INFECTIOUS DISEASES IN HAEMATOLOGY PATIENTS:

Clinical severity, pathophysiology and therapeutic strategies in a high-burden South African setting

Justin Rudolph du Toit



Infectious Diseases in Haematology Patients: Clinical Severity, Pathophysiology, and Therapeutic Strategies in a High-Burden South African Setting

Propositions

1. The prevention of community-acquired respiratory viral infections prior to transplantation represents the most cost-effective and safest strategy in a low-resource South African setting. (this thesis)

2. Bloodstream infections in patients undergoing transplantation in the private sector will be associated with higher rates of multidrug-resistant bacterial organisms. (this thesis)

3. The use of low-dose etoposide for chemo-mobilisation of peripheral blood stem cells in patients with multiple myeloma is associated with reduced morbidity, adequate stem cell yields, and cost savings. (this thesis)

4. Haploidentical stem cell transplantation is a feasible treatment modality; however, optimal outcomes depend on careful patient and donor selection. (this thesis)

5. Enhanced focus on undergraduate education in transfusion medicine is likely to result in improved patient outcomes. (this thesis)

6. Improving access to transplantation in parallel with building capacity for CAR (chimeric antigen receptor)-based cellular therapies in South Africa is more appropriate than a stepwise approach.

7. The South African–adapted ex vivo alemtuzumab GVHD prophylaxis strategy may be associated with higher relapse rates and impaired immune reconstitution in the matched unrelated donor setting.

8. The distinction between mismatched related and unrelated donors is becoming increasingly less relevant in the era of post-transplant cyclophosphamide.

9. The use of haematopoietic stem cells from well-controlled HIV-positive donors may represent a viable future option.

10. The introduction of quality management programmes in haemato-oncology training hospitals will improve patient outcomes.

11. “Not everything that can be counted counts, and not everything that counts can be counted.” (William Bruce Cameron)

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Infectieziekten bij hematologiepatiënten: Ernst van ziekte, pathofysiologie en therapeutische strategieën in een Zuid-Afrikaanse setting met hoge ziektelast

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Part I: Introduction

1

General Introduction and Outline of Thesis

1.1 THE SOUTH AFRICAN CONTEXT

South Africa (SA), classified as an upper-middle-income country [1], had an estimated population of approximately 63 million people in July 2024 [2]. The country is burdened with a unique and complex quadruple disease profile. This includes a persistent burden of communicable diseases, particularly HIV and tuberculosis (TB); a growing incidence of non-communicable diseases (NCDs), such as diabetes, cardiovascular disease, obesity and cancer; unacceptably high rates of maternal, neonatal, and child morbidity and mortality; and high levels of trauma and violence-related injuries [3,4].

According to existing health data, non-communicable diseases now account for approximately 40% of the total disease burden, while HIV and TB together were responsible for 26.7% of all deaths in 2015 [3,4]. The 2009 *Lancet* Series on Health in South Africa [5] attributed much of the country's poor health outcomes to its legacy of colonialism and apartheid, which entrenched systemic racial segregation, socioeconomic inequity, and poor access to healthcare and education – factors that continue to influence public health today.

South Africa maintains a two-tiered healthcare system comprising public and private sectors. While the country allocates around 8.5% of its gross domestic product to health, there is a significant imbalance in human resource distribution. More than 70% of medical doctors are employed in the private sector, which serves less than 20% of the population [6,8]. In response to these disparities, the government is in the process of implementing a National Health Insurance (NHI) scheme aimed at providing quality healthcare to all citizens, regardless of income or social class [7]. However, this remains a work in progress.

1.2 HAEMATOLOGY AND BONE MARROW TRANSPLANTATION IN SOUTH AFRICA – A HISTORICAL PERSPECTIVE

The field of haematology and haematopoietic stem cell transplantation (HCT) in South Africa developed within a broader context of medical innovation. A significant milestone was the world's first heart transplant, performed by Professor Christiaan Barnard in 1967 at Groote Schuur Hospital (GSH), affiliated with the University of Cape Town (UCT). This achievement fostered a culture of clinical advancement and established the necessary infrastructure, including

tissue typing laboratories, that later supported the development of haematology services.

It was in the early 1970s that Professor Peter Jacobs established the first haematology department at GSH. At that time, there was minimal infrastructure, blood was stored in glass bottles, and platelets were only available as single-unit concentrates. Despite these limitations, GSH became the first centre in Africa to acquire and use apheresis technology, a major development that eventually influenced practices at national blood banks.

