89 | 0.806 |
| First | 25 (28.4) | 21 (23.6) |         |
| Second | 23 (26.1) | 27 (30.3) |         |
| Third | 19 (21.6) | 22 (24.7) |         |
| Fourth | 21 (23.9) | 19 (21.3) |         |

SAM severe acute malnutrition, MUAC mid upper arm circumference, IQR interquartile range, WASH water, sanitation and hygiene

<sup>a</sup> Using 2006 world Health organisation multicentre child growth references

<sup>b</sup> Based on 24 h recall of breastfeeding, dietary diversity and meal frequency

<sup>c</sup> Summarised as recommended by WHO and UNICEF methodology [62]

<sup>d</sup> Generated using principal component analysis as described in [61]
Discussion

Undernutrition in early life is associated with a number of adverse health consequences, including impaired growth and neurocognition, long-term body composition abnormalities and persistent immune system dysfunction [27–30]. Little is known about how the CMAM approach can be improved so that long-term benefits are added to the well-documented short-term benefits already

Table 2 Results of delayed-type hypersensitivity reaction on tuberculin antigen

| Skin test categories | Baseline | Follow-up (6 months) | p value |
|----------------------|----------|----------------------|---------|
|                      | n (%)    | n (%)                |         |
| Proportion of anergic|          |                      |         |
| Post-SAM (n = 93/86) | 83 (89.2)| 79 (92.0)            | 0.551   |
| Community control (n = 94/89) | 90 (95.7)| 84 (94.4)            | 0.742   |
| p value              | 0.091    | 0.604                |         |
| Proportion with any induration|          |                      |         |
| Post-SAM (n = 93/86) | 10 (10.8)| 7 (8.0)              | 0.551   |
| Community control (n = 94/89) | 4 (4.3)| 5 (5.6)              | 0.742   |
| p value              | 0.091    | 0.604                |         |
| Positive test (induration ≥ 10 mm) |          |                      |         |
| Post-SAM (n = 93/86) | 3 (3.2)| 6 (7.0)              | 0.316   |
| Community control (n = 94/89) | 2 (2.1)| 3 (3.4)              | 0.676   |
| p value              | 0.091    | 0.324                |         |

Skin test categories | Baseline | Follow-up (6 months) | p value |
|----------------------|----------|----------------------|---------|
|                      | Mean ± SD| Mean ± SD            |         |
| Average diameter of induration |          |                      |         |
| Post-SAM (n = 10/7) | 10.2 ± 9.8| 14.9 ± 4.4          | 0.256   |
| Community control (n = 4/5) | 7.7 ± 6.4| 11.2 ± 3.5          | 0.327   |
| p value              | 0.648    | 0.150                |         |
| Average induration for positive test |          |                      |         |
| Post-SAM (n = 3/6) | 21.7 ± 1.0| 15.9 ± 3.9          | 0.044   |
| Community control (n = 2/3) | 13.0 ± 3.5| 13.7 ± 1.0        | 0.748   |
| p value              | 0.398    | 0.376                |         |

Proportions are compared using Pearson Chi square or Fisher exact test as appropriate, means are compared using unpaired Student t test. SAM severe acute malnutrition, delayed-type hypersensitivity skin reaction considered positive if induration diameter ≥ 10 mm

Fig. 1 Diameter of induration at enrolment and follow up for the children exhibiting an induration during at least one assessment time-point. Post-SAM post-severe acute malnutrition treatment (cases)
being achieved. Thus, it is important to start to focus on improving our knowledge on the impact of current treatment protocols on the quality of immune system normalisation as it is clear that cell mediated immunity and resistance to infection are not being adequately recovered.

This short report presents our attempts to characterize the quality of immune system repair at the time of being discharged as recovered from CMAM programmes. Using the TST we were not able to confirm whether cell-mediated immunity was adequately or inadequately repaired in post-SAM cases as the TST appeared to be inappropriate for assessing immune system functioning in our study setting. However, the difference in incidence of symptoms of common infectious diseases suggests cases did have lowered immunity than controls, since impairment in cellular mediated immunity is independently associated with increased incidence of these diseases [31–33].

