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Master of Science in Epidemiology and Biostatistics

Clinical characteristics and survival analysis of aplastic anaemia patients at the University Teaching Hospitals, Lusaka

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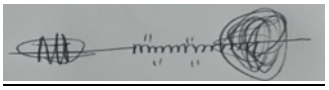
A dissertation submitted to the University of Lusaka in partial fulfillment of the requirements for the award of a Master of Science in Epidemiology and Biostatistics degree.

DECLARATION

I, Milimo Joseph, do hereby certify that this dissertation is my sole research. It has not been submitted for another master's programme.

SIGNATURE DATE



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APPROVED

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ABSTRACT

Background: The current study aimed at assessing the characteristics of patients with aplastic anaemia and determining survival rates and predictors of survival for patients diagnosed with aplastic anaemia at the University Teaching Hospitals (UTH), Lusaka, Zambia.

Methods: A retrospective cohort study was conducted at UTH, Lusaka, Zambia. The included cases were patients diagnosed with aplastic anaemia from 2014 to 2023. Data was collected through review of patients' medical records. Kaplan-Meier plots were used in the analysis of time-to-event data for estimation of survival probabilities. The log-rank test was utilized to make comparisons between survival curves. Cox proportional hazards regression was used to analyze risk factors for survival. Data analysis was done using R version 4.3.2.

Results: A total of 126 cases were included, 96 paediatric cases and 30 adult cases, from 2014 to 2023. For paediatric patients, 52.08%(n=50) were females while for adult patients, 73.33% (n =22) were male. The median age at diagnosis was 11 years (IQR =12) and 22.5 years (IQR = 36) in paediatrics and adults, respectively. The most common cause of aplastic anaemia was unknown in both paediatrics and adults. Most of the participants had NSAA followed by SAA and VSAA in both groups. The mortality rate was 21.88% and 46.67% in paediatrics and adults, respectively. The median survival time from diagnosis was found to be 65 months in the paediatric cases. The 1-year survival rate was found to be at 43% and 53% in paediatrics and adults, respectively. The study did not find any factors to be independent predictors of survival among paediatrics patients with aplastic anaemia in both groups. Analysis of all the patients revealed a slight male predominance, a median age of 12 years and a median survival time of 65 months. The study reports a mortality rate and a 1-year survival rate of 27.78% and 42%, respectively. The study found that being a paediatric conferred superior survival while (HR = 0.33, 95%CI 0.17 – 0.66, p = 0.002) and presenting with bleeding was an independent predictor of inferior survival (HR = 2.90, 95% CI 1.25 – 6.71, p = 0.013).

Conclusion: Paediatric aplastic anaemia patients have better survival and haemorrhage remains a major predictor for mortality. An improvement in the management of adult aplastic anaemia patients in needed. Prevention, early identification and treatment of bleeding is needed. Availability, accessibility and affordability of treatment are recommended.

Key words: Aplastic anaemia, Survival analysis, Immunosuppressive therapy, pancytopaenia, Zambia

DEDICATION

This work is dedicated to family, friends, colleagues and lecturers who gave unwavering support.

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Abbreviations

ATG	Antithymocyte Globulin
CsA	Cyclosporine
CyA	Cyclosporine
E-PAG	Eltrombopag
HCT	Hematopietic Cell Transplantation
HSCT	Hematopietic Stem Cell Therapy
IST	Immunosuppressive Therapy
NSAA	Non-Severe Aplastic Anaemia
rATG	Rabbit Antithymocyte Globulin
SAA	Severe Aplastic Anaemia
SCT	Stem Cell Therapy
UTH	University Teaching Hospitals
VSAA	Very Severe Aplastic Anaemia

CHAPTER ONE

INTRODUCTION

1.1. Background

Aplastic anaemia is a rare life-threatening bone marrow condition. It is a syndrome of bone marrow failure with varying severity of bone marrow hypoplasia and significant pancytopenia *(Clucas et al., 2019)*. It is due to hypocellular bone marrow failure and characterized by pancytopenia (Keel and Geddis, 2021). Aplastic anaemia is categorized as either inherited or acquired aplastic anaemia. Acquired aplastic anaemia represents most of the cases (Ahmed *et al.,* 2020).

Aplastic anaemia is a significant cause of morbidity and mortality. This is due to its natural history and the devastating complications as a result of unsatisfactory management. The mortality at 2 years due to aplastic anaemia can be as high as 70 percent, if it is not adequately managed (Tichelli *et al.,* 2020).

The incidence of aplastic anaemia is varied with higher incidence in Asia and Africa compared to Europe and North America, which have an incidence of about 2.0 million per year (Kojima, 2017; Ally *et al.,* 2019). A bimodal incidence peak has been reported, with young adults comprising the first peak and the elderly comprising the second peak (Vaht *et al.,* 2017).

There are various aetiologies that have been associated with aplastic anaemia. These include exposure to chemicals such as benzene and pesticides; infections such as Epstein-Barr virus infection, hepatitis, HIV; autoimmune conditions such as systemic lupus erythromatosis as well as drugs such as anti-epileptics (Schoettler *et al.* 2018; Shallis *et al.* 2018; De-Melo *et al.* 2020). However, in most cases, the aetiology is not known (Young, 2018).

The clinical presentation of aplastic anaemia include pallor; easy fatigability; bleeding such as epistaxis, bleeding from the gums, bleeding from the skin; and

fever. In females, aplastic anaemia can also present with menorrhagia (Peslak *et al.* 2017).

The treatment modalities of aplastic anaemia may comprise of supportive therapy only, immunosuppressive therapy (IST) or hematopietic cell transplantation (HCT). The human leukocyte antigen (HLA)-matched sibling-donor HCT is the definitive curative treatment for severe or very severe aplastic anaemia, particularly in patients younger than 50 years (Killick *et al.* 2016). However, HCT has various drawbacks such as lack of suitable matched donors, risk of graft-versus-host disease and it being expensive (Youssef *et al.* 2023).

IST, mostly consisting of antithymocyte globulin (ATG) and cyclosporine (CsA), is the alternative to HCT for patients who are ≤50 years old and the first line of treatment for patients older than 50 years or patients between 35 – 50 years old with comorbidities. IST is also the recommended first line of treatment for patients with non-severe aplastic anaemia (Killick *et al.* 2016). Supportive therapy includes blood transfusion, iron chelation and treatment of infections through antibiotic prophylaxis, empirical antibiotic treatment, antiviral therapy, antifungal agents and cytokine support (Killick *et al.* 2016; Youssef *et al.* 2023).

Survival analysis is an important set of statistical procedures that can be utilized in assessing various factors that influence and impact the outcomes and survival of patients with aplastic anaemia. This study thus employed survival analysis in understanding aplastic anaemia at the University Teaching Hospitals (UTH).

1.2. Statement of the problem

Despite the substantial advancements in the treatment options for aplastic anaemia, aplastic anaemia remains a huge problem (Georges *et al.* 2018). This calls for an extensive understanding of the incidence, associated factors and survival analysis of patients with aplastic anaemia.

Optimal management of aplastic anaemia is a cardinal determinant of survival of patients with aplastic anaemia. Treatment of aplastic anaemia still remains a big

challenge, particularly in resource limited settings. This could partly be due to unaffordability of more advanced and definitive treatment modalities (Zhang *et al.* 2021). As a result, practitioners do not treat patients according to the standard management guidelines as they opt for alternative treatment modalities.

A lack of understanding of the survival time and the associated factors will lead to a lack of improvement in the health indicators of patients with aplastic anaemia. This entails that there would be no reduction in the case fatality, mortality as well as no improvement in the life expectancy of patients with aplastic anaemia.

With advancement in treatment modalities for aplastic anaemia, it is imperative to assess the survival of patients with aplastic anaemia and the clinical characteristics that predict their survival. Additionally, there is paucity of research on aplastic anaemia in Zambia. Thus, this study sought to investigate and determine the clinical characteristics and survival of aplastic anaemia patients in Zambia.

1.3. Research objectives

1.3.1. Main objective

The main objective was to assess the clinical characteristics and survival of patients with aplastic anaemia at the University Teaching Hospitals.

1.3.2. Specific objectives

1. To establish the clinical characteristics of patients presenting with aplastic anaemia.
2. To assess the treatment types for aplastic anaemia.
3. To determine the prognosis and survival rate of patients with aplastic anaemia based on their age, sex, disease severity, disease aetiology, cause of death and type of treatment at UTHs.

1.4. Research questions

1. What are the clinical characteristics of patients presenting with aplastic anaemia?

2. What are the treatment types for aplastic anaemia?

3. What is the prognosis and survival rate of patients with aplastic anaemia based on their age, sex, disease severity, disease aetiology, cause of death and type of treatment?

1.5. Justification of the study

Aplastic anaemia is a fatal condition. In spite of the relative rarity of aplastic anaemia, the incidence has been reported to be higher in developing nations (Kushwaha & Singh, 2024). An understanding of the clinical characteristics of patients with aplastic anaemia is vital as certain characteristics and factors influence the survival time of patients with aplastic anaemia.

Knowing the survival of aplastic anaemia patients and the associated factors for survival would help in the endeavour to improve the quality of life of aplastic anaemia patients.

This research will help provide information on survival of patients with aplastic anaemia and the related influencing characteristics. The knowledge obtained from this study will be important to practitioners in order to make improvements in the management of aplastic anaemia in Zambia.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The chapter is a review of literature on the incidence, treatment, patient characteristics and survival analysis of aplastic anaemia. The theoretical and conceptual frameworks are also presented.

2.2 Aplastic anaemia Incidence

The incidence of aplastic anaemia differs in different populations. A Swedish study found that the incidence of aplastic anaemia was 2.35 cases per million persons per year (Vaht *et al.*, 2017). Additionally, the study found that was no significant difference in the incidence between the time periods 2000 – 2005 (2.19 (95% CI: 1.80–2.29)) and 2006 – 2011(2.5 (95% CI: 2.08–2.91)). The study findings also presented a biphasic age distribution; one peak in patients aged 15–20 years (2.87 (95% CI: 1.72– 4.03)), and another in patients >60 years old (4.36 (95% CI: 3.55–5.18)).

The incidence of aplastic anaemia has been reported to be higher in Asia. The incidence of aplastic anaemia in Japan was found to be 8.2 per million person-years (Ohta *et al.*, 2015). The incidence in males was found to be 7.6 per million person-years while the incidence in females was found to be 8.8 per million person-years with a female to male sex ratio of 1.16. Furthermore, the age specific incidence showed biphasic peak at age 10–20 years and 70–80 years, with a larger peak seen at 70–80 years. The age of onset of aplastic anaemia in Japan was found to be higher in females than males (Nagai, 2015). The incidence of aplastic anaemia in China has been documented to be 7.4 per million persons per year, has equal distribution by sex and a bimodal age distribution with a peak between 15 – 25 years and another peak at >60 years (Liu & Shao, 2018).

A Taiwan study investigating the incidence and survival of aplastic anaemia patients who were diagnosed between 2001 and 2010 found the incidence of 5.67 per million persons per year (Li *et al.*, 2018). There was a biphasic age distribution of the incidence, highest in patients ≥70 years (19.83 per million persons per year) and another peak in patients aged 2 – 9 years (5.26 per million persons per year). Unlike findings from Japan (Ohta *et al.*, 2015) which had a female predominance and results from India (Mahapatra *et al.*, (2015) and Pakistan (Ahmed *et al.*, 2020), which found a male predominance, Li *et al.* (2018) did not observe a difference in the sex-specific incidence rates of aplastic anaemia. Similar findings have been

observed in Spain (Montane *et al.*, 2008) and Sweden (Vaht *et al.*, 2017) where no difference in the incidence rates between males and females was observed.

The incidence of aplastic anaemia in Thailand has been reported to be 4.6 cases per million persons per year (Norasetthada *et al.*, 2021). The study observed that peak incidence was in the elderly of age group 80 to 89 years. The article further indicates that the incidence of SAA and VSAA (3.8 cases per million) was found to be higher than NSAA (0.8 cases per million). However, Ashwini *et al.* (2016) found that there were more non-severe aplastic anaemia cases than severe and very severe cases. Ashwini *et al.* (2016) also reported a biphasic age distribution.

A Nigerian study investigating patients who were diagnosed with aplastic anaemia over a 10 year period of 2012 – 2021 found an incidence of 1.625 cases per year (Osho *et al.*, 2022) while an incidence of 6.4 cases per year was observed in South Africa (Ramsamy *et al.*, 2022). The incidence rate of aplastic anaemia in Tanzania has been reported to be 6 cases per million persons per year (Ally *et al.*, 2019).

2.3 Treatment of aplastic anaemia

Treatment plays a crucial role in the outcomes of patients with aplastic anaemia. However, recommended treatment modalities are not readily available or accessible to everyone. For example, Mahapatra *et al.* (2015) found that 32.5% of patients with aplastic anaemia either did not receive any treatment or were treated with androgens only due to unaffordability of stem cell therapy (SCT) or IST. Similarly, Zhu *et al.* (2019) found that despite most of the patients in China being managed with IST, few were managed according to the recommended combination of ATG and CsA due to financial constraints. Notwithstanding that, the study reported that most of the patients that received any IST had partial or complete response.

Various factors may affect patients' responsiveness to treatment. A meta-analysis of clinical trials found that there was no statistically significant association between the aplastic anaemia severity and absolute neutrophil count and unresponsiveness of patients to IST (Wang *et al.*, 2020). However, age (≥60 years), cytogenetic abnormalities and human leukocyte antigen (HLA)-DR2 negativity had statistical significant association with unresponsiveness to IST.

Ramsamy *et al.* (2022) investigated the response to immunosuppressive therapy by aplastic anaemia patients. The study found that only 18% had complete response to therapy, 49% had partial response and 29% were refractory to immunosuppressive therapy. Additionally, there were no significant differences in treatment response according to age, sex, ATG type or the HIV status of the patient.

Fattizzo *et al.* (2023) found that the majority of aplastic anaemia patients were treated with cyclosporine (CyA) alone or in combination with ATG or eltrombopag (E-PAG), E-PAG alone, or others including androgens. However, similar outcomes were observed across different strategies, with a 6-month overall response rate of 73% for CyA, 74% for ATG plus CyA, 68% for CyA plus E-PAG, 87% for E-PAG, and 79% for others. In resource limited settings, E-PAG+ CsA was found to be a safe and effective alternative treatment for children with SAA (Youssef *et al.*, 2023).

In contrast to the findings by Fattizo *et al.* (2023), Norasetthada *et al.* (2021) observed that different treatment strategies yielded statistically significant different outcomes. The study found that patients with SAA/VSAA who were treated with rabbit ATG with/without cyclosporin A (rATG± CsA) had a significantly superior response rate than those treated with CsA alone, or anabolic steroids (44.4% vs 36.4% and 31.2%, respectively, $P < 0.001$).

2.4 Aplastic Anaemia Patient Characteristics

A Zimbabwean case control study found that HIV/AIDS, viral hepatitis and exposure to chloramphenicol were not significantly associated with aplastic anaemia (Makaza *et al.* 2023). However, there was significant association with pancytopenia. Similarly, a study by Syed *et al.* (2021) which investigated the relationship between drugs and aplastic anaemia found that chloramphenicol and other drugs such as trimethoprim/sulfamethoxazole, benzodiazepines, antihistamines, oral contraceptives, and herbal medicine were not significantly associated with aplastic anaemia. The study also found that pesticide exposure, carbamazepine, thiazides and mebendazole had a positive association to aplastic anaemia. Unlike Makaza *et al* (2023), Waja *et al.* (2018) found a significant association between HIV/AIDS and aplastic anaemia.

On the other hand, the study by Wu *et al.* (2019) found that hepatitis was a significant risk factor for aplastic anaemia. Additionally, prematurity, low birthday weight, were found to be significant risks for aplastic anaemia. Furthermore, drinking alcohol, smoking, unhealthy dietary practices (unbalanced diet), drinking pond or lake water, use of hair dye and occupational chemical exposure were found to be significant risk factors. This underscores the important role that the environment and individual behaviours play in aplastic anaemia.

Some studies have attempted to determine associations between socio-demographic factors and aplastic anaemia. A study investigating the importance of socio-demographic factors associated with aplastic anaemia found that family history of anaemia, being illiterate, occupation, age above 30 years and living in a rural setup

were significantly associated with aplastic anaemia (Syed *et al.*, 2021). These findings are consistent with another study in which low socio-economic status and rural residence was found to be significantly associated with aplastic anaemia (Akram *et al.*, 2019). This is also similar to the study by Ahmed *et al.* (2020) who found that most of patients with aplastic anaemia were from low or middle class socio-economic status.