To address infrastructure constraints, the unit initiated the creation of a dedicated platelet donor panel and established specialised laboratories for clonogenic assays, flow cytometry, and the cryopreservation of haematopoietic stem cells. A collaborative partnership between GSH and Oxford University led to the early adoption of alemtuzumab ("Campath-in-the-bag") against graft-versus-host disease (GVHD), a strategy that still remains a cornerstone of GVHD prevention in SA [9].

Professor Jacobs performed South Africa's first allogeneic HCT in 1974. In 1991, Professor Ernette du Toit established the South African Bone Marrow Registry (SABMR), which represented another important step in expanding transplant access. As of 2025, South Africa has nine allogeneic bone marrow transplant units. Despite this progress, access remains limited, with only two of these centres serving an estimated 84% of the national population through the public healthcare sector. As a result, transplant activity in South Africa remains low compared to high-income countries, owing to ongoing financial, infrastructural and systemic challenges.

1.3 CURRENT CHALLENGES IN HAEMATOLOGY IN SOUTH AFRICA

Haematology in South Africa is inextricably linked to haematopoietic stem cell transplantation. Unfortunately, most public hospitals lack the infrastructure required to conduct bone marrow transplants safely and consistently. There is also a significant shortage of nurses trained in haematology and transplant care, and this sub-specialisation is not yet formally recognised as a distinct discipline within the national health workforce.

The SABMR had 78,027 registered local donors as of 2017. The majority of these donors are of Caucasian descent, which presents a major challenge in a country

where most patients are of African or mixed-race origin [10]. Furthermore, the high prevalence of HIV infection, estimated at 13.1% nationally, further limits the potential donor pool [11]. The Human Leukocyte Antigen (HLA) profiles of African populations are genetically highly diverse, making donor matching both complex and resource-intensive. The combination of limited donor availability and financial constraints often makes international donor searches prohibitively expensive.

Compounding these challenges is the underdiagnosis and underreporting of haematological illnesses in South Africa, along with the absence of a central bone marrow transplant registry. These gaps hinder our ability to accurately assess national transplant needs and to develop best-practice care pathways for patients requiring complex haematological interventions.

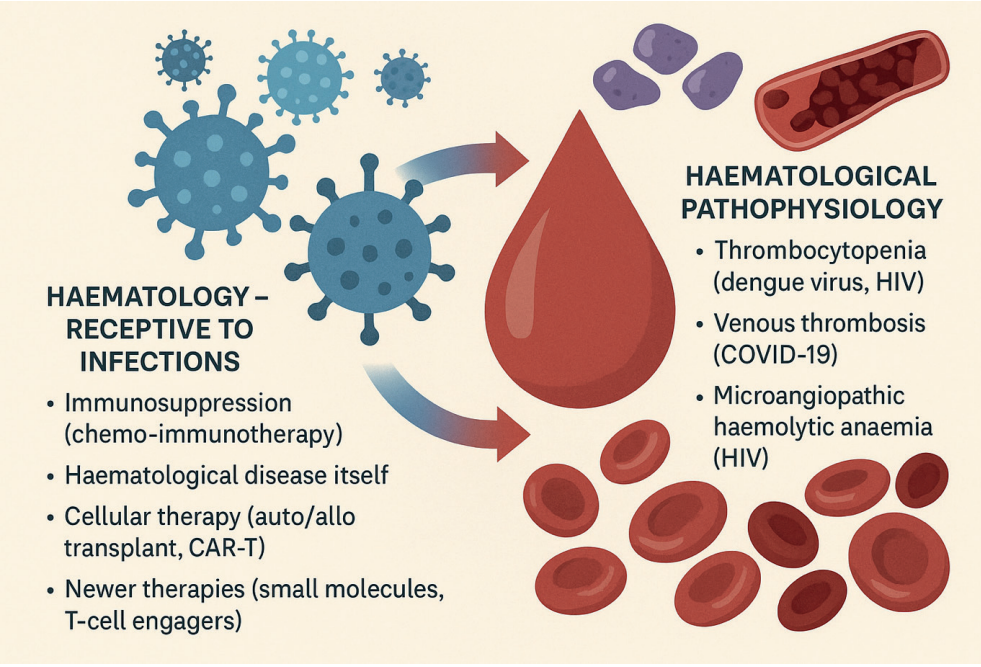


Figure 1. The relationship between haematology and virology is intrinsically interconnected. Individuals with haematological diseases are particularly vulnerable to infectious complications, while viral infections themselves can induce or exacerbate haematological pathology through diverse pathophysiological mechanisms.Figure 1. The relationship between haematology and virology is intrinsically interconnected. Individuals with haematological diseases are particularly vulnerable to infectious complications, while viral infections themselves can induce or exacerbate haematological pathology through diverse pathophysiological mechanisms.

