CLINICAL PROFILE AND MORTALITY AMONG CHILDREN WITH APLASTIC ANEMIA ADMITTED AT MUHIMBILI NATIONAL HOSPITAL - TANZANIA

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CLINICAL PROFILE AND MORTALITY AMONG CHILDREN WITH APLASTIC ANEMIA ADMITTED AT MNH

By

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CERTIFICATION

The undersigned, certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation titled: **‘’Clinical profile and mortality among children with aplastic anemia admitted at Muhimbili National Hospital – Tanzania”** Master of Medicine in Paediatrics and Child Health at the Muhimbili University of Health and Allied Sciences

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DECLARATION

I **Nazareth M. Mbilinyi**declare that this dissertation is my original work and has not been presented and will not be presented to any other Higher Learning Institution for a similar or any other degree award.

Signature: …… Date: …….

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DEDICATION

This piece is dedicated to my family, who have been the pillars of my path with their everlasting support.
To my beloved wife, Happy Emmanuel, your unending love and compassion have been my beacon of hope and support through every hardship and victory.

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.

ABSTRACT

Background: Aplastic anemia (AA) is a relatively rare hematological disease, presenting with pancytopenia and unexplained hypo-cellularity in bone marrow. It is a condition with a high mortality rate if left undiagnosed and untreated. There is inconsistency in findings from previous studies regarding the clinical profile and factors associated with mortality among patients with AA.

Objectives: The main aim was to find out the clinical profile and factors associated with mortality among children with AA admitted to Muhimbili National Hospital, Dar es Salaam, Tanzania.

Methods: An analytical retrospective hospital-based cross-sectional study patients with AA at MNH admitted between 2016 and 2023. Records of 82 patients aged less than 18 years old were reviewed. Information obtained included age, sex, date of diagnosis, laboratory results, treatment given, and status of living or death. Descriptive analysis was summarized using proportions and medians with interquartile ranges (IQR). The overall clinical profile was calculated with a 95% confidence interval. Chi-square and, where applicable. Logistic regression was used to determine the independent factors associated with mortality among patients with AA. A p value of < 0.05 was considered statistically significant.

Results: The majority of patients were male (62.2%), with a median age at diagnosis of 9 years. Common clinical presentation included bleeding tendencies (61.0%) and pallor (96.3%), while laboratory findings revealed severe anemia (60.9%), neutropenia (41.4%), and severe thrombocytopenia (75.6%). Treatment strategies primarily consisted of immunosuppressive therapy with ATG, cyclosporine, Danazole, with supportive measures such as blood products and antibiotics. The mortality rate among AA patients was 48.7%. statically significant factors associated with mortality were bleeding tendency, Absolute neutrophil count (ANC) and thrombocytopenia. No association with sex nor age was found.

Conclusions: Despite advances in treatment, mortality remains a significant concern, emphasizing the need for early detection, prompt initiation of therapy, and close monitoring of high-risk patients. Identification of prognostic factors can aid in risk stratification and targeted interventions to improve survival rates in AA.

Recommendations: Based on the findings, recommendations include enhanced education and awareness programs for healthcare providers and further research to explore novel therapeutic approaches and prognostic markers in AA.

MUHTASARI

Utangulizi: "Aplastic anemia" (AA) ni miongoni mwa magonjwa adimu ya damu unaosababishwa na chembechembe za damu kutozalishwa kwenye uroto wa mifupa. Ni ugonjwa wenye hatari kubwa cha kifo ikiwa hautatambuliwa mapema na kutibiwa.

AA inaweza kuainishwa kadiri ya sababu za ugonjwa yaani kuwa wa kurithi au wa kuupata baada ya kuzaliwa. Pia huweza kuanishwa kulingana na makali yake kuwa sio mkali , mkali na mkali sana. Kuna utaofauti wa matokeo katika tafiti mbalimbali za awali kuhusu maelezo ya dalili za ugonjwa na viashiria vinavyohusiana na vifo kati ya wagonjwa huu. Katika tafiti za awali, baadhi zinaonyesha kuwa matokeo yanatofautiana kulingana na umri wa kuanza kuugua, aina ya ugonjwa, ukali wa ugonjwa au aina ya matibabu zilizotolewa. Kitaifa, kuna upungufu wa taarifa kuhusu ya maelezo ya dalili na viashiria vinavyohusiana na vifo kati ya wagonjwa.

Malengo: Lengo kuu la utafiti huu ni kubainisha aina za dalili na viashiria vinavyohusiana na vifo kati ya watoto walio na ugonjwa huu waliolazwa katika Hospitali ya Taifa ya Muhimbili, Dar es Salaam, Tanzania**.**

Njia: Taarifa za wagonjwa kutoka katika majarada ya hospitali na zile za mfumo wa kielektroniki za wagonjwa 82 walio na umri chini ya miaka 18 na ugonjwa wa "Aplastiki anemia" waliolazwa katika hospitali ya taifa MNH zilichunguzwa. Taarifa zilizochukuliwa kwa kila mgonjwa ni pamoja na umri, jinsia, tarehe ya utambuzi wa ugonjwa, matokeo ya maabara, matibabu yaliyotolewa, na tarehe ya kifo.

Matokeo: Wagonjwa wengi walikuwa wa jinsi ya kiume (62.2%) na umri wa kati wa utambuzi wa miaka 9. Dalili kuu za wagonjwa zilizo gunduliwa zilikuwa ni za kutokwa damu (61.0%) na kupauka mikono (96.3%). Matokeo ya maabara yalionyesha upungufu mkali wa damu (60.9%), na upungufu wa chembe kinga (41.4%), na upungufu mkali wa chembe sahani (75.6%). Matibabu yalihusisha dawa za *ATG* ikifuatiwa na *Cyclosporine*, na *Danazole*.Asilimia ya vifo kati ya wagonjwa wa ilikuwa 48.7%, na viashiria kama chembe kinga na chembe Sahani chache iligunduliwa kama vichocheo vya vifo.

Hitimisho: Licha ya maendeleo katika matibabu, uwiano wa vifo bado ipo juu,

Mapendekezo: Kutokana na matokeo, mapendekezo ni pamoja na mamlaka za afya kuongeza elimu na ya ufahamu kwa watoa huduma za afya kuhusu ugonjwa huu, na kuendelea kufanya utafiti zaidi kuchunguza njia mpya za matibabu.

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ABBREVIATIONS

AA	Aplastic anemia
ALC	Absolute lymphocyte count
ANC	Absolute Neutrophil Count
ARC	Absolute Reticulocyte Count
ATG	Anti-thymocyte globulin
BM	Bone-Marrow
CBC	Complete Blood Count
CPL	Central Pathology Laboratory
FFP	Fresh Frozen Plasma
G-CSF	Granulocyte colony-stimulating factor
GPI	Glycophosphatidylinositol
HLA-DR	Human Leukocyte Antigen- antigen D Related
HSCs	Hematopoietic stem cells
IST	Immunosuppressive Therapy
MNH	Muhimbili-National Hospital
MUHAS	Muhimbili-University of Health and Allied Sciences
PBS	Peripheral blood smear
PNH	Paroxysmal Nocturnal Haemoglobinuria
PRBC	Packed Red Blood Cells
SD	Standard deviation
TNF	Tumor Necrosis Factor
W.H. O	World-Health-Organization.
FA	Fanconi anemia
SAA	Severe Aplastic Anemia
VSAA	Very severe Aplastic Anemia
NSAA	Non severe Aplastic anemia
GVHD	Graft versus Host Disease

DEFINITION OF TERMS

Aplastic anemia is hematological disease, presenting with pancytopenia and unexplained hypo-cellularity in bone marrow which is replaced by fat cells.

Severe aplastic anemia: Decrease in blood cells counts involving ≥2 hematopoietic lineages i.e., absolute reticulocyte count $<60 \times 10^9$/L, absolute neutrophil count between 0.2 to $<0.5 \times 10^9$/L, or platelet count $<20 \times 10^9$/L.

Very severe Aplastic Anemia: Decrease in blood cells counts involving ≥2 hematopoietic lineages i.e., absolute reticulocyte count $<60 \times 10^9$/L, absolute neutrophil count $<0.2 \times 10^9$/L, or platelet count $<20 \times 10^9$/L.

Non-severe Aplastic Anemia: is the decrease of blood cells counts not fulfilling the definition of severe disease

Pancytopenia is the presentation of a reduction in the number of cellular components of the blood cells, i.e., neutropenia, low hemoglobin level, and thrombocytopenia.