A study in India found that aplastic anaemia was most common in the age group of 10 – 20 years and a median age of 25 years (Mahapatra *et al.*, 2015). Additionally, aplastic anaemia had a male preponderance. Most of the patients had SAA, followed by VSAA and NSAA. Zhu *et al.* (2019) also found that most of the aplastic anaemia patients had SAA, followed by VSAA and the least being NSAA. On the contrary, Khalid *et al.* (2022) and Ramsamy *et al.* (2022) found that most patients presented with NSAA, followed by SAA and VSAA.

Similar to the findings by Mahapatra *et al.* (2015), Ahmed *et al.* (2020) found that aplastic anaemia was more common in males and the younger population. Additionally, those with SAA constituted the majority of the cases followed by those with VSAA and lastly NSAA. Furthermore, history of consanguinity among aplastic anaemia patients was found to be higher than in the general population, a finding consistency with a study from Japan (McGowan *et al.*, 2019). Syed *et al.* (2021) found a significant positive association between family history of aplastic anaemia and aplastic anaemia. Wu *et al.* (2019) found an association between aplastic anaemia and family history of other haematological diseases and malignancies. The early onset of aplastic anaemia, the high degree of consanguinity and the family history observed in these studies suggests the possible role genetics play in aplastic anaemia.

The clinical presentation of aplastic anaemia is quite variable and depends on the severity. The study by Mahapatra *et al.* (2015) found that the most common clinical findings of aplastic anaemia include fever, pallor and bleeding manifestations such as gum bleeding, epistaxis, bleeding from the skin and abnormal uterine bleeding. These findings were replicated by other studies such as Ashwini *et al.* (2016), Waja *et al.* (2018) and Khalid *et al.* (2022).

2.5 Survival analysis of aplastic anaemia

Several studies that investigated prognostic indicators and survival of patients with aplastic anaemia have been conducted. A study in South Africa conducted by Ramsamy *et al.* (2022) did not find statistical differences in the outcome by sex, age, immunosuppressive treatment type and HIV status. Contrary, Fattizzo *et al.* (2023) found that poor survival was associated with older age, male sex, elevated

lactate dehydrogenase levels, somatic mutations and blood transfusion need. Additionally, a higher absolute neutrophil count at diagnosis, presence of paroxysmal nocturnal hemoglobinuria clone, trilineage response to treatment at 6 and 12 months were associated with better survival outcomes.

The severity of the aplastic anaemia has been found to have an impact on the survival of patients. A Chinese study found being in the age group 36 – 65 years, having SAA or VSAA and having not received therapy as independent risk factors for inferior survival (Zhang *et al.*, 2021). Norasetthada *et al.* (2021) found that the 2-year overall survival in NSAA, SAA, and VSAA patients were 65.5%, 49.3%, and 20.1%, respectively (P<0.001). Additionally, the study found that the 2-year overall survival in SAA/VSAA patients treated with rATG ± CsA, CsA, and anabolic steroids was 54.8%, 54.5%, and 37.6%, respectively (P=0.037). As such, it demonstrated that rATG ± CsA provided superior survival. Li *et al.* (2018) also showed that severity and treatment affected survival as the study found that VSAA, SAA and receiving treatment other than HSCT, IST, or androgens conferred inferior survival. This emphasizes that the type of treatment has an impact on the survival of aplastic anaemia patients.

In patients receiving matched sibling donor allogeneic transplantation, age has been found to be the most important predictor of survival. This is supported by the study conducted by Gupta *et al.* (2010) which showed that patients younger than 20 years had better 5-year survival compared to those between 20 – 40 years and those older than 40 years (82% vs 72% vs 53%, respectively, p<0.001). Similar findings have also been reported by Kim *et al.* (2012) where patients ≤40 years had a better 5-year survival rate compared to those older than 40 years (76.6% vs 55.9%, p=0.007).

In another study comparing the outcomes of transplantations in patients with SAA between 2001 – 2010 and 2011 – 2021, it was found that there was no significant improvement in the survival of aplastic anaemia patients (Zielinska *et al.,* 2023). This finding is collaborated with an earlier study which found no improvement in survival of aplastic anaemia patients who received transplants (Giammarco *et al.*, 2018).

Giammarco *et al.* (2018) compared the outcome of patients older than 40 years with SAA who had received HSCT between 2001 – 2009 and 2010 – 2015. The study found that there was no significant change in the survival of aplastic anemia patients between the two eras, showing a lack of improved survival in 2010 to 2015, as compared with 2001 to 2009. The study also found that those between the age 40 – 49 years had better 5-year survival than those between 50 – 59 years, and those above the age of 60 years (67%, 58%, and 45%, respectively (P <0.0001)). Survival was also better in patients who had received either ATG or alemtuzumab in the

conditioning regimen compared to those that had not received any (63% vs 48% ($P < 0.0001$)).

Vaht *et al.* (2017) investigated the survival of aplastic anaemia patients in Sweden. The study found that there was a survival rate of 96% in patients who had undergone HSCT, 68.9% in those that had IST and 29.6% in those that had received CSA alone or no therapy. Survival was not different between age groups ≤18 years and 19 – 39 years who had HSCT or IST. However, patients aged 60 years and above had the worst survival compared to those ≤18 years (P=0.003) and 19 – 39 years (P=0.001) but not with those aged 40 – 59 years (P=0.15). Furthermore, the Cox regression analysis revealed that age (40-59 and ≥60 years age groups), VSAA and treatment with CSA alone/no therapy were independent risk factors for inferior survival.

A study which investigated the efficacy of treatment in patients with SAA which progressed from NSAA discovered that patients with SAA which progressed from NSAA who underwent allo-HSCT experienced a better clinical course than with IST, and even though the overall survival remained similar, failure-free survival was superior (Liu *et al.* 2021). Additionally, IST was found to have a lower efficacy in SAA that progressed from NSAA compared to patients with SAA that had met the criteria for SAA at the time of diagnosis.

Kekre *et al.* (2017) documented that a delay in doing HSCT by over 3 months from the time of diagnosis was associated with higher mortality. This underscores the importance of early diagnosis and treatment initiation in order to improve the survival of aplastic anaemia patients.

The major causes of mortality in aplastic anaemia patients have been documented in various studies. Giammarco *et al.* (2018) reported major cause of death to be infections, followed by graft-versus-host disease (GvHD) and organ toxicity. Likewise, Waja *et al.* (2018) found infection, particularly sepsis to be the leading cause of death. This indicates the importance of early treatment and prevention of infections. Bleeding has also been found to be a common cause of mortality in aplastic anaemia patients (Vaht *et al.*, 2017).

Despite studies having been conducted on aplastic anaemia, much of the data and evidence on aplastic anaemia are from other continents. Additionally, there is a paucity of published research on aplastic anaemia done in Zambia. To the best of our knowledge, this is the first study to be done in Zambia assessing the survival of aplastic anaemia patients. Aplastic anaemia epidemiology has been documented to exhibit marked geographical variability and period of observation. Due to the geographical, genetic and racial differences, findings from foreign studies may not

give a true reflection of the epidemiology, treatment and survival of aplastic anaemia patients in the Zambian context.

Additionally, management of aplastic anaemia in Zambia could also be affected by financial constraints of the country, Zambia being a developing country. Consequently, some patients may not be managed according to the standard treatment guidelines which affect their survival. Therefore, in order to bridge these existing gaps, this study sought to investigate the clinical characteristics, treatment and survival of aplastic anaemia patients in the Zambian population.

2.6 Conceptual framework

The conceptual framework for this research is depicted in figure 2.1

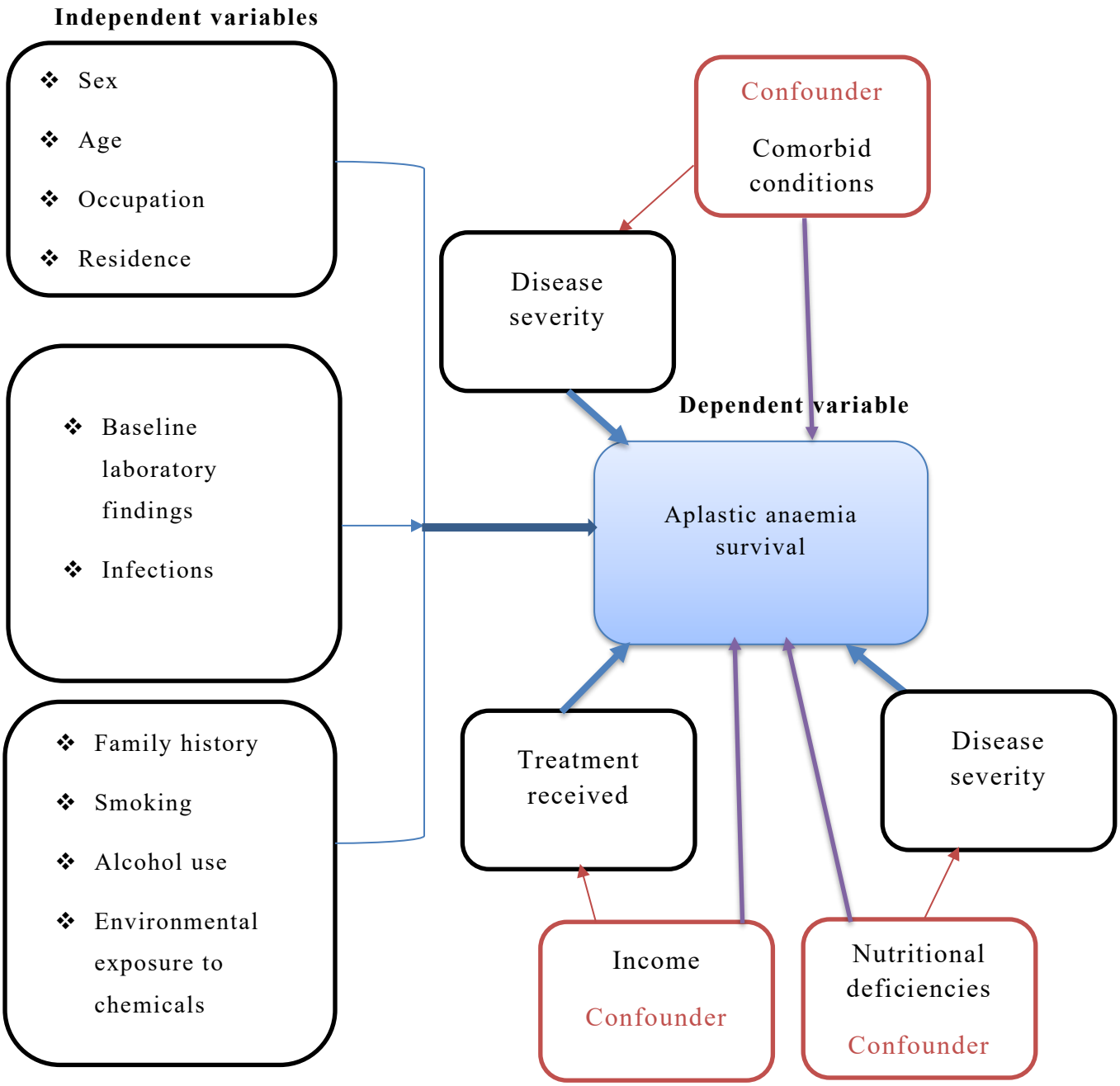

Figure 2.1 Conceptual framework

Source: Researcher (2024)

2.7 Chapter Summary

This chapter reviewed previous literature and the conceptual framework. It presented the incidence of aplastic anaemia, the characteristics of aplastic anaemia patients, the various treatment modalities and the survival data on aplastic anaemia.

It further presented the interactions between various variables in a conceptual framework.

CHAPTER THREE: METHODOLOGY

3.0. Introduction

This section describes the methodology for the proposed research. It includes the research approach, research design, research setting, study population, sampling, data collection, data analysis, data validity and reliability, and ethical considerations.

3.1. Research Approach

The study utilized a quantitative research approach. The quantitative research involves gathering and employing statistical techniques to analyse numerical data by describing participant characteristics, studying trends, evaluating associations or testing hypotheses (Park *et al.*, 2020).

The study was grounded in the Positivism Paradigm which asserts that clinical outcomes in aplastic anaemia are objective phenomena that can measured and analyzed through quantitative methods. By utilising standardised hematological indices and survival metrics, the study sought to identify cause-and-effect relationships between clinical presentation and patient longevity at UTH, Lusaka, ensuring that the findings are both replicable and statistically significant. A Positivist Paradigm was highly appropriate for this study. It treats the disease and its outcomes as objective, measurable, and independent of the researcher's perspective. Using the Positivist paradigm, aplastic anaemia exists as a concrete biological reality and survival is viewed as an absolute binary outcome (alive or deceased) and the clinical characteristics are objective facts that can be measured without bias. Additionally, knowledge is acquired through the rigourous process of collection of numerical data from laboratory results and medical files. Finally, the research remains emotionally and personally detached from the participants. The researcher did not influence the survival of the patients; they merely recorded and recorded the “truth” found in the medical records.

3.2. Research design

The study utilized a retrospective cohort study design. In retrospective study design, individuals with a known exposure are selected and followed back in time to assess the occurrence of outcomes. In this study, individuals with a diagnosis of aplastic anaemia were followed to assess the occurrence of outcomes such as survival using available medical records.

3.3. Research setting

This study was conducted at the University Teaching Hospitals, Lusaka, Zambia. UTH is the largest public tertiary hospital in Zambia. It is a health care service provider to much of Lusaka's population as well as referrals from other parts of Zambia.

3.4. Study population

The study population were individuals diagnosed with aplastic anaemia from 2014 to 2023 whose records are with UTH. A 10-year period gave sufficient time to understand the trends in the survival of aplastic anaemia patients.

3.5. Sampling

3.5.1 Sample size

The sample size determination was done based on available data in the institution's medical records. Suitable cases, cases with data on the required variables, over the follow up period were selected into the study. The total number of patients whose records were reviewed and selected constituted the study's sample size. Approximately 15 – 20 aplastic anaemia patients are seen at UTH in a year.

3.5.2 Sample Selection

a) Inclusion criteria

Individuals diagnosed with aplastic anaemia with available medical records.

b) Exclusion criteria

1. Individuals with missing key data.

2. Individuals who were pregnant.

3.6. Data Collection

The study utilized secondary data sources to obtain participants' information. The data was collected through review of medical records of aplastic anaemia patients kept at UTH. Prior to data collection, permission was obtained from UTH management. This facilitated the investigator to have access to the medical records. The data collected included demographic and clinic characteristics, treatment received and outcomes. Table 3.1 shows key variables that will be used and their descriptions.

Table 3.1 Description of key variables

Variables	**Descriptions**	**Scale of measurement**
Dependant variables		
Date of diagnosis	Year of aplastic anaemia diagnosis	Nominal
Age	Patient's age at diagnosis	Ratio
Sex	Patient's sex	Nominal
Location	The patient's place of residence	Nominal
Family History	Patient's family history of aplastic anaemia	Nominal
Smoking	Patient's history of smoking	Nominal
Alcohol	Patient's history of alcohol consumption	Nominal
Chemical exposure	Exposure to drugs or environmental agents	Nominal
Severity	Aplastic anaemia severity at diagnosis	Ordinal
Treatment	Treatment received after diagnosis	Nominal
Outcome Variable		
Survival status	Patient's survival status	Nominal

3.7. Data analysis

Data management and statistical analysis was done using R version 4.3.2. Descriptive data analysis was presented in frequency tables and graphs. Kaplan-Meier plots were used in the analysis of time-to-event data for estimation of survival probabilities. Furthermore, the log-rank test was utilized to make

comparisons between survival curves. Cox proportional hazards regression was used to analyze risk factors for survival.

3.8. Scientific Rigor

Information of the participants was uniformly recorded in order to ensure data integrity and accuracy. Additionally, a speciality in haematology was consulted to provide input on the data variables to be measured. Additionally, a pilot study was conducted to identify any potential problems with the data collection process and provide familiarization of protocol procedures.

3.9. Ethical considerations

The study took into account ethical principles. The researcher obtained ethical clearance from the University of Lusaka School of Medicine and Health Sciences Research Ethics Committee. Additionally, approval was obtained from the National Health Research Authority (NHRA).

