Universitat de Girona

ASSESSING THE PREVALENCE OF INFECTION AMONG CHILDREN WITH MODERATE ACUTE MALNUTRITION

FINAL DEGREE PROJECT

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"Many things can wait. Children cannot. Today their bones are being formed, their blood is being made, their senses are being developed. To them we cannot say 'tomorrow'. Their name is today."

Gabriela Mistral.

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ABSTRACT

Background: The global burden of acute malnutrition remains unacceptably high, mainly in poor resource settings, and the most affected group are the children.

Two groups of acute malnutrition can be differentiate: moderate and severe.

For those children with severe acute malnutrition, the recommendations are clear, they receive an effective nutritional treatment and are checked and treated for some of the most important comorbidities. Nevertheless, when we are in front of a case of moderate acute malnutrition, none of those are taken into account.

It has been seen that children with moderate acute malnutrition are at a high risk of remain malnourished or even worsen their condition the year after they receive the recommended nutritional treatment. That means that their management is not working well. We believe that this issue can be not only about finding the optimal nutritional support, but also about the well managing of the possible concomitant conditions. Some infectious diseases that are very common in poor resource settings can be playing a critical role in their capability of recovery. So, it will be important to study the possible presence of those conditions, so that in the future further research could be done about this issue and its impact on the malnourished children's health.

Objective: The goal of this study is to assess the prevalence of HIV, tuberculosis, malaria, geohelminths and schistosomiasis in children with moderate acute malnutrition, and to compare it to the prevalence in those children with severe acute malnutrition.

Design: This is a cross-sectional study carried out at the Hospital Nossa Senhora da Paz in Angola, that will last two years in total.

Methods: Diagnostic tests to determine the presence of HIV, tuberculosis, malaria, geohelminths and schistosomiasis will be performed in children under the condition of acute malnutrition that arrive to the Nutritional Service of the Hospital Nossa Senhora da Paz. Clinical and analytical additional data will also be recorded.

Keywords: malnutrition, moderate acute malnutrition (MAM), World Health Organization (OMS), comorbidities, infection.

ABBREVIATIONS

Ab: antibody

AFB: acid-fast bacilli

Ag: antigen

CEIC: clinical research ethical committee

CHW: community health workers

GH: growth hormone

HIV: human immunodeficiency virus

IC: informed consent

IMCI: integrated management of childhood illness

LED: light-emitting diode

MAM: moderate acute malnutrition

RDTs: rapid diagnostic tests

RUFT: ready-to-use therapeutic foods

SAM: severe acute malnutrition

SRAA: renin-angiotensin-aldosterone system

TB: tuberculosis

UNICEF: United Nations International Children's Emergency Found

WHO: World Health Organization

INTRODUCTION

MALNUTRITION

The term *malnutrition* is multifaceted. It encompasses both overnutrition, associated with overweight and obesity, and undernutrition, referring to multiple conditions including acute and chronic malnutrition and micronutrient deficiencies.

Focusing on undernutrition, by 2016, 52 million children under 5 years of age were wasted, 17 million were severely wasted and 155 million were stunted.

This fact is important because around 45% of deaths among children under 5 years of age are linked to **undernutrition**, and these mostly occur in low- and middle-income countries.

Furthermore, this high burden of malnutrition has serious economic, medical and social impacts at the individual, family, community and country level.(1)

Underweight (weight-for-age below –2SD) is an anthropometric indicator that can indicate wasting, or much more commonly, stunting. These two conditions can have different determinants and manages. So that, considering wasting and stunting is more useful than consideration of underweight.(2)

Stunting (height-for-age below –2 SD) is the disastrous result of poor nutrition in early childhood. It is also known as *chronic malnutrition*.

Stunted children may never grow to their full height and their brains may never develop to their full cognitive potential. They begin their lives at a marked disadvantage: they are at greater risk of have learning difficulties in school, earn less as adults, and be less integrated at their communities.

Globally, approximately 155 million children under 5 suffer from this condition. The number of stunted children is decreasing worldwide, but Africa is still the only region where this number has risen. Western Africa accounts for half of the stunting increase in Africa; there were 4 million more stunted children in Western Africa in 2016 than in 2000.

Wasting (weight-for-height below –2SD) in children is the life-threatening result of hunger and/or disease. It is also known as *acute malnutrition*, and it is divided into moderate (between -2 and -3SD) and severe (less tan -3SD). Global acute malnutrition refers to MAM and SAM together; it is used as a measurement of nutritional status at a population level and as an indicator of the severity of an emergency situation.

Those under this condition have a weaker immunity, they are susceptible to long term developmental delay, and face a heightened risk of death: they usually require urgent treatment and care to survive. In 2016, nearly 52 million children under 5 were wasted and 17 million were severely wasted. By 2016, in Africa, 14.0 million children were wasted, of which 9.9 million were moderately wasted.(3)
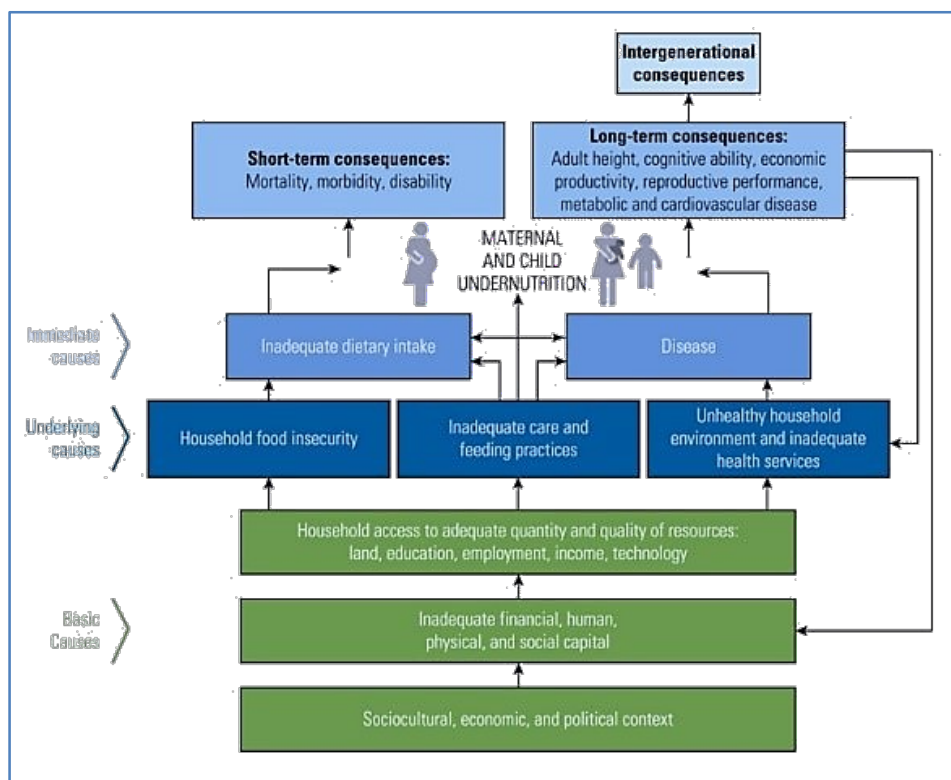
Figure 1. Conceptual Framework of Determinants of Undernutrition.(4)

SEVERE AND MODERATE ACUTE MALNUTRITION

The clinical presentation of SAM includes kwashiorkor (or edematous malnutrition) and severe wasting (or marasmus).

Diagnoses of severe and moderate forms of malnutrition are being made with anthropometric criteria and clinical examination for the presence of edema. Recently, in order to make the diagnose simpler and faster to the community health workers

(CHW), measurement of mid-upper arm circumference can be used instead weight for height, which is the classical anthropometric criteria.(5)

Table 1. Criteria for SAM or MAM in young children*. Adapted from (5)

Criteria	SAM	MAM
Weight for height	z-score <-3SD; or weight <70% reference median for height	z-score <-2 SD and >-3SD; or weight <80% and ≥70% reference median for height
Mid-upper arm Circumference	<115 mm	<125mm and ≥115mm
Bipedal edema	Present or absent	Absent
*Based on a WHO and UNICEF document on the application of WHO 2006 Growth standards for the identification of SAM in infants and children. The WHO 2006 growth standard is the recommended source of anthropometric reference data.(6) SAM may be considered to be present if one of the anthropometric criteria is fulfilled or bipedal edema is present. MAM may be considered to be present if one of the anthropometric criteria is fulfilled and bipedal edema is absent.		

IMPORTANCE OF MODERATE ACUTE MALNUTRITION

Despite the considerable difference in the approximate number of children affected by MAM (33 million) compared with SAM (19 million), there is currently no standardized approach to the management of MAM.

The importance of MAM should not be neglected. Although moderate wasting is not a condition of medical urgency, it can easily deteriorate. If some of these undernourished children with moderate wasting do not receive adequate support, they may progress toward SAM, which is a life-threatening condition. The provision of complementary food to prevent and treat moderate malnutrition among children under 2 years of age is also less costly (35-75€ per child) than the treatment of SAM (190€ per episode and per child).(7) Therefore, the management of MAM should be a public health and development priority, also because preventing MAM could go hand-in-hand with preventing stunting.

Children successfully treated for MAM remain vulnerable, it's been demonstrated that just 63-67% of them remain well-nourished the year after treatment.(8) Thus, there is a clear need to improve the outcomes of their management.

When assessing children with SAM, some infectious diseases as VIH, malaria and parasites are systematically checked and treated in low-income countries.(9)

Nonetheless, when we are in front of a case of MAM, very little health prevention is done. They are given supplementary food to eat at their homes and, sometimes, parasites prophylaxis, most of times in insufficient quantity to really eradicate the parasitism.

Not much research has been made on this important issue on the past 30 years, and it is crucial to improve our knowledge in the management of this huge group of children.(10)

PHYSIOPHATOLOGY

There are two main ways to develop undernutrition.

First one is by *decreased food intake*, this can be a result of a *limited availability of food*, or a result of *anorexia*, which means a diminished appetite. The second one is by increased energy expenditure due to a metabolic disorder, that is *cachexia*.

Those often occur together in many types of cancer or in the 'wasting syndrome' observed in patients with acquired immunodeficiency syndrome (AIDS) and chronic inflammatory disorders.

Acute malnutrition in children initially stops the weight gain keeping the growth speed. If the process continues, weight is lost until arriving to wasting. When this goes on for a long time and turns into chronic, a halt in the growth take place, resulting in a decreased height or stunting.(11)

An adaptive response takes place at different levels:

- *Corporal composition*: Our bodies, in average conditions, prefer using the carbohydrates to obtain energy over protein and lipids. Nevertheless, the carbohydrates stored in the body can only supply the energy for about half a day. When we run out of carbohydrates, a progressive depletion of lipids and proteins take place. The fat is the prime source of energy, so that, the rate of depletion remains unabated, until most of the body fat stores are gone. On

opposition, protein undergoes three phases of depletion: rapid depletion at first, then greatly slowed depletion, and, finally, rapid depletion again, shortly before death (Figure 2).(12)

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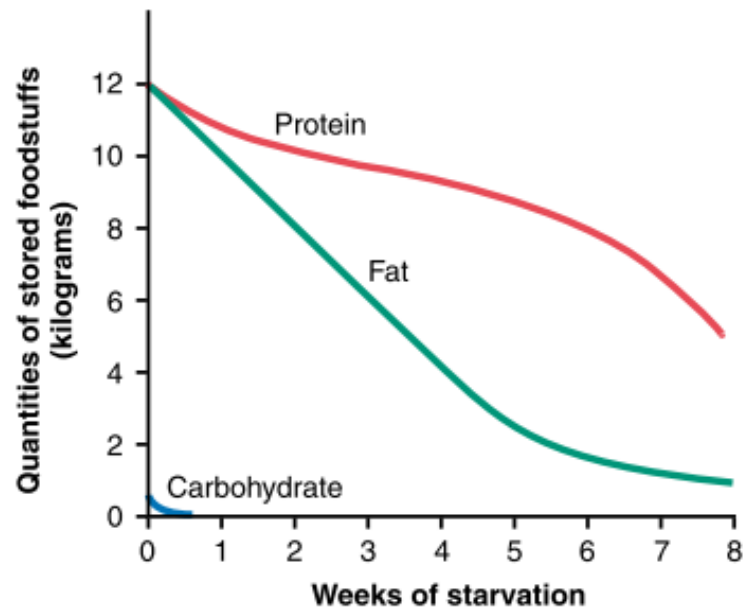
**Figure 2.** Effect of starvation on the food stores of the body (12)

- *Endocrine-metabolic response*: there is a reduction on the plasmatic glucose and, as a consequence, a reduction of the insulin secretion and an augment of glucagon. The glucogenolysis and lipolysis are stimulated.

  As a response to the stress, the levels of adrenalin and cortisol are heightened, which results in more lipolysis and protein catabolism. This increases the free fatty acids that favor the peripheral resistance to insulin. The increased cortisol and free amino acids suppress the GH axis, inhibiting the growth. There is an increase on the SRAA function because of the lowered blood volume and renal perfusion, increasing the retention of water and sodium, which will favor the formation of edema (more in Kwashiorkor) and the depletion of potassium. Gonadotropin levels will also decrease so that there will be a pubertal delay.