Immunosuppressive therapy is a medical intervention that involves the use of medications that inhibit or suppress the immune response to control the immune system's hyperactivity. These medications can reduce inflammation and prevent further damage to Hematopoietic stem cells (HSCs)

A clinical profile refers to a comprehensive summary or description of a patient's medical history, current health condition, and relevant investigational characteristics. It is a detailed overview of the patient's health status.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Aplastic anemia (AA) is an uncommon and potentially life-threatening condition. It is identified by a lack of bone marrow activity and low levels of all blood cells. In 1888, Paul Ehrlich detailed the postmortem examination of a young woman who experienced a brief, fatal illness marked by anemia, bleeding, and infection. This was accompanied by yellow rather than red bone marrow in her femur (1).

Over the past three decades, there has been a significant improvement in the survival of individuals with AA following extensive use of immunosuppressive medications and allogeneic bone marrow transplantation (2).The child with AA most commonly presents with recurrent infections due to a low absolute neutrophil count (ANC), bleeding tendency due to a low platelet count, fatigue, and cardiopulmonary findings associated with progressive anemia. Infections are typically caused by bacteria, leading to pneumonia and urinary tract infections. Invasive fungal infection is a common cause of death, especially in subjects with prolonged and severe neutropenia (3).

Based on etiology, AA can be categorized into primary (congenital or inherited) and secondary (acquired) causes. Congenital AA is due to genetic defects that impair the usual functions of hematopoietic stem cells (HSCs). Children with congenital AA usually have somatic features associated with specific inherited syndromes (e.g., short stature, microcephaly, developmental delay, skin and nail lesions). The secondary cause, which is also called acquired AA, is usually the result of an autoimmune attack that appears to be directed at hematopoietic stem and progenitor cells (21).

The prognosis of patients with acquired AA has significantly improved over time due to improving treatment options, including the introduction of immunosuppressive therapy (IST). This improvement is not just limited to those who experienced partial or complete remission following a particular course of treatment. This most likely reflects improved treatment's effect on patients' overall survival, mainly in high-income countries where the survival rate for AA patients has been increased to 65–85% (4-5).

The global incidence of aplastic anemia is estimated to be 0.7–4.1 cases per million people, with males and females showing approximately equal frequency. [44] Asia has a two- to three-times higher rate of AA than Western countries (6).

In a retrospective study done in the metropolitan area of Barcelona that described the trend of the diseases, it was found that in cases of AA identified between 1980 and 1999, the survival rate at three months was 73%, 57% at 2 and 5 years, and 51% at 15 years. Patients diagnosed after 1990 had a higher 2-year survival rate. Patients with severe AA showed lower survival rates (7).

The number of white blood cells (WBC), monocytes, and neutrophils influences a patient's unique risk for life-threatening infection complications. Although infection-related mortality and invasive fungal infections have declined over the last two decades, infections remain the most serious hazard for individuals with severe or very severe AA. Bacterial and fungal infections are the most common complications and primary causes of death (4).

We have minimal data about the factors associated with mortality among patients with AA in children in our setting. These highlight the importance of descriptive and analytical studies in understanding the disease better.

1.2 Statement of the Problem

Literature on epidemiological studies documenting the population-based incidence of AA is scarce in African countries. Furthermore, findings from previous studies regarding the clinical profile and factors associated with mortality among patients with AA are inconsistent. Several studies in various settings have shown high mortality rates, although the results of these studies vary significantly due to variances in risk factors and sociocultural systems. In a study done in West Africa involving both children and adults, thrombocytopenic hemorrhage was the leading cause of mortality in 60% of the patients. In comparison, the burden of severe sepsis had a mortality rate of 20% of the patients (8).

According to studies, the increased mortality recorded within one year after the presentation was related to a late presentation and insufficient supportive treatment. This raises the matter of managing patients with AA (9). Supportive treatment among patient depends on the clinical presentations.

Most studies regarding AA have been done involving adults. Due to the disease being rare in nature has further limited the sample size. There is limited data in the country regarding the clinical profile and mortality among children with AA; however, the observational study of the frequency of acquired AA in adults at MNH in 2018 showed a mortality rate of 48%. It was further demonstrated that, compared to international guidelines, the management of AA in MNH has been suboptimal (10).

1.3 Rationale of the Study

The results of this investigation will give insight into the clinical profile of children with AA in Tanzania therefore providing early diagnosis and treatment.

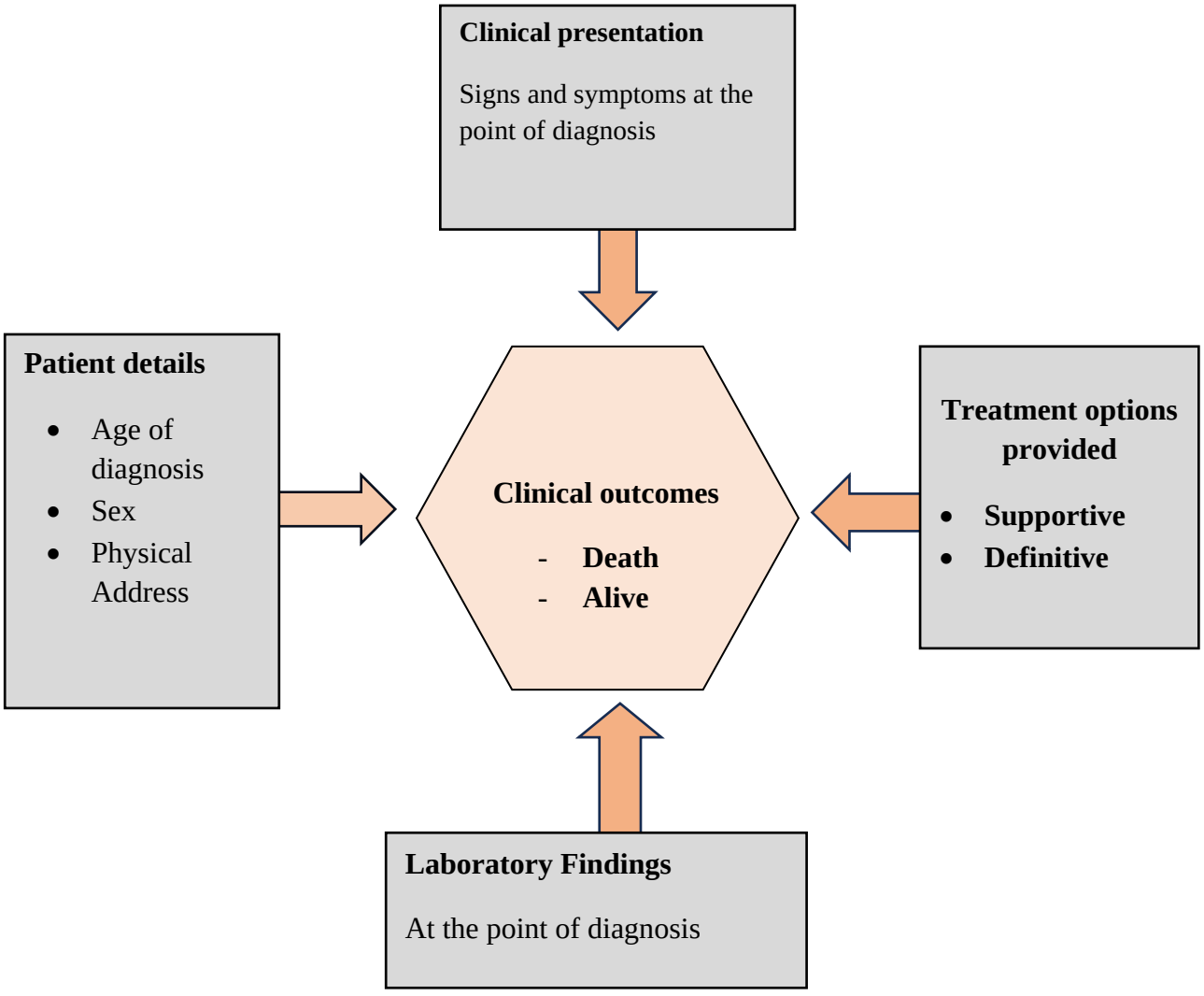
Information obtained from this study will provide a trend in AA (presentation, diagnostic modalities, supportive care provided, and outcomes), therefore better planning of resource allocations for better care of patients

Specifically, the findings will provide knowledge on factors associated with mortality among patients with AA, that will raise awareness of the disease severity hence improve the patient care in Tanzania.