Anonymity and confidentiality were maintained. Personal identifying information was not recorded. Furthermore, measures were put in place to prevent unauthorized access to the data. This was ensured through the storage of the data using a password secured system as well as only personnel involved in the study having access to the data.

CHAPTER FOUR: PRESENTATION AND ANALYSIS OF RESULTS

4.0 Introduction

The section presents the results and their analyses. The section presents results for the paediatric respondents, followed by adult respondents and finally, data for all the respondents.

4.1 Paediatric participants

4.1.1 Demographic characteristics of paediatric participants

Of the 96 paediatric patients with aplastic anaemia that were included in our analysis, they had a median age of 11 years (IQR=12). About 52% of the participants were females, 57.29% were from outside Lusaka (Table 4.1).

Table 4.1 Demographic characteristics of paediatric population

Characteristic		**Patients included n(%)**
Age (n=96)	Median (IQR)	11 (12)
Sex (n=96)	Male	46 (47.92)
	Female	50 (52.08)
Residence (n=96)	Lusaka	41 (42.71)
	Outside Lusaka	55 (57.29)

4.1.2 Clinical Characteristics of paediatric participants

From the 96 participants, 21(21.88%) died and 75 (78.13%) were alive at the end of follow up. The commonest cause of mortality was bleeding (n=17, 80.95%). The median weight of the participants was 25Kg (IQR=43.2).

With regards to severity, there were 45 (46.88%), 26 (27.08%) and 25 (26.04%) patients with NSAA, SAA and VSAA, respectively. The majority of the patients presented with features of anaemia (n=92, 95.83%), while 65.63% (n=63) presented with bleeding and 45.83% (n=44) presented with fever. Approximately 23% of the participants had dysmorphic features. The commonest aetiology was unknown (n=56, 58.33%) followed by Fanconi anaemia (n=24, 25.00%).

Of the 96 participants, 4 (4.17%) underwent HSCT, 5 (5.21%) had HSCT combined with other treatment modalities. Steroids alone were used in 39.58% of participants (n=38) while 28.13% (n=27) of the participants were managed conservatively (Table 4.2).

Table 4.2 Clinical Characteristics of paediatric population

Characteristic		**Patients included n(%)**
Survival Status (N=96)	Alive	75 (78.13)
	Dead	21 (21.88)
Aplastic Anaemia Severity (N=96)	NSAA	45 (46.88)
	SAA	26 (27.08)
	VSAA	25 (26.04)
Weight (n=86)	Mean	28.01977±13.3267
	Median	25 (IQR=43.2)
	Range	4.7 – 66.4
Features of anaemia (N=96)	Yes	92 (95.83)
	No	4 (4.17)
Bleeding (N=96)	Yes	63 (65.63)
	No	33 (34.38)
Fever (N=96)	Yes	44 (45.83)
	No	52 (54.17)
Presence of Dysmorphic Features (N=96)	Yes	22 (22.92)
	No	74 (77.08)
Aetiology (N=96)	Unknown	56 (58.33)
	Fanconi anaemia	24 (25.00)
	Infections	9 (9.38)
	Chemical Exposure	7 (7.29)
Family History of aplastic anaemia (N=58)	Yes	15 (25.86)
	No	43 (74.14)
Chemical Exposure	Yes	9 (15.52)

(N=58)	No	49 (84.48)
Cause of death (N=21)	Bleeding	17 (80.95)
	Sepsis	1 (4.76)
	Sepsis & bleeding	3 (14.29)
Treatment (N=96)	Conservative	27 (28.13)
	Steroids	38 (39.58)
	Steroids and IST	9 (9.38)
	HSCT	4 (4.17)
	HSCT in combination with other modalities	5 (5.21)
	Other treatment or treatment combinations	13 (13.54)

4.1.3 Laboratory features of paediatric participants

The median white blood cell count was 2.22 (IQR = 8.07) X10⁹/L, median platelet count was 13.5 (IQR = 140) X10⁹/L, the median absolute neutrophil count was 0.335 (IQR = 3.06) (X10⁹/L), haemoglobin level median was 5.9 (IQR=9.19) g/dL and the median Reticulocyte count (%) was 1.56 (IQR = 3.3) (Table 4.3).

Table 4.3 Laboratory features of paediatric population

Characteristic		**Summary statistics**
White Blood Cell Count (X10⁹/L) (n=96)	Median (IQR)	2.22 (8.07)
Haemoglobin (n=96)	Median (IQR)	5.9 (9.19)
Platelet count (X10⁹/L) (n=96)	Median (IQR)	13.5 (140)
Absolute Neutrophil count (X10⁹/L) (n=96)	Median (IQR)	0.335 (3.06)
Reticulocyte count (%) (n=22)	Median (IQR)	1.56 (3.3)

4.1.4 Survival Analysis of paediatric participants

4.1.4.1 Overall survival and 1-year survival of paediatric patients

The median survival time of the paediatric participants was found to be 65 months from diagnosis of aplastic anaemia (Figure 4.1). The one-year survival was found to be 43% (Table 4.4).

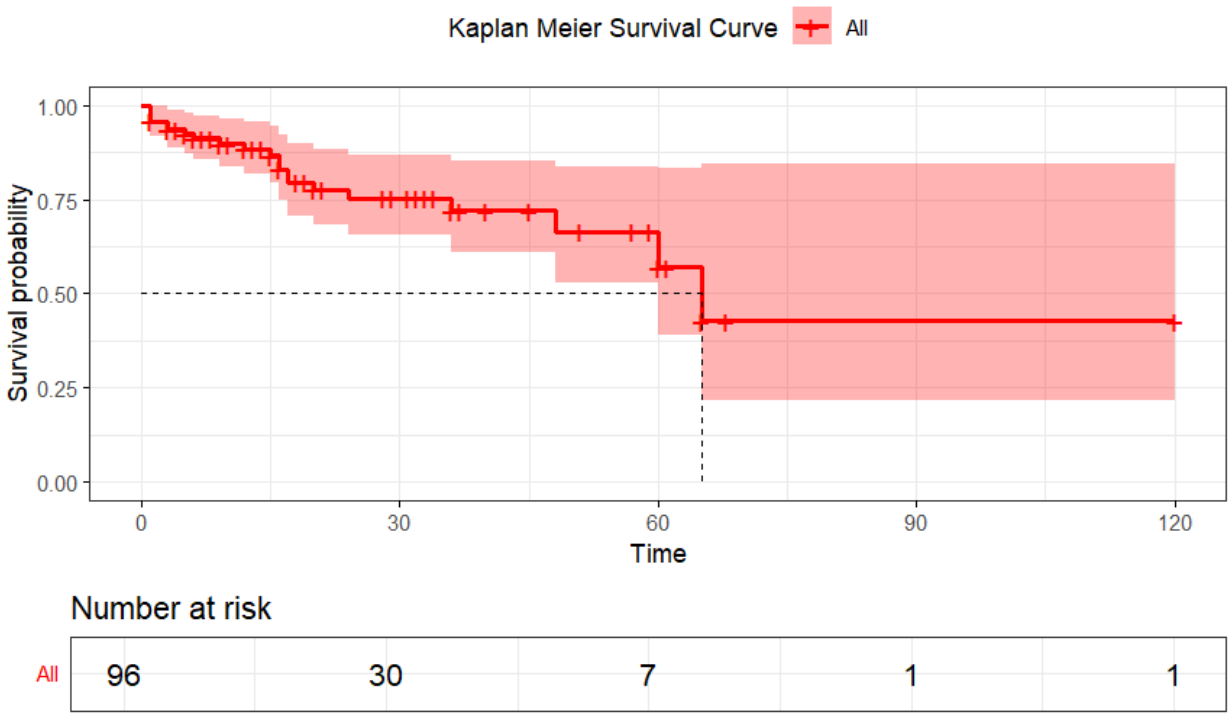

Figure 4.1: Overall survival for paediatric patients

Table 4.4 1-Year survival for paediatric population

Characteristic	1-year survival (95%CI)
Overall	43% (22%, 84%)

4.1.4.2 Survival outcome of paediatric participants and age

The cox proportional hazard analysis of patient age was not significant for survival of paediatric patients (HR = 0.98; 95% 0.88 – 1.09, p = 0.7) (Table 4.5).

Table 4.5 cox proportion hazard analysis in paediatric patients and age

Characteristic	HR	95% CI	p-value
Age	0.98	0.88, 1.09	0.7

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

4.1.4.3 Survival outcome of paediatric participants and weight

The cox proportional hazard analysis of patient weight was not significant for survival of adult patients (HR = 1.00; 95% 0.97 – 1.04, p = 0.8) (Table 4.6).

Table 4.6 cox proportion hazard analysis in paediatric patients and weight

Characteristic	HR	95% CI	p-value
Weight	1.00	0.97, 1.04	0.8

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

4.1.4.4 Survival outcome of paediatric participants and sex

There was no significant difference in survival between sex (p=0.92) (Figure 4.2).

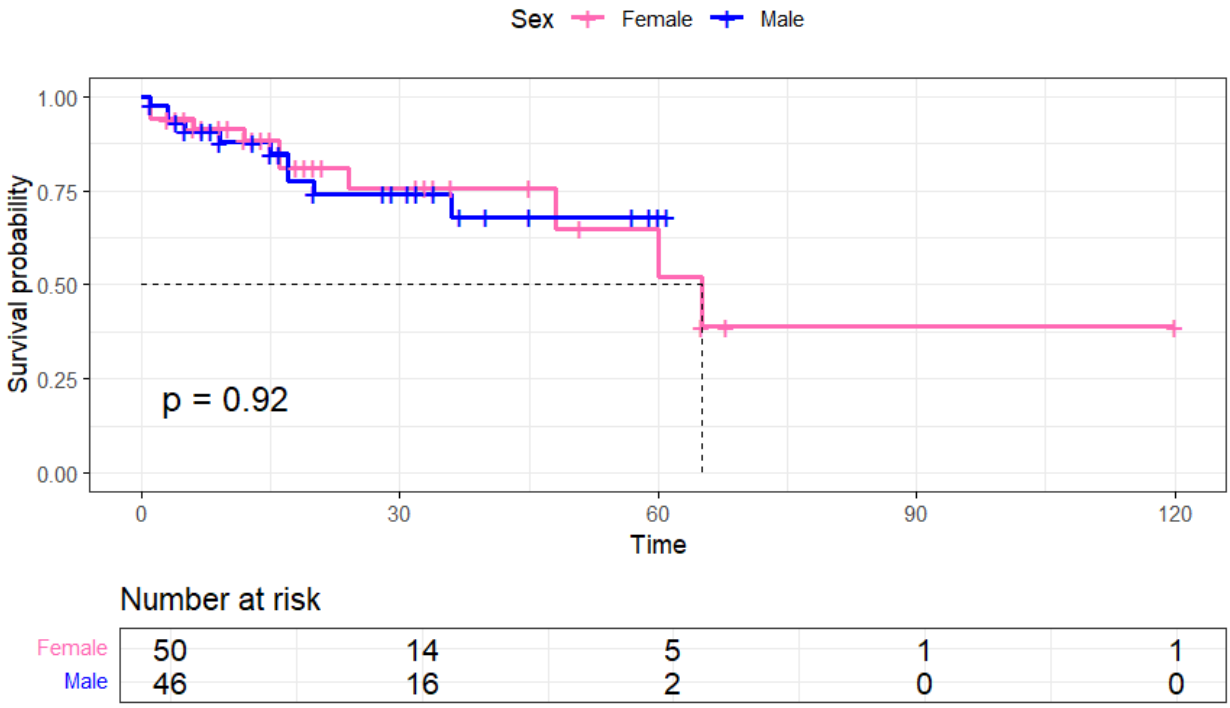

Figure 4.2: Overall survival of paediatric participants according to sex

4.1.4.5 Survival outcome of paediatric participants and disease severity

When participants were stratified by the severity of the disease at diagnosis, there was no significant difference in survival among the different disease severity (p=0.5) (Figure 4.3).

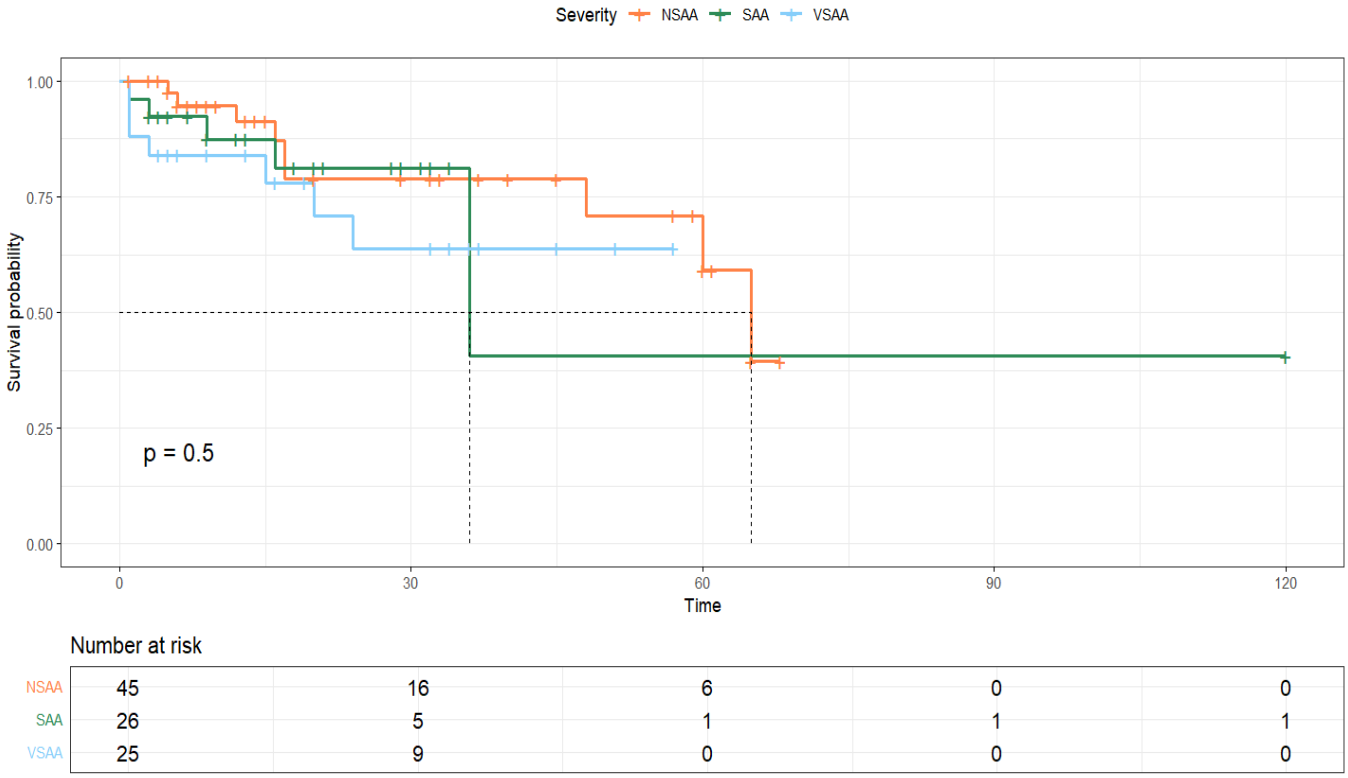

Figure 4.3: Overall survival of paediatric participants according to disease severity at diagnosis (Non-severe, severe, very severe)

4.1.4.6 Survival outcome of paediatric participants and disease aetiology

There was no significant difference in the survival outcome by disease aetiology (p=0.44) (Figure 4.4).