Despite the still limited understanding of the interplay between the immune function and SAM, existing evidence points toward impairment of cell-mediated immunity during SAM episode including DTHR [34–39]. Also, it has been suggested that DTHR return to normal after successful treatment of SAM [40]. Thus, we understood that checking DTHR using TST was logical to assess the immune system recovery in children who were treated under the current CMAM programme as virtually all children are Bacille Calmette–Guérin (BCG) vaccinated in the region and BCG vaccinated children with low risk of tuberculosis infection can have a positivity of up to 80%, as observed in Turkey [41]. Unfortunately, contrary to our hypothesis of high prevalence of positive DTHR in matched controls, almost all exhibited anergy making it difficult to link the impaired DTHR in cases solely to the insufficiency of nutrition recovery.

Our results differ from that of an early study conducted in Colombia that showed a rapid increase in TST positivity from 0% for kwashiorkor children and 5.5% for marasmic children at admission to 50% for kwashiorkor children and 90.9% for marasmic children after 6 weeks of therapeutic feeding [37]. They also differ from that of Forse et al. who showed in 257 adults on pre-surgical total parenteral nutrition that reduced cell mass was associated with impaired DTHR, while nutrition recovery and accompanying increased body mass cells improved the likelihood of positive DTHR [42]. However, our results are in accord with some studies conducted in African children that showed negative DTHR even in presence of both BCG scar and confirmed active tuberculosis pulmonary infections [23, 24, 43, 44]. They are also consistent with results of a study among Ethiopian adults that reported a lower prevalence of tuberculosis infection when using TST than when using T-cell based interferon-g release assays [45]. Thus, even if TST may be used in certain contexts to assess immune system competence, our study suggests it cannot be universally recommended for children (whether these are children with SAM or those who are apparently well-nourished) living in countries with similar characteristics as Ethiopia. We tentatively suggest that in rural Ethiopia the high frequency of environmental enteric dysfunction and high incidence of viral, bacterial and parasitic infection may adversely affect the DTHR test, including in non-malnourished children, by maintaining children in a state of chronic inflammation [22, 34, 46–48]. Also, despite not being wasted, the control group may have undiagnosed micronutrient deficiencies that can affect the DTHR [49]. These factors may also explain why many of the children did not have a BCG scar despite being BCG vaccinated. Nonetheless, TST negative results are also common among BCG vaccinated children from United Kingdom, who are unlikely to have micronutrients deficiencies and who are most likely to be immune-competent, suggesting that factors unrelated to immunity contribute to the presence of the negative TST [50].

Despite the null TST results the fact that cases had a higher reported incidence of common diseases than controls suggests that at time of reaching the anthropometric discharge criteria currently recommended by the Ethiopian national guidelines for management of SAM, the immunity has not completely recovered. This is in accord with several previous studies that showed that immune recovery measured by thymus size, serum immunoglobulin level or CD4 count is delayed comparatively to anthropometric recovery [51–54]. To date, all these results have not yet been considered for defining practical criteria for judging recovery that combine body mass catch up and immune recovery [20, 55].

In conclusion, this study did not permit a conclusion on immune recovery following CMAM treatment for SAM, whether at discharge or at 6 months follow up but suggests persistence of lower resistance to infection after the anthropometric criteria of nutrition recovery are reached. Research is needed to identify a clinical proxy of immunity recovery that can be used in community-based programmes in low income countries.

**Limitations**

Having a laboratory blood test confirming immunity disturbance could have strengthened our conclusion. Rytter et al. have proposed laboratory tests to use in the assessment of immune system recovery, but most of the proposed tests are difficult to conduct in the low income settings where most SAM children are found [34]. Future research should include the quantification of T cell receptor excision circles using Dried Blood Spot specimens to
measure the quantity of circular deoxyribonucleic acid molecules formed during rearrangement of the T cell receptor genes during lymphocyte development [56–58]. The test is increasingly used in screening newborns for primary immunodeficiency and for assessing immune response to the antiretroviral treatment of HIV infected children [59, 60]. The simplicity of the sampling allows prospective sampling at several points during management and follow-up after discharge and may help validate the choice of clinical and anthropometric parameters best used as a proxy of immune system recovery.