1.4 Conceptual Framework

This conceptual framework depicts the study's organization, order, and direction. It focuses on independent variables such as socio-demographic characteristics. The clinical presentation of children with AA includes symptoms such as bleeding tendency, fever, and general body weakness, as well as signs such as pallor, bleeding, and body dysmorphic features. It also includes treatment options, including Hematopoietic stem cells (HSC) transplantation, blood products, antibiotics, and growth-stimulating factors. The key outcome in this relationship is the number of children with AA admitted to Muhimbili National Hospital (MNH) who survived or died.

Figure 1: Conceptual framework



1.5 The Research Questions

1.5.1 The **broad research question**

What is the clinical profile and factors associated with mortality among children admitted to MNH between 2016 and 2023?

1.5.2 Specific Research Questions

1. What are the clinical presentations of children with AA admitted to MNH between 2016 and 2023?
2. What are the treatment options among children with AA admitted to MNH between 2016 and 2023?
3. What is the mortality rate among children with AA admitted to MNH between 2016 and 2023?
4. What factors are associated with mortality among children with AA at MNH between 2016 and 2023?

1.6 Objectives

1.6.1 The Main objective

To determine the clinical profile and factors associated with mortality among children with AA admitted at MNH between 2016 and 2023.

Specific objectives

1. To determine the clinical presentations of children with aplastic anemia admitted to MNH between 2016 and 2023.
2. To determine the treatment for children with aplastic anemia admitted to MNH between 2016 and 2023.
3. To determine the mortality rate among children with AA admitted to MNH between 2016 and 2023.
4. To determine factors associated with mortality among children with AA admitted to MNH between 2016 and 2023.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Magnitude of AA.

AA is a relatively rare but life-threatening disease. The World Health Organization (WHO) defines a rare disease as one that affects less than 6.7 per 1 million people (41).

The occurrence of AA varies significantly across different regions worldwide and has changed over time. Historical data from Europe and the United States during the 1960s and 1970s presented a wide range of incidence rates, reaching 6–10 AA cases per million individuals annually (38, 39). Some of these studies indicated a correlation with agricultural toxins. However, discrepancies arose due to the utilization of different diagnostic criteria, leading to the misclassification of some instances (38).

A study done in Barcelona, Spain, between 1980 and 2003, identified 235 cases of AA. The overall incidence was 2.34 per million inhabitants (40).

The incidence of aplastic anemia is estimated to be 0.7–4.1 cases per million people worldwide, with the prevalence between men and women being approximately equal (6).

The incidence rate of AA in Asia is 2–3 times higher than in the West; the incidence in the United States is 300–900 cases per year. AA has a bimodal peak that most commonly affects patients aged 15–25 and over 60, but it can be observed in all age groups. (6).

2.2 Pathophysiology and etiological classification of AA

The underlying pathophysiology depends on the etiological type of AA, whether it is primary (congenital or inherited) or secondary (acquired) in origin (21). This etiological classification is summarized in Table 1. However, patients with AA, regardless of etiology, typically present with anemia symptoms, bleeding, and infections (32). The underlying pathophysiology depends on the etiological type of AA (21). This etiological classification is summarized in Table 1.

Acquired AA

Acquired AA often involves the T cell-mediated destruction of bone marrow hematopoietic cells by secondary causes (18). Hematopoietic failure can also result from direct physical or chemical injury to the HSC.

While radiation and cytostatic drugs used in cancer treatment are well-known causes of marrow aplasia, other medications like nonsteroidal anti-inflammatory drugs and antibiotics have also been linked to AA development (19, 20). In children, AA may follow viral exposure. For example, the Epstein-Barr virus and seronegative hepatitis can cause AA, likely by activating the immune system (18, 22).

Immunological mechanisms play a role in the pathophysiology of acquired AA. Previous research revealed abnormalities in early progenitor cells and the activation of cytotoxic T cells, which produce cytokines like interferon-γ (IFN-γ) and trigger cell apoptosis. Macrophages also play a critical role in IFN-γ-related stem cell destruction by producing tumor necrosis factor-alpha (TNF-α) and engaging cytotoxic T cells. Another important aspect involves regulatory T cells (Tregs), which are decreased in AA (23, 24, 25).

Congenital AA

Congenital (inherited) AA, which accounts for about 70% of cases, can be inherited marrow failure or idiopathic. Children with congenital AA often have both physical and hematologic findings, but depending on the disease, they may not have any physical abnormalities.

Clonal hematopoiesis is a well-established feature of AA, with up to 47% of patients harboring clonal populations. Mutations in specific genes have been identified, including DNMT3A, ASXL1, BCOR, BCORL1, and PIGA (30).

Human leukocyte antigens (HLA) and acquired copy number-neutral loss of heterozygosity (CNN-LOH) on chromosome 6 are also implicated in AA development. Reduced HLA antigen expression allows stem cells to evade autoimmune attacks, facilitating clonal outgrowth and potentially maintaining hematopoiesis in AA patients (42).

Telomere shortening, although primarily associated with immune-mediated destruction of hematopoietic cells, has been observed in up to one-third of AA patients, often due to mutations in the telomerase gene complex. (26,27). Nevertheless, discoveries in congenital AAs, such as Dyskeratosis congenital (DC), Fanconi anemia (FA), and Schwachman-Diamond syndrome helped to connect genetic lesions and telomere shortening of hematopoietic cells. (28).

Of these congenital causes of AA, FA is the most common inherited cause of bone marrow failure. Genes mutated in FA patients are termed FANC, and more than 20 have been identified; FANCA, FANCC, FANCG, and FANCD2 are the most common (33, 34). FA patients have a cumulative hematopoietic dysfunction likely related to excess genetic instability, cellular stress, p53 activation, and cell death (35). Patients often present with congenital abnormalities, such as short stature, microphthalmia, thumb and radius deformities, skin hyperpigmentation, and other signs. Still, approximately 25% have no physical signs, and the disease can be missed during childhood. Up to 80% of FA patients have been reported to develop bone marrow failure, and it usually occurs during the first or second decades of life (36, 37).

Table 1: Classification of AA according to etiology (21)

Primary causes	**Secondary causes**
CONGENITAL/INHERITED AA • Fanconi's anaemia • Dyskeratosis congenita • Shwachman–Diamond syndrome • Reticular dysgenesis • Amegakaryocytic thrombocytopaenia • Familial AA	1. Chemical exposure • Benzene • Pesticides 2. Drugs • Carbimazole • Chloramphenicol • Chlorpromazine • Phenytoin 3. Ionizing radiation 4. Infection • Epstein Barr virus (EBV) • Cytomegalovirus (CMV) • Parvovirus B19 • Human immunodeficiency virus (HIV) • Hepatitis virus

2.3 The clinical presentations and modality of diagnosis of AA in children

Clinical presentations

Most children with AA present with signs and symptoms resulting from advanced pancytopenia, while others are diagnosed by incidental laboratory findings. The patient with AA most commonly presents with recurrent infections due to neutropenia, mucosal hemorrhage, or menorrhagia due to thrombocytopenia or fatigue and cardiopulmonary findings associated with progressive anemia (45).

Infections are typically bacterial, including sepsis, pneumonia, skin infections (cellulitis, abscess), and urinary tract infections; invasive fungal infection is a common cause of death, especially in subjects with prolonged and severe neutropenia (3).

Hepatosplenomegaly and lymphadenopathy are typically absent. A history of jaundice, often occurring on average 2–3 months prior to the discovery of pancytopenia, is consistent with hepatitis-associated AA (45).

Younger children with congenital AA may have somatic manifestations associated with specific inherited syndromes like short stature, microcephaly, developmental delay, skin pigmentation (café au lait), and nail lesions. They may also have renal, cardiac, and gastrointestinal (GI) abnormalities, microcephaly, microphthalmos, hypogonadism, and skeleton anomalies. However, some older children with AA may also manifest characteristic somatic abnormalities such as fingernail dystrophy associated with congenital dyskeratosis. This is due to a previously unrecognized inherited disorder (11).

Diagnosis of AA

To establish the diagnosis of AA, a thorough medical history should encompass exposure to medications, recreational substances, and chemicals, along with any preceding signs of infection. Delving into family medical records is crucial, examining conditions and indicators suggestive of congenital AA (45).

A set of laboratory tests is necessary to confirm the AA diagnosis, determine its severity, and investigate potential causes. Conducting a bone marrow aspiration and biopsy is essential for diagnosis. A full blood count, reticulocyte count, and examination of the peripheral blood smear are essential to identify existing cytopenia and rule out dysplasia (44, 45).