- *Immune system*: impaired gut-barrier function, reduced exocrine secretion of protective substances, and low levels of plasma complement. Lymphatic tissue, particularly the thymus, undergoes atrophy, and delayed-type hypersensitivity responses are reduced. (13)

-   *Temperature regulation*: the subcutaneous fat loss reduces the capacity to regulate the temperature and the corporal water, so that, it is common to see hypothermia and dehydration.
-   *Digestive system*: the liver is not able to mobilize the fat, which leads to fat accumulation that produces hepatomegaly. The gastric wall is thickened and there is an atrophy of the mucous, lowering its secretion and facilitating bacterial overgrowth in the small bowel.
-   *Hematopoietic system*: the levels of hemoglobin and red blood cells are diminished
-   *Cardiovascular*: decreased cardiac output, blood pressure and venous return. If the heart fat disappears, the consequence can be a heart failure.
-   *Nervous system*: changes such as the decrease of myelination, neurotransmitters, nervous conduction speed and intellectual development.(11)
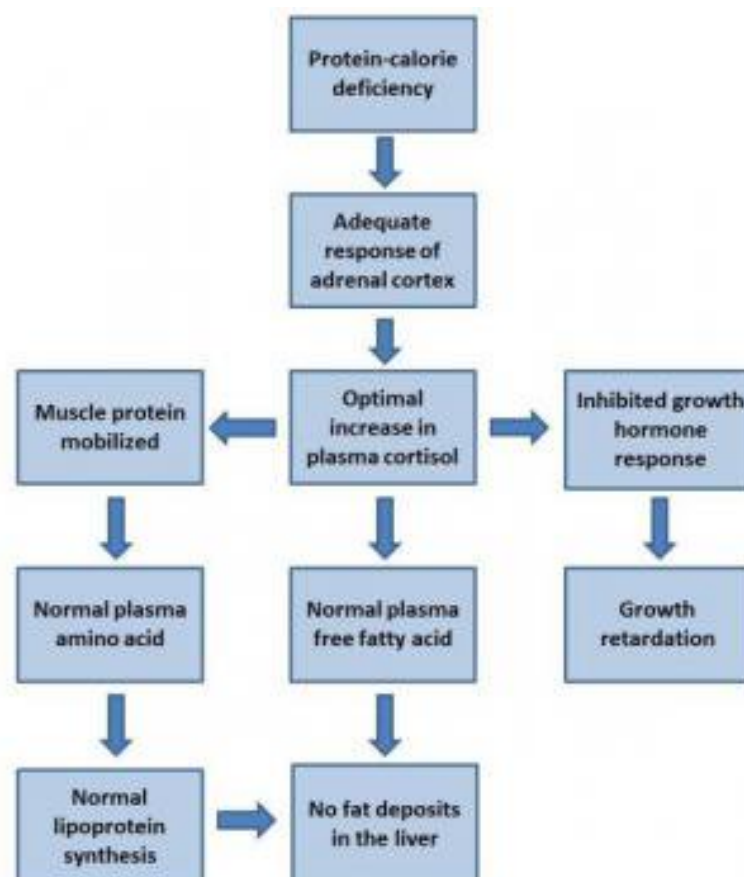
Figure 3. Hormonal adaptation to the stress of malnutrition: The evolution of marasmus.(14)

CLINICAL DIAGNOSIS OF MALNUTRITION

When assessing malnutrition, the key findings are those described at table 1.

The measure of MUAC and the presence of edema are quickly assessed and give a lot of information. To determine the presence of edema, normal thumb pressure is applied to both feet for three seconds. If when the thumbs are lifted a shallow print pit persists on both feet, then the child presents edema. Also the lower legs and the face of the child are checked to view the severity of the child's swelling.(15)

Table 2. Classification of edema. (adapted from (15))

No edema	0
Edema below the ankles	+
Edema in both feet and legs, below the knees	++
Edema on both feet, legs, arms and sacral pad and eye lids	+++
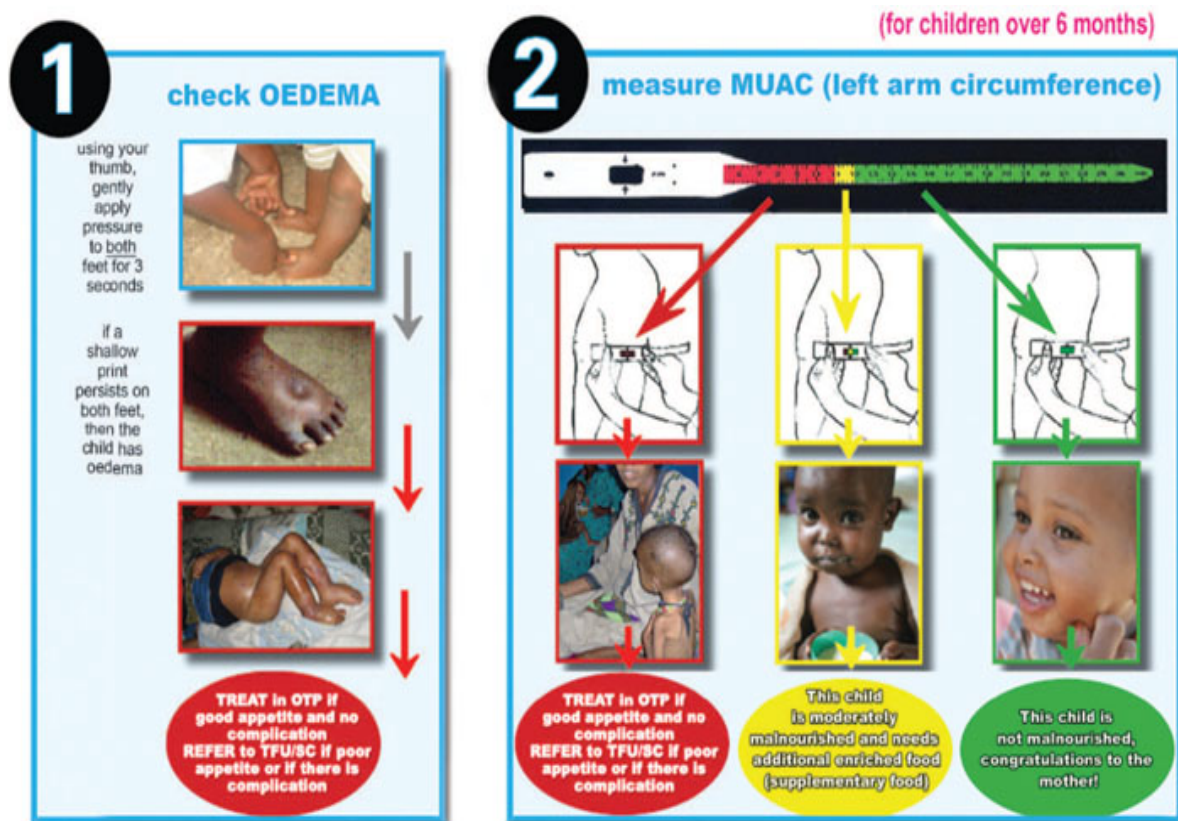
Figure 4. How to identify acute malnutrition in a child. (16)

Other physical findings:

- Decreased subcutaneous tissue: Areas that are most affected are the legs, arms, buttocks, and face.
- Oral changes: Cheilosis, angular stomatitis, and papillary atrophy.
- Abdominal findings: Abdominal distention secondary to poor abdominal musculature and hepatomegaly secondary to fatty infiltration.
- Skin changes: Dry, peeling skin with raw, exposed areas; hyper pigmented plaques over areas of trauma (see figure 7 and 8).
- Nail changes: Fissured or ridged nails.
- Hair changes: Thin, sparse, brittle hair that is easily pulled out and that turns a dull brown or reddish color(14) (see figure 6).

Figure 6. Blonde hair, is a sign of nutritional insufficiency, but without great significance of prognosis (17)

Figure 7. Peeling skin (11)

Figure 8. Peeling skin (17)

GENERAL MANAGEMENT

The first step is the community mobilization and screening, in order to prevent, detect and treat the cases of acute malnutrition. This is mainly carried out by the health facilities that are closer to the population, but also by the hospitals.

The children are divided depending on their nutritional status, and then they have specific approaches.

Those with MAM or SAM without complications can be treated at a local treatment center for outpatient dietary management and treatment of concurrent infections. However, those with SAM and severe complications are treated as inpatients.

Nowadays, the weight gain of 15-20% over the baseline weight and the absence of edema for at least 2 weeks are considered the recovery criterion.
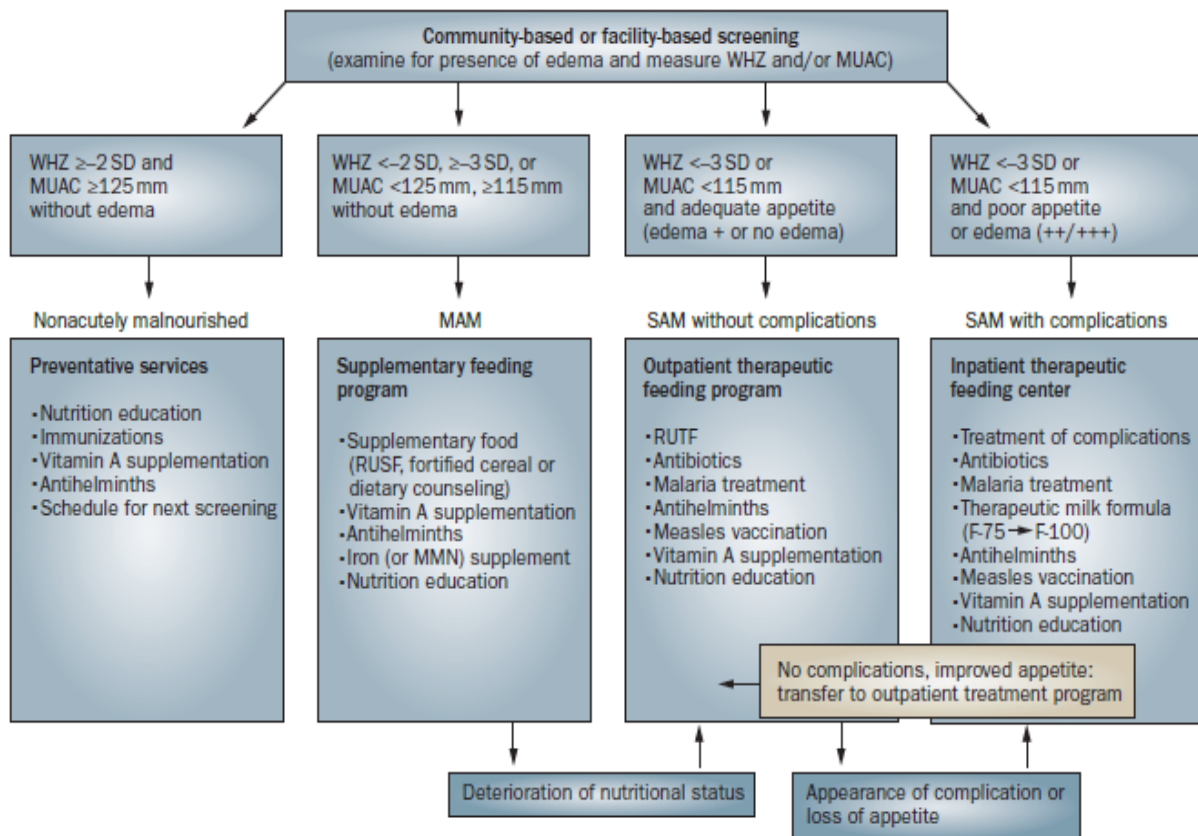
Figure 9. Screening and treatment algorithm for acutely malnourished children, 6-59 months of age. (5)

Reduced lactose, milk-based formulas (F-75 and F-100) are used to meet the nutritional needs of children.

RUTF have been a major advance in treatment of SAM. Those are usually prepared from peanut paste, milk powder or soy protein, vegetable oil, sugar, and vitamin and mineral premixes, are energy dense (about 4.5 kcal/g) and well accepted by children. Because of their low water activity they're resistant to bacterial proliferation and do not require refrigeration. (18) Several studies have proven the benefit of using RUFT among other nutritional treatments (milk-based formulas or cereal-legume blends) in the nutritional rehabilitation of SAM. (19–22)

Nonetheless, regarding the treatment of MAM, RUFT has shown very little advantage versus other options as maize-soy flour blend(19) or corn-soy blend(23), but the price is two to four times higher than those options.

Requirements in children with MAM must fall between the needs in children with SAM and those with a good health condition, that is, they need increased intake of energy

and essential nutrients over and above those required by non-malnourished children and, when necessary, treatment for any associated medical conditions. (24)

There is a supplementary food composition recommendation made by WHO (see table 2), however, it is not yet known which the appropriate approach is, and the outcomes of the available treatments aren't satisfying.

Table 3. Proposed nutrient composition of supplementary foods for use in the management of moderate acute malnutrition in children(24)

TABLE 1
Proposed nutrient composition of supplementary foods for use in the management of moderate acute malnutrition in children[a,b]

| Nutrient per 1000 kcal	Unit	Minimum	Maximum
Protein[c]	g	20	43
Fat	g	25	65
Minerals			
Sodium (Na)	mg	—	500
Potassium (K)	mg	1500	2200
Magnesium (Mg)	mg	280	420
Phosphorus (P)[d]	mg	850	1400
Zinc (Zn)	mg	20	35
Calcium (Ca)	mg	1000	1400
Copper (Cu)	mg	1	3.5
Iron (Fe)[e]	mg	18	30
Iodine (I)	µg	150	350
Selenium (Se)[f]	µg	35	90
Manganese (Mn)	mg	1	2[g]
Vitamins, water soluble			
Thiamin (B$_1$)	mg	> 1	—
Riboflavin (B$_2$)	mg	> 4	—
Pyridoxine (B$_6$)	mg	> 2	—
Cobalamine (B$_{12}$)	µg	> 5	—
Folate (dietary folate equivalent)	µg	> 400[h]	—
Niacin	mg	> 25	—
Ascorbate (vitamin C)	mg	> 150	—
Pantothenic acid	mg	> 5	—
Biotin	µg	> 20	—
Vitamins, fat soluble			
Retinol (vitamin A)	µg	2000	3000
Cholecalciferol (vitamin D)	µg	20	60
Vitamin E (DL-α tocopherol acetate)	mg	>30	—
Phytomenadione (vitamin K)	µg	>50	—
Fatty acids			
ω-6 fatty acid	% energy	>4.5	<10
ω-3 fatty acid	% energy	>0.5	<3
Trans-fatty acids	% total fat		3
Ratios of nutrients (based on weight)			
Ca/P ratio		1.0	1.5
Zn/Cu ratio		5	20
Zn/Fe ratio		0.8	3.5
Vitamin C/Fe		3	16

INTERACTION BETWEEN MALNUTRITION AND DISEASE

Infection and malnutrition have always been complexly linked. Malnutrition is the leading cause of immunodeficiency worldwide, but, at the same time, infectious diseases lead to malnutrition and immune breakdown.