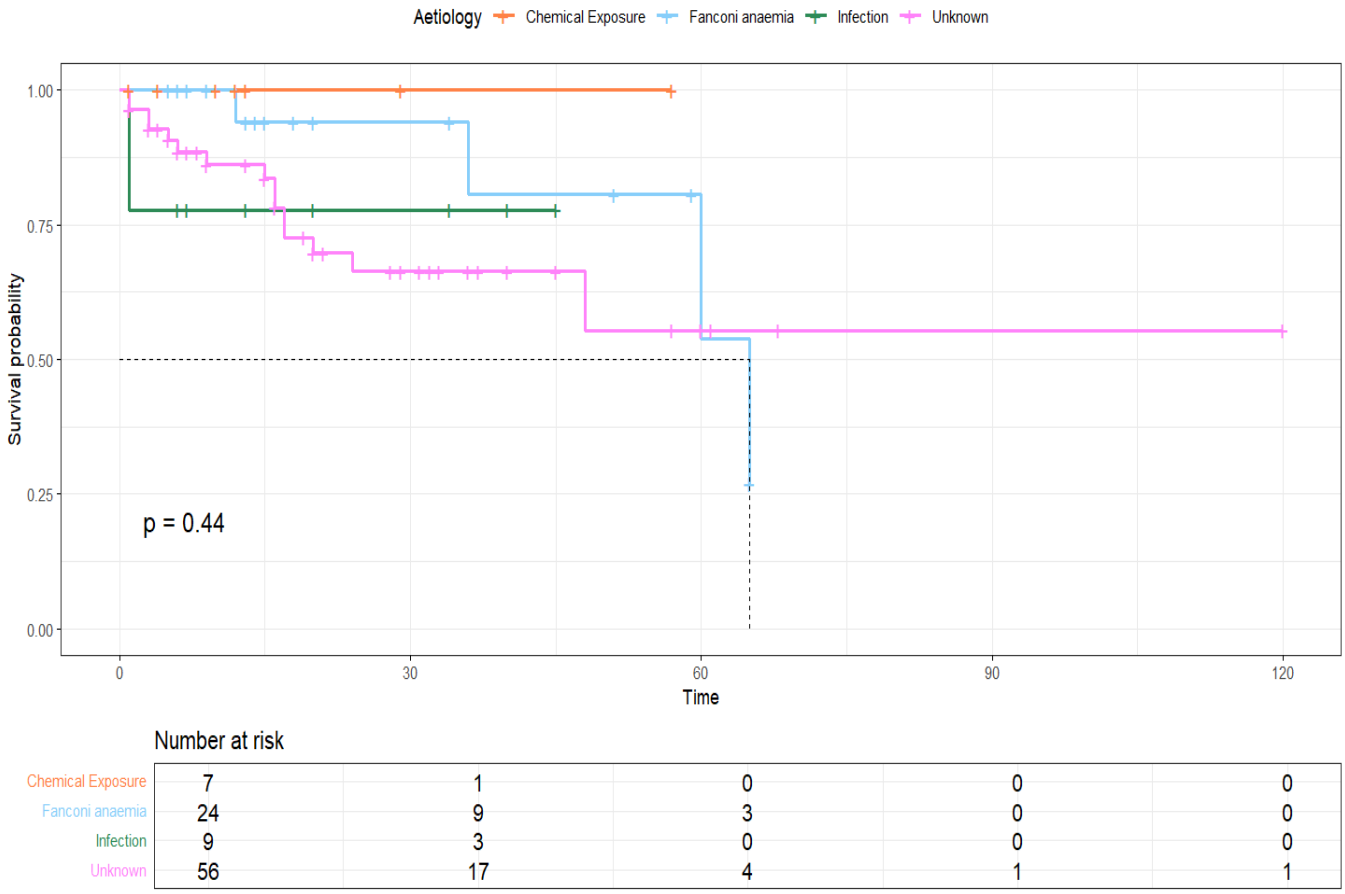

Figure 4.4: Overall survival of paediatric participants according to aetiology

4.1.4.7 Survival outcome of paediatric participants and cause of death (COD)

The study found that there statistically significant difference in the survival of the participants by the cause of death (p=0.034) (Figure 4.5). Cox proportional hazard regression found that there was a 5.96 increased hazard of dying due to sepsis and haemorrhage compared to participants that died due to haemorrhage alone (HR = 5.96, p =0.021) while no significant difference in the hazard was found between those that died as a result of haemorrhage alone and those that died due to sepsis alone (p=0.7) (Table 4.7).

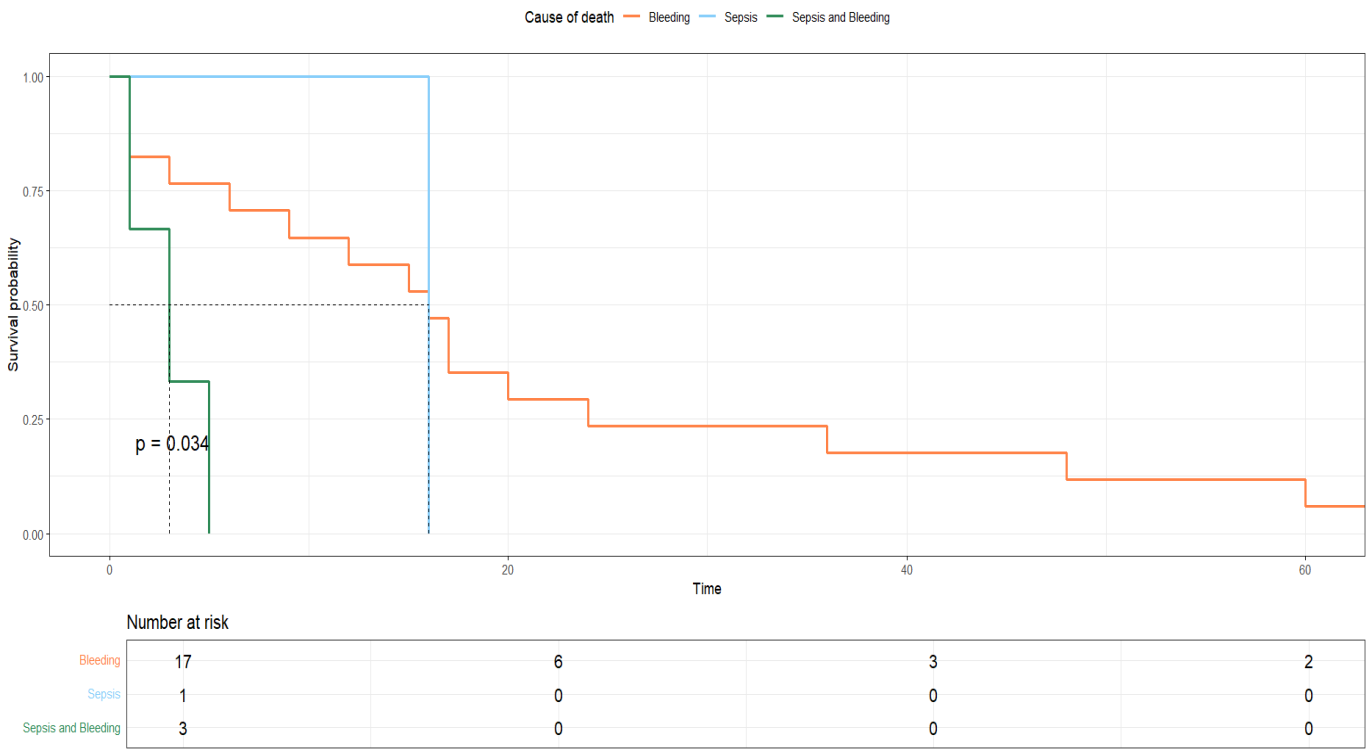

Figure 4.5: Overall survival of paediatric participants according to cause of death

Table 4.7 cox proportion hazard analysis for cause of death in paediatrics

Characteristic	HR	95% CI	p-value
COD			
Haemorrhage	—	—	
Sepsis	1.40	0.18, 11.1	0.7
Sepsis/Haemorrhage	5.96	1.30, 27.3	0.021

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

4.1.4.8 Survival outcome of paediatric participants and Family history of aplastic anaemia

There was no significant association between survival and family history of aplastic anaemia (p =0.55) (Figure 4.6).

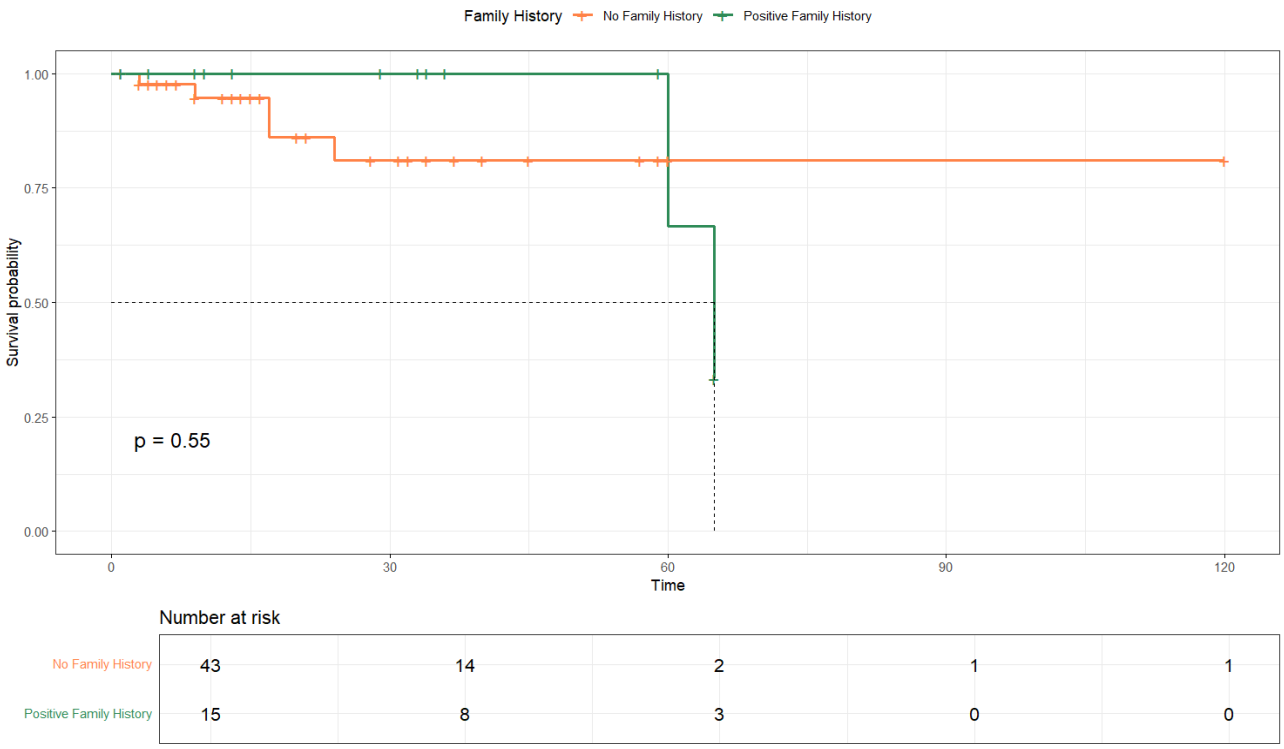

Figure 4.6 Overall survival of paediatric participants according to family history

4.1.4.9 Survival outcome of paediatric participants and treatment

There was no significant association between survival and primary treatment (p =0.70) (Figure 4.7).

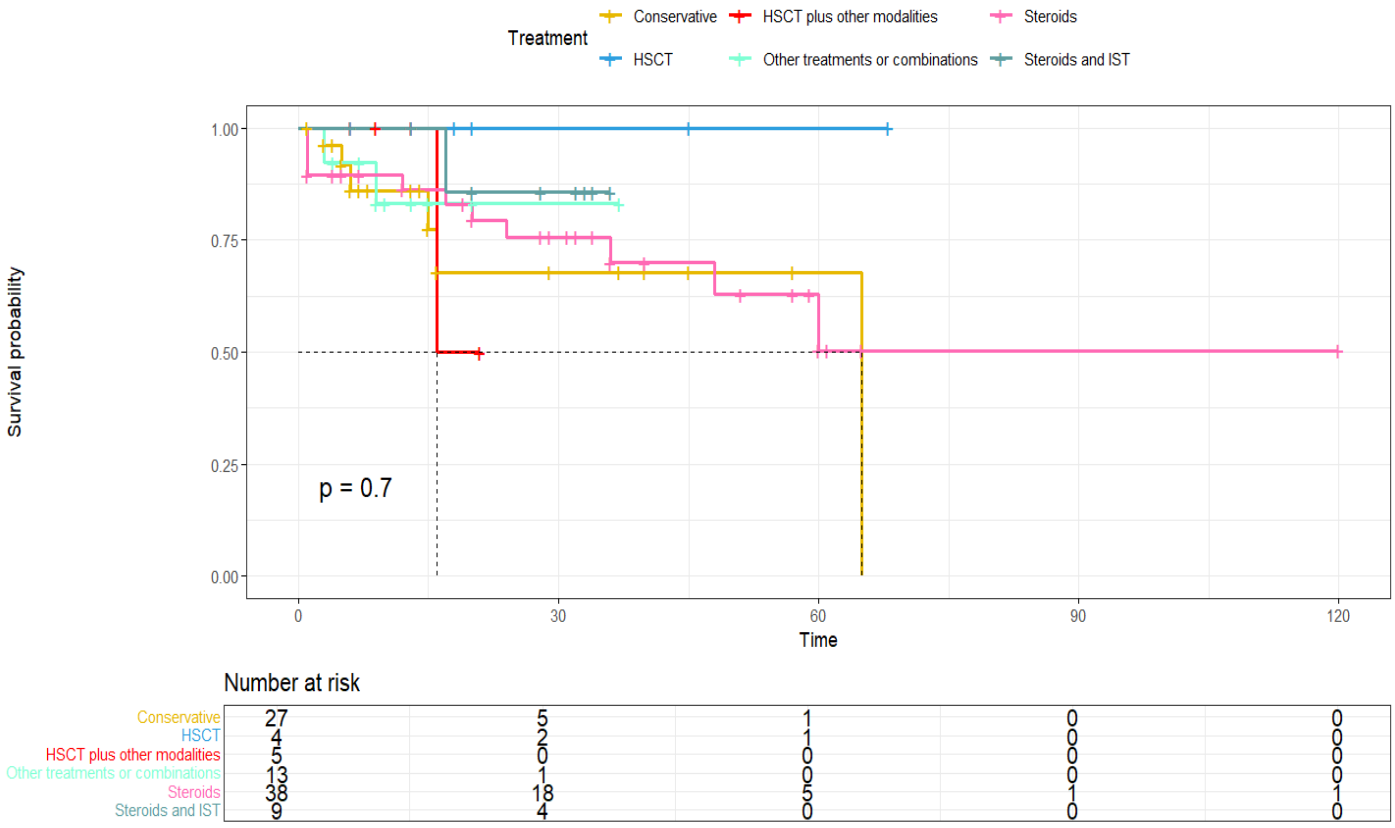

Figure 4.7: Overall survival of paediatric participants according to primary treatment

4.2 Adult participants

4.2.1 Demographic characteristics of adult population

The median age of the adult participants was 22.5 years (IQR = 36). The majority of adults with aplastic anaemia were males (n=22, 73.33%) (Table 4.8).

Table 4.8 demographic characteristics of adult population

Characteristic		**Patients included n (%)**
Age (n=30)	Median (IQR)	22.5 (36.00)
Sex (n=30)	Male	22 (73.33)
	Female	8 (26.67)
Residence (n=30)	Lusaka	12 (40.00)
	Outside Lusaka	18 (60.00)

4.2.2 Clinical Characteristics of adult patients

Of the 30 participants, 46.67% (n=14) had died by the end of the follow up period. The major cause of death was sepsis (n =10, 71.43%). In terms of severity at diagnosis, 56.67% (n=17) had NSAA, 23.33% (n=7) had SAA while 20.00% (n=6) had VSAA. All the participants presented with features of anaemia, 60.00% (n=18) presented with bleeding and 26.67% (8) with fever. The majority of the adults with aplastic anaemia received conservative treatment only (n=21, 70.00%). None of the participants received HSCT. The aetiology included unknown (n=12, 40.00%), infection (n=9, 30.00%) and chemical exposure (n=7, 23.33%) (Table 4.9).

Table 4.9 Clinical characteristics of adult population

Characteristic		**Patients included n(%)**
Survival Status (N=30)	Alive	16 (53.33)
	Dead	14 (46.67)
Aplastic Anaemia Severity	NSAA	17 (56.67)
	SAA	7 (23.33)
	VSAA	6 (20.00)
Features of anaemia	Yes	30 (100)

(N=30)	No	0 (0.00)
Bleeding (N=30)	Yes	18 (60.00)
	No	12 (40.00)
Fever (N=30)	Yes	8 (26.67)
	No	22 (73.33)
Presence of Dysmorphic Features (N=30)	Yes	2 (6.67)
	No	28 (93.33)
Aetiology (N=30)	Unknown	12 (40.00)
	Chemical Exposure	7 (23.33)
	Infection	9 (30.00)
	Others	2 (6.67)
Family History of aplastic anaemia (N=30)	Yes	1 (3.33)
	No	29 (96.67)
Chemical Exposure (N=30)	Yes	11 (36.67)
	No	19 (63.33)
Smoking(N=30)	Yes	1 (3.33)
	No	29 (96.67)
Alcohol intake (N=30)	Yes	2 (6.67)
	No	28 (93.33)
Cause of death (N=14)	Bleeding	4 (28.57)
	Sepsis	10 (71.43)
Treatment (N=30)	Conservative	21 (70.00)
	Steroids	1 (3.33)
	CsA	3 (10.00)
	ATG	1 (3.33)
	Steroids, CsA	1 (3.33)
	ATG, Steroids, CsA	3 (10.00)

4.2.3 Laboratory features of adult patients

The median white blood cell count was 2.055 (IQR = 1.8) X10⁹/L, median platelet count was 31 (IQR = 104) X10⁹/L, the median absolute neutrophil count was 0.455 (IQR = 1.42) (X10⁹/L), haemoglobin level median was 4.4 (IQR = 4.1) g/dL and the median Reticulocyte count (%) was 0.71 (IQR = 0.35) (Table 4.10).