Grading the severity of the disease requires reviewing the biopsy findings. It's noteworthy that AA may manifest with a hematopoietic cell clone lacking glycosylphosphatidylinositol (GPI)-anchored proteins akin to those found in paroxysmal nocturnal hemoglobinuria (PNH) at the time of diagnosis. Detecting an early PNH cell clone entail utilizing flow cytometry to assess the absence of GPI-anchored proteins (46,47).

To confirm congenital AA, include the assessment of peripheral lymphocytes with chromosome breakage studies for FA and telomere length analysis for dyskeratosis congenita (45).

2.4 Treatment Modalities of AA

Following a confirmed diagnosis, bone marrow transplantation (BMT) and immunosuppressive therapy (IST) stand as the foremost treatment modalities for both young and older children with AA patients (45). For inherited AA, androgens continue to be a treatment option, of which danazol, which is less toxic and virilizing, is the most widely used medicine for FA and DC (48). Patients with AA are further kept on supportive therapies.

2.4.1 HSCs Transplantation

Transplantation is the definitive treatment for AA. Management of transplant-eligible children and adolescents with sAA or vsAA depends on the availability of a human leukocyte antigen (HLA)-matched sibling donor. Compared with IST, allogeneic HCT with an MSD graft provides faster restoration of blood counts, less early treatment failure, fewer relapses, and prevents clonal evolution to hemolytic paroxysmal nocturnal hemoglobinuria (PNH), acute myeloid leukemia (AML), and myelodysplastic syndromes (45).

2.4.2 IST therapy

The IST with either anti-thymocyte globulins (ATG) followed by cyclosporine or high-dose cyclophosphamide is the therapy for patients unable to access HSC transplant (32).

ATGs are complex mixtures of antibodies targeting human proteins, with their composition not entirely delineated. While ATGs exhibit a moderate effect on lymphocyte depletion, nuanced variations in their mechanisms of action seem pivotal for their effectiveness. For instance, findings from a randomized controlled trial revealed that horse-derived ATG demonstrated superior efficacy compared to rabbit-derived ATG. Notably, this discrepancy in efficacy was attributed to a more pronounced depletion of CD4 cells and T regulatory cells following the administration of rabbit-derived ATG (55). One prospective study involving cyclophosphamide was terminated early due to a high incidence of mortality from severe infections as a result of prolonged neutropenia (50).

2.4.3 Androgen therapy

Androgen therapy for AA was introduced in 1959 by Shahidi and Diamond, and early studies from this and other institutions showed a high frequency of remissions in treated patients (49). Danazol (2,3-isoxazol-17α-ethynyl testosterone) is a synthetic androgen compound inhibiting pituitary gonadotropins. It also has mild immunosuppressive properties via inhibition of TNF-alpha and IL-1 (36).

Danazol has shown activity in treating anemia in inherited bone marrow failure syndromes like FA and DC. It has also been used in other hematological conditions, including myelodysplasia, myelofibrosis, and pure red cell aplasia (43).

2.4.4 Eltrombopag

Eltrombopag Is the thrombopoietin receptor agonist which increase platelets cells production. The drug is used in patients with severe AA who fail to respond adequately to IST. However, the use of Eltrombopag is controversial due to risks of relapse and late-onset clonal disease, such as PNH, MDS, or leukemia (54).

2.4.5 Supportive therapy

Blood transfusion

The initial goal of treatment for anemia should be to correct or avoid cardiopulmonary complications. When symptomatic, most patients without significant comorbidities should be transfused with packed red blood cells. The goal of a platelet transfusion should be to maintain a high enough platelet count to prevent spontaneous bleeding. For most patients, platelet transfusions are indicated when platelet levels are below 10,000/ul or if the patient is experiencing bleeding (56). Patients with severe cytopenia require urgent support with blood products. Blood products should be irradiated to prevent GVHD (44).

Cytokine support

Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) may be considered in refractory infections. However, the benefit of this therapy should be weighed against its cost and efficacy (53).

Treatment and prevention of infections

Among the main supportive therapies in both congenital and acquired AA, patients diagnosed with severe aplastic anemia (SAA) face a heightened susceptibility to various infections, encompassing both gram-positive and gram-negative strains (57). A recent case study indicates that the continuous infusion of β-lactam antibiotics presents a promising approach to managing Pseudomonas aeruginosa infections that show resistance to conventional therapies among immunocompromised patients (58). Additionally, patients with SAA are prone to viral infections, given these risks, maintaining vigilance and adopting a proactive stance by prescribing prophylactic antibiotics, antivirals, and antifungals (59).

2.6 Mortality among Patients with AA and Associated Factors

Survival rates for stem cell transplants vary depending on factors like age and donor match. Patients with matched sibling donors have better survival outcomes than those with unrelated donors (51).

Overall, the five-year survival rate exceeds 75% among individuals undergoing transplantation (52). The fate of individuals with severe AA is impacted not only by patient characteristics such as the severity of the condition and age but also by the first therapy chosen. (14). Globally there is neither sex nor age predilection regarding factors associated with mortality among patients with AA (7).

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Study Design and Setting

The analytical retrospective hospital-based cohort study was done at MNH, the national hospital in Tanzania and East Africa. MNH has a bed capacity 1,500 and serves as a national referral hospital, research center, and university teaching hospital, attending approximately 2,000 outpatients daily. MNH is a tertiary health facility in Tanzania. Therefore, there is a high rate of referral from different parts of the country for receiving children with signs and symptoms of aplastic anemia who are diagnosed and managed. The definitive treatment for AA, which is a stem cell transplant, is not yet provided at MNH as of the time of this work in 2024.

3.2 Study population

The sample for this study was selected among children aged less than 18 years, with signs and symptoms of AA and on hematological investigation had pan- or bi-cytopenia for the period of 2016-2023.

3.3 Sample size.

The study's sample size (n) was calculated using a finite population formula proposed by Yamane's formula to maintain a 95% confidence interval.

$$\mathbf{n = N / \{1 + N (e)^2\}}$$

Where by:

n - Minimum required sample size

N Population size (from data in the hospital electronic system and hard copy files, which were reported to be 429 between 2016 and 2023)

e=Margin of error (5%

Therefore: $155 / \{1+ 155(0.05)^2\}$

n= 110

Adjusting, 10% of the estimated sample size was added

$$(110+11) = 121$$

The maximum estimated sample size was 120 patients with aplastic anemia younger than 18 years for an 8-year period.

3.4 Sample Selection

3.4.1 Inclusion Criteria

Children with a confirmed diagnosis of AA who were less than 18 years old at the point of diagnosis admitted at MNH between the period of eight (8) years (between January 2016 and December 2023).

3.4.2 Exclusion criteria

1. Pancytopenia in children due to treatment-related treatments (chemotherapy, radiation therapy)
2. Children with pancytopenia due to infiltrative conditions (leukemia, lymphoma).
3. Children with AA who were lost follow-up and with unknown vital status.

3.5 Variables

3.5.1 Independent variables

1. Socio-demographic characteristics: Age, Sex, physical address
2. Clinical characteristics of AA: Age at the point of diagnosis, severity
3. Type of treatment provided. Including both treatment with IST followed by cyclosporin, Danazole and supportive treatment.
4. Laboratory findings Absolute neutrophil count (ANC), Platelets, Hemoglobin level, Creatinine, Urea, Alanine transferase, C-reactive proteins)

3.5.2 Dependent variables

Mortality rate among patients with AA admitted to MNH between 2016 and 2023.

3.6 Data collection procedures

The data collection methodology adopted in this study primarily relied on hospital records as the principal means of case ascertainment. Medical records at MNH are saved in both written and electronic formats.

All records with Medical Subject Headings (MeSH) terms of AA were searched as indicated by the International Classification of Diseases, 10th Revision (ICD-10) codes D61.9, D61.81, D50-D77, and D61.09. They were systematically retrieved and subjected to thorough review to ensure diagnostic accuracy and minimize the potential for misdiagnosis.

The study cohort included patients presenting with pancytopenia evident in FBP and PBS, subsequently confirmed through hypocellular bone marrow identified via bone marrow aspiration or trephine biopsy.

Data collection was facilitated through the utilization of the Open Data Kit (ODK) Collector platform. To ensure standardized data acquisition procedures, trained field assistants were provided with comprehensive instructions on proficiently using the data collection tool. The data collection methodology adopted in this study primarily relied on hospital records as the principal means of case ascertainment. Medical records at MNH are saved in both written and electronic formats.

The data-gathering instrument was structured into five distinct sections, each designed to capture essential aspects of the study:

1. Socio-demographic Characteristics.
2. Clinical Aspects of AA.
3. Diagnostic Modalities and Investigations.
4. Treatment Modalities.
5. Clinical Outcomes (Death or alive).