1.4 INTERPLAY BETWEEN HAEMATOLOGY AND VIROLOGY

The interplay between haematology and virology is bidirectional: patients with haematological disorders are at increased risk of acquiring infections (viral, bacterial and fungal), while viral pathophysiological mechanisms can directly and indirectly affect the haematopoietic system, shaping disease manifestation and clinical presentation. This heightened susceptibility is driven by profound immune dysfunction involving both the cellular and humoral arms of immunity. Cellular immune compromise results from quantitative and functional deficiencies in T- and B-lymphocyte populations, leading to impaired antiviral immune responses and dysregulated cellular immunity [12,13,16]. Concurrently, humoral immune dysfunction, characterised by hypogammaglobulinaemia and impaired antibody production, undermines effective pathogen neutralisation and immune memory [13,15,16]. These immune abnormalities may arise from the underlying haematological disease itself or secondarily from therapeutic interventions including cytotoxic chemotherapy, monoclonal antibodies, targeted agents, and conditioning regimens for haematopoietic stem cell transplantation, all of which further exacerbate both cellular and antibody-mediated immune deficiency [12,15,16].

1.5 RELEVANCE AND IMPACT OF THIS THESIS

Against the backdrop of multifactorial challenges facing the South African healthcare system, including those within the discipline of haematology, there is an urgent need for data-driven, contextually appropriate strategies to improve patient care and outcomes. The intersection of a high infectious disease burden, constrained healthcare resources, substantial donor limitations arising from the marked HLA diversity of our population and high HIV seroprevalence, and the growing demand for HCT and complex cellular therapies, creates a uniquely challenging clinical environment. This thesis addresses these challenges through an integrated programme of research focused on the burden of infectious diseases in immunocompromised hosts, the mechanistic interplay between viral infections and haematological pathology, and the optimisation of treatment pathways within a resource-limited setting.

Key clinical decisions, such as determining eligibility for HCT or cellular therapies, require meticulous assessment of host resilience and fitness for transplantation, specific disease biology and disease status prior to HCT, immune competence and infectious risk, as well as optimal donor selection based on availability. In

this setting, understanding how the burden of community-acquired respiratory viral infections and bloodstream infections influences transplant outcomes, complication rates and survival is critical to informing patient selection, timing of transplantation and supportive care strategies. Furthermore, viral infections exert direct effects on the haematopoietic and haemostatic systems through immune-mediated and immunothrombotic mechanisms, contributing to cytopenias, thrombosis and virus-associated autoimmune bleeding disorders. Elucidating these processes, and defining associated biomarkers and clinical correlates, is essential to improving diagnostic accuracy, risk stratification and therapeutic decision-making across haematology practice.

In parallel, optimisation of therapeutic processes represents a major opportunity to enhance feasibility and outcomes within South Africa's constrained healthcare environment. Improving haematopoietic stem cell mobilisation strategies has the potential to increase transplant success while minimising procedural toxicity and costs. Given the local scarcity of fully matched donors, attributable to the factors outlined above, evaluation of haploidentical transplantation using post-transplant cyclophosphamide (PTCY) as a practical and safe alternative is particularly relevant, with important implications for equitable access to curative therapies. Additionally, understanding current patterns of transfusion prescribing and blood-product modification practices among South African haematology providers is a necessary step toward developing patient blood-management frameworks tailored to the realities of resource-limited settings, with potential applicability to other low-resource environments.

International literature underscores the relevance of these themes. Global inequities in access to transplantation among ethnically diverse populations have been highlighted by Joshua et al., Fiala et al., Ailawadhi et al., and Winestone et al. [16–19], emphasising barriers directly applicable to the South African context. Graham et al. [20] have demonstrated the expanding role of haploidentical transplantation as a feasible and cost-effective alternative when matched donors are unavailable, while Sahu et al. [21], Ruiz-Argüelles et al. [22], and Jaimovich et al. [23] have stressed the central importance of infection surveillance and infection-control strategies in transplant populations, particularly in resource-constrained settings. Similarly, Sadana et al. [24], Mueller et al. [25], Gammon et al. [26], and the World Health Organization [27] have shown that optimisation of transfusion practice through patient blood-management programmes can substantially reduce complications, wastage and healthcare costs, outcomes highly relevant to local clinical practice.