Table 4.10 laboratory features of adult population

Characteristic		**Summary statistics**
White Blood Cell Count (X10⁹/L) (n=30)	Median (IQR)	2.055 (1.8)
Haemoglobin (n=30)	Median (IQR)	4.4 (4.1)
Platelet count (X10⁹/L) (n=30)	Median (IQR)	31 (104)
Absolute Neutrophil count (X10⁹/L) (n=30)	Median (IQR)	0.455 (1.42)
Reticulocyte count (%) (n=10)	Median (IQR)	0.71 (0.35)

4.2.4 Survival Analysis

4.2.4.1 Overall survival of adult patients

There was no median survival time for the adult population as it was not reached (Figure 4.8). The one-year survival was 53% (Table 4.11).

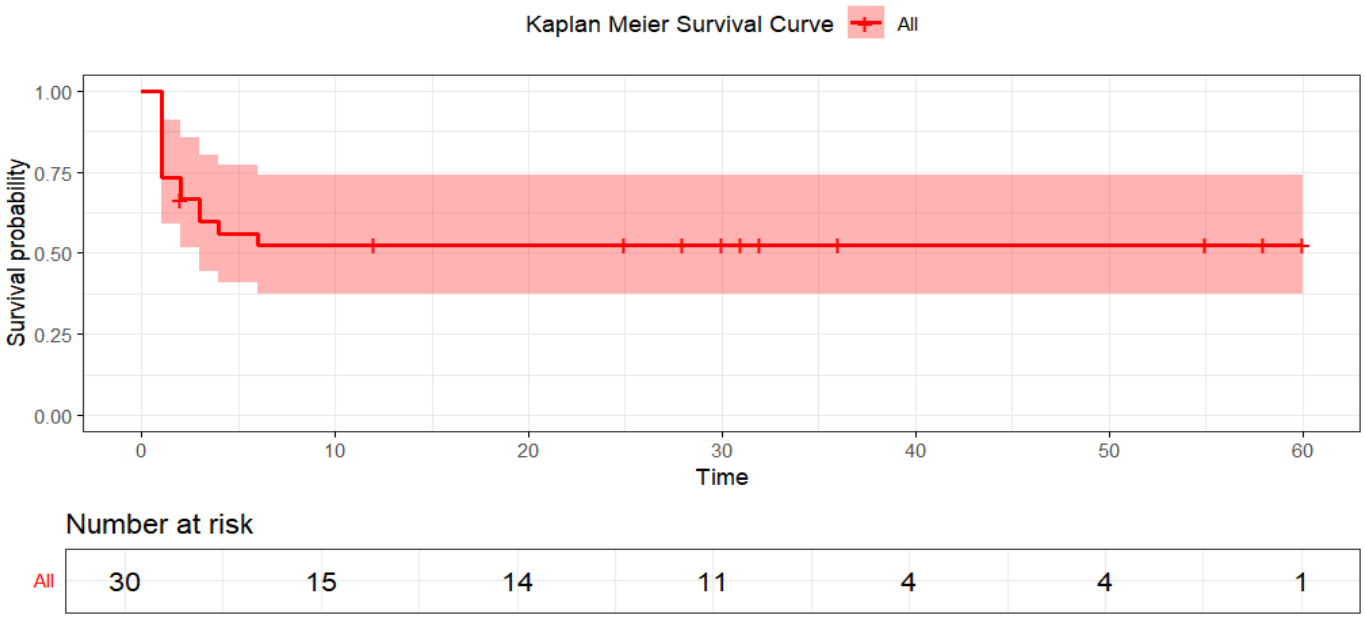

Figure 4.8: Overall survival for all adult patients

Table 4.11 1-Year survival for adults

Characteristic	1-year survival (95%CI)
Overall	53% (37%, 74%)

4.2.4.2 survival outcome of adults and age

The cox proportional hazard was not significant for survival of adult patients (HR = 1.01; 95% 0.98 – 1.04, p = 0.5) (Table 4.12).

Table 4.12 Cox proportion hazard analysis for age in adults

Characteristic	HR	95% CI	p-value
Age	1.01	0.98, 1.04	0.5
Abbreviations: CI = Confidence Interval, HR = Hazard Ratio			

4.2.4.3 Survival outcome and sex for adult population

There was significant difference in survival between sex (p=0.025) (Figure 4.9). However, cox proportional hazard analysis found that there was no significant difference in the hazard of dying between males and females (Table 4.13).

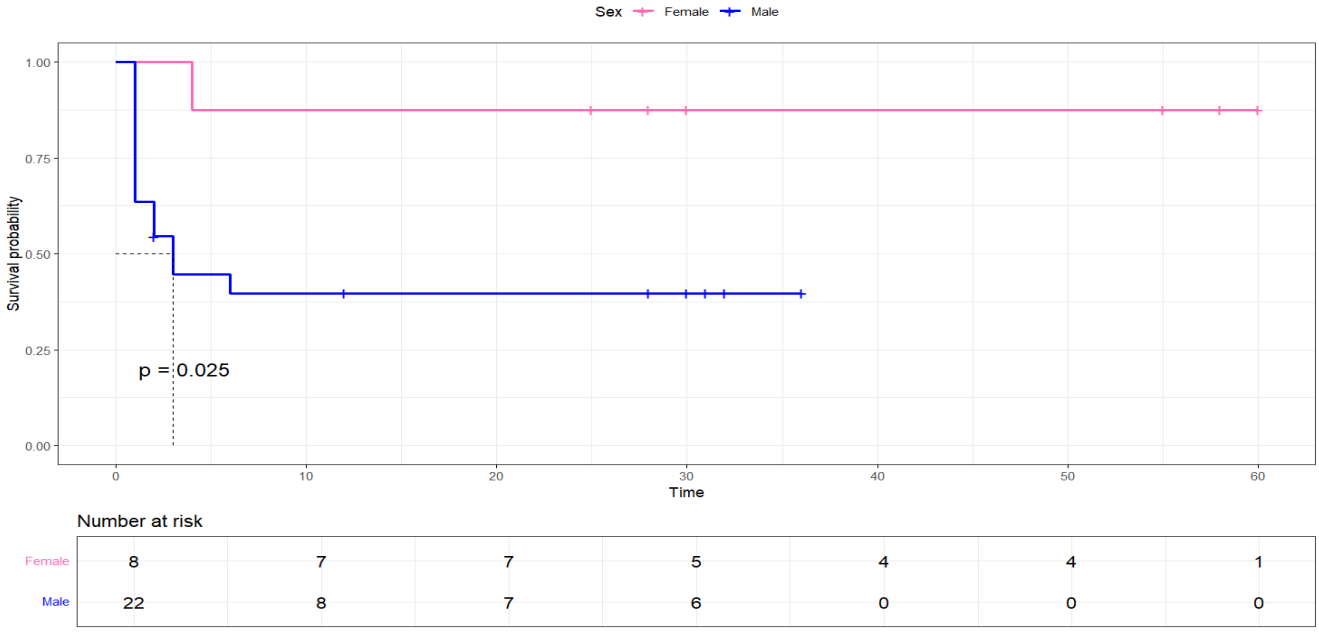

Figure 4.9: Overall survival of adults according to sex

Table 4.13 cox proportion hazard analysis for survival in adults and sex

Characteristic	HR	95% CI	p-value
Sex			
F	—	—	
M	7.29	0.95, 56.0	0.056

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

4.2.4.4 Survival outcome and disease severity for adult population

When participants were stratified by the severity of the disease at diagnosis, there was no significant difference in survival among the different disease severity (p=0.42) (Figure 4.10).

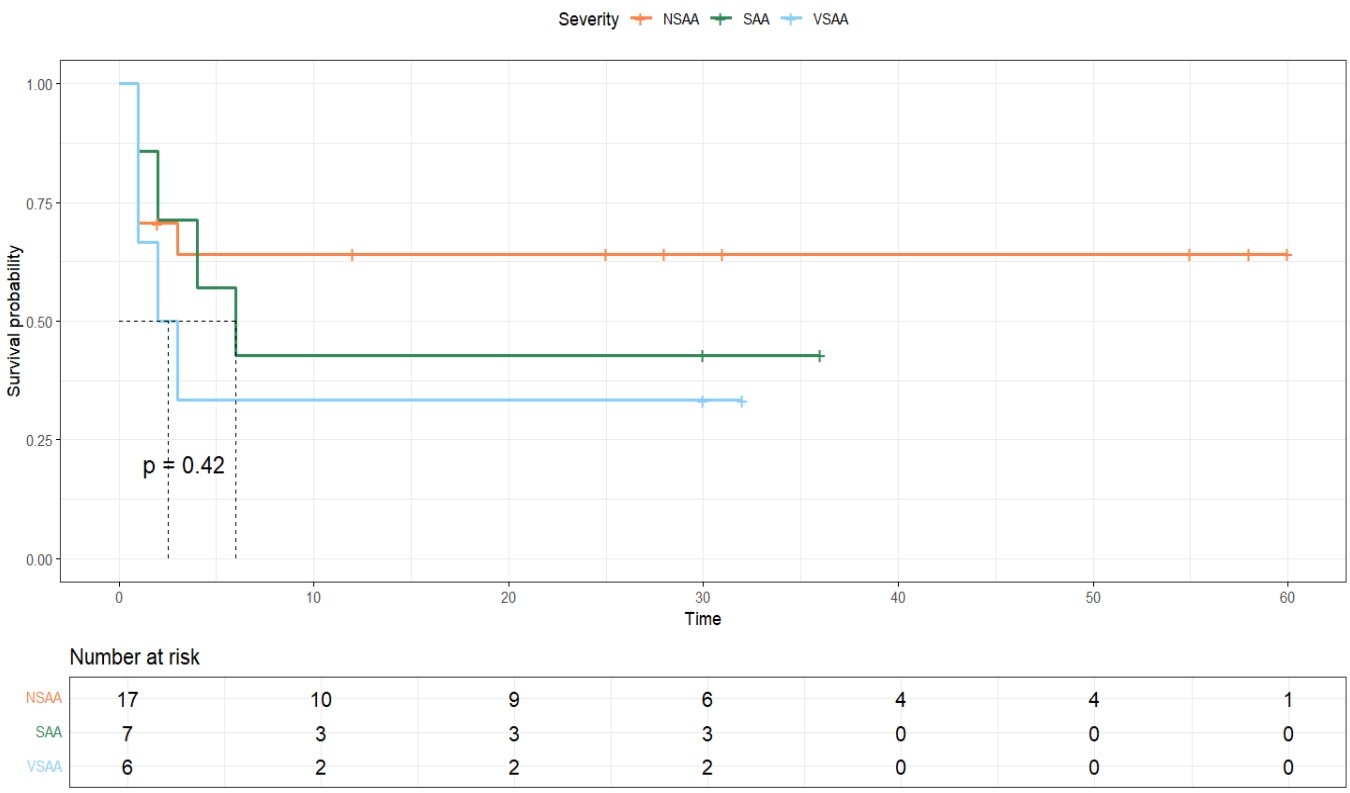

Figure 4.10: Overall survival of adults according to disease severity

4.2.4.5 Survival outcome and disease aetiology for adult population

There was no significant difference in survival among the participants according to the disease aetiology (p=0.81) (Figure 4.11).

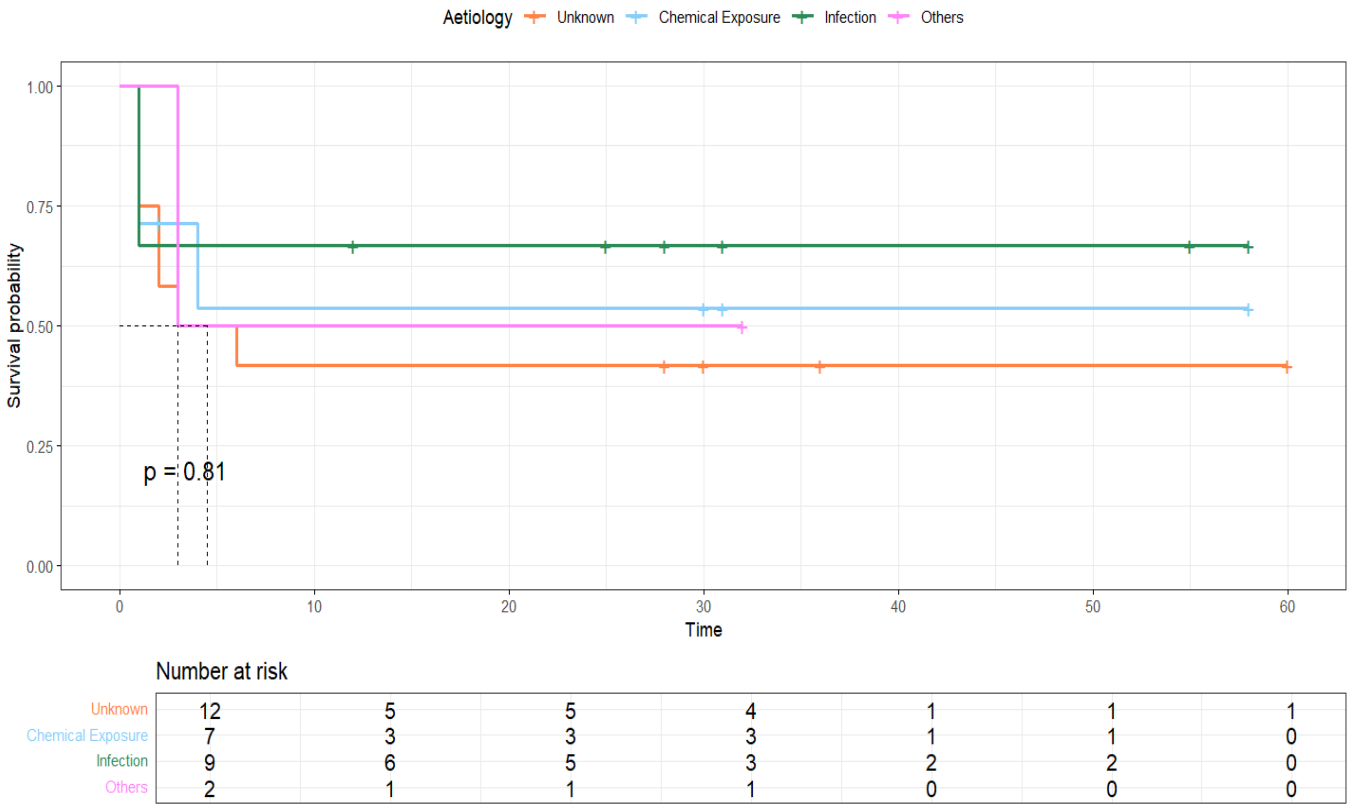

Figure 4.11: Overall survival of adults according to aetiology

4.2.4.6 Survival outcome and Cause of death for adult population

The study found that there statistically significant difference in the survival of the participants by the cause of death (p=0.048) (Figure 4.12). Cox proportional hazard regression found that there was a 0.23 decreased hazard of dying due to sepsis compared to participants that died due to haemorrhage (HR = 0.23, p = 0.046) (Table 4.14).