**Abbreviations**

BCG: Bacille Calmette–Guérin; CMAM: community-based management of acute malnutrition; DTHR: delayed-type hypersensitivity reaction; ENGINE: empowering new generations to improve nutrition and economic opportunities; MUAC: mid-upper-arm circumference; PPD: tuberculin purified protein derivative; SAM: severe acute malnutrition; TST: tuberculin skin test.

**Authors’ contributions**

PB, PJ, AA, KS and TG designed the study, supervised data collection, analysed and interpreted the findings and participated in the writing of this manuscript. YeG and YG carried out data collection, data entry, participated in data analysis and, interpretation and contributing during write up. All authors read and approved the final manuscript.

**Author details**

1 Valid International, 35, Leopold Street, Oxford OX4 1TW, UK. 2 Research Centre in Epidemiology, Biostatistics and Clinical Research, School of Public Health, Free University of Brussels, Brussels, Belgium. 3 London School of Hygiene and Tropical Medicine, London, UK. 4 Department of Medical Laboratory Sciences and Pathology, Jimma University, Jimma, Oromia, Ethiopia. 5 Save Children Federation, Addis Ababa, Ethiopia. 6 Department of Paediatrics and Child Health, Jimma University, Jimma, Oromia, Ethiopia.

**Acknowledgements**

We thank the mothers and children who consented to give their time and support freely throughout this study. We are grateful for the hard work of the field data collectors and the data entry clerks for their essential contribution. We thank the ENGINE, Jimma University and Valid International management teams for their vast contribution to the ongoing supervision: Beyene Wondafash, Habtamu Fekadu, Cherinet Abuye, and Benti Geleta.

**Competing interests**

The authors declare that they have no competing interests. However, Alemseged Abdissa who is one of the authors works for BMC Research Notes as an Associate Editor.

**Availability of data and materials**

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

The study protocol was approved by the Jimma University Ethics Review Board (Reference RPCG/J/130/2013). Enrolment into the study was voluntary and data collection was initiated after obtaining written consent or thumbprint from the caregiver of the child that authorised both data collection and anonymous publication of findings.

**Funding**

This research work was funded by the United States Agency for International Development under Agreement No. AID-663-A-11-00017, Empowering New Generations to Improve Nutrition and Economic opportunities (ENGINE). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Received: 25 May 2017 Accepted: 1 November 2017**

**Published online: 07 November 2017**

**References**

1. Collaborators GBDCM. Global, regional, national, and selected sub-national levels of stillbirths, neonatal, infant, and under-5 mortality, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2016;388(10053):1723–74.

2. You D, Hug L, Ey delimiter S, Idele P, Hogan D, Mathers C, Gerland P, New JR, Alkema L. United Nations Inter-agency Group for Child Mortality. Global, regional, and national levels and trends in under-5 mortality between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Inter-agency Group for child mortality estimation. Lancet. 2015;386(10010):2275–86.

3. Wang H, Liddell CA, Coates MM, Mooney MD, Levitz CE, Schumacher AE, Apfelf H, Iannarone M, Phillips B, Lofigren KT, et al. Global, regional, and national levels of neonatal, infant, and under-5 mortality during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9947):957–79.

4. Collins S, Yates R. The need to update the classification of acute malnutrition. Lancet. 2003;362(9379):249.

5. Collins S, Sadler K, Dent N, Khara T, Guerrero S, Myatt M, Saboya M, Walsh A. Key issues in the success of community-based management of severe malnutrition. Food Nutr Bull. 2006;27(3):594–82.

6. Collins S, Sadler K. The outpatient treatment of severe malnutrition during humanitarian relief programmes. Lancet. 2002;360:1824–30.