For participants lost to follow-up, a supplementary methodology was employed: phone calls, detailed in Appendix 2, were conducted to ascertain the current status of these individuals. For deceased participants, additional information was solicited regarding the

date of death and clinical presentations at the time of demise. Surviving participants were queried regarding the number of hospital admissions since the last recorded follow-up date.

3.7 Data analysis plan

Description analysis was done on categorical variables, which were described using frequencies and proportions. The difference in proportions was analyzed using the chi-squared or Fisher's exact test, as appropriate. This was included in the objectives to determine the clinical presentation of AA, the type of supportive treatment provided, and the trend of the disease, and it was presented in terms of tables and figures.

Univariate logistic regressions were used to determine risk variables associated with mortality in children with AA. The multiple regression model included all variables with a P value of 0.05 or less. A p-value of less than 0.05 was deemed statistically significant. **R-Software version 4.3.2** was used for data analysis.

3.8 The Ethical Consideration

The Muhimbili University of Health and Allied Science (MUHAS) provided formal ethical permission to conduct the study before it was conducted. Further permission was sought from MNH.

Guidelines on maintaining the confidentiality of information provided by participants were effectively adhered to, and no patient identification was used on the questionnaire. Participants who were traced through phone calls provided verbal consent. Information was stored in a password-secured computer database, and the principal investigator secured the hard copies of the written important details in a locked cabinet.

CHAPTER FOUR

4.0 RESULTS

4.1 Recruitment of study participants

A total of 249 cases were identified by MeSH of the ICD-10 codes of AA, of which 159 (63.8%) were excluded because they had no laboratory findings of bi or pancytopenia. Seventy-seven (77) participants were not involved in the study due to various reasons, such as death before confirmation of diagnosis were 9 (5%). Also 54 (33.9%) had other diagnoses. 14 cases (8.8%) were lost to follow-up and could not be traced by phone calls.

The study included 82 participants with either acquired or inherited AA confirmed by bone marrow and/or trephine biopsy admitted to MNH between 2016 and 2023.

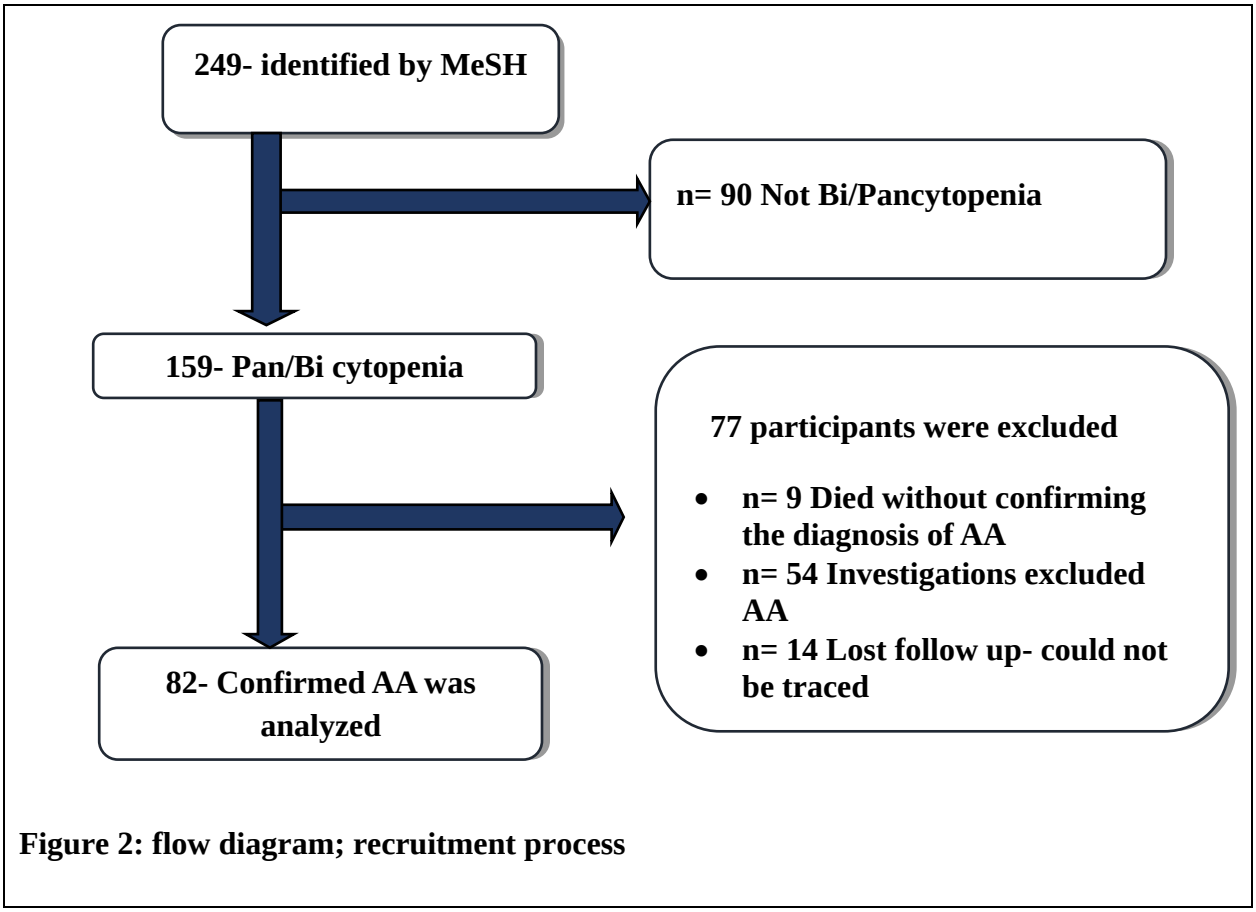


Figure 2: flow diagram; recruitment process

4.2 Social demographic and clinical characteristics of the study participants

A total of 82 patients with confirmed diagnoses of AA were recruited for the study. The study revealed a predominantly male population, with 51 males (62.2%) compared to 31 females. About 40% of participants were in the age group of 6 to 10 years, with the median age at first diagnosis admission being nine (9) years (IQR = 5-11). 41.4% of these patients' caregivers had a secondary education level. 27% of patients had a permanent physical address in Dar es Salaam, where MNH is located. Others were from other regions, including both the Mainland and Zanzibar. (Table2)

Table 2: Demographic data among participants

Variable and category	N	Frequency (%)
Sex		
Male	51	62.2
Female	31	37.8
Age group (Years)		
≤ 5	17	20.7
6- 10.9	33	40.3
11-15.9	27	32.9
16-18	5	6.1
Caregiver Education Level		
Primary	12	14.6
Secondary	34	41.4
Tertiary (Certificate, Diploma, Degrees)	26	31.8
Not documented	10	12.2
Regions		
Dar es Salaam	27	32.9
Zanzibar	3	3.6
Other mainland regions	52	63.5

4.3 Clinical Profile Among Participants

When the diagnosis of AA was established, a clinical profile including symptoms, signs, and laboratory parameters was studied. The most common symptoms were bleeding tendencies (61.0%), body weakness (50.0%), and fever (42.7%). A history of blood transfusions was present in 30.5% of the patients. (Table 3)

The most observed clinical sign was pallor (96.3%), while 36.5% had notable dysmorphic features on examination (microcephaly, microphthalmos, hypogonadism, skeletal anomalies). Other notable signs included petechiae, reported in 20.7% of cases. (Table 3)

Table 3: Clinical presentation Symptoms and signs of AA

Symptoms	**N**	**Frequency %**	**Signs**	**N**	**Frequency %**
Bleeding tendency	50	61.0	Pallor	79	96.3
Fever	35	42.7	Dysmorphic features	30	36.5
Body weakness	41	50.0	Lymphadenopathy	1	1.2
Difficulty in breathing	7	8.5	Jaundice	1	1.2
Cough	4	4.9	Splenomegaly	2	2.4
Dark urine	2	2.4			
Yellowish coloration	1	1.2			
Headache	3	3.7			
Body swelling	3	3.7			
Weight loss	3	3.7			

4.4 Laboratory results at the point of diagnosis.

Using the WHO classification of anemia by severity, it was found that 61% (N = 50) of participants had severe anemia, and 26.8% (N = 22) had very severe anemia. The distribution of levels of ANC was as follows: 25% (N = 21) had ANC of less than 0.2 x 10^9, 41.4% (N = 34) had neutropenia between 0.2 x10^9 and 0.5 x10^9, and 29.2% (N = 24) had a neutrophil count above 0.5 x 10^9/L. Most participants had thrombocytopenia of 75.6% (N = 62) with platelets count of less than 20 x 10^9 /L. (Table 4)