Immune changes that take place due to malnutrition predispose children to severe and chronic infections, most commonly infectious diarrhea, which further compromises nutrition, causing anorexia, raised metabolic needs, decreased nutrient absorption, and straight nutrient losses. Activation and sustenance of immune responses during infection requires increased energy consumption, so, infection itself contributes to malnutrition. The relationship of malnutrition on immune suppression and infection is complicated by the profound effects of a number of infections on nutrition itself. There are different examples of how infection can contribute to malnutrition: gastrointestinal infection can lead to diarrhea; HIV/AIDS, tuberculosis, and other chronic infections can cause cachexia and anemia; and intestinal parasites can cause anemia and nutrient deprivation.

Stimulation of an immune response by infection increases the demand for metabolically derived anabolic energy and associated substrates, leading to a synergistic vicious cycle of adverse nutritional status and increased susceptibility to infection.

This combination is a leading cause of infant mortality. So that, nowadays we are learning more and more about the pathogenesis of this interaction.
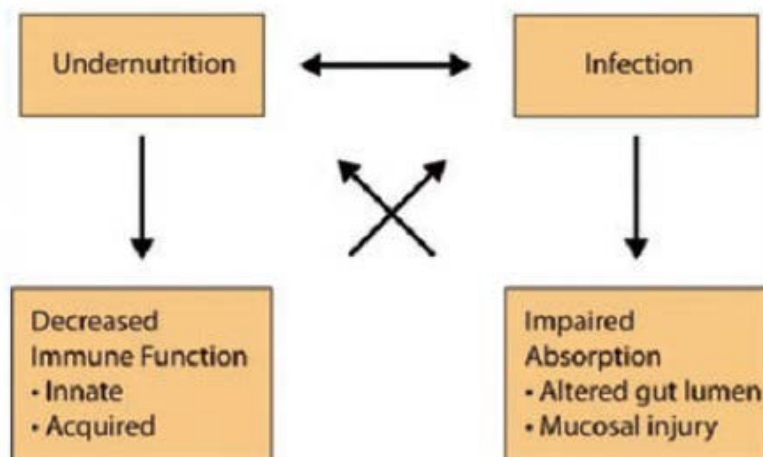
Figure 10. Interaction between malnutrition and infection (25)
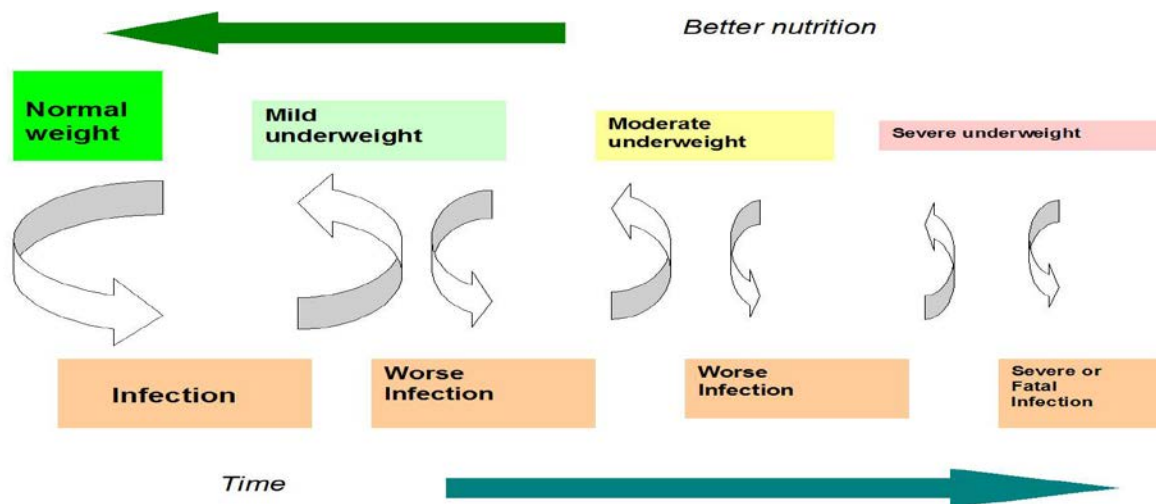
Figure 11. Spiral of malnutrition and infection

The worldwide magnitude of parasite infections is huge. One billion people are infected with soil-transmitted helminths, 200 million people are infected with schistosomes, and 400 million school-aged children are infected with various other intestinal parasites.

Intestinal parasite infection may be associated with decreased food intake, malabsorption, endogenous nutrient loss and anemia. Helminth infection are also associated with cognitive impairment in school-aged children.

It is understood that parasites may lead to malnutrition, but the extent to which malnutrition causes increased parasite infestation is not known; thus, the conditions need to be addressed together. (25).

Some research has been made about this issue, demonstrating facts such as malnourished children are deficient for protective IgE antibodies against Ascaris lumbricoides(26), or as malnourished suffering from helminth infections have high concentrations of total IgE, but these are neither worm-specific nor protective, and their memory T cells do not recognize helminth antigens.(27)

Another concrete parasitism, which is Malaria, is also endemic in the locations where malnutrition mostly beats, because in this areas there are uncontrolled Anopheles mosquito. The concept of malaria is often synonymous with its most obvious symptoms: a characteristic recurrent cycle of fever and chills. This mostly occur in partially immune or non-immune individuals, especially children in high-transmission settings and visitors to endemic areas. There exists also an 'asymptomatic' malaria infection that result from partial immune response, which controls but does not

completely eliminate the infection. Nevertheless, both forms worsen the nutrition situation of the person badly, one in an acute way and the other in an chronic way, increment the needs of supplementary feeding, and are also more likely to happen in a person with a debilitated immune system.(28)

Malnutrition and nutritional alterations, common complications of human immunodeficiency virus infection, include disorders of food intake, nutrient absorption, and intermediary metabolism and play a critical and independent role in morbidity and mortality.(25)
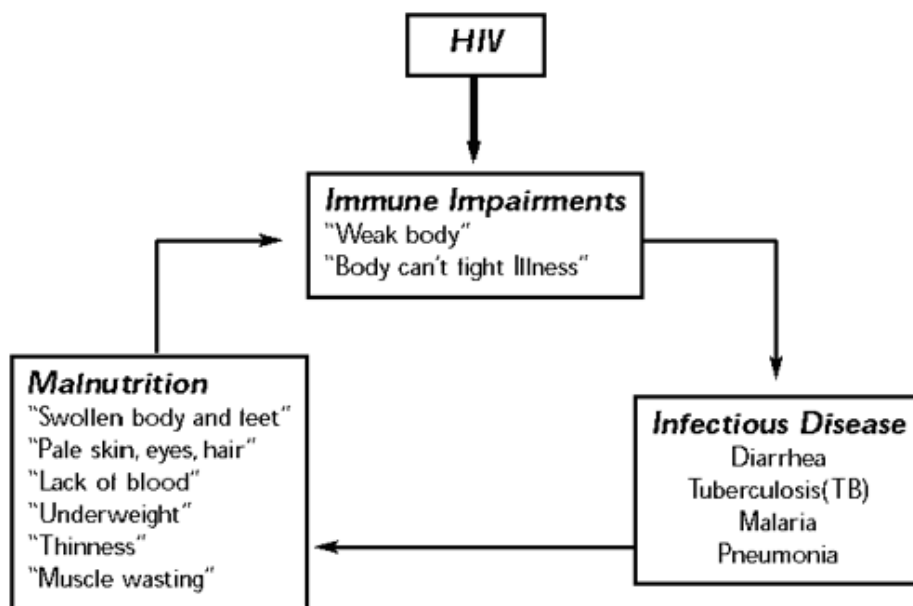
Figure 12. Relationship between HIV and malnutrition (29)

The human immunodeficiency virus is usually linked to tuberculosis, even more if we are talking about developing countries. Tuberculosis (TB) is an infectious disease caused by a bacteria called Mycobacterium tuberculosis. Tuberculosis generally affects the lungs, but can also affect other parts of the body. In children it differs from adults, because the disseminated form is more usual, which is very dangerous. Even so, the children usually have low bacillary charges, so that they are less infective, more difficult to detect, and, finally, misdiagnosed. An estimated number of 230.000 children under 15 years died from tuberculosis worldwide in 2015; 80% of these deaths were in children younger than 5 years. More than 70% of deaths occurred in WHO Southeast Asia and Africa regions, and nearly 20% of the deaths were in children with

HIV infections, most of them in the WHO Africa region. In addition, more than 96% of all tuberculosis deaths occurred in children not receiving tuberculosis treatment. (30) The relationship of malnutrition and tuberculosis is clear but still being studied. The lack of breastfeeding leads to a weakened innate immune response since the first months of life. For the adaptive response, it is known that the Th1-type immune response ruled by the thymus has a great role defending against intracellular bacterium, and its atrophy in malnourished children plays a key role in the risk of infection. Those and other findings that are common in the pathophysiology of malnutrition exposes those children in a higher degree to this debilitating illness.(31)
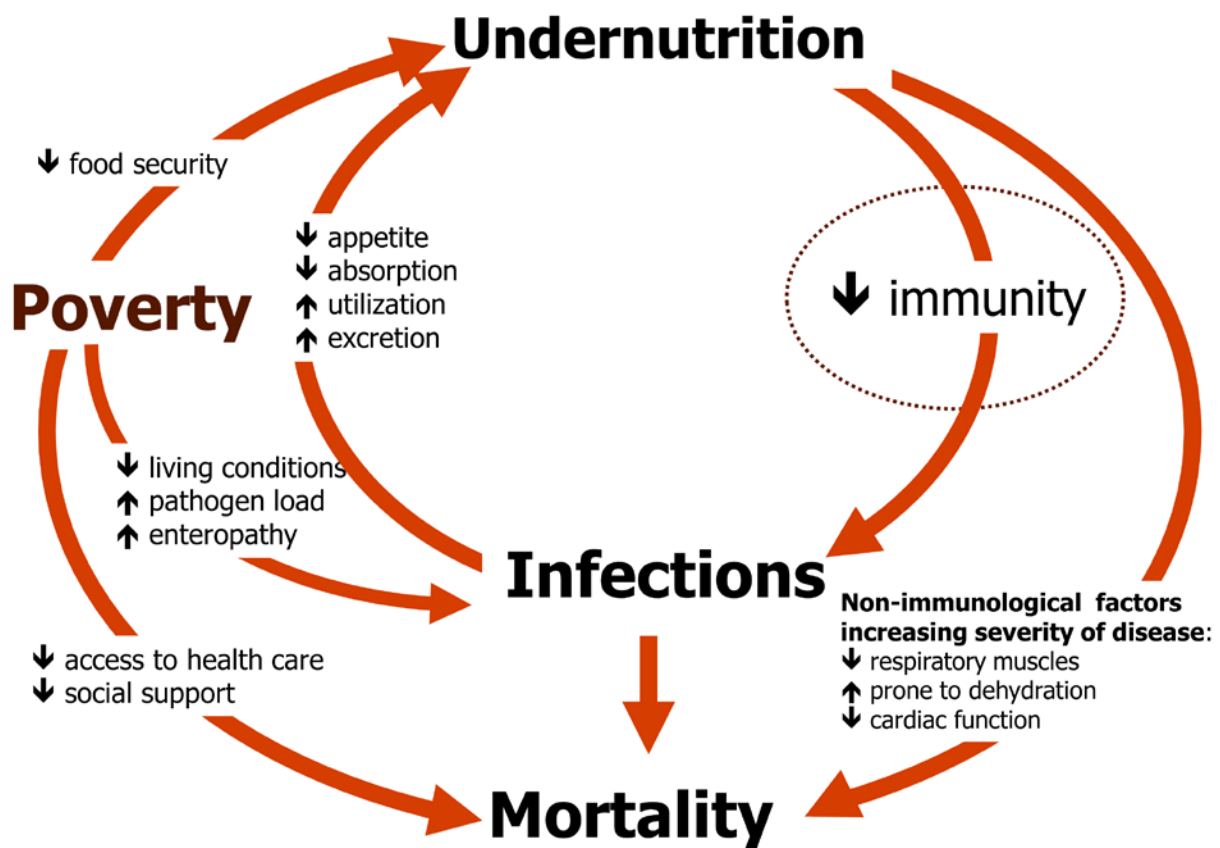
Figure 13. Relationship between malnutrition, infections and poverty (13)

Concluding, malnutrition is responsible, directly or indirectly, for 54% of the 10.8 million deaths per year in children under five and contributes to every second death (53%) associated with infectious diseases among children under five years of age in developing countries. Infection causes energy loss on the part of the individual, which reduces productivity on the community level and perpetuates the alarming

spiral of malnutrition, infection, disease, and poverty.(32)

ANGOLA CONTEXT

Angola is an emerging country located at the Sub-Saharan region of Africa. Bordered by Democratic Republic of the Congo to the north, Zambia to the east, Namibia to the south and the Atlantic Ocean to the west, it is the seventh-largest country in Africa. It has a total population of 25.022.000 people and its capital and largest city is Luanda.(33)

The country was a Portuguese colony until 1975. From then and until 2002, the country faced a four decades period of conflict. Since the end of the conflict, a growing and stabilizing period started. Angola has enjoyed a rapid and sustained expansion of its economy and an enormous increase in government resources. From 2004 to 2012, Angola had the fastest growing economy in Sub-Saharan Africa.