7. Collins S, Dent N, Binns P, Bahwere P, Sadler K, Hallam A. Management of severe acute malnutrition in children. Lancet. 2006;368(9551):1992–2000.

8. Collins S. Treating severe acute malnutrition seriously. Arch Dis Child. 2007;92(S):453–61.

9. Collins S. Community-based therapeutic care—a new paradigm for selective feeding in nutritional crises. In: Network HP, editor. HPN Network Papers. London, 2004.

10. Collins S. Changing the way we address severe malnutrition during famine. Lancet. 2001;358(9280):498–501.

11. WHO, WFP, UNICEF & UNSCN. Joint statement on the community-based management of severe malnutrition in children. 2007.

12. Kerac M, Bunn J, Chagaluka G, Bahwere P, Tomkins A, Collins S, Seal A. Follow-up of post-discharge growth and mortality after treatment for severe acute malnutrition (FuSAM study): a prospective cohort study. PLoS ONE. 2014;9(6):e96030.

13. Bahwere P, Mitimuni A, Sadler K, Banda T, Collins S. Long term mortality after community and facility based treatment of severe acute malnutrition: analysis of data from Bangladesh, Kenya, Malawi and Niger. J Public Health Epidemiol. 2012;4(8):215–25.

14. Hennart P, Beghin D, Bossuyt M. Long-term follow-up of severe protein-energy malnutrition in Eastern Zaire. J Trop Pediatr. 1987;33(1):10–2.

15. Roosmalen-Wiebenga MW, Kuwin JA, de With C. Nutrition rehabilitation in hospital—a waste of time and money? Evaluation of nutrition rehabilitation in a rural district hospital in South-west Tanzania. II. Long-term results. J Trop Pediatr. 1987;33(1):24–8.

16. Khanum S, Ashworth A, Huttley SR. Growth, morbidity, and mortality of children in Dhaka after treatment for severe malnutrition: a prospective study. Am J Clin Nutr. 1996;63(6):940–5.

17. Pecoul B, Soutif C, Hounkpevi M, Ducos M. Efficacy of a therapeutic feeding centre evaluated during hospitalization and a follow-up period, Tahoua, Niger, 1987–1988. Ann Trop Paediatr. 1992;12(1):47–54.

18. Penia A, Costello AM. Efficacy of outreach nutrition rehabilitation centres in reducing mortality and improving nutritional outcome of severely malnourished children in Guinea Bissau. Eur J Clin Nutr. 1995;49(S):353–9.
19. Chang CY, Trehan I, Wang RJ, Thakwalakwa C, Maleka K, Deitchler M, Manary MJ. Children successfully treated for moderate acute malnutrition remain at risk for malnutrition and death in the subsequent year after recovery. J Nutr. 2013;143(2):215–20.

20. Ethiopia Federal Ministry of Health. Protocol for the management of severe acute malnutrition. 2007.

21. Lee H, Cho SN, Kim HJ, Anh YM, Choi JE, Kim CH, Ock PJ, Oh SH, Kim DR, Floyd S, et al. Evaluation of cell-mediated immune responses to two BCG vaccination regimes in young children in South Korea. Vaccine. 2011;29(38):6564–71.

22. Bonilla FA, Stehmel ER, Wood RA, Feldweg AM. Laboratory evaluation of the immune system. 2008.

23. Van der Zalm MM, van Soelen N, Mandalakas AM, Jacobsen M, Detjen AK, Marx FM, Grewal HM, Cotton MF, Walzl G, Hesseling AC. The effect of deworming on tests of tuberculosis infection in children with recent tuberculosis exposure: a randomized controlled trial. Pediatr Infect Dis J. 2016;35(6):622–6.

24. van Soelen N, Mandalakas AM, Kirschner HL, Walzl G, Grewal HM, Jacobsen M, Hesseling AC. Effect of Ascaris lumbricoides specific IgE on tuberculin skin test responses in children in a high-burden setting: a cross-sectional community-based study. BMC Infect Dis. 2012;12:211.