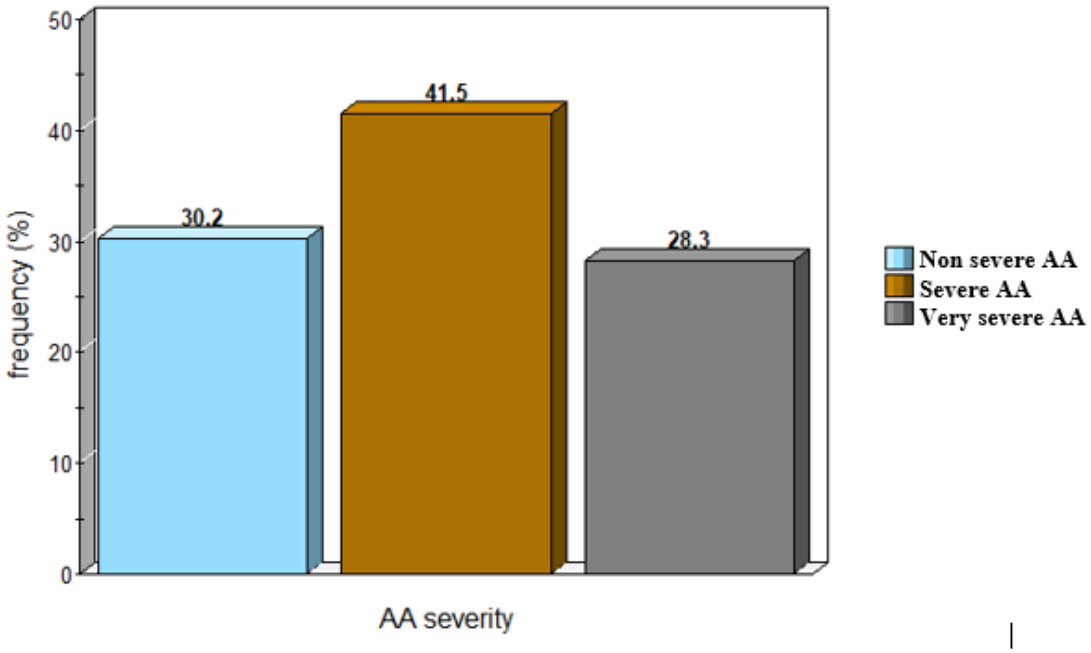
Further classification by Camitta: (Severe AA - decrease in blood counts involving ≥2 hematopoietic lineages i.e., absolute reticulocyte count <60 × 10^9/L, absolute neutrophil count <0.5 × 10^9/L, or platelet count <20 × 10^9/L. Very severe AA has an absolute neutrophil count <0.2 × 10^9/L, none severe AA is by the depression of blood counts not fulfilling the definition of severe disease).

Among participants 41.5% had severe AA, 30.2% had non severe AA, and 28.3% had very severe AA. (Figure 2)

Table 4: Laboratory profile among participants;

Laboratory investigations	N	Frequency (%)
ANC LEVEL (x 10^9/L)		
< 0.2	21	25.6
0.2 – 0.49	34	41.4
> 0.5	24	29.2
HB LEVELS (g/dL)		
>7	32	39.0
4- 6.9	28	34.1
<4	22	26.8
PLATELETS (x 10^9/L)		
< 20	62	75.6
>20	20	24.3

Figure 3: Classification of AA by severity



4.5 The treatment option provided

A total of 79 (96.3%) received treatment for AA according to international protocols. Treatment strategies included ATG, cyclosporine for 47.5% of the patients, and Danazole for 51.2%. 6% received both ATG, followed by cyclosporin and later Danazole. Also, 3.7% added Eltrombopag to their treatment plans.

No patient was treated with cyclophosphamide, and three patients were referred abroad for the definitive treatment of an HSC transplant. Supportive treatment included blood products, treatment for infections with antibiotics, and antifungal treatments. The majority, 86.6%, received packed red blood cells (PRBC), and 59.7% received platelet concentrate.

Table 5: Treatment options

Patient characteristics	N	Frequency %
Treatment		
ATG + Cyclosporine	39	47.5
Danazol	42	51.2
SCT	3	3.7
ELTROMBOPAG	3	3.7
Supportive		
PRBC	71	86.6
FFP	47	57.3
Platelets	49	59.7

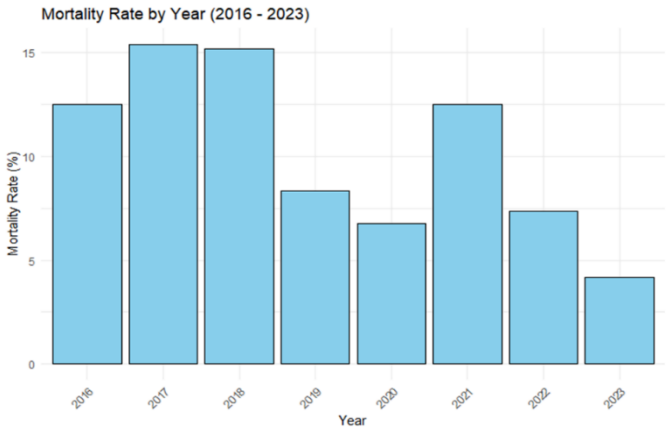
4.6 Mortality rate among patients with AA

The mortality rate among patients with AA was 48.7% (N = 40). There is an overall yearly decrease in the proportion of deaths except for the year 2021.

Figure 4: Proportion of mortality among patients between 2016 and 2023



Figure 5: Mortality rate in years among patients between 2016 and 2023



4.7 Factors associated with the mortality rate

In logistic regression analysis, factors associated with mortality among children with AA (P-value < 0.05) were low ANC, thrombocytopenia of platelets of less than 20 $x10^9$, and increased fat space on bone marrow analysis.

Patients with platelet levels below 20 x 10^9 have a mortality risk that is 20 times higher compared to those with platelet levels above 20 x 10^9. Patients with ANC above 0.5 x 10^9 had a 97% chance of surviving compared to those with less. Of the three patients who were referred for definitive treatment of HSC transplants, one died.

In chi-square analysis, there was statistical significance, with patients with bleeding tendencies having a higher risk of mortality, while those kept on Danazol appeared to offer a protective benefit. Low platelet counts and severe ANC were associated with higher mortality; however, mortality was not statistically significant. AA mortality is not associated with age, sex, or a history of multiple blood transfusions. (Table 6 and 7)

Table 6: Distribution of factors associated with mortality calculated by chi-square.

Variable	Dead	Living	P value
Sex of patient			
Male	24 (47.1)	27 (52.9)	**0.751**
Female	16 (51.6	15 (48.4)	
Age group (years)			
<5	9 (52.9)	8 (47.1)	
5-10	13 (39.4)	20 (60.6)	**0.740**
>11	18 (56.3	14 (43.7)	
Physical address			
Dar es Salaam	12(44.4)	15 (55.6)	**0.710**
Other regions	28 (50.9)	27 (49.1)	
Bleeding tendency			
History present	38(62.2)	23 (37.8)	**<0.001**
No history	2 (9.52)	19 (90.4)	
Platelets levels (x 10^9/L)			
< 20	36 (56.3)	28 (43.7)	**0.024**
>20	4 (40)	6 (60)	
ANC levels (x10^9/L)			
<0.2	13 (52)	12 (48)	
0.2- 0.49	17 (48.6)	18 (51.4)	**0.062**
>0.5	10 (45.5	12 (54.5)	

Table 7: Logistic Regression Factors associated with Mortality

		Univariate analysis				Mult variety analysis			
Category	**Variable**	**OR**	**2.5% CI**	**97.5% CI**	**P**	**OR**	**2.5% CI**	**97.5% CI**	P
Sex	Male	Reference							
	Female	0.5	0.27	1.05	0.077	0.29	0.06	1.09	0.084
Age category	< 10 years	Reference							
	>10 years	0.92	0.49	1.71	0.792	1.4	0.38	5.55	0.609
History of bleeding	No history of bleeding	Reference							
	History	8.85	3.11	29.73	<0.001	16.89	3.23	13.37	<0.001
HB levels (g/dl)	HB < 4	Reference							
	HB 4- 6.9	0.80	0.25	2.51	0.707	0.825	0.087	8.91	0.866
	HB > 7	0.23	0.06	0.68	0.012	0.956	0.123	7.87	0.965
FAT SPACE	No fat space	Reference							
	Increased	2.08	0.73	6.17	0.174	14.390	1.836	206.40	0.021
ANC	ANC <0.2	Reference							
	ANC0.20.49	0.24	0.07	0.74	0.015	0.102	0.005	0.86	0.063
	ANC >0.5	0.08	0.01	0.29	<0.001	0.032	0.001	0.300	**0.007**
Platelets	Platelets >20	Reference							
	Platelets <20	13.67	4.50	52.06	0. 019	27.31	3.645	445.57	**0.004**
History of BT	No history of BT	Reference							
	History of BT	0.91	0.34	2.337	0.858	0.586	0.090	3.340	0.550

*** BT- Blood transfusion, ANC x10^9/L Platelets x 19^3 /L*

CHAPTER FIVE

5.0 DISCUSSION

The study aimed to identify the clinical profile and risk factors for deaths among children with AA hospitalized at MNH for eight years (2016-2023). The study population consisted primarily of males, accounting for 62% of the study population. This data contrasts with the global sex ratio for patients with aplastic anemia (AA), which is about 1:1 (15) but corresponds with another multicenter study indicating a male prevalence of 57% (57). This disparity may result from environmental or genetic variables that increase a child's susceptibility to AA.