By integrating these clinical, mechanistic, and operational domains, this thesis addresses three central questions:

1. How infectious disease burden influences outcomes among haematology patients undergoing transplantation in the South African setting;
2. How viral infections drive haematological pathology through immune-mediated and immunothrombotic mechanisms; and
3. How key treatment processes, namely haematopoietic stem cell mobilisation, donor selection via haploidentical transplantation strategies, and transfusion practices, can be optimised to improve patient outcomes and resource utilisation in South Africa.

Collectively, the findings of this work aim to strengthen evidence-based clinical practice, inform local policy and contribute to the development of pragmatic care models applicable not only within South Africa but also across other low- and middle-income countries facing comparable healthcare system constraints.

1.6 OUTLINE OF THIS THESIS

This thesis is organised into three major parts, each addressing a key thematic area within haematology, with particular emphasis on its interplay with virology and the specific clinical challenges faced in South Africa.

Part II examines the epidemiology of infections commonly affecting haematology patients. Chapter 2 focuses on the burden of respiratory syncytial virus (RSV) infection by comparing data from three different centres in South Africa and the Netherlands. Chapter 3 explores the incidence of bloodstream infections and patterns of microbial colonisation among haematopoietic stem cell transplant recipients in South Africa. Chapter 4 investigates testing policies and clinical knowledge regarding community-acquired respiratory viruses (CARVs) among healthcare professionals working across different treatment centres internationally.

Part III shifts focus to the pathophysiological mechanisms by which viral infections influence haematological disease, particularly examining how changes in haematological manifestations, such as thrombocytopenia, procoagulant monocyte activation, and coagulation abnormalities, contribute to disease severity and viral pathogenesis. Chapter 5 presents a comprehensive review of viral infections associated with clinically significant thrombocytopenia and

their potential underlying mechanisms. Chapter 6 explores the role of monocyte tissue factor expression in severe COVID-19, focusing on its contribution to the procoagulant state and the development of pulmonary embolism. This chapter highlights how the presence of procoagulant peripheral blood mononuclear cells correlates with ventilation–perfusion mismatch, an imbalance that may precede pulmonary embolism in critically ill patients. Chapter 7 describes a case series of patients with HIV-associated and idiopathic acquired haemophilia, highlighting these rare but clinically significant immune-mediated haematological disorders.

Part IV addresses treatment approaches and outcomes for haematology patients in South Africa, a region characterised by a high burden of infectious disease that contrasts with high-income settings of the United States and Western Europe, where most available cohort data and clinical guidelines originate. This section focuses on optimisation of clinical resources and practices in haematology through evaluation of stem cell mobilisation protocols, outcomes of haploidentical stem cell transplantation, and knowledge and practice patterns among haematology providers. Chapter 8 describes the outcomes and optimisation of the local haematopoietic stem cell mobilisation protocol, aiming to improve stem cell yields while reducing treatment-related toxicity. Chapter 9 discusses the implementation of haploidentical (half-matched) allogeneic transplantation using a specific conditioning regimen, proposed as a feasible solution to donor scarcity. Finally, Chapter 10 evaluates clinician knowledge and practices pertaining to red cell and platelet transfusion, and blood product modification requests in haematology patients, identifying opportunities for improved education and policy development to enhance patient care and healthcare resources.

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Part II: Burden of Infectious Diseases in Haematology Patients

2

Contemporary Respiratory Syncytial Virus Epidemiology, Outcomes and Practice Variation after Haematopoietic Cell Transplantation – A multicontinental cohort study

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2.1 Abstract

Background: Respiratory syncytial virus (RSV) is an important cause of respiratory morbidity in haematopoietic stem cell transplant (HCT) recipients, yet comparative data across different healthcare settings remain limited. We aimed to evaluate contemporary RSV burden, real-world management variation, and clinical outcomes across three HCT centres in South Africa and the Netherlands.

Methods: This retrospective multicentre cohort included adults (≥ 18 years) with polymerase chain reaction (PCR)-confirmed RSV infection in the HCT care pathway between January 2015 and December 2024. Observed RSV case burden was expressed as the proportion of all transplants and as detected cases per 100 person-years. RSV testing was clinician-driven rather than protocolised, and the number of transplant recipients tested for RSV was not uniformly available across centres.