As Angola is completing its transition from a period of reconstruction to a phase of stable development, the country has a great chance to build on successes to date and consolidate its status as an upper-middle income country, where all the society enjoy the benefit of the increased wealth. Of the 24 million Angolans, the majority (13 million) are children. Ensuring that these children and their families benefit from Angola's social and economic development is the best investment that the country can make for the sustainability of growth and success in its future. To do so requires special consideration and targeted investment for the most vulnerable and impoverished members of Angolan society. Focusing on children is also a legal responsibility that Angola committed to fulfil when it was among the first countries to sign the Convention on the Rights of the Child in 1990.(34)

Despite all the above, the reality in the country is far from achieving the expectations. Poor sanitation, difficult access to health facilities, lack of health education and food resources poor management leads to a situation in which still a high amount of population is at a high risk of death and disability. All this hold up the speed growth of the society and expand the gap between social ladders.

Child mortality in Angola is gradually declining, however far too many children continue to die before the age of five. Due to a lack of comparable data over time, child mortality estimates for Angola must be taken with caution but can still be regarded as indicators of the health and wellbeing of children and of society as a whole.
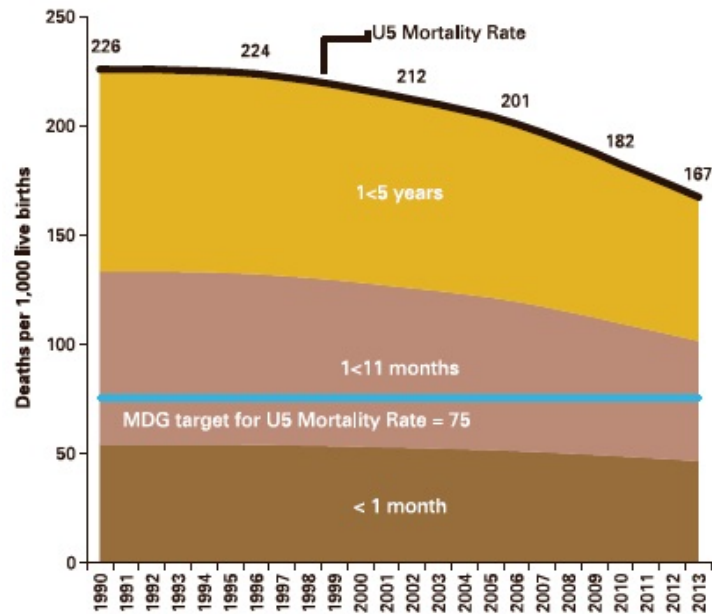
Figure 14. Child mortality estimates in Angola, 1990-2013: Deaths per 1000 live births(34)

Data about malnutrition among the country is still worrying, even more in the group of children below 5 years old, who have a percentage of acutely malnourished children that exceeds 20% in some areas, and a tax of stunting that arrives to 60%.(35) Although no recent data is available, it is likely that acute malnutrition increased during the last years due to a drought emergency in 2012 that affected much of the country and in particular the southern provinces.
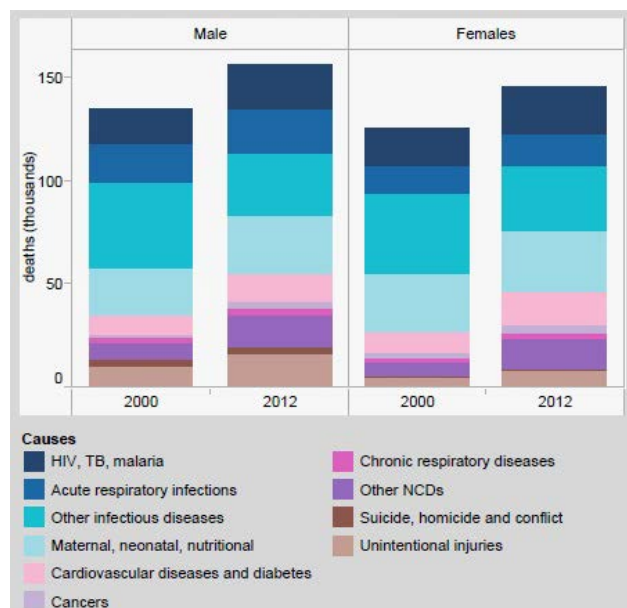
Figure 15. Deaths by broad case groups in Angola(36)

As we can see in figure 15, 'the big three' (HIV, TB, malaria) cause a high burden of the deaths in this country, but even more have the infectious diseases in general, and maternal, neonatal and nutritional factors. This data leave in evidence the kind of health problems that faces this population and supports the credence that still a lot has to be done in order to achieve an equal and health society. Even more, life expectancy is of 51/54(36) years of age, which means that the country is still far from upper-middle income countries rates.

JUSTIFICATION

Acute malnutrition is still a huge problem worldwide, especially in developing countries. This condition also entails different risks, at short and long term, which most of times aren't well assessed because of the background of the population in which this situation mainly occurs.

Moreover, those who are at a greater risk are the children. This means that not only the future of the person but also the future of the society depends on a certain grade on how this issue is solved.

A lot of efforts are being made in order to ameliorate the management and prognosis of children with SAM. Nevertheless, MAM is still a bit neglected by the investigators. This happens as a result of the higher risk of death in the first group, but it's time to start moving forward in the acknowledgement of MAM because it is a needed step through SAM. In addition, improving the assessment of MAM will not only prevent the evolution to SAM, but also will decrease the risk of death that it has itself, that equals this on SAM because of its higher prevalence.(8)

When assessing children with SAM, apart from the nutritional treatment, the WHO recommends discarding certain comorbidities. (9) Notwithstanding, in children with MAM, there is no consensus or recommendation regarding comorbidities.(10) We propose that these comorbidities can be also present in children with MAM, and the knowledge of its implication is expected to improve the progress of these patients.

It will also be interesting to record clinical and analytical variables, which can be object of further investigation to improve our knowledge about the malnourished children who arrive to the hospital and their management.

HYPOTHESIS

In children, similarly to severe acute malnutrition, moderate acute malnutrition is associated with the presence of certain comorbidities (VIH, tuberculosis, malaria, geohelminths, schistosomiasis).

OBJECTIVES OF THE STUDY

Primary objective:

Assess the prevalence of comorbidities (VIH, tuberculosis, malaria, geohelminths, schistosomiasis) in patients with moderate acute malnutrition.

Secondary objectives:

Assess the prevalence of comorbidities (VIH, tuberculosis, malaria, geohelminths, schistosomiasis) in patients with severe acute malnutrition.

Compare the prevalence of comorbidities (VIH, tuberculosis, malaria, geohelminths, schistosomiasis) between patients with moderate acute malnutrition and patients with severe acute malnutrition.

Identify clinical and/or analytical data correlated with the comorbidities.

MATERIAL AND METHODS

STUDY DESIGN

This is a cross-sectional study carried out at Nossa Senhora da Paz Hospital in Cubal, city that pertains to the province of Benguela in Angola.

STUDY POPULATION

Children between 6-59 months attending the nutritional service of the Nossa Senhora da Paz Hospital in Cubal, Angola. Those with acute malnutrition who fall within the inclusion criteria and excluding the exclusion criteria, will be consecutively selected.

Figure 16. Angola map (37)

As largely explained before, Angola is a developing country which grows fast and has a great potential. In its 1.246.700 km^2 of extension lives 25.022.000 people, half of them children. The languages of Angola are predominantly Bantu and Portuguese, with a small minority of Kung and Khoe speakers. Life expectancy there is around 51 years old.(36)

Regarding objective data for development, Angola is number 150 out of 188 in the Human Development Index (HDI), prepared by the United Nations Development

Program (UNDP). It measures human development in each country, based on advances in: long and salient life, knowledge, and decent standard of living. Angola Human Development Index value for 2015 is 0.533, putting the country in the low human development category. Even worse, if adjusted by inequality, it falls to 0.336.

Children have a key role in their population analysis. The major part of the population are children with a median age of 16 years old, and the mortality rate under 5 years old (83 per 1.000 live births)(38) is high, mainly because of infectious and diarrheal diseases.
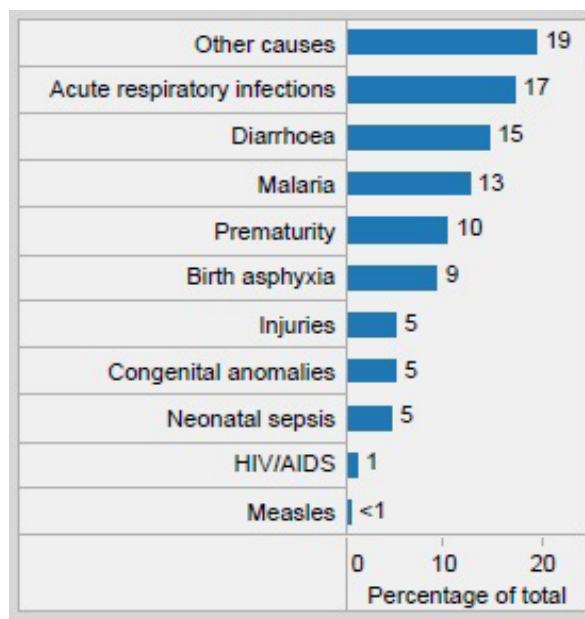
Figure 17. Distribution of causes of deaths in children under 5 years old, 2013 (36)

SAMPLE SIZE

With an alpha risk of 5%, assuming maximum indeterminacy, infinite population, statistical power 80% assuming a drop out of 10%, in a bilateral test, the sample size was 668. This figure corresponds of a precision of at least 4%.

The computation of the sample size was done with the software of professor Marc Saez, based on the package 'pwr' of the free statistical environment R (version 3.4.3).

Sampling method

A systematic and consecutive sampling will be done to pick the patients arriving to the nutritional service of the Hospital who meet the study criteria.

The sample will be divided in two groups of 334 children each one, one for the children with moderate acute malnutrition, and another for the children with severe acute malnutrition.

Estimated time of recruitment

Based on previous information about the amount of patients arriving to the hospital with our studied condition, we estimate that an amount of 6 months could be enough for taking the sample. Nevertheless, we will extend this time to 9 months to ensure that even if any unexpected event occurs we will have time enough to recruit the needed number of patients.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria

Weight/height or length less than -2 SD in relation with the WHO child growth standards. (*Annex I*)

Age between 6 – 59 months of age.

Caregivers consent to participate. (*Annex II*)

Exclusion criteria

Refusal to participate.

Presence of disability including congenital or acquired disorders affecting growth.

Previous diagnosis of cancer (solid or hematological).

Immunosuppressive treatment.

VARIABLES OF THE STUDY

Because it is a cross-sectional study and we're not exactly defining causality, we will not denominate the variable as dependent or independent, our variables will the anthropometrical measurement, the comorbidities and the covariates.

1. Anthropometrical measurement

1. 1. *Weight*

There are many types of scales currently in use. The UNISCALE (made by UNICEF) has the recommended features for a scale and is the one that will be used in the study. It is powered by a lithium battery that is good for a million measurement sessions.

If the child is less than 2 years old or is unable to stand, tared weighing will be done.

Explain the tared weighing procedure to the mother:

1. The mother will remove her shoes and step on the scale to be weighed alone first. She may need to adjust any long garments that could cover the display and solar panel of the scale.
2. After the mother's weight appears on the display, tell her to remain standing on the scale. Re-set the reading to zero by covering he solar panel of the scale
3. Give the mother her child to hold
4. The child's weight appear on the scale: records child's weight.

Mother's weight alone   Taring the scale   Baby's weight appears on display.
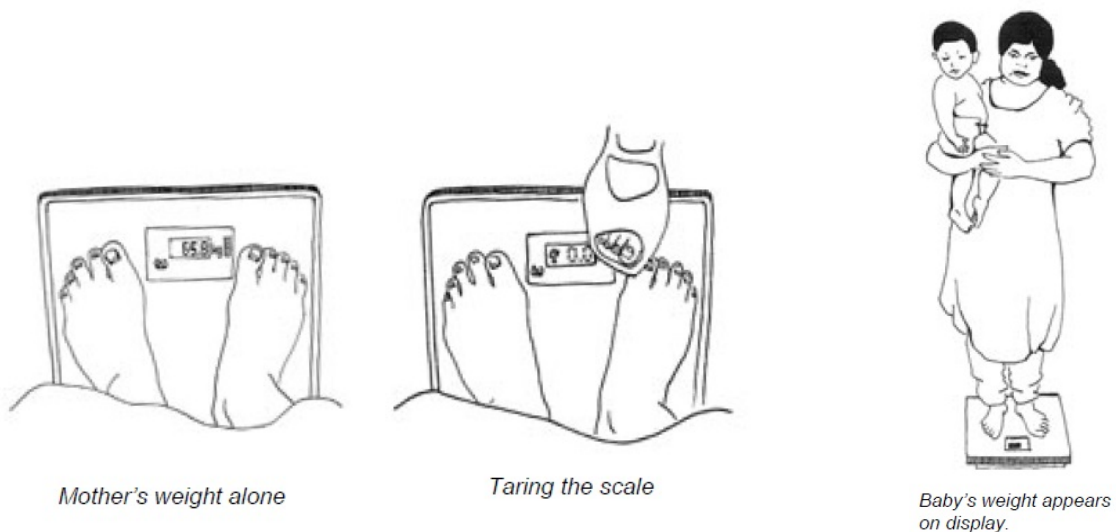
Figure 18. Tared weighing procedure. (39)

If the child is 2 years or older, the weighing of the child will be done alone if the child will stand still.

1. Explain that the child will need to step on the scale alone and stand very still.
2. Undress the child. Explain that child needs to remove outer clothing in order to obtain an accurate weight. Babies should be weighed naked; wrap them in a

blanket to keep them warm until weighing. Older children should remove all but minimal clothing, such as their underclothes.

1. 2. *Length or height.*

Depending on a child's age and ability to stand, measure the child's length or height. A child's length is measured lying down (recumbent). Height is measured standing upright.