5.1 Clinical profile among patients with AA

The study's reported symptoms and signs align with typical presentations of AA, including bleeding tendencies, weakness, fever, and pallor. These findings corroborate existing literature on the clinical manifestations of the disease (10). Among participants 36.5% had dysmorphic features on examination (microcephaly, microphthalmos, hypogonadism, skeletal anomalies), suggesting an inherited/congenital nature of AA.

The finding on the distribution of severity of AA, where most participants had severe AA followed by very severe AA, was similar to another study done in Pakistan, which had a large sample size and showed that non-severe, severe, and very severe AA were 17.4%, 45.2%, and 37.4%, respectively (60). The observed patterns of disease severity in our setting might be related to the fact that most of the patients had already received therapy before referral, hence shifting from very severe AA to severe AA. Further investigation is warranted to elucidate the factors contributing to the observed differences in severity distribution.

Among the participants who had dysmorphic features and underwent abdominal ultrasound as a routine investigation, 45.4% of them had renal malformations. Renal and urinary tract abnormalities in FA are common, with previous studies suggesting that around one-third of patients have structural renal abnormalities (58, 59). The findings in this study are similar to those in another study that involved participants with FA. It found that of the thirty participants who underwent abdominal ultrasound, fourteen (46.7%) had evidence of anatomical abnormalities of the kidneys (5).

5.2 Treatment options provided to patients with AA

The decision to choose treatment options was made by clinical judgment; those with dysmorphic features were kept on Danazole, while those with acquired AA were kept on ATG, followed by cyclosporin. A total of 79 (96.3%) received treatment for AA. Two of them died before starting medications, while one patient did not receive treatment as the parents denied the treatment plan.

Treatment options included ATG, followed by cyclosporine for 47.5% of the patients, and danazol for 51.2%. 6% received both ATG, followed by cyclosporin and later Danazole. This treatment option was similar to what was found in a previous multicenter study; however, the treatment option was based on AA severity. Severe AA and very severe AA included ATG (equine or rabbit), Androgens, IST (calcineurin inhibitors, Daclizumab or Mycophenolate Mofetil), Eltrombopag, and HSC transplant (61).

In previous studies, the decision to choose treatment options depended on the severity of AA. In one study, patients with NSAA were treated with cyclosporine and danazol. In contrast, patients with severe SAA, as well as patients with non-severe AA who progressed to SAA after the beginning of the treatment, were candidates for receiving ATG in addition to cyclosporin and Danazole. After three months, the overall response to treatment was 58.6% in patients with NSAA and 12.9% in patients with severe AA. (54)

Also, 3.7% added Eltrombopag to their treatment plans for IST. Further investigation is needed into the reasons behind the low utilization of Eltrombopag, especially considering its efficacy as a platelet production stimulator. Apart from being expensive the use of Eltrombopag remains controversial as some studies show potential risks include relapse and late-onset clonal disease, such as Paroxysmal nocturnal hemoglobinuria (PNH), Mylo dysplastic syndrome (MDS), or leukemia (54).

The finding in this study of not treating AA with cyclophosphamide is on the same lines as the previous study, which revealed potential side effects of neutropenia (50).

Three patients were referred for HSC transplants, but one eventually died due to complications of HSC Transplant. However, Findings from previous studies still recommend HSC transplant as the definitive management of AA (4, 50).

Supportive treatment included blood products, prophylaxis with antibiotics, and antifungal treatments. The majority, 86.6%, received packed red blood cells (PRBC), and 59.7% received platelet concentrate.

5.3 Mortality rate among patients with AA

The observed mortality rate of 48.7% underscores the severity of AA, particularly in resource-limited settings. This finding is consistent with previous studies worldwide across all age groups (10). However, in advanced settings, the mortality rate of AA is low due to earl diagnosis and advanced treatment. In a previous study done in Canada, the survival rate at 5 years for the age groups (0–18 years) was 86.2% (62).

The overall mortality trend seems to decrease yearly for eight years of study duration. This finding is similar to the reported improved survival rates from modern clinical trials with ATG or SCT registry data (17). The exception to the trend of mortality decrease was the observed variation in 2021.

This warrants exploration into potential contributing factors, such as the impact of the COVID-19 pandemic on healthcare delivery and access to essential services. In one study, the severity of AA increased with COVID-19 infection, HIV and other chronic illness with the hypothesis that since these patients are immunocompromised. However, whether there is a higher risk of contracting infection or they are susceptible to severe COVID-19 illness is unclear. (63)

5.4 Factors associated with mortality among patients with AA.

The findings of this study showed that severe thrombocytopenia, low ANC, and bleeding propensity were all related to mortality. It is consistent with other analytical investigations that demonstrate bleeding, anemia, and infection burden are the main causes of death for patients with AA with no correlation between mortality and neither sex nor age, suggesting that several factors may influence mortality in AA and underscoring the need for a thorough risk assessment. (50, 52, 14). The study's logistic regression analysis confirms the importance of particular clinical factors in AA patients' mortality prediction.

5.5 Study limitations

AA is among the rare diseases, and a small sample size of 82 participants for eight years might make it difficult to conclude these findings and their general applicability.

Also, the study's retrospective nature further limits the accuracy of information.Due to a lack of details from peripheral health facilities, some patients who were referred to MNH had no proper details on their clinical profiles. Furthermore, upon discharge, some patients could not report back for regular clinic follow up.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study found several notable findings, including a predominance of males in the study population, a wide range of clinical presentations of bleeding tendency and fever consistent with known manifestations of AA, and an increasing trend in AA diagnoses over the study period. Treatment strategies employed at MNH were largely in line with standard protocols for AA management, with high utilization of supportive measures such as blood product transfusions. Currently, there is no definitive treatment AA with HCT at MNH. Factors associated with mortality included patients with severe AA and severe neutropenia were in line with general knowledge of AA.

Despite these interventions, the observed mortality rate of 48.7% reinforces the severity of AA and highlights the need for continued efforts to optimize treatment strategies and improve patient outcomes. Also this mortality implies a prognostic factors of having reasonable expectation for severe AA

6.2 Recommendations

1. The Ministry of Health should establish comprehensive care for patients with AA, involving treatment plans from primary health facilities to the national level. This will ensure standard quality of care.
2. Healthcare providers should insure early identification from clinical presentation of AA and hence provide early initiation of treatment so as to improve the quality of care and decreases mortality.
3. A prospective longitudinal study should be conducted to follow up on patients found to have AA to obtain a more precise clinical profile and factors associated with mortality among patient with AA.

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APPENDICES

Appendix 1: Collection Tool.