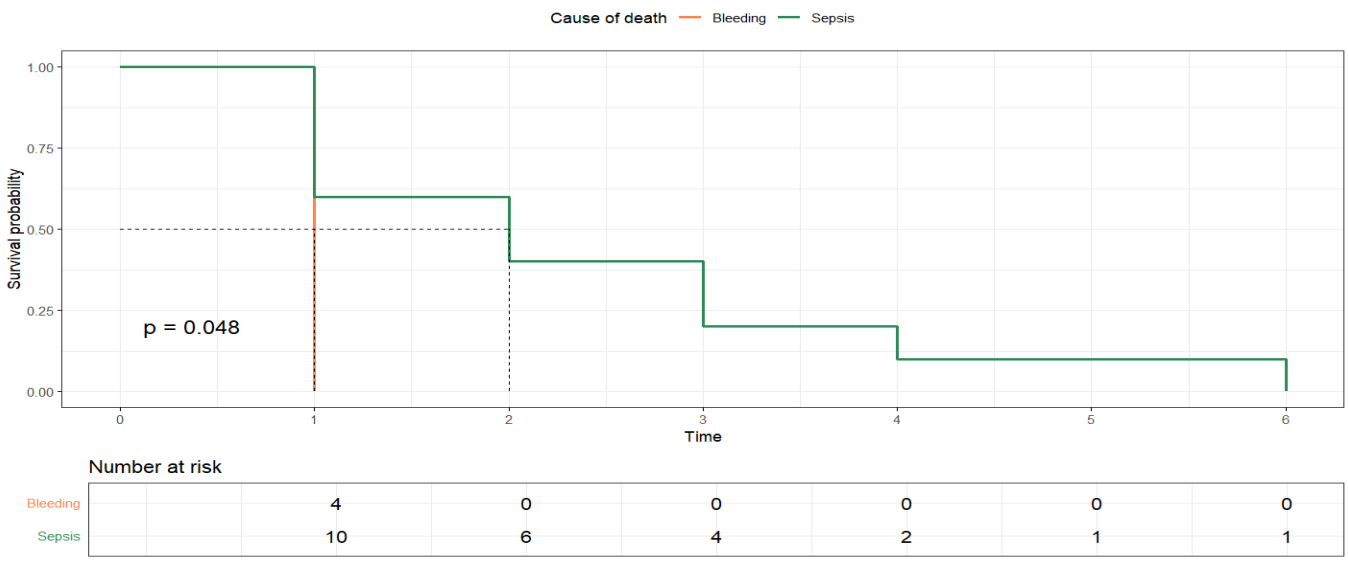

Figure 4.12: Overall survival of adults according to cause of death

Table 4.14 cox proportion hazard of cause of death in adults

Characteristic	HR	95% CI	p-value
COD			
Haemorrhage	—	—	
Sepsis	0.23	0.06, 0.97	0.046

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

4.2.4.7 Survival outcome and Treatment for adult population

There was statistically significant difference in the survival of the participants according to the treatment they received (p =0.032) (Figure 4.13). The cox regression model indicated that the hazard of dying was 11.8 more for adults who were on ATG only (p=0.029), 5.52 more for adults who were on CsA, ATG and steroids (p=0.015) and 11.8 more for adults who were on steroids (p=0.029) (Table 4.15).

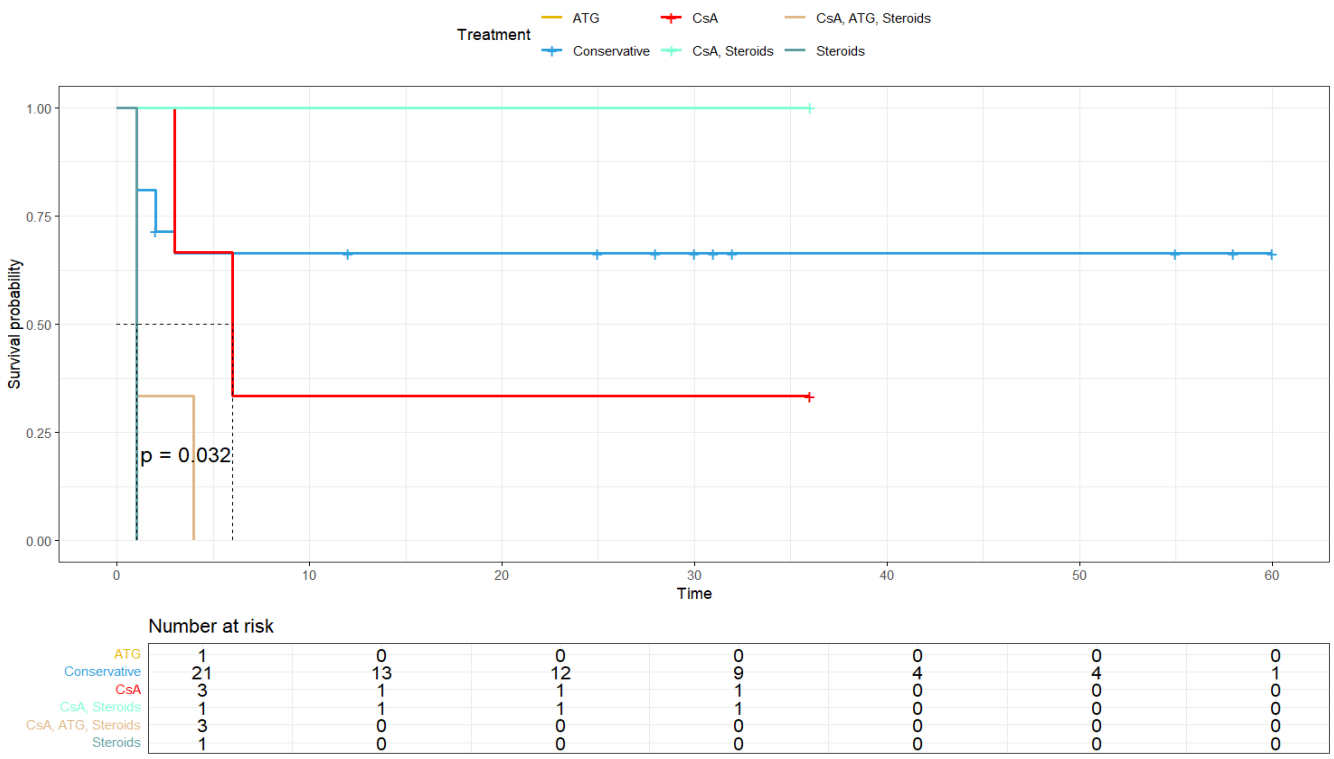

Figure 4.13: Overall survival of adults according to primary treatment

Table 4.15 cox proportional regression for primary treatment in adults

Characteristic	HR	95% CI	p-value
data$Treatment			
Conservative	—	—	
ATG	11.8	1.29, 108	0.029
CsA	1.70	0.35, 8.19	0.5
CsA, Steroids	0.00	0.00, Inf	>0.9
CsA,ATG,Steroids	5.52	1.38, 22.0	0.015
Steroids	11.8	1.29, 108	0.029

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

4.2.4.8 Multivariable Cox proportion hazard analysis for adults

Multivariable cox proportion hazard analysis found that none of the variables were independent predictors of the hazard of dying (Figure 4.14).

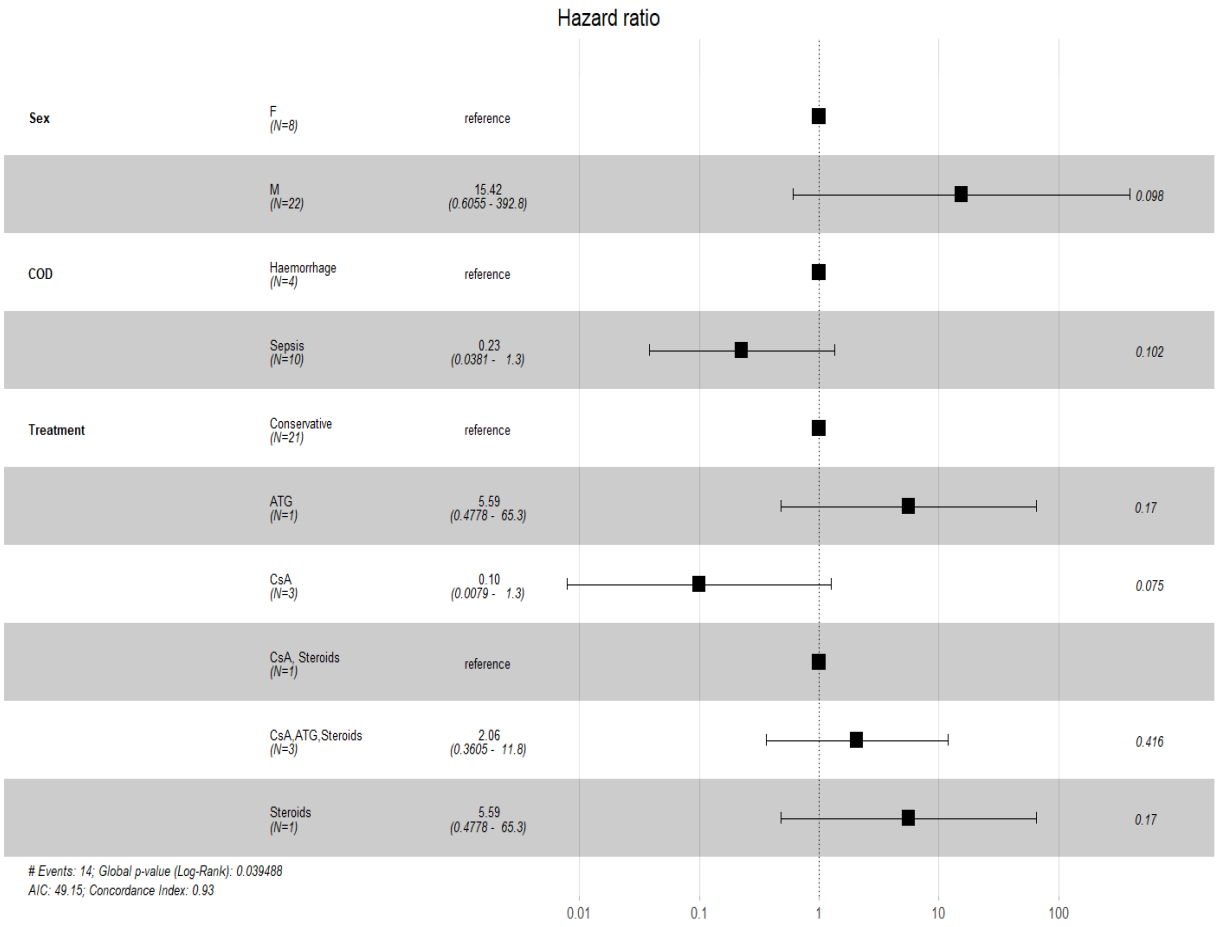

Figure 4.14: forest plot for multivariable cox proportion hazard analysis for adult participants

4.3 Combined population statistics

4.3.1 Demographic characteristics of combined paediatric and adult participants

For both paediatric and adult populations, the median age was 12 (IQR = 51) and 53.97% (n=68) were male (Table 4.16).

Table 4.16 demographic characteristics of combined paediatric and adult participants

Characteristic		**Patients included n(%)**
Age (n=126)	Median (IQR)	12 (51)

Sex (n=126)	Male	68 (53.97)
	Female	58 (46.03)
Residence (n=126)	Lusaka	53 (42.06)
	Outside Lusaka	73 (57.94)

4.3.2 Clinical Characteristics of combined paediatric and adult participants

At the end of the follow up period, 27.78% (n=35) had died. About 48% of cases had NSAA, 26% with SAA and 24.6% with VSAA. The causes of death included bleeding (n=21, 60.00%), sepsis (n=11, 31.43%) and 8.57% (n=3) died due to both sepsis and bleeding. The most common aetiology was unknown (n=68, 53.97%) (Table 4.17).

Table 4.17 Clinical characteristics of combined paediatric and adult participants

Characteristic		**Patients included n(%)**
Survival Status (n=126)	Alive	91 (72.22)
	Dead	35 (27.78)
Aplastic Anaemia Severity	NSAA	62(49.21)
	SAA	33 (26.19)
	VSAA	31 (24.60)
Features of anaemia (n=126)	Yes	122 (96.83)
	No	4 (3.17)
Bleeding (n=126)	Yes	81 (64.29)
	No	45 (35.71)
Fever (n=126)	Yes	52 (41.27)
	No	74 (58.73)
Presence of Dysmorphic Features (n=126)	Yes	24 (19.05)
	No	102 (80.95)
Aetiology (n=126)	Unknown	68 (53.97)

	Fanconi anaemia	24 (19.05)
	Infection	19 (15.08)
	Chemical Exposure	13 (10.32)
	Others	2 (1.59)
Family History of aplastic anaemia (n=88)	Yes	16 (18.18)
	No	72 (81.82)
Chemical Exposure (n=88)	Yes	20 (22.73)
	No	68 (77.27)
Cause of death (n=35)	Bleeding	21 (60.00)
	Sepsis	11 (31.43)
	Sepsis & bleeding	3 (8.57)
Treatment (n=126)	Conservative	48 (38.10)
	Steroids	39 (30.95)
	ATG	1 (0.79)
	CsA	3 (2.38)
	Steroids, CsA	8 (6.35)
	ATG, Steroids, CsA	5 (3.97)
	ATG, Steroids, CsA, Eltrombopag	12 (9.52)
	Eltrombopag	1 (0.79)
	HSCT	4 (3.17)
	Steroids, HSCT	3 (2.38)
	Steroids, ATG, CsA, HSCT	1 (0.79)
	CsA,ATG,Eltrombopag, HSCT	1 (0.79)

4.3.3 Laboratory features of combined paediatric and adult participants

The median white blood cell count was 2.2 (IQR = 9.59) X10^9/L, median platelet count was 15 (IQR = 140) X10^9/L, the median absolute neutrophil count was 0.34

(IQR = 6.13) ($X10^9$/L), haemoglobin level median was 5.59 (IQR = 4.1) g/dL and the median Reticulocyte count (%) was 1.41 (IQR = 3.3) (Table 4.18).

Table 4.18 laboratory features of combined paediatric and adult participants

Characteristic		**Summary statistics**
White Blood Cell Count ($X10^9$/L) (n=126)	Median (IQR)	2.2 (9.59)
Haemoglobin (n=126)	Median (IQR)	5.1 (9.19)
Platelet count ($X10^9$/L) (n=126)	Median (IQR)	15 (140)
Absolute Neutrophil count ($X10^9$/L) (n=103)	Median (IQR)	0.34 (6.13)
Reticulocyte count (%) (n=32)	Median (IQR)	1.41 (3.3)

4.3.4 Survival analysis for all the participants

4.3.4.1 Overall survival and 1-year survival for all participants

The median survival time for both the paediatric and adult participants was found to be 65 months from diagnosis of aplastic anaemia (Figure 4.15). The one-year

survival was found to be 42% (Table 4.19).