- **If a child is less than 2 years old**, measure recumbent length.
- **If the child is aged 2 years or older and able to stand**, measure standing height.

In general, standing height is about 0.7 cm less than recumbent length. This difference was taken into account in developing the WHO growth standards.

Therefore, it is important to adjust the measurements if length is taken instead of height, and vice versa.

- If a child less than 2 years old will not lie down for measurement of length, measure standing height and **add 0.7 cm** to convert it to length.
- If a child aged 2 years or older cannot stand, measure recumbent length and **subtract 0.7 cm** to convert it to height.

Equipment needed to measure length is a length board (sometimes called an infantometer) which should be placed on a flat, stable surface such as a table. To measure height, use a height board (sometimes called a stadiometer) mounted at a right angle between a level floor and against a straight, vertical surface such as a wall or pillar.
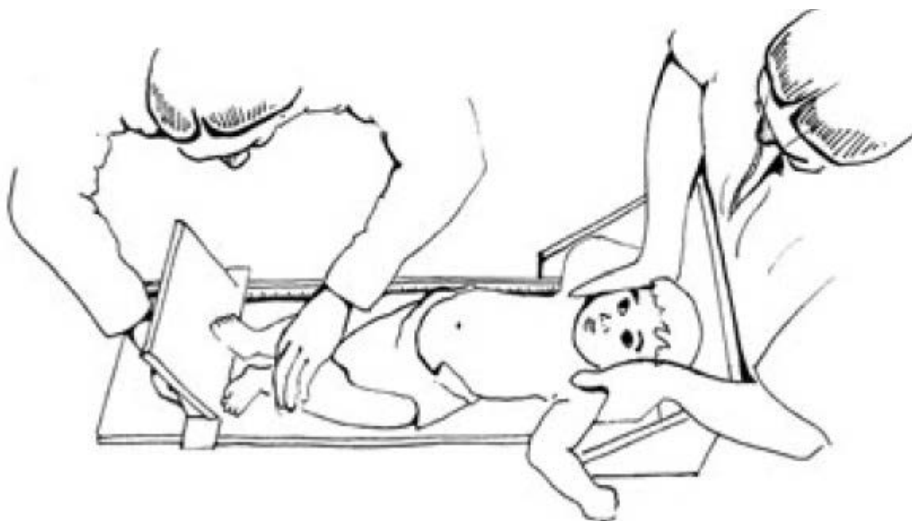
Figure 19. Measuring length (39)

Image 20. Measuring child's height. (39)

Proper care for the scale and length/height boards is important to ensure that measurements are as accurate as possible. The equipment will be kept clean and stored at a normal indoor temperature, protected from humidity and wetness. It will be checked by the personnel daily to ensure its correct functioning.(39)

1. 3. _MUAC_

MUAC is the circumference of the left upper arm and is measured at the mid-point between the tips of the shoulder and elbow. It is divided in colors: green is normal, yellow means 'at risk', orange is related with MAM, and red means SAM. To measure:

1. Bend the left arm, find and mark with a pen the olecranon process and acromium.
2. Mark the mid-point between these two marks.
3. With the arm hanging straight down, wrap a MUAC tape around the arm at the midpoint mark.
4. Measure to the nearest 1 mm.(40)
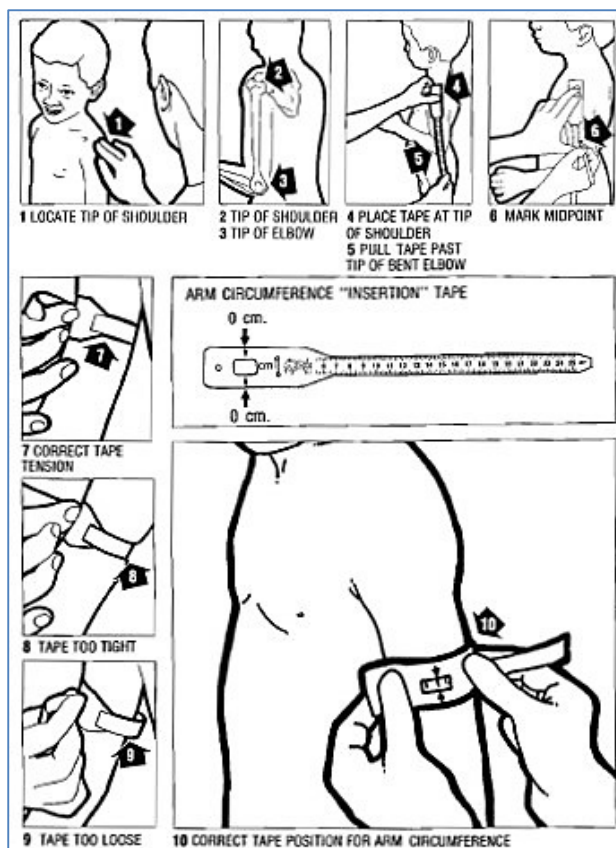
Figure 21. MUAC assessment (41)   **Figure 22.** MUAC tape (40)
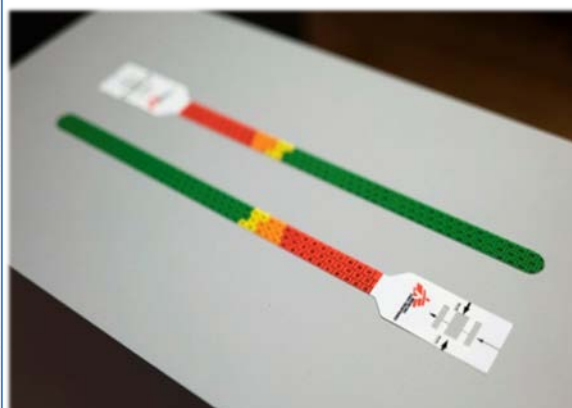
2. Comorbidities

The presence or absence of each comorbidity will be assed as a qualitative dichotomous variable.

2. 1. _HIV_

First, we will perform Determine™ test and then the result will be confirmed with Uni-Gold™ test. This sequence of positive test will be recorded as positive for this comorbidity.

Alere Determine™ HIV–1/2 Ag/Ab Combo is an in vitro, visually read, qualitative immunoassay for the simultaneous detection of Human Immunodeficiency Virus Type 1 (HIV-1) p24 antigen (Ag) and antibodies (Ab) to HIV Type 1 and Type 2 (HIV-1 and HIV-2).(42) The test is able to distinguish between an acute HIV-1 infection and an

established HIV-1 infection when the blood sample is positive for the HIV-1 p24 antigen but negative for HIV-1 and HIV-2 antibodies.

Interpretation:

a)Ab reactive   **b)**Ag reactive   **c)**Ag and Ab reactive   **d)**Non-reactive
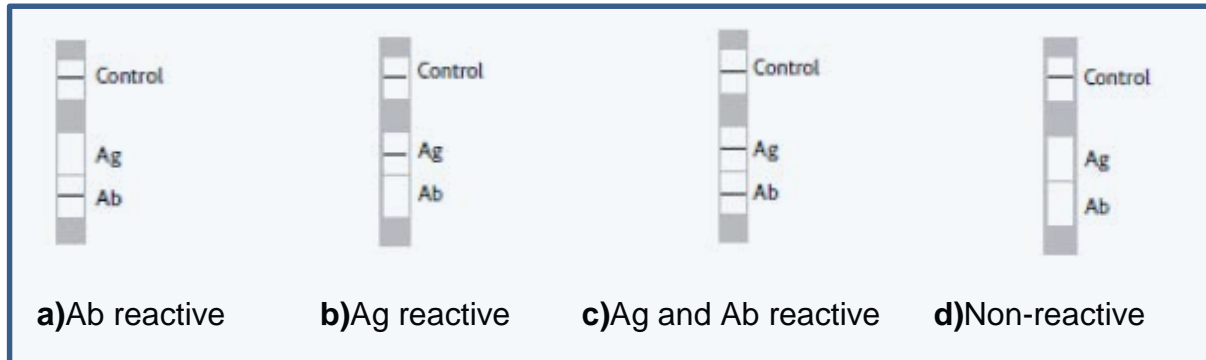
Pink/red color line appears in the areas. The intensity of the lines may vary, any visible pink/red color, regardless of intensity, is considered REACTIVE.

a) Antibody reactive: a line appears in the control area and in the lower test area (ab). It is interpreted as PRELIMINARY POSITIVE for HIV-1 and HIV-2 antibodies

b) Antigen (HIV-1 p24) reactive: a line appears in the control area and in the upper test area (ag). It is interpreted as PRELIMINARY POSITIVE for HIV-1 p24 antigen. A test result that is PRELIMINARY POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV-1 infection in the test subject. In this case the acute HIV-1 infection is distinguished from an established HIV-1 infection in which antibodies to HIV-1 are present.

c) Antibody reactive and antigen (HIV-1 p24) reactive: a line appears in the control area, in the upper test area and in the lower test area. It is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies and HIV-1 p24 antigen

d) Non-reactive: a line appears just in control area. It means that HIV-1 or HIV-2 antibodies and HIV-1 p24 antigen were not detected in the specimen.

e) Invalid: no control line. The test should be repeated.

a), b) and c) results will be considered positive for HIV. d) will be considered as negative for HIV. e) will be considered Indeterminate if repeating the test is not possible.

Uni-Gold™ HIV is an immunoassay of only one use for the qualitative detection of HIV-1 and HIV-2 antibodies in serum, plasma and blood. It is designed as a helping tool for the diagnosis of HIV-1 and HIV-2 infections. The kits will be stored at 2 – 30⁰. (see *Annex III*) Result:

- Reactive: 2 lines of any intensity appear in both the control and test areas.
- Non-reactive: 1 line appears in the control area and no line in the test area.
- Invalid: No line appears in the control area. Test will be repeated with a new test device even if a line appears in the test are

Reactive result will be considered as positive for HIV infection. Non-reactive result will be considered as negative for HIV infection. Invalid result will be considered as Indeterminate if repeating the test is not possible

2. 2. *Tuberculosis:*

The positive result for tuberculosis diagnosis in any of the following will be considered as positive for tuberculosis.

Although conventional light microscopy of Ziehl-Neelsen-stained smears directly from sputum is the most widely available test for diagnosis of tuberculosis in resource-limited settings we chose LED fluorescence microscopy because of it's better performance stain in the diagnosis of tuberculosis (6% more sensitivity) and in patients with disseminated form or with HIV.(43) Gastric lavage aspiration sample is preferable in children because it's difficult to obtain a right sputum sample.

LED fluorescence microscopy and geneXpert will be assessed in gastric aspiration samples. Three gastric aspiration lavage will be done to take three samples. The patient will provide a specimen on the spot upon first attendance at the health care facility, a second specimen one hour after the first specimen, and a third specimen the following morning.

LED fluorescence microscopy will be conducted on standard microscopes fitted with adaptors consisting of a specialized objective with an attached LED light source. LED-FM smears were examined at 200x, with confirmation of positive smears at 400× magnification.

Smears will be classified as positive when ≥1 AFB was detected per 100 fields, and patients will be considered smear-positive if they had ≥1 positive smear.

Smear-positive patients will be classified as positive for tuberculosis infection. Smear-negative patients will be classified as negative for tuberculosis infection. If the sample is lost or damaged or invalid for any reason it will be classified as indeterminate for tuberculosis infection

Xpert MTB/Rif test is a cartridge-based fully automated NAAT (nucleic acid amplification test) for TB case detection and rifampicin resistance testing, suitable for use in disease-endemic countries. It purifies, concentrates, amplifies (by rapid, real-time PCR) and identifies targeted nucleic acid sequences in the TB genome, and provides results from unprocessed samples in less than 2 hours, with minimal hands-on technical time.

If Mycobacterium tuberculosis DNA is detected, the patient will be classified as positive for tuberculosis infection, if Mycobacterium tuberculosis DNA is not detected, the patient will be classified as negative for tuberculosis infection. If the result is invalid, the test has to be repeated and, if not possible, it will be classified as indeterminate.(44)

Because it's difficult to completely rule out the diagnosis of tuberculosis by the diagnostic test, clinical criteria should also be taken into account. We will use the **Modified Edward's score for diagnosis of Tuberculosis in children** (see *Annex IV*). In order to fulfill the criteria a tuberculin test will also be assayed. A punctuation of 7 or more in the score suggest a tuberculosis infection.

Also a **chest radiography** will be assessed. Those are the interpretations:

1) Lung condensation without hiliar or mediastinal adenomegaly and without pleural evident involvement will be called pneumonical form.
2) Lung condensation associated to an incremented hiliar or mediastinal ganglia will be called pneumoganglionar form.
3) Lung condensation associated to a pleural leak will be called pleuropulmonar form.
4) Hiliar or mediastinal adenomegaly without involvement of the lung or the pleura will be called ganglionar form.

5) Diffuse lung infiltrate, like disseminated micronodulary injuries, will be called miliar form.

6) Another patterns will be called miscellanea.

It will be considered tuberculosis forms the ganglionar, pneumoganglionar and miliar forms.

2. 3. *Malaria*

The positive result of any of the following test will be recorded as positive for malaria infection.