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF PEDIATRICS

Data Collection Tool

Title: CLINICAL PROFILE AND MORTALITY AMONG CHILDREN WITH APLASTIC ANEMIA ADMITTED AT MNH

QUESTIONAIRE No:

	PART 1 REGISTRATION AND SOCIAL DEMOGRAPHIC DATA.
i.	Hospital Reg. Number
ii.	Date of Birth
iii.	Calculated age. (years)
iv.	Sex
v.	Date of first admission
vi.	Permanent physical address
vii.	The highest Level of education of caregivers
viii.	Occupation of caregiver

PART 2: PHYSICAL EXAMINATION

i. Pulse rate (beats/min)
ii. Respiratory rate (breath/minute)
iii. Oxygen saturation (%)
iv. Temperature (C)
v. Height/length (cm)
vi. Weight (Kg)

In the following, Put Y for YES, N for NO

	YES	**NO**	**SPECIFY**
i. Pallor (if YES, the severity of paleness)			
ii. Cyanosis			
iii. Jaundice			
iv. Lymphadenopathy			
v. visible bleeding/ Petechiae			
vi. Edema			
vii. Dysmorphic features (Microcephaly, Microphthalmos, Hypogonadism, Skeletal anomalies, etc.) If YES specify			
viii. Abdominal tenderness			
ix. Hepatomegaly			
x. Abdominal			
xi. Splenomegaly			
xii. Kidneys ballotable?			
xiii. Others (Specify)			

PART 4: TREATMENT PROVIDED DURING THE COURSE OF ILLNESS			
	YES	**NO**	**SPECIFIED**
i. ANTIBIOTICS (if YES specify)			
ii. ANTIFUNGAL (if YES specify)			
iii. ATG (followed by Cyclosporine)			
iv. DANAZOLE			
i. CYCLOPHOSPHAMIDE			
ii. PLATELETS			
iii. PRBC			
iv. FFP			
v. Referred abroad for allogeneic HSCT			

PART5; IS THE SUBJECT ALIVE? YES …… NO……

- **IF NO, WHAT WAS THE PRESENTATION AT THE TIME OF DEATH (Fever, Bleeding tendencies, DIB, chest tightening, Abdominal pain, Sudden death) ……. Others (specify)…**

- **DATE OF DEATH ……**
- **WHERE THE DEATH OCCURRED (MNH Hospital, Other hospital, Home, On the way to the hospital, …….**

Other informal treatment centers (specify) …….

PART 3. INVESTIGATIONS DONE AT THE ADMISSION OF DIAGNOSIS

1. COMPLETE BLOOD COUNT

i. White cell count
ii. Absolute neutrophil count
iii. Absolute Lymphocyte count
iv. Hemoglobin
v. Red blood cell count
vi. Mean corpuscular volume
vii. Mean Hemoglobin concentration
viii. Platelets count

2. PERIPHERAL BLOOD SMEAR

Reported findings. (Abnormal cells, Cytopenia (bi/pan)) ……

3. BONE MARROW ASPIRATE/TREPHINE BIOPSY

Reported findings. (Hypocellular, increased fat space, Bi/Pancytopenia) …….

4. OTHER LABORATORY INVESTIGATIONS

i. Reticulocyte count …….
ii. Telomere length test…… Flow cytometry DC55/59 ……
iii. Hams test……. LDH …….
iv. Liver enzymes ALT……. AST…… GGT…. ALP……...
v. Creatinine ……..., UREA ……....
vi. Bleeding indices PT……. PTT ……., INR …….
vii. Bilirubin TOTAL, DIRECT
viii. HIV Test ……. Hepatitis panel, B/C ……...

5. RADIOLOGICAL INVESTIGATIONS

Name of investigation …… Findings …….

Appendix 2: phone call; English version

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF PAEDIATRICS

TITLE: CLINICAL PROFILE AND MORTALITY AMONG CHILDREN WITH APLASTIC ANEMIA ADMITTED AT MNH

Hospital Reg. No…… Contacts ……...……

Hello. My name is Nazareth, and I'm calling from Muhimbili Hospital. I'm a medical student at MUHAS conducting a study on patients with aplastic anemia admitted to our hospital but unfortunately lost to follow-up.

I understand that you were involved in caring for a patient named ……. with aplastic anemia who was admitted to our hospital. I'm reaching out to gather some important information about their health status.

Before proceeding, I want to assure you that any information you provide is confidential and used solely for research purposes.

Confirming the Patient Identification:	Full name ……. Age (Years) …… Gender……
Follow-up Status:	• Date last seen or contact made • Current Status at last contact (alive, deceased)
If deceased,	• Presentations at time of death ……
If alive	• Number of admissions at peripheral hospital …… • Any significant changes in health status since last contact • Any change of medications …….

Thank you for taking the time to speak with me today.

Appendix 3 Phone Call; Swahili Version

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES SCHOOL OF

MEDICINE

DEPARTMENT OF PAEDIATRICS

TITLE: CLINICAL PROFILE AND MORTALITY AMONG CHILDREN WITH APLASTIC ANEMIA ADMITTED AT MNH

Na. ya usajili hospitali …….. Mawasiliano ……

Habari, jina langu ni Nazareth, na ninapiga simu kutoka hospitali ya Muhimbili. Mimi ni mwanafunzi wa matibabu katika chuo cha MUHAS na nina fanya utafiti kuhusu wagonjwa wenye ugonjwa wa kupungukiwa damu wa Aplastic anemia waliolazwa katika hospitali yetu lakini kwa bahati mbaya hawakuendelea na matibabu kadiri walivyo pangiwa.

Ninatambua kuwa ulihusika katika kuuguza mgonjwa aitwaye …….. ambaye alilazwa katika hospitali yetu, na nimekupigia simu ili kukusanya taarifa muhimu kuhusu hali yake ya afya kwa sasa.

Kabla hatujaendelea, ningependa kupata idhini yako na kukuhakikishia kuwa taarifa yoyote utakayotoa itahifadhiwa kwa siri na kutumiwa kwa madhumuni ya utafiti pekee.

Kuthibitisha taarifa za Mgonjwa:		Jina kamili: ..... Umri (Miaka): ..... Jinsia: ..... mahali anapoishi ……..
Taarifa za ufuatiliaji		• Tarehe ya mwisho kutibiwa hospitali kwetu……. • Hali ya Sasa (yupo hai, alifariki)
	Ikiwa alifariki	• Dalili zake wakati wa kifo: .....
	Ikiwa yupo hai	• Amelazwa mara ngapi hospitali zingine ..... • Mabadiliko yoyote katika hali ya afya..... • Mabadiliko ya dawa: .....

Asante kwa muda wako kuzungumza nami

Appendix 4; The Approval letter for ethical clearance

UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR – RESEARCH, PUBLICATIONS AND INNOVATION

Ref. No DA 282/298/01.C/1865 Date: 20/08/2023

MUHAS-REC-08-2023-1865
NAZARETH M. MBILINYI
SCHOOL OF MEDICINE
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: CLINICAL PROFILE AND MORTALITY AMONG CHILDREN WITH APLASTIC ANEMIA ADMITTED AT MNH: 2012 TO 2022.

Reference is made to the above heading.

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research

APPROVAL DATE 20/08/2023
EXPIRATION DATE OF APPROVAL 20/08/2024

STUDY DESCRIPTION:
Purpose:
The purpose of this analytical retrospective hospital-based cross-sectional study is to find out the Clinical Profile and factors associated with mortality among children with aplastic anemia admitted at MNH during the period of years 2012-2022

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided:
https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%2014844.pdf and in the MUHAS archives.

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain data transfer agreement (DTA) from NIMR if data will be transferred to a foreign country
7. Apply for and obtain material transfer agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country.
8. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
9. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
10. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://dor.muhas.ac.tz/

Dr. Nahya Salim Mabsoud,
Chairperson, MUHAS Research and Ethics Committee



Appendix 5: Permission Letter

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

MUHIMBILI NATIONAL HOSPITAL

In reply please quote;

Ref. No.: MNH/CRTCU/Perm/2023/515 Date: 29th September, 2023

Head Department,
Paediatrics and Child Health
Muhimbili National Hospital

RE: PERMISSION TO COLLECT DATA AT MNH.

Name of Student	**Nazareth M. Mbilinyi**
Title	**"Clinical Profile and Mortality Among Children with Aplastic Anemia Admitted at MNH"**
Institution	Muhimbili University of Health and Allied Sciences
Supervisor	Dr. Lulu Chirange (MUHAS)
Co - Supervisor	Dr. Peter Swai (MNH)
Period	29th September, 2023 to 30th June, 2024

Approval has been granted to the above mentioned student to collect data at MNH. **A final study report should be lodged with Clinical Research, Training and Consultancy Unit.** Dissemination of study findings to MNH stakeholders shall be overseen by Dr. Peter Swai (MNH) Co – supervisor.

Kindly ensure that the student abide to the ethical principles and other conditions of the research approval.

Yours Sincerely,

Dr. Deus Buma
For: Head of Clinical Research, Training and Consultancy Unit

HEAD, TEACHING, RESEARCH & CONSULTANCY UNIT P. O. Box 65000 DAR-ES-SALAAM MUHIMBILI NATIONAL HOSPITAL

c.c DMS
c.c Dr. Peter Swai
c.c **Nazareth M. Mbilinyi**

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