25. Stop TB, Partnership ChildhoodTB Subgroup, World Health Organization.

26. van Soelen N, Mandalakas AM, Kirchner HL, Walzl G, Grewal HM, Jacobsen M, Detjen AK, Marx FM, Grewal HM, Cotton MF, Walzl G, Hesseling AC. The effect of deworming on tests of tuberculosis infection in children with recent tuberculosis exposure: a randomized controlled trial. Pediatr Infect Dis J. 2016;35(6):622–6.

27. Galler JR, Bryce CP, Zichlin ML, Fitzmaurice GM, Eaglesfield D, Waber DP. Immune responses in malnourished guinea pigs. J Nutr. 1982;112(1):167–74.

28. Galler JR, Bryce CP, Zichlin ML, Fitzmaurice G, Eaglesfield GD, Waber DP. Improvement of RES and cellular immunity to bacterial antigens in Northern Senegal. Am J Trop Med Hyg. 2014;90(3):566–73.

29. Lelijveld N, Seal A, Wells J, Heyderman R, Nyirenda M, Kerac M. P05 Long-term effects of acute malnutrition on growth and body composition in children successfully treated for moderate acute malnutrition with no access to supplementary feeding programmes experience high rates of deterioration and no improvement: results from a prospective cohort study in rural Ethiopia. PLoS ONE. 2016;11(4):e0153350.

30. Galler JR, Bryce C, Waber DP, Zichlin ML, Fitzmaurice GM, Eaglesfield D. Socioeconomic outcomes in adults malnourished in the first year of life: a 40-year study. Pediatrics. 2012;130(1):e1–7.

31. Victoria CG, Adar L, Fall C, Hallal PC, Martorell R, Richter L, Sachdev HS. Maternal and child undernutrition: consequences for adult health and human capital. Lancet. 2008;371(9609):340–57.

32. Lelijveld N, Seal A, Wells J, Heyderman R, Nyirenda M, Kerac M. P05 Long-term effects of acute malnutrition on growth and body composition in children successfully treated for moderate acute malnutrition with no access to supplementary feeding programmes experience high rates of deterioration and no improvement: results from a prospective cohort study in rural Ethiopia. PLoS ONE. 2016;11(4):e0153350.

33. Zaman K, Baqui AH, Yunus M, Sack RB, Chowdhury HR. Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. Acta Paediatr. 1997;86(9):923–7.

34. Zaman K, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. Eur J Clin Nutr. 1996;50(9):909–14.

35. Baqui AH, Sack RB, Black RE, Chowdhury HR, Yunus M, Siddique AK. Cell-mediated immune deficiency and malnutrition are independent risk factors for persistent diarrhea in Bangladeshi children. Am J Clin Nutr. 1995;58(4):543–8.

36. McMurray DN, Yetley EA. Immune responses in malnourished guinea pigs. J Nutr. 1982;112(1):167–74.

37. McMurray DN, Watson RR, Reyes MA. Effect of renutrition on humoral and cell-mediated immunity in severely malnourished children. Am J Clin Nutr. 1981;34(10):217–26.

38. Watson RR, McMurray DN. The effects of malnutrition on secretary and cellular immune processes. CRC Crit Rev Food Sci Nutr. 1979;12(2):113–59.

39. Satyanarayana K, Bhaskaram P, Seshu VC, Reddy V. Influence of nutrition on postvaccinal tuberculin sensitivity. Am J Clin Nutr. 1980;33(1):2334–7.

40. Fakhir S, Ahmad P, Faridi MA, Rattan A. Cell-mediated immune responses in malnourished host. J Trop Pediatr. 1989;35(4):175–8.

41. Çalış I. Comparison of QuantiFERON-TB gold in-tube test with tuberculin skin test in children who had no contact with active tuberculosis case. Tuberk Toraks. 2014;62(2):116–21.

42. Fose RA, Christou N, Meakins JL, MacLean LD, Shugal HM. Reliability of skin testing as a measure of nutritional state. Arch Surg. 1981;116(10):1284–8.