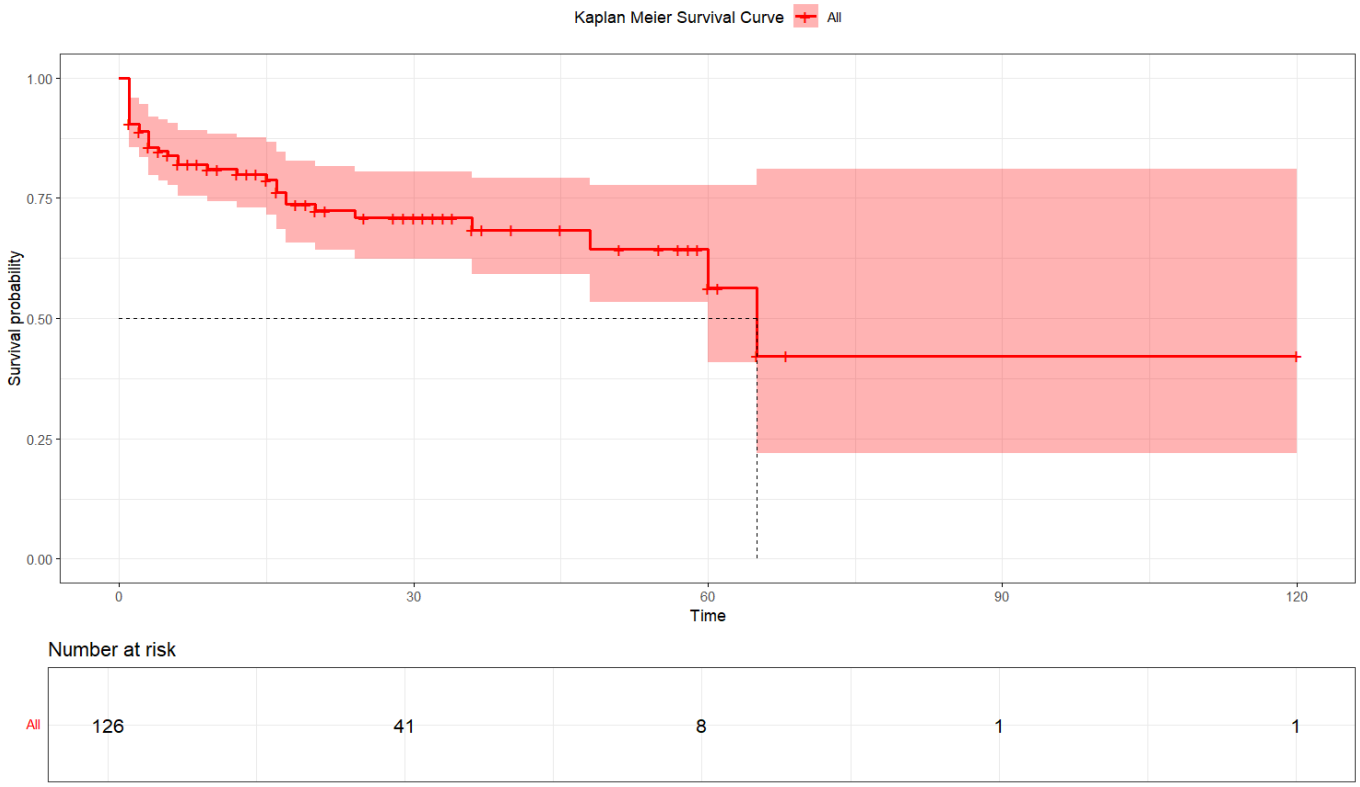

Figure 4.15: Overall survival for all the participants

Table 4.19 1-year survival

Characteristic	1-year survival (95%CI)
Overall	42% (22%, 81%)

4.3.4.2 Survival by population category (paediatric vs adult population)

The study a statistically significant association between survival status and the category (p = 0.011) and presenting with bleeding (p =0.024) However, no significant association was found between survival status and sex, treatment modalities (p=0.374), presenting with anaemia features (p = 1.000) or fever (p= 0.420).

Table 4.20 Association between survival status and categorical independent variables

Variable	*P*-value
Category	0.011
Sex	0.114
Anaemia features	1.000
Bleeding	0.024
Fever	0.420
Treatment	0.374

Using the log rank test, the study found a statistically difference in survival by whether participants presented with bleeding or not (p =0.019) (Figure 16). Cox proportional hazard analysis found that patients that presented with bleeding had a 2.61 higher hazard of dying compared to those that did not present with bleeding (HR =2.61, 95%CI 1.13 – 6.02, p = 0.024) (Table 4.21).

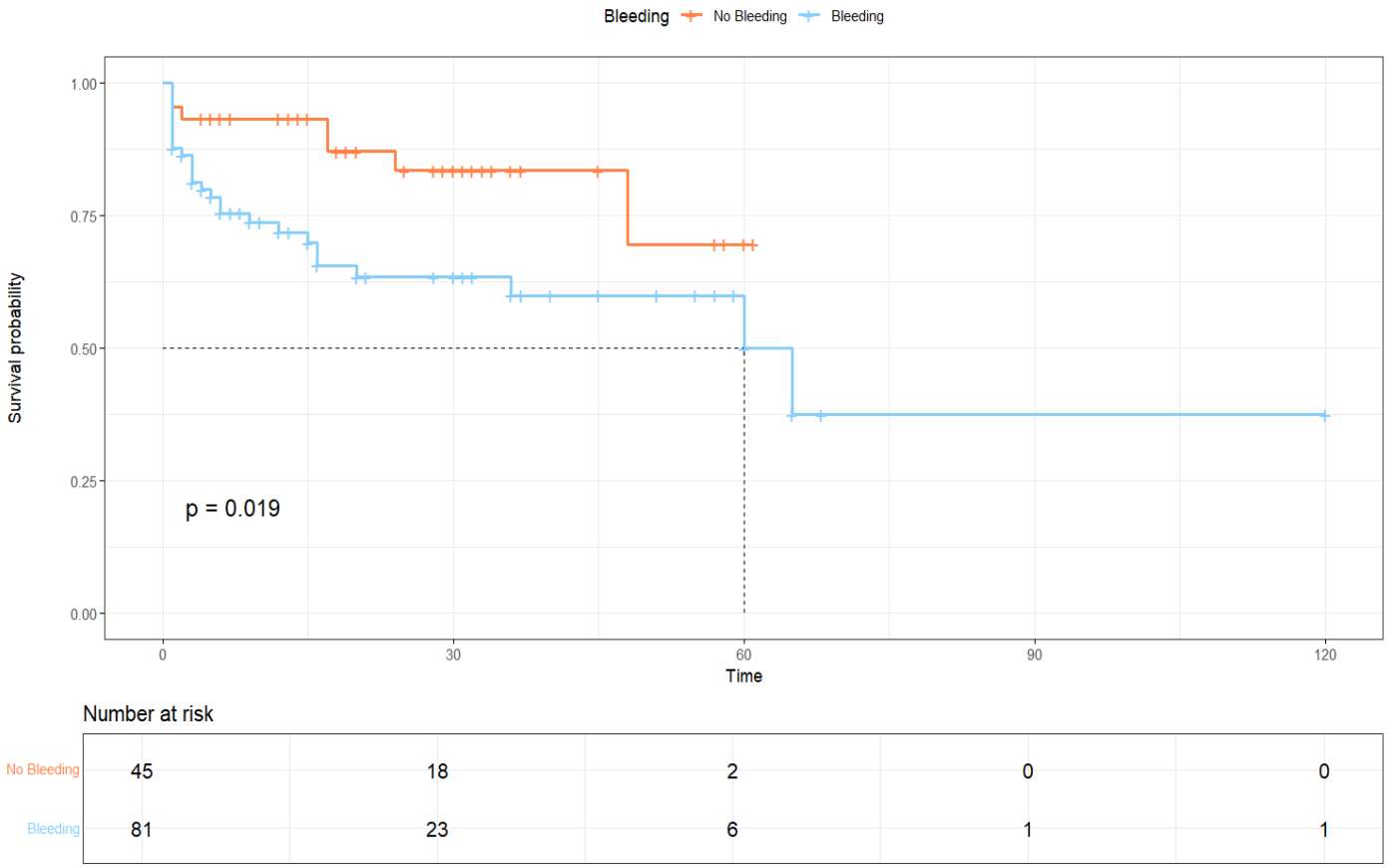

Figure 4.16 Survival and presenting with bleeding

Table 4.21 Cox proportional hazard analysis for combined paediatric and adult patients for presence of bleeding

Characteristic	HR	95% CI	p-value
Bleeding			
N	—	—	
Y	2.61	1.13, 6.02	0.024
Abbreviations: CI = Confidence Interval, HR = Hazard Ratio			

The study found that there statistically significant difference in the survival of the participants by the population category using the log rank test (p=0.0038) (Figure 4.17). Cox proportional hazard analysis found that being an adult had a 2.73

increased hazard of dying compared to being a paediatric (HR = 2.73, p =0.004) (Table 4.22).

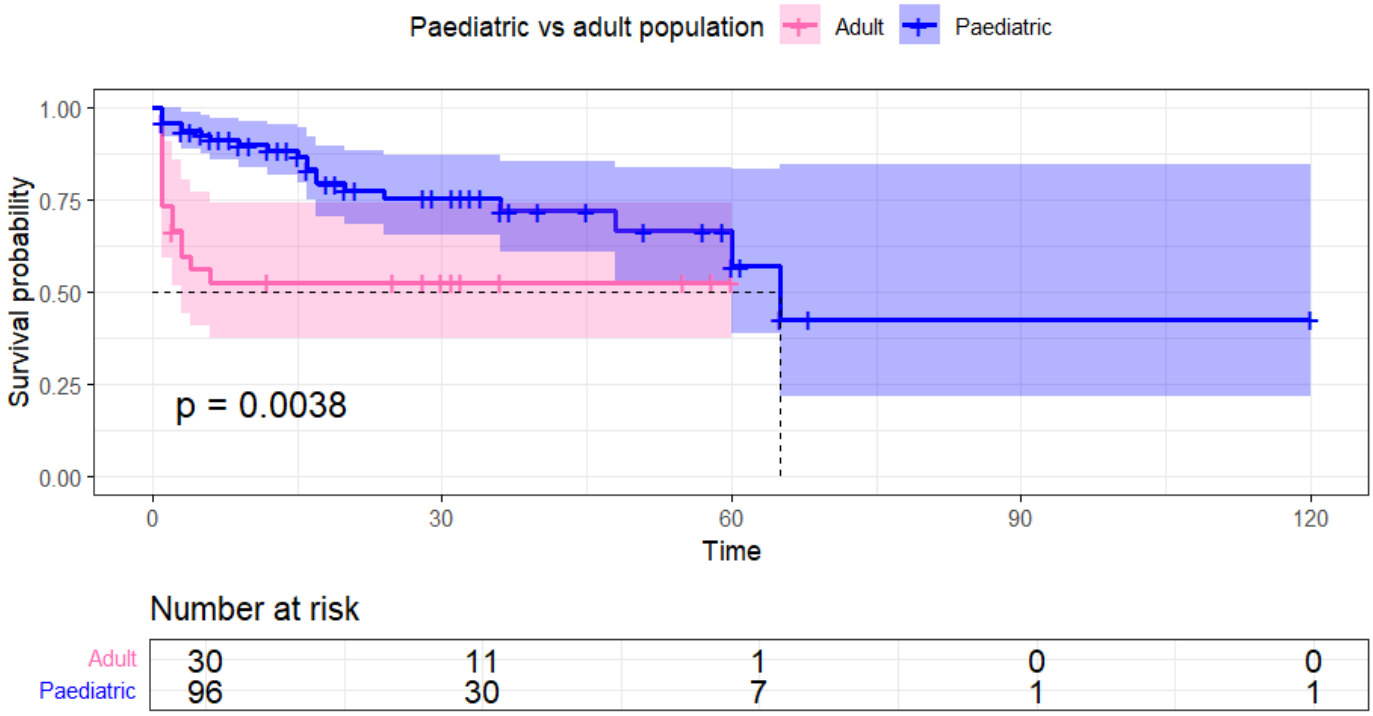

Figure 4.17 Survival by population category

Table 4.22 Cox proportional hazard analysis for combined paediatric and adult patients according category

Characteristic	HR	95% CI	p-value
Category			
Paediatric	—	—	
Adult	2.73	1.37, 5.41	0.004

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

Multivariable cox proportional hazard analysis revealed both the category and bleeding to be statistically significant prognostic factors in aplastic anaemia in this study with presenting with bleeding conferring inferior survival (HR = 2.90, 95%CI

1.25 – 6.71, p = 0.013) while being a paediatric patient conferring superior survival (HR = 0.33, 95%CI 0.17 – 0.66, p = 0.002) compared to not presenting with bleeding and being an adult, respectively. These results are depicted in table 4.23. The frailty model was also fitted for the two variables and the results are shown in table 4.24

Table 4.23 Multivariable cox regression analysis for survival in both paediatric and adult patients

Characteristic	HR	95% CI	p-value
Bleeding			
N	—	—	
Y	2.90	1.25, 6.71	0.013
Category			
Adult	—	—	
Paediatric	0.33	0.17, 0.66	0.002

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

Table 4.24 Frailty model for survival and category and bleeding status

Characteristic	HR	95% CI	p-value
Bleeding			
N	—	—	
Y	4.23	1.39, 12.9	0.011
Category			
Adult	—	—	
Paediatric	0.14	0.05, 0.41	<0.001
frailty(PATIENT)			0.12

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio

CHAPTER FIVE: DISCUSSION

This chapter discusses the findings of the study. It firstly discusses the findings for the paediatric patients followed by findings for the adult patients and finally findings for all the patients.

5.1 Paediatric participants

5.1.1 Demographic characteristics of paediatric participants

For the period under study, there were 96 new paediatric cases with a diagnosis of aplastic anaemia, giving an average of approximately 10 new cases per year. Conversely, a UK study that run from 2017 to 2022 found an average of 25 cases per year (Famokunwa *et al.* 2023).

The median age at diagnosis was found to be 11 years and the majority of participants were females. While the median age is in keeping with the findings from Lithuania (Rutkauskaite *et al.*, 2023), India (Shah *et al.*, 2019), South Africa (Nest, 2020), the studies from India and South Africa found that the majority of the patients were males. Additionally, a study from UK found a slight male predominance of 52% in the paediatric population (Famokunwa *et al.* 2023). The discrepancies could be attributed to differences in the methodologies as the study from UK studied paediatric cases for the period 1st April, 2017 to 31st March, 2022 while the study from India investigated only patients that were on immunosuppressive therapy.

5.1.2 Clinical Characteristics

The study found that in 58.33% of cases, the cause of the aplastic anaemia was unknown followed by Fanconi anaemia. This finding is consistent with findings from a UK study which reported that 56% of paediatric cases had a diagnosis of unspecified (Famokunwa *et al.* 2023). Similarly, a retrospective study of paediatric cases from 1992 to 2021 in Lithuania found that the majority (81.6%) of cases were diagnosed with idiopathic aplastic anaemia (Rutkauskaite *et al.*, 2023). In keeping with this finding, a study from South Africa also reported most cases as being idiopathic (Ramsamy *et al.*, 2022). The finding of a good percentage of children presenting with dysmorphic features (22.92%) and being diagnosed with Fanconi anaemia (25.00%) underscores the importance of Fanconi anaemia in our setting. Further studies on Fanconi anaemia may need to be conducted in our setting.

The study found that a higher percentage of paediatric patients had non-severe aplastic anaemia follwed by severe aplastic and very severe aplastic anaemia being

least. However, a study conducted in China (Huang *et al.*, 2021) reported higher percentage of paediatric patients having severe aplastic anaemia followed by very severe aplastic and non-severe aplastic anaemia. Early diagnosis of aplastic anaemia before the disease advances may explain the higher proportion of children being diagnosed with non-severe aplastic anaemia in our study.

The present study only had about 9% of paediatric patients undergoing stem cell transplant. On the other hand, (Famokunwa *et al.* 2023) found that 20% of UK paediatric patients from 2017 – 2022 had undergone stem cell transplants. This difference could be attributed to differing socio-economic profile of the two countries, with Zambia being a resource limited nation and stem cell transplants being expensive.

The present study found that 25.86% of paediatric patients had a positive family history of aplastic anaemia. This underscores the important role that genetics may play in disease predisposition. The high degree of consanguity that was reported in a Pakistan study could further support genetic factors having a role to play in disease predisposition (Ahmed *et al.*, 2020).

5.1.3 Laboratory features

The study found the median white blood cell count of 2.22 $X10^9$/L, median platelet count of 13.5 $X10^9$/L, the median absolute neutrophil count of 0.335, haemoglobin level median of 5.9 g/dL and the median Reticulocyte count (%) of 1.56. These results are similar with results from China (Huang *et al.*, 2021).

5.1.4 Survival Analysis

This study found that 21.88% of paediatric patients died during the study period, giving an overall survival of 78.12%. In contrast, a study in Lithuania reported that in the first period (199 – 2001) of study during which no HSCT was done, 71.4% of children with aplastic anaemia died and only 28.6% died in the second period (2002 – 2021) of its study in which HSCT was performed (Rutkauskaite *et al.*, 2023). The findings underscore the improvement in treatment modalities over the years for aplastic anaemia and shows that survival of aplastic anaemia patients was low prior to HSCT treatment era and supports HSCT as the first line treatment for aplastic anaemia. A study in USA that investigated 1140 children found that only 11% of children with aplastic anaemia died during the follow up (Hossain & Xie, 2018). In addition, an Indian study found a 61.6% survival rate in paediatric cases (Kharya *et al.*, 2023). On the other hand, a study in Nigeria found the mortality rate as high as 83.3%. The difference could be attributed to the very small sample size as well as the unavailability of HSCT in the Nigerian study (George *et al.*, 2020)

The present study found a median survival time of 65 months. Similarly, Rodgers *et al.* (2019) found a median survival time of 62 months in a USA study while a study in China reported a median survival time of 50.2 months (Huang *et al.*, 2021). The 1-year survival of paediatric patients in our study was found to be 43%. This is lower than the findings reported from a Swedish study that found a 5-year survival among children of 90.7% (Vaht *et al.*, 2017) and from China that reported a 5-year survival among children of 85.7% (Huang *et al.*, 2021). The differences in results could be due to differences in the study populations.