Malaria RDTs will be assessed in a blood sample:
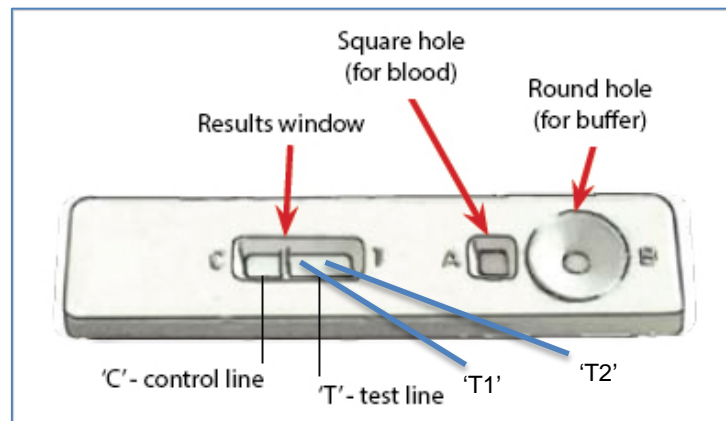
Figure 23. Malaria RDTs cassette (adapted from (45))

Visible test and control line will be recorded as positive for malaria infection. A line in T1 zone means falciparum malaria, and a line in T2 zone means non falciparum malaria. If both are present it means a mixed infection. Visible control line but empty test line will be recorded as negative for malaria infection. If control line is not present the result is invalid and the test has to be repeated. If the test can't be repeated it will be recorded as indeterminate. (46)

Thick and thin blood smear:

The visualization of the parasites will be considered as positive for thick and thin blood smear. Not visualizing the parasites will be considered as negative for thick and thin blood smear.

An invalid blood smear or lost or damage of the smear will be considered as indeterminate.

The thin blood smear is more likely to distinguish between *Plasmodium* specimens. The expected one is *Falciparum*, because it is the most prevalent in Angola (99%). Even so, if another specimen is detected it will be also recorded.
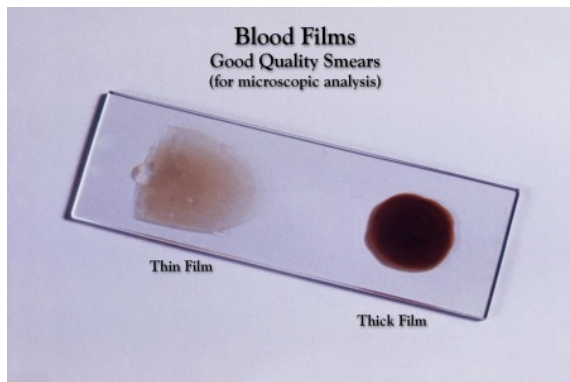
Figure 24. Thin and thick blood smear (from(47))
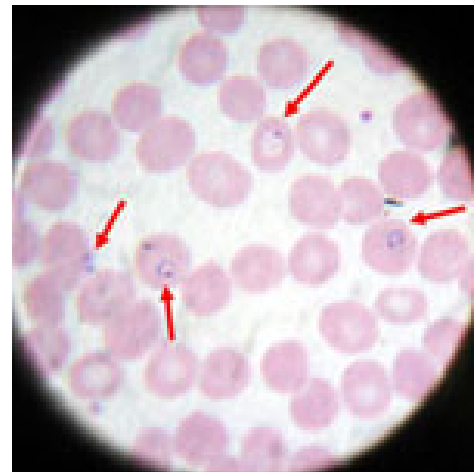
Figure 25. Infected red blood cells (from(47))

2. 4. _Geohelminths_

The finding of pathogenic helminthic eggs/larvae will be recorded as positive for geohelminth infection.

Microscopic examination of stool sample:

Two techniques are routinely done at the laboratory. First, a formol-ether concentration technique and, secondly, the Baermann technique based on the active migration of larvae from fresh stool samples (when faeces are suspended in water, the larvae move into the water, sink to the bottom and can be collected for identification).

The most expected species are Strongyloides stercoralis, Hymenolepis spp,, hookworm, Enterobius vermicularis, Ascaris lumbricoides, Taenia spp, Fasciola hepatica and Trichuris trichiuria. Basing our assumption in a cross-sectional study made at school-aged children in Angola(48)

Found pathogenic helminth eggs/larvae will be recorded.

2. 5. *Schistosomiasis*

The presence of this parasite eggs will be recorded as positive for schistosomiasis infection.

Urine sample will be **microscopically examined** for parasite eggs (Schistosoma haematobium eggs)

Stool sample will also be **microscopically examined** for parasite eggs (Schistosoma mansoni)

The microscopic findings will be recorded. The visualization of the eggs in urine will be recorded as positive for urinary schistosomiasis infection. The visualization of the eggs in stool will be recorded as positive for intestinal schistosomiasis infection. The visualization in both samples will be recorded as positive for urinary and intestinal schistosomiasis infection. Not visualizing eggs in any sample will be recorded as negative for schistosomiasis infection. Losing or damaging a sample or lack of information will be recorded as indeterminate for schistosomiasis infection.

3.   Covariates
3. 1. *Analytical varibles*

In a blood sample of the patient the prepared laboratory that the hospital has will assess:

- Complete blood count:
    Number of red blood cells measured in millions/mm3, hematocrit (proportion between red blood cells and plasma volumes) in percentage

(%), hemoglobin measured in g/dL, mean corpuscular volume in fl, mean corpuscular hemoglobin concentration in g/dL, mean corpuscular hemoglobin in pg.

- o Number of leukocytes per mL, number of neutrophils per mL and %, eosinophils per mL and in %, basophils per mL and in %, lymphocytes per mL and in %, monocytes in %.
- o Platelets per mL
- Glucose in blood measured in mg/dL
- Sodium in blood measured in mEq/L
- Potassium in blood measured in mEq/L
- Calcium in blood measured in mg/dL
- Urine rapid test. High leukocytes or nitrites may be a sign of bacterial infection. This will be recorded as urinary tract infection.

3. 2. *Clinical variables*

The presence of the main signs and symptoms of the studied comorbidities observed in the patient will be recorded:

- Swollen lymph nodes: yes/no
- Tiredness: yes/no
- Diarrhea: yes/no
- Cough: yes/no
- Hemoptysis: yes/no
- Dyspnea: yes/no
- Profuse night sweating: yes/no
- Headache: yes/no
- Shaking chills: yes/no
- Abdominal pain: yes/no
- Hematuria: yes/no
- Thrush: yes/no
- Hepatomegaly: yes/no and write down the length in centimeters
- Hepatomegaly: yes/no and write down the length in centimeters

- Fever: record with an approved and calibrated thermometer in Celsius degrees.

Note that some of them may be difficult to exactly assess in the patient, but the skilled health professional in the field will do the best examination.

3. 3. *Other covariates*

- Age: this variable will be recorded in months and will be expressed as a quantitative discrete variable.
- Gender: this variable will be presented as a qualitative dichotomous variable (male or female).

PROCEDURE

1. When arriving to the nutritional health service of the hospital the children will be measured and weighed as previously explained.
2. If the children meets the inclusion criteria he or she will be invited to participate. The conditions of the study will be explained to the caregivers and they will be asked to sign the IC.
3. Children who have medical complications, severe edema (+++), or poor appetite (fail the appetite test) or present with one or more IMCI danger signs should be treated as inpatients.
4. The doctor and the CHW, at their times, will have a sheet to fulfill with all the test and examinations that have to be done and recorded about the child's health and characteristics needed for the study (*Annex V*). The doctor is responsible of the first physical examination and its recording. The nurses will be responsible of assessing the anthropometrical measurement and of taking the samples (gastric lavage, blood, urine and stool samples).
5. The anthropometric data will be analyzed by WHO Anthro 2010 software to generate Z-score values.
6. The laboratory of the hospital Nossa Senhora da Paz will be the responsible of doing the laboratory tests for diagnosing the comorbidities, and for the analytical examination. The procedures per each one are standardized and are

also done routinely in the laboratory so they will follow the same steps when assessing the samples in our study. The health worker in charge will take notes of the results facilitated by the laboratory in the given sheet.

7. The X-ray machine is also available at the hospital and will be assessed as per their protocols. The results will be recorded by the health worker in charge.

8. All children will receive their correspondent treatment based on the WHO protocols and will also receive treatment for any associated medical condition that can be potentially found during the study.

9. The data will be analyzed by the statistician anonymously, giving them the identification number of the patient.

STATISTICAL ANALYSIS

Descriptive analysis

The comorbidities associated with moderate and severe acute malnutrition (objective 1 and objective 2 respectively) will be summarized by means of proportions (prevalence).

The relationship between the analytical and clinical covariates and moderate and severe acute malnutrition (objective 4) will be assessed using means (standard deviations) and medians (interquartile range) in case of quantitative variables (with symmetric or asymmetric distributions respectively) and proportions for the qualitative variables

These analysis will be stratified by the covariates. Quantitative covariates will be categorized.

Bivariate inference

The estimation of the prevalence of the comorbidities associated with moderate and severe acute malnutrition (objective 1 and objective 2 respectively) will be estimated with proportions and 95% of confidence interval.

Student's t and Mann-Whitney's U will be used to test the difference on means and medians (respectively) of analytical and clinical covariates in moderate and severe acute malnutrition groups. Difference in proportions will be tested by the Pearson chi-square (objective 4)

These analysis will be stratified by the covariates. Quantitative covariates will be categorized

The differences of the prevalence of the comorbidities between moderate and severe acute malnutrition (objective 3) will be tested by means of the Pearson chi-square.

Again, the analysis will be stratified.

Multivariate analysis

The difference of the prevalence of the comorbidities between moderate and severe acute malnutrition (objective 3) will be adjusted by the covariates in a multivariate model (logistic regressions).

ETHICAL CONSIDERATIONS

This study will be carried out taking into account the basic principles established by:

- ✓ The World Medical Association in the 'Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects' (last reviewed in October 2013) which is universal and everybody has to follow
- ✓ The Ethics of Research Related to Health care in Developing Countries. Nuffield Council on Bioethics (2005)
- ✓ The Standards and Operational Guidelines for Ethical Review of Health-Related Research with Human Participants. World Health Organization, WHO (Geneva, 2011)
- ✓ The International Ethical Guidelines for Biomedical Research involving Human subjects. Council for International organization of Medical Sciences, CIOMS (Geneva, 2016)

The research protocol should be presented, evaluated and submitted to the Clinical Research Ethical Committee (CEIC) at the Hospital Nossa Senhora da Paz in Cubal, Angola, before the beginning of the study. After its ending, the final report should be also presented to the CEIC.

Note that our study will also follow the "Ley de Autonomía 41/2002, de 14 de noviembre" about the basic regulation of the autonomy of the patient and of rights and obligations in the matter of information and clinical documentation.

According to "Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal", personal and clinical information of participants will be confidential and only used for the purpose of the research. Moreover, all data will be analyzed anonymously. The participant (or the responsible person of the participant) will always be allowed to modify or destroy any of their collected data.

The study will also have to follow the "Ley 14/2007, Real Decreto 1716/2011", that shall apply to the use of biological samples for the purposes of scientific and technical research, which includes innovation and development as the principal or secondary purpose of obtaining, storing or transferring them. The rights of the subjects must be respected whenever their biological material is used to obtain new scientific knowledge, confirm hypotheses, or carry out activities of technological adaptation, quality controls, teaching, and others.

Those responsible for the participants will be personally informed by researchers and an informative document about the study will be given to them, where they will find all the information of the process of data collection, measurement of anthropometry, the necessary additional tests (basic tests for HIV, tuberculosis, including X-ray, malaria, geohelminths and schistosomiasis; blood analysis, urine test) to which he/she should be submitting, and objectives of the study will be explained in detail. Thus preserving the right of autonomy of the participant. Parents or guardians of all participating children must give informed consent, and have signed it before the children is included in the study (*Annex II*). Information must be given both orally and written, in Portuguese and Bantu, and it has to be confirmed that the information has been well received and understood through the interpreter.

STUDY LIMITATIONS

Because it is a cross-sectional study we are not able to establish causality between the studied factors.

The diagnostic tools used are those available at the field, which means that many of the test are not the gold standard ones because the most used test there are quicker and cheaper, but also have a lower sensitivity and specificity. So that, it is possible to misdiagnose or over diagnose some cases, but it will be attempted to solve with the sample size and the statistical analysis.

The clinical meanings of our study (if the hypothesis is confirmed) can't be assessed and would have to be a basis for further investigation.

The team will be exposed to possible political and/or weather problems. If so, the research coordinator is in charge of maintaining them in a safe facility, and all the data of the study would be stored until the study could keep going through.

The Hospital Nossa Senhora da Paz is not completely computerized, so that, many information will have to be stored in paper until the Forms are completed and data is dumped into a database, which increases the possibility of information loss. The research team will be told about this issue to maximize the care and minimize the problem.

WORK PLAN AND CHRONOGRAM

The reference points of our study are commented below, and a chronogram is presented after the explanation for easier visualization of the whole process. The following sequence of activities will be carried out by the main investigator and its team.

- **PHASE 1**. Preparation and coordination (4 months)
  - o Activity 1.1. Protocol consideration and elaboration. Once ready, the protocol will be evaluated by the Ethical Committee of the Hospital Nossa Senhora da Paz in Angola. The objectives and covariates chosen

for our study are the result of a clinical need detected by the clinical researchers and a bibliography revision of the subject.

- o Activity 1.2. Coordination and preparation of the research team. Five training days will be necessary, 8 hours a day, to ensure the well performing of the data collection. During this phase also a pilot study will be carried out to check that the Form is perfectly understood and assessed.

  A research coordinator will be in charge of maintaining the team, solving the problems that could appear and supervise the process

  Community Health Workers will help the team to adapt and understand the environment and the community, and will be in charge of filling the Data collection Form.

  Nurses will be the ones who do the anthropometrical measurement and who take the test samples of the children (blood, stool, urine, gastric lavage)

  Laboratory workers will be ready to an incremented number of test assessing and x-ray technician will also be part of the team and introduced in the forms that the thorax radiographies are going to be recorded.

  The interpreter will ensure that all the information in the study is comprehended by the participants.

  Doctors will perform the initial visit, and will be in charge of ensure the well-being of the participant. If the doctor finds a dangerous situation or an emerging disease condition, the needed treatment will be performed or even the hospitalization of the children if required.

- **PHASE 2**: Field work and data collection (1 year)
  - o Activity 2.1. Data collection. Based on previous data of the Nutrition Service of the hospital, we expect to have completed our sample data of 668 children in 9 months. Once everything is ready and the material is checked, anthropometric measures will be taken for each child who arrives to the service. If the children are within the inclusion criteria and do not have exclusion criteria, it is proposed to enter the study through the information sheet and informed consent is offered (Annex II), and the

battery of tests will be carried out by the nurses, CHW, the doctors and the translator.