43. Lala SG, Parbhoo KB, Verwey C, Khan R, Danger Z, Moore D, Pettifor JM, Martinson NA. The effect of topical calciopetrol or zinc on tuberculin skin tests in hospitalised South African children. Int J Tuberc Lung Dis. 2014;18(4):398–93.

44. Warsie L, Aseffa A, Abebe M, Gebeyehu MZ, Zewedie M, Mihret A, Erenso G, Chanyalew M, Tilahun H, Yamiuk LC, et al. Parasitic infection may be associated with discordant responses to QuantiFERON and tuberculosis skin test in apparently healthy children and adolescents in a tuberculosis endemic setting. Ethiopia. BMC Infect Dis. 2013;13:265.

45. Legesse M, Ameni G, Mamo G, Medhin G, Bujie G, Abebe F. Community-based cross-sectional survey of latency tuberculosis infection in Afar pastoralists, Ethiopia, using QuantiFERON-TB gold in-tube and tuberculin skin test. BMC Infect Dis. 2011;11:89.

46. Prendergast A, Kelly P. Enteropathies in the developing world: neglected effects on global health. Am J Trop Med Hyg. 2012;86(5):756–63.

47. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. Lancet. 2009;374(9694):1032–5.

48. Watanabe K, Petri WA. IR. Environmental enteropathy: elusive but significant subclinical abnormalities in developing countries. EllioMedicine. 2016;10:25–32.

49. Gaayeb L, Sarr JB, Cames C, Pincon C, Hanon JB, Ndiath MO, Seck M, Herbert F, Sagna AB, Schacht AM, et al. Effects of malnutrition on children’s immunity to bacterial antigens in Northern Senegal. Am J Trop Med Hyg. 2014;90(3):566–73.

50. Seddon JA, Paton J, Nademi Z, Keane D, Williams B, Williams A, Welch SB, Liebeschutz S, Riddell A, Bertonevie, et al. The impact of BCG vaccination on tuberculin skin test responses in children is age dependent: evidence to be considered when screening children for tuberculosis infection. Thorax. 2016;71(10):932–9.

51. Chevalier P. Zinc and duration of treatment of severe malnutrition. Lancet. 1995;345(8956):1046–7.

52. Chevalier P, Sevilla R, Sejas E, Zalles L, Belmonte G, Patent G. Immune recovery of malnourished children takes longer than nutritional recovery: implications for treatment and discharge. J Trop Pediatr. 1998;44(5):304–7.

53. Nasar MF, Younis NT, Tohamy AG, Dalam DM, El Badawy MA. T-lymphocyte subsets and thymic size in malnourished infants in Egypt: a hospital-based study. East Mediterr Health J. 2007;13(5):1031–42.

54. Lesourd BM, Mazari L. Immune responses during recovery from protein-energy malnutrition. Clin Nutr. 1997;16(Suppl 1):37–46.

55. WHO. Guideline: updates on the management of severe acute malnutrition in infants and children. Geneva: World Health Organisation; 2013.

56. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol. 2005;115(2):391–8.

57. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circle. J Allergy Clin Immunol. 2012;129(3):607–16.

58. Purwadi D, Gonzalez-Espinosa D, Comeau AM, Dutra A, Pak E, Puck J. Cellular calibrators to quantitate T-cell receptor excision circles (TRECs) in clinical samples. Mol Genet Metab. 2012;107(3):586–91.

59. Quiros-Roldan E, Serana F, Chiarini M, Gotti D, Zanotti C, Sottini A, Caimi G, et al. Characterizing immune reconstitution after long-term highly active antiretroviral therapy in pediatric AIDS. AIDS Res Hum Retroviruses. 2002;18(18):1395–406.

60. Vyas S, Kumararayake L. Constructing socio-economic status indices: how to use principal components analysis. Health Policy Plann. 2006;21(6):459–68.

61. WHO. UNICEF. Core questions on drinking water and sanitation for household surveys, 2006.