The present study did not find a statistically significant association between sex and survival time. This is in keeping with studies conducted in the USA and South Africa that found that sex was not a statistically significant predictor of mortality (Hossain & Xie, 2018; Ramsamy *et al.*, 2022).

The study also found no statistically significant difference in survival according to the disease severity. On the contrary, Huang *et al.* (2021) conducted a study in Chinese children reported that there were significant differences between the non-severe aplastic anaemia group and the very severe aplastic anaemia and between the non-severe aplastic anaemia group and the severe aplastic anaemia group. However, no statistically significant difference was found between the severe aplastic anaemia and the very severe aplastic anaemia. A plausible explanation to the discrepancy in the findings would be due differences in the study population as the study from China recruited children post-immunosuppressive therapy.

Additionally, the study did not find a difference in the survival of patients according to the treatment they received. This is similar to findings from Ramsamy *et al.* (2022), Vaht *et al.* (2017) and Bacigalupo *et al.* (2016) who also reported no significant difference in survival in paediatric patients regardless of the treatment they received. However, Rodgers *et al.* (2019) found that HSCT conferred superior survival in paediatric patients who had relapsed/refractory aplastic anaemia compared to second immunosuppressive therapy in a USA study.

The study did not find age in the paediatric patient to be a prognostic factor. This is consistent with results from South Africa (Ramsamy *et al.*, 2022). The study also did not find significant association between weight and survival. This is inconsistent with a study from Thailand that reported the BMI to be a predictor to treatment response (Somprasertkul *et al.*, 2024). The discrepancy could be due differences in the study population and as the study from Thailand investigated adult aplastic anaemia patients who had severe and very severe aplastic anaemia and had received immunosuppressive therapy.

Additionally, the aetiology of aplastic anaemia was not associated with the survival of patients. This could entail that aplastic anaemia has a particular disease progression regardless of the aetiology.

5.2 Adult participants

5.2.1 Demographic characteristics of adult patients

The adult patients had a median age of 22.5 years. Similarly, the median age for adult patients in South Africa was 24.5 years (Waja *et al.*, 2018). This is lower than the median age that was found in studies conducted in Thailand (Norasetthada *et al.*, 2021), India (Shah *et al.*, 2019) and France (Contejean *et al.*, 2022) which reported median ages of 59 years, 29 years, and of 68.5 years respectively. Nevertheless, a French study reported a median follow up time of 2.7 years (Contejean *et al.*, 2022). The discrepancy could be attributed to the differences in the methodology. For example, while his study was a retrospective cohort study involving a single hospital, the study from Thailand was a prospective multi-centre study.

The study found a male predominance. This is similar to findings from South Africa (Ramsamy *et al.*, 2022) and Thailand (Norasetthada *et al.*, 2021).

5.2.2 Clinical Characteristics of adult patients

With regards to the disease severity, the study found that the majority of the patients had non-severe aplastic anaemia. This is in keeping with findings from China in which most of the patients were diagnosed with non-severe aplastic anaemia (Zhang *et al.*, 2021). This in contrast to the study findings in Thailand where most of the patients had very severe aplastic anaemia (Norasetthada *et al.*, 2021). The differences in the findings could be due to differences in the populations that were studied as the current study and the study from China were a single institutional study while the study in Thailand was a nationwide population-based study.

5.2.3 Laboratory features of adult patients

The median white blood cell count was 2.2 (IQR = 9.59) $X10^9/L$, median platelet count was 15 (IQR = 140) $X10^9/L$, the median absolute neutrophil count was 0.34 (IQR = 6.13) ($X10^9/L$), haemoglobin level median was 5.59 (IQR = 4.1) g/dL and the median Reticulocyte count (%) was 1.41 (IQR = 3.3). This is similar to results by Contejean *et al.* (2022) though it reported a slightly higher haemoglobin level and a slightly lower platelet level.

5.2.4 Survival Analysis of adult patients

In this study, the one-year survival for the adult patients was 53%. This is lower than the findings from a French study which reported a 3-year survival rate of 74.7% (Contejean *et al.*, 2022) and Swedish study which reported higher 5-year survival rates in adult patients (Vaht *et al.*, 2017).

The study did not find any association between age and sex and survival. Similarly, a study in Pakistan did not find an association between survival and age and sex (Awan *et al.*, 2024). Rice *et al.* (2020) also found that the age at which a patient received treatment was not associated with survival. On the other Norasetthada *et al.* (2021), Vaht *et al.* (2017) and Zhang *et al.* (2021) reported that older age was a predictor of inferior survival.

According to disease severity, the study did not find differences in the survival rates. However, Norasetthada *et al.* (2021) reported significant survival rates among patients in Thailand with NSAA having a superior 2-year survival followed by those with SAA and finally those with VSAA. Vaht *et al.* (2017) also reported statistically significant higher mortalities among VSAA and SAA compared to NSAA. Contejean *et al.* (2022) also reported that age and severe aplastic anaemia were significantly associated with mortality.

Similarly, (Contejean *et al.*, 2022) found no significant impact of different treatment modalities on the mortality and survival of patients. Similar to our findings, a study in China did not find a difference in the 5-year survival time of aplastic anaemia patients on different treatment modalities (Shen *et al.*, 2023). Norasetthada *et al.* (2021) reported statistically differences in survival outcomes depending on the treatment received.

5.3 Combined population

5.3.1 Demographic characteristics of combined paediatric and adult participants

The majority of aplastic anaemia patients were found to be paediatric patients (76%). Similarly, a study from Pakistan reported a high degree of younger age at age of disease diagnosis (Ahmed *et al.*, 2020). In contrast, a study in India found the majority (75%) of aplastic anaemia patients being adult patients (Shah *et al.*, 2019). The difference in the findings is attributed to the study population as the study from India investigated aplastic anaemia patients that received immunosuppressive treatment only while the current study included all aplastic anaemia patients regardless of the treatment modality they received. The younger

age for disease onset observed in our study underscores a plausible genetic factor being involved in predisposition to aplastic anaemia.

The study found a median age of 12 years at diagnosis and the younger population accounting for higher proportion of individuals diagnosed with aplastic anaemia. Similarly, Khalid *et al.* (2022), found a median age at diagnosis of 10 years. The finding reveals that aplastic anaemia is more common in the younger population in our setting. This is in contrast to a study that was conducted in China which found a median age at diagnosis of 27 years (Zhang *et al.*, 2021). Additionally, Vaht *et al.* (2017) reported a median age at diagnosis of 60 years and Zhu *et al.* (2019) reported median age at diagnosis of 21 years in a Chinese study. The differences are possibly due to the differences in methodologies used as well as environmental exposures.

Additionally, it found a male predominance. Similarly, Khalid *et al.* (2022), found a male predominance of aplastic anaemia patients in a Pakistan study and Shah *et al.* (2019) also reported a male predominance of male to female ration of 2:1 in an Indian study. This is also consistent with findings from UK that reported a male predominance (Famokunwa *et al.*, 2023). Similarly, Osho *et al.* (2022) found a male to female ratio of 0.54:0.46 similar to our 0.54:0.46 male to female ratio. On the other hand, Li *et al.* (2019) reported female predominance while Vaht *et al.* (2017) did not find a difference in incidence rates between males and females. The differences are possibly due to the differences in methodologies used as well as socio-demographic profiles.

5.3.2 Clinical Characteristics for all the participants

Aetiologically, the study found that most of the participants were diagnosed with idiopathic aplastic anaemia. This is consistent with previous studies (Waja *et al.*, 2018; Kojima, 2017). The most common presentation was features of anaemia followed by bleeding and then fever. This is in keeping with findings from previous studies (Ashwini *et al.*, 2016; Mandal *et al.*, 2016).

Additionally, the study found that most patients were diagnosed with the non-severe form of aplastic anaemia. This is in keeping with findings from Pakistan (Khalid *et al.*, 2022; Dutta *et al.*, 2021), Nigeria (Osho *et al.*, 2022) and South Africa (Ramsamy *et al.*, 2022). On the hand, studies in India (Shah *et al.*, 2019) and China (Zhu *et al.*, 2019) found that most of the patients had severe aplastic anaemia followed by very severe aplastic anaemia and non-severe aplastic anaemia. The discrepancies could be due diagnostic related factors such as early presentation and diagnosis in our setting.

Our study found that only 7% of all the aplastic anaemia patients during the study period received HSCT. This is supported by previous studies that have reported that ≤10% of aplastic anaemia patients receive HSCT (Famokunwa *et al.*, 2023; Vaht *et al.*, 2017; Zhu, 2019; Mahapatra *et al.*, 2015). This low percentage of patients undergoing HSCT could be attributed to high costs associated with the procedure as well lack of suitable donors.

5.3.3 Laboratory features for all the participants

The median white blood cell count was 2.2 $X10^9$/L, median platelet count was 15 $X10^9$/L, the median absolute neutrophil count was 0.34 $X10^9$/L, haemoglobin level median was 5.59 g/dL and the median Reticulocyte count (%) was 1.41. These results are similar from a study conducted in South Africa (Akpan *et al.*, 2021) and India (Mandal *et al.*, 2016).

5.3.4 Survival analysis for all the participants

The study found the median survival time of 65 months from the diagnosis of aplastic anaemia. In contrast, a Swedish study found median survival time of 76 months (Vaht *et al.*, 2017). Additionally, a Chinese study found the median survival time of 57.2 months (Yang *et al.*, 2023) while Zhang *et al.*, 2021 reported a median survival time of 38 months.

The study found a mortality rate of about 28% with the most common cause of death being haemorrhage. This is further established that presenting to the health facility with bleeding was associated with survival status. This is consistent with results from previous results (Akpan *et al.*, 2021; George *et al.*, 2020). The finding indicates the importance of early management, treatment and prevention of bleeding in aplastic anaemia patients. On the contrary, Shah *et al.* (2019) found that the most common cause of death was infection followed by intracranial heamorrhage, with a mortality rate of 31%. Fattizzo *et al.* (2023) and Vaht *et al.* (2017) also reported infection as the most common cause of mortality.

The study found a 1-year survival rate of 42%. In contrast, studies in China found higher 5-year survival rates as high as 83.7% (Shen *et al.*, 2023; Yang *et al.*, 2023; Zhang *et al.*, 2021), a study in Sweden found a 5-year survival time of 60.7% (Vaht *et al.*, 2017), a study in Taiwan reported a 5-year survival rate of 60.0% (Li *et al.*, 2017) while a study from Poland reported a 2-year overall survival rate of 73% (Zielińska *et al.*, 2023). These discrepancies could be due to differences in the study population as well as availability and affordability of treatment modalities in different countries.

The study reports a statistically association between survival and being either a paediatric patient or an adult patient. The study found that there was a statistically difference in survival of paediatric patients and adult patients with being an adult having a higher likelihood of mortality compared to being from the paediatric cohort. This could be attributed to better and faster responses to treatment exhibited in the paediatric patients compared to adult patients. This is consistence with results from India that found that children patients with aplastic anaemia had overall faster and better responses to treatment (Shah *et al.*, 2019) and a multi-nation study of Italy, UK and USA which reported that older age was associated with poorer survival (Fattizzo *et al.*, 2023).

The study did not find significant association between survival and treatment modalities. This is consistent with findings from Indian study which reported no significant differences in response to different treatment modalities (Mahapatra *et al.*, 2015). Additionally, a study in India did not find statistically difference in survival between patients that received different treatment modalities (Dutta *et al.*, 2021). Similarly, Li *et al.* (2017) also did not find treatment modalities as independent predictors of survival. Conversely, Khan et al (2022) found that there was a significant difference in the survival distributions of different treatment modalities in Pakistan study and Vaht *et al.* (2017) reported that being treated with CSA alone or receiving no therapy were independent risk factors for inferior survival. This deviation from our findings could be explained by the difference in the study population as the Pakistan study was conducted on patients that had received immunosuppressive treatment modalities only.

Furthermore, the study did not find significant association between survival and sex and disease severity. This is keeping with findings by Dutta *et al.* (2021). Despite reporting that sex was not a risk factor for inferior survival like in our study, Vaht *et al.* (2017) reported that having very severe aplastic anaemia was an independent risk factors for inferior survival. Additionally, in contrast to our findings, Li *et al.* (2017) also reported patients in Taiwan with very severe aplastic anaemia and those with severe aplastic anaemia had inferior survival compared to those who had non-severe aplastic anaemia. In addition, Mahapatra *et al.* (2015) found that patients in India who had severe aplastic anaemia had better treatment responses than patients who had very severe aplastic anaemia. Zhang *et al.* (2021) also reported a higher 5-year overall survival in patients with non-severe aplastic anaemia in comparison to severe aplastic anaemia and very severe aplastic anaemia.

5.4 Study limitations and strengths

The study had several limitations. Firstly, this was a retrospective medical record-based study and as such a lack of control for any factors that could have influenced the outcome such as, duration to treatment initiation from diagnosis, concomitant medications and management practices at the facility. Prospective studies to address this medical need are recommended. Additionally, this was a single institution study. Notwithstanding the limitations, the study has a strength of having a long-term follow up of patients and being conducted. Additionally, the study has provided the demographic, clinical and laboratory profiles of patients with aplastic anaemia at UTH. It has provided important information on the survival of aplastic anaemia patients at the facility. To our knowledge, this is the first study conducted in Zambia on survival analysis of aplastic anaemia patients.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The study aimed to study the characteristics and survival of aplastic anaemia patients at UTHs. It investigated the demographics of aplastic anaemia patients, clinical profile as well as the laboratory characteristics. Survival analysis techniques were employed to determine the survival rates and factors that predict survival in patients with aplastic anaemia at UTHs. A total of 126 aplastic anaemia patients were recruited with 96 of these being paediatric patients.

In paediatric patients, the study found a slight female predominance. The median age at diagnosis was found to be 11 years. The most common cause of aplastic anaemia was unknown. Most of the participants had NSAA followed by SAA and VSAA. The mortality rate was 21.88% with haemorrhage being the most common cause of mortality. The median survival time from diagnosis was found to be 65 months. The 1-year survival rate was found to be at 43%. The study did not find any factors to be independent predictors of survival among paediatric patients with aplastic anaemia.

In the adult patients, the study reports a male predominance and a median age at diagnosis of 22.5 years. Similarly to paediatric patients, the most cause of aplastic anaemia in adult patients was unknown. Most of the participants had NSAA followed by SAA and VSAA. The mortality rate in adults was higher than in the paediatric patients at 46.67%. Haemorrhage was the most common cause of mortality. The 1-year survival rate was at 53%. None of the factors under study were found to be independent predictors of survival among adult patients as well.

Analysis of all the patients revealed a slight male predominance, a median age of 12 years and a median survival time of 65 months. The study reports a mortality rate and a 1-year survival rate of 27.78% and 42%, respectively. The study found that being adult and presenting with bleeding were independent predictors of inferior survival.

6.2 RECOMMENDATIONS

In view of the study findings, the following are recommendations:

6.2.1 **Improvements in management**: The current study revealed that most patients with aplastic anaemia at UTH are treated conservatively. There is need for improvements in management of aplastic anaemia patients, especially adults, by clinicians. Aplastic anaemia

complications especially bleeding should be prevented and detected early and prompt management instituted by clinicians.

6.2.2 **Availability, accessibility and affordability of treatment**: There is need for the government to ensure the availability and accessibility of HSCT centres in the country. Additionally, immunosuppressive drugs such as cyclosporin and antithymocyte immunoglobulin should be made affordable.

6.2.3 **Further research**: Prospective studies may need to be conducted. Additionally, multi-centres studies can be conducted in Zambia.

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Appendix I: Study timeline

Month / Activity	APR 2024	MAY 2024	JUNE – SEPT 2025	OCT 2025	NOV 2025	DEC 2025	JAN 2026
Research proposal Writing							
Research proposal submission for ethical approval							
Data collection							
Data analysis and report writing							
Final report submission							

Appendix II: Budget

The budget for the study is as depicted below.

Budget Items	**Number of items**	**Cost per item (ZMW)**	**Total cost (ZMW)**
Printing costs	100 pages	2.00	200.00
Binding of final project report	1	50.00	50.00
Travel expenses	-	-	500.00
Internet connectivity	-	-	500.00
Food expenses	-	-	500.00
UNILUSREC Clearance NHRA Approval	1	1,000.00	1,000.00
Research assistants	2	2,000.00	4,000.00
Total			6,750.00
Contingency (5%)	-	-	337.50
Grand Total Cost	**-**	**-**	**7,087.50**