Any child that requires treatment, will begin to be treated, according to the protocols of MSF and WHO.

- o Activity 2.2. Once the laboratory test and the evaluation is performed, the data collected on the Form presented in Annex 5 will be digitalized and saved waiting for future analysis.

- **PHASE 3**: Data analysis and final evaluation (3 months).
  - o Activity 3.1. A statistician will be involved in the study, so that, statistical analysis is performed with excellent guarantees. Once the results of the tests of the comorbidities are ready, a statistician hired by the research team will interpret the results and prepare an analysis.
  - o Activity 3.2. The coordinator of the team will prepare a fine report with the results, discussion and conclusion of the study.

- **PHASE 4**: Publication and dissemination of the results (5 months)
  - o Activity 4.1. The results obtained by the statistical analysis must be confirmed by a steering committee and a statistician.
  - o Activity 4.2. Finally, when data is obtained and processed, results can be published and promulgated in written publications and international congresses such as the International Congress of Nutrition.

Project activity	PERSONNEL	2018												2019										
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
PHASE 1. Preparation and coordination																								
1.1. Protocol consideration and elaboration	*Research coordinator CEIC*	■	■																					
1.2. Coordination and preparation of the research team	*All research team*			■	■																			
PHASE 2. Field work and data collection																								
2.1. Participants recruitment and samples collection and examination	*All research team*								■	■	■	■	■	■										
2.2. Data recording	*All research team*														■	■	■							
PHASE 3. Data analysis and final evaluation																								
3.1. Statistical analysis	*Statician*																		■					
3.2. Interpretation of the results and final report	*Research coordinator*																		■	■				
PHASE 4. Publication and dissemination of the results																								
4.1. Scientific publication	*Research coordinator and statician*																				■	■	■	■
4.2. Congress assistance	*Research coordinator*																				■	■	■	■

FEASIBILITY

This work could not be possible without the implication of the Nossa Senhora da Paz Hospital, in particular the Nutritional service, where we will carry out the patient selection, and the laboratory, that will receive all the samples and make the diagnostic tests and analytical check-up with the tools they usually have. Thanks to their experience in the field the work will be assessed with the maximum guarantees.

The possibility of using the resources and basic materials of the hospital such as MUAC bracelets, scales, measurement board, thermometers, etc. (no extra purchase) will be of great help for our investigation.

The community health workers will be the connection with the field for our investigators, and will be the ones introducing us to the community and its understanding. Doctors and nurses from the hospital will also teach us how to manage different and concrete situations that may have not been taken into account.

Those professionals will be paid for their expected extra-work caused by taking part of this study, and will also have meetings at the start to make team building and to ensure that all that has to be done is understood.

Also a statistician will be needed for our work, to ensure the best information analysis.

Nossa Senhora da Paz Hospital is formed by people interested in research, and they have many agreements with Vall d'Hebron Hospital in order to improve their knowledge and management of the diseases that mainly affects their population. Because of that and because of the lack of improvement that they saw among moderately malnourished children they are very interested in performing this study.

In case of something unexpected happens, as political or climatological issues, the security of the workers and the participants are primordial, so the study will stop until the situation gets better and allows it to continue.

BUDGET

For this study we are expecting to need (in addition to those materials already available at the hospital):

Personnel:

- Four Community Health Workers will be given an extra salary of 100€ per month, in the 9 months that they will be needed that is 900€ per each one, 3,600€ in total

- Three laboratory technicians will be given an extra salary of 200€ per month, in the 9 months that they will be needed, that will be 1,800€ each one, 5,400€ in total.

- Three nurses enrolled at the team, in charge of taking measures and collecting samples, will be given an extra salary of 300€ per month, in the 9 months that they will be needed, that will be 2,700€ each one, 8,100€ in total.

- Three doctors will be needed to perform the first physical examination, they will be given an extra salary of 500€ per month, in the 9 months that they will be needed, that will be 4,500€ each one, 13,500€ in total.

- Two interpreter will be needed for the nine months of recruitment and will receive 150€ per their work, 1,350€ each one, 2,700€ in total.

- One radiologist technician will perform the chest radiographies. He/she will be given an extra salary of 200€ per month, per 9 months, that will be 1,800€.

- One statistician will be needed to perform the statistical analysis to make the analysis easier and more reliable. The estimated salary will be 35€/hour. We estimate approximately that it will take 72 hours to complete the work (he or she will work three hours, three days per week, during two months) In total, 2,520€ is the estimated cost for this service.

- One data manager will be hired to make a good data base into which put the data, the estimated cost of that is 250€.

- The research coordinator, who will be the responsible during these two years of follow-up and supervise the process, will receive a salary of 35,000€ per year. Since the study lasts two years, he or she will receive a total of 70,000€.

The training of the staff required for the proper development of the study is estimated to take place over two months. There will be 5 sessions of 8 hours each. A total of 40 hours (27€/ hour). With an expense for it of 1,080 €.

The staff expenses make a total of 108,950€

Diagnostic tests per child:

- HIV: Determine™ and Uni-gold™ will cost 4€
- Tuberculosis: LED fluorescence microscopy, geneXpert testing, chest radiography and tuberculin test will cost 25€
- Malaria: RDT and thick and thin blood smear will cost 10€
- Geohelminths: stool examination will cost 1.75€
- Schistosomiasis: stool and urine examination will cost 2.5€
- Blood count: 5 €
- Biochemical analysis: 5€
- Urine strip: 1€

Total cost per child included in our study: 54.25€.

The number of children studied will be 668. Nevertheless, the hospital is routinely doing these test in children with severe acute malnutrition. So that, the cost of the testing for children with severe acute malnutrition will be zero.

The testing will have to be paid to the 334 children included in our study with moderate acute malnutrition. That is 18,119.5€

Publication and dissemination expenses:

- The publication in a scientific journal will cost 1.500€ approximately.
- With the idea of disseminating knowledge, we will go to the next International Nutrition Congress, which will cost about 2.500€ taking in account the travel, the inscription, accommodations, etc.

The total cost of publication and dissemination will be 4,000€

EXPENSES			COST
1.Personnel			
CHW	4	100€ x 9 months	3,600€
Laboratory technicians	3	200€ x 9 months	5,400€
Nurses	3	300€ x 9 months	8,100€
Doctors	3	500€ x 9months	13,500€
Interpreter	2	150€ x 9 months	2,700€
Radiologist technician	1	200€ x 9 months	1,800€
Statician	1	35€/hour x 72 hou	2,520€
Data manager	1	250 €	250€
Research coordinator	1	35,000/year x 2 ye	70,000€
Research team training		27€/h x 40 hours	1,080€
2. Diagnostic tests and analytical material			
HIV	4 €		
Tuberculosis	25 €		
Malaria	10 €		
Geohelminths	1,75 €		
Schistosomiasis	2,50 €	x334	18,119.5€
Blood count	5 €		
Biochemical analysis	5 €		
Urine strip	1 €		
3. Publication and dissemination expenses			
Publication in a journal			1,500€
Attendance to congresses			2,500€
		TOTAL	**131,069.5€**

CLINICAL AND HEALTH CARE IMPACT

The improving on the comprehension of moderate acute malnutrition is expected to be useful to enhance the management and prognosis of this group of children. It is important not to neglect the situation and see it as normal, and to keep doing research.

If the study demonstrate clear relationship between MAM and any of the studied comorbidities, and especially if this association is equal to that expected in children with SAM, further hypothesis and research can be done to give a meaning to this relationship and to meliorate the treatment of this conditions.

The clinical and analytical recorded variables can be widely investigated with new models in relationship with the comorbidities if necessary, in order to widen the knowledge about the malnourished children who arrive to the hospital and their management.

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ANNEXES

Annex I. WHO weight per height or length graphics
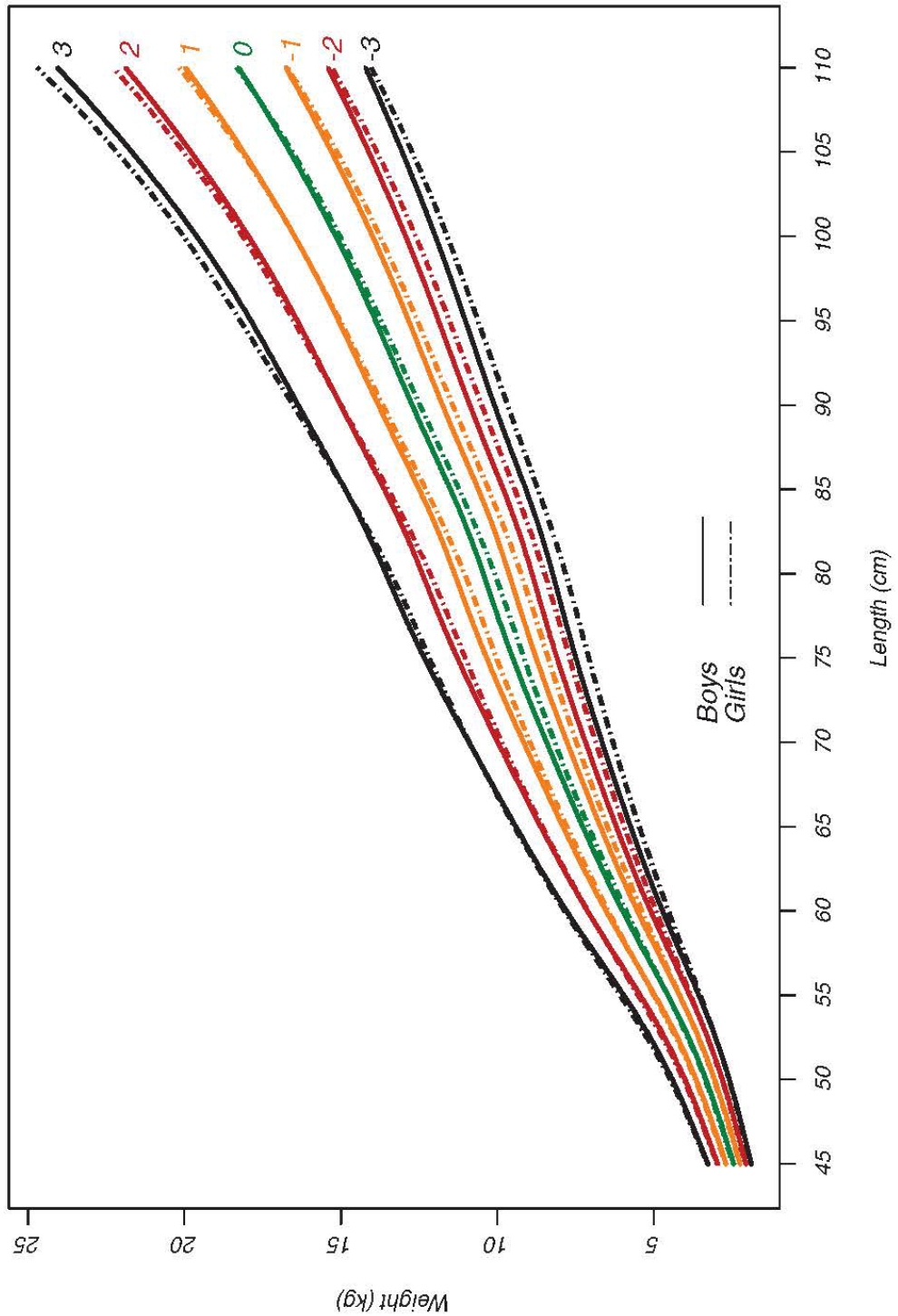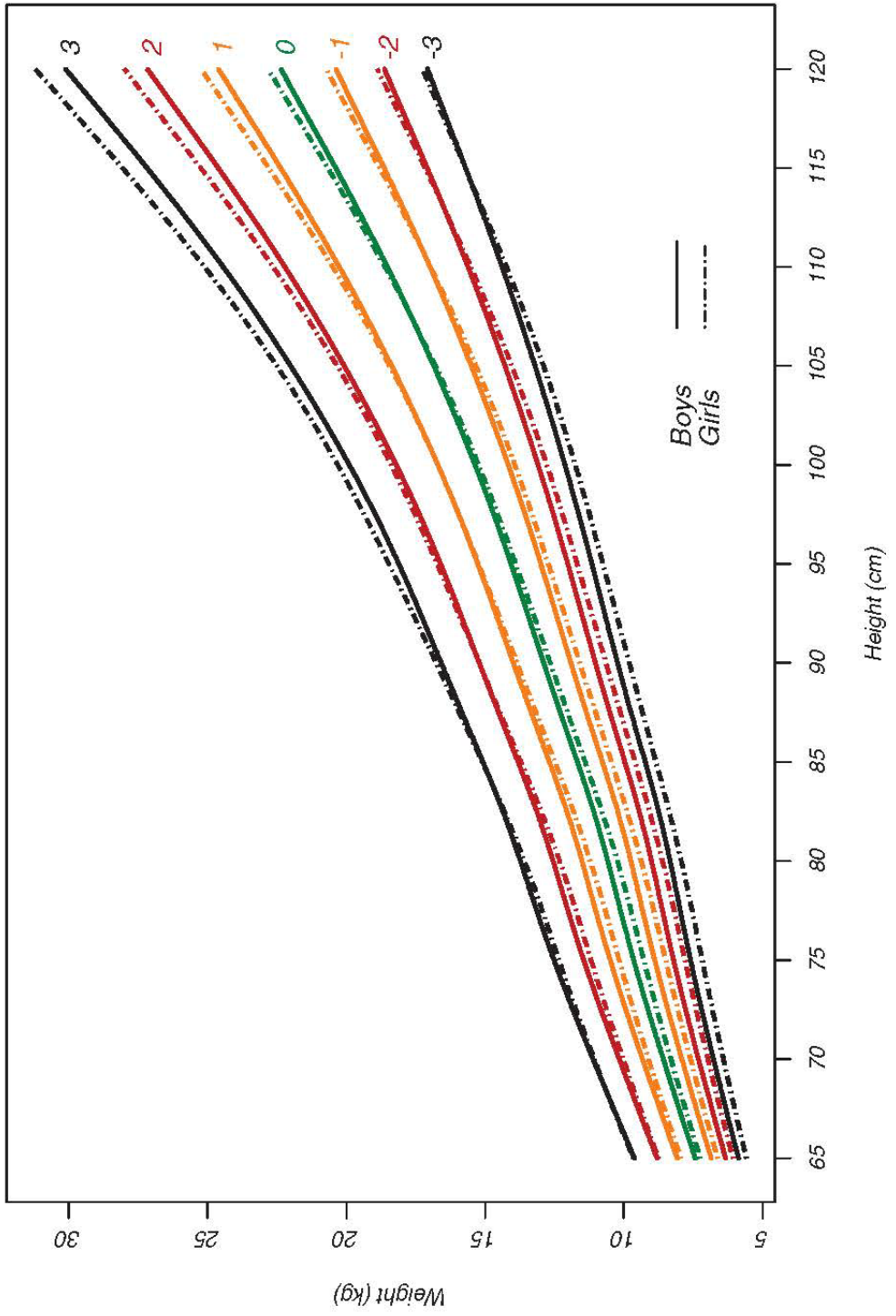
INFORMATION SHEET AND CERTIFICATE OF CONSENT

This informed consent form is for the parents of children between 6 and 59 months of age who attend the nutritional health service of the hospital Nossa Senhora da Paz, and who we are asking to participate in the research about the comorbidities of acute malnutrition.

Principal investigator identification:

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the study with you)**
- **Certificate of Consent (for signatures if you agree that your child may participate)**

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

I am the researcher working for the Vall d'Hebron Hospital. We are doing research on acute malnutrition, which is very common in this country

I am going to give you information and invite you to have your child participate in this research. You do not have to decide today whether or not you agree that you child may participate in the research. Before you decide, you can talk to anyone you feel comfortable with.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will tike time to explain. If you have questions later, you can ask them of me, the study doctor or the staff

Purpose

Wasting or acute malnutrition is a dangerous condition that leaves children very weak and exposed for diseases. Furthermore, these diseases worsen the

malnutrition situation. There are two types of acute malnutrition, the severe and the moderate ones. The purpose of this research is to assess the proportion of children with moderate acute malnutrition that have some infectious diseases, and to do so in the group of severe malnourished children, so that we can compare the groups. This can help us in the future to improve the treatment of those children. We will also write down clinical and analytical data, also in order to improve in the future the acknowledgement and treatment of these patients.

Type of Research Intervention

Assess diagnostic tests for HIV, tuberculosis, malaria, geohelminths and schistosomiasis, and take note for clinical and analytical variables.

Participant selection

The condition of being acutely malnourished is specially dangerous in children under 5 years old. Their immune or protective system is still not fully ready and their bodies that need to grow are more sensible to the lack of food, nutrients, and to the presence of diseases.

We are inviting you to take part in this research because it is important to know the relationship of this condition with other diseases than could be potentially treated and we can only do it in children that are under this situation, as your child does.

Voluntary Participation

Your decision to have your child participate in this study is entirely voluntary. It is your choice whether to have your child participate or not. If you choose not to consent, all the services you and your child receive at this hospital will continue and nothing will change. You may also choose to change your mind later and stop participating, even if you agreed earlier, and the services you and/or your child receives at the hospital will continue

Description of the Process

You may stay with your child during the visits and the procedures. In the first visit, two small amounts of blood, equal to about a teaspoon, will be taken from your

child's arm. Your child will feel some discomfort when the needle stick goes into her/his arm but this will go away very quickly. There may be slight bruising but this will disappear in a few days. In the same visit we will take a fingerstick sample and a urine and a stool sample. Also a prick will be done in the skin of your child's arm, it will not be painful. The region where this prick was done can flame up within the next days, don't worry, this is a test that will be helping us to detect diseases. The last sample taken this day will be a gastric lavage, this consists on taking by a tube fluids from the stomach of your child and will be done twice. This may is uncomfortable for your child but it's not painful and will be helpful for us. The doctor will ensure that your children receive the best care and will ask you and him/her some questions about possible symptoms, and also will make a review of the children in order to find some signs that will be written down.

The day after this visit you will have another visit, in which we will take another sample of blood for counting the cells and checking your child's inside situation. Another gastric lavage and another urine and stool samples will be taken. Finally a chest radiography will be assessed to your children. This procedure is like looking on the inside of your child in order to assess its lungs and is not painful for the children. The radiation can be dangerous for health at a long term view, but the radiation of just one radiography is a small amount and we think is justified in this case.

Two days after the first visit, you will have a little follow up, just to check the result of the test that we did on the arm of your children. You can ask questions in any moment and for any procedure. All the samples taken from your child will help us to know whether your child is infected with HIV, tuberculosis, malaria, schistosomiasis (parasites) and/or any kind of geohelminths (worms in the bowel)

If you don't want us to do some of the above let us know. Because all the named before is important for the study, if we cannot assess this tests, we will not be able to include you in this study.

You will be informed about all the results involving your children's health.

There are guidelines to follow to treat and manage the conditions found in your children, and we will follow them for her/his best assessment.

The biological samples obtained during this research procedure will be used only for this research, and will be destroyed after 2 years, when the research is completed.

Duration

The research takes place over 2 years in total, from which 9 months we will be taking information about children like yours. During that time we will test the samples taken from your child and you will be informed of any medical condition that we found. If any treatment is needed you will be asked to come to the hospital for the correct management.

Side Effects

The interventions are not expected to have any side effects or risks, but if something happens, your children can come to the hospital and the health professionals will do the better treatment for your children, and will carry with the money burden of this management.

The radiation of the chest radiography can have consequences at a long term, but this is very unlikely due to the small amount of radiation that just one chest radiography has.

Discomforts

By participating in this research it is possible that your children may experience some discomfort such as the discomfort of the injections. There may be a slight hardening and/or swelling where the needle stick goes into the skin. This should disappear in one day. The gastric lavage can also lead to discomfort in the children but this will stops as the sample is taken and the tube is retired. Your child may also be fussier than usual or more tired. These behaviors usually stop within one day but if you are concerned, please call me or come to the hospital.

Benefits

If your child participates in this research, he/she will have the following benefits: any interim illnesses will be treated at no charge to you. If we detect any of the associated conditioned mentioned above, your child will be treated at no charge at you, and you

will also be offered to be tested and treated for this infectious diseases. There may not be any benefit to the society at this stage of the research, but future acutely malnourished children are likely to benefit.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about your child that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about your child will have a number on it instead of his/her name. Only the researchers will know what his/her number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except the health care workers, nurses, doctors or researchers that needed it for assess her/him specific information.

Sharing of the results

The knowledge that we get from this study will be shared with you before it is made widely available to the public. Confidential information will not be shared. There will be small meeting in the community and these will be announced. Afterwards, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to agree to you child taking part in this research if you do not wish to do so and refusing to allow your child to participate will not affect your treatment or your child's treatment at this Hospital in any way. You and your child will still have all the benefits that you would otherwise have at this hospital. You may stop you child from participating in the research at any time that you wish without either you or your child losing any of your rights as a patient here. Neither your treatment nor your child's treatment at this Centre will be affected in any way.

Who to Contact

If you have any questions you may ask them now or later, even after the study started. If you wish to ask questions later, you may contact with the number and/or e-mail of the nutritional service of the hospital, or came to the hospital, and they will put you in contact with the main researcher.

PART II: Certificate of Consent

Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child to participate as a participant in this study.

Print Name of Participant_____
Print Name of Parent or Guardian_____

Signature of Parent or Guardian _____
Date _____
     Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the consent form to the parent of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____   AND   Thumb print of parent

Signature of witness _____

Date _____

   Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the parent of the potential participant, and to the best of my ability made sure that the person understands that the following will be done:

1. Physical examination

2. Different samples collection

3. Testing the samples

I confirm that the parent was given an opportunity to ask questions about the study, and all the questions asked by the parent have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

         Day/month/year

An Informed Assent Form will_____ OR will not _____ be completed.

➡ **This informed consent will be translated into Portuguese and Bantu by a professional.**

Annex III. Uni-Gold rapid test performance

Uni-Gold HIV Rapid Test
For use with whole blood, serum, or plasma
Store Kits: 2 - 30°C

- Check kit before use. Use only items that have not expired or been damaged.
- Bring kit and previously stored specimens to room temperature prior to use.
- Always use universal safety precautions when handling specimens. Keep work areas clean and organized.

This outline is not intended to replace the product insert or your standard operating procedure (SOP).

1. Collect test items and other necessary lab supplies.

2. Remove device from package and label device with client identification number.
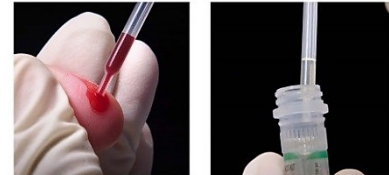
3. Collect specimen using the disposable pipette.

4. Add 2 drops (approx. 60µl) of specimen to the sample port in the device.

5. Add 2 drops (approx. 60µl) of the appropriate wash reagent to sample port.

6. Wait for 10 minutes (no longer than 20 min.) before reading the results.

7. Read and record the results and other pertinent info on the worksheet.

Uni-Gold HIV Rapid Test Results

Reactive
2 lines of any intensity appear in **both** the **control** and **test** areas.

Non-reactive
1 line appears in the **control** area and no line in the test area.

Invalid
No line appears in the **control** area. Do not report invalid results. Repeat test with a new test device even if a line appears in the test area.

Use of trade names and commercial sources is for identification only and does not imply endorsement by WHO, the Public Health Service, or by the U.S. Department of Health and Human Services (2005).

WHO

CDC

Annex IV. Modified Edward's clinical score for tuberculosis

Feature	Score			
	0	1	3	4
Duration of illness (weeks)	<2	2-4	>4	
Nutrition (%weight for height/length)	>80	60-80	<60	
Family history of tuberculosis	None	Reported by family	Proven sputum positive	
Unexplained fever (>2 weeks)		No response to treatment		
Tuberculin test			Positive	
Lymph nodes			Large, painless, firm, soft sinus 3 in neck/axilla	
Malnutrition			Not improving after 4 weeks	
Central nervous system			Change in temperament	
Joint, bone, sinuses			Swelling	
Abdomen			Mass, ascites	
Spine				Angle deformity of spine
A score of 7 or more is indicative of tuberculosis				

Annex V. Data collection Form

DATA COLLECTION FORM

Health worker identification: Patient number identification:

Patient name: Age(months):   Sex: Male ☐ / Female ☐

Anthropometric features

Weight (kg):   Height or length (cm):   MUAC (mm):

Weight for height or length: ▭   Z-score: ▭

Edema: ☐ YES (+ / ++ / +++)

   ☐ NO

Malnutrition: ☐ MAM

    ☐ SAM

Height or length for age: ▭   Z-score: ▭

Comorbidities	+	-	IND
• **HIV**	☐	☐	☐
Tuberculosis	☐	☐	☐
Malaria	☐	☐	☐
• **Geohelminths**	☐	☐	☐
• **Schistosomiasis: Urinary**	☐	☐	☐
Intestinal	☐	☐	☐

Determine: ☐ Ab reactive   ☐ Ag reactive ☐ Ab and ag reactive

    ☐ Non reactive ☐ Invalid

Uni-Gold: ☐ Reactive ☐ Non-reactive ☐ Invalid

LED fluorescence microscopy (No. of AFB per 100 fields) ▭
GeneXpert: ☐ Positive ☐ Negative ☐ Invalid
Thorax radiography features: ....
Clinical score: ▭

Malaria RDT: ☐ Positive for falciparum malaria ☐ Positive for non falciparum malaria

    ☐ Negative ☐ Invalid

Thick and thin blood smear: ☐ Positive for falciparum malaria ☐ Negative

    ☐ Positive for non falciparum malaria: .... ☐ Invalid

Type: ....

Analytical findings

• Complete blood count: ▭
Number of red blood cells (million/mm³) ▭
Hematocrit (%) ▭
Hemoglobin (g/dL) ▭
Mean corpuscular volume (fl) ▭

DATA COLLECTION FORM

Mean corpuscular hemoglobin concentration (g/dl) [   ]
Mean corpuscular hemoglobin (pg) [   ]
Leukocytes (per mL) [   ]
   Neutrophils (per mL) (%) [   ]
   Lymphocytes (per mL) (%) [   ]
   Eosinophils (per mL) (%) [   ]
   Basophils (per mL) (%) [   ]
   Monocytes (%) [   ]
Platelets (per mL) [   ]
- Glucose (mg/dL) [   ]
- Sodium (mEq/L) [   ]
- Potassium (mEq/L) [   ]
- Calcium (mg/dL) [   ]
- Rapid urine test:
     pH: [   ]
     Nitrites: [   ]
     Leukocytes: [   ]
     *Urinary tract infection: ☐YES ☐NO
     Red blood cells: [   ]
     Proteins: [   ]
     Other substances not usually found in urine: [   ]

Clinical variables	YES	NO	
• Swollen limph nodes	☐	☐	
• Tiredness	☐	☐	
• Diarrhea	☐	☐	
• Cough	☐	☐	
• Hemoptysis	☐	☐	
• Dyspnea	☐	☐	
• Profuse night sweating	☐	☐	
• Headache	☐	☐	
• Shaking chills	☐	☐	
• Abdominal pain	☐	☐	
• Hematuria	☐	☐	
• Thrush	☐	☐	
• Splenomegaly (cm)	☐	☐	[ ]
• Hepatomegaly (cm)	☐	☐	[ ]
• Fever (ºC)	[ ]		