Results: Among transplant populations comprising 1,189 HCTs, 82 PCR-confirmed RSV infections were identified (65 Erasmus Medical Centre (EMC); 9 Spaarne Gasthuis Hospital (SG); 8 Wits Donald Gordon Medical (WDG)). Observed centre-level case proportions ranged from 4.2% to 7.7%, corresponding to 1.2–1.5 detected cases per 100 person-years. Most infections (65%) occurred within 15 months of HCT, with 43% occurring between 0 and 9 months. Respiratory viral co-infections were documented in 16–33% of cases. Hospitalisation, ICU admission and in-hospital mortality ranged from 25–56%, 3–22% and 3–13%, respectively. Ribavirin-based treatment was used only at EMC.

Conclusion: PCR-confirmed RSV infection represented a relatively small observed case burden in these HCT populations and was associated with generally favourable short-term outcomes. Most infections occurred early after HCT, suggesting a clinically relevant period of increased vulnerability. Because testing was clinician-driven and testing denominators were unavailable, direct comparisons of incidence between centres require caution.

2.2 INTRODUCTION

RSV, a negative-sense ribonucleic acid (RNA) virus of the *Pneumoviridae* family, is a major cause of lower respiratory tract infections (LRTIs) worldwide. Although disease burden is highest in infants and young children, RSV also causes substantial morbidity and mortality in older adults and immunocompromised populations, including HCT and solid organ transplant recipients [1–5].

Among HCT recipients, RSV is one of the most frequently detected community-acquired respiratory viruses, with reported incidence rates ranging from 2% to 30%, particularly after allogeneic transplantation [6,7]. Progression from upper respiratory tract infection to RSV lower respiratory tract disease (LRTD) is associated with considerable mortality, reported up to 25–30% in high-risk populations, as well as chronic pulmonary sequelae such as bronchiolitis obliterans [8,9].

Management remains challenging and is largely based on supportive care, infection prevention measures and selective antiviral treatment. Although early ribavirin therapy may reduce progression to severe disease in selected patients, evidence remains limited, practice varies substantially between centres, and the additional role of intravenous immunoglobulins (IVIG) is uncertain [10–12]. As a result, RSV management in transplant recipients is frequently guided by local expertise rather than robust comparative data.

The preventive landscape is rapidly evolving. Long-acting monoclonal antibodies such as nirsevimab and clesrovimab, and RSV vaccines including Arexvy, Abrysvo and mRESVIA, have demonstrated efficacy in older adults and paediatric populations. However, their effectiveness, timing and cost-effectiveness in HCT recipients remain largely unknown [13–16].

Comparative data on RSV epidemiology and management across different healthcare systems, particularly including sub-Saharan Africa, remain scarce. Better understanding of real-world practice patterns may help identify patients most likely to benefit from targeted prevention and harmonised management strategies.

Therefore, we conducted a multicentre cohort study to evaluate contemporary RSV burden, management practices and clinical outcomes in adult HCT recipients across three centres in South Africa and the Netherlands.

2.3 MATERIALS AND METHODS

2.3.1 Study design

This retrospective, multicentre observational cohort study was conducted across three institutions: Wits Donald Gordon Medical Centre (WDG) (Johannesburg, South Africa), Spaarne Gasthuis Hospital (SG) (Haarlem, the Netherlands) and Erasmus Medical Centre (EMC) (Rotterdam, the Netherlands). The cohort included all adults (≥ 18 years) with PCR-confirmed RSV infection in the HCT care pathway between 1 January 2015 and 31 December 2024. RSV detections during admission immediately preceding a planned HCT were retained as peri-transplant cases; this included the WDG patient responsible for the negative lower bound of the centre-specific time interval.

Both inpatients and outpatients with laboratory-confirmed RSV infection were included. Hospital or intensive care unit (ICU) admissions were considered RSV-related if the virus was detected in a respiratory sample using PCR-based diagnostic assays (described below) up to two days before or during admission. Patients were excluded if the admission was deemed unrelated to the respiratory infection.

Ethical approval was obtained from the Local Institutional Review Board of Spaarne Gasthuis Hospital, the Netherlands (reference: 2024.0082; approval date: 4 October 2024), and from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg, South Africa (reference: M250658; approval date: 11 July 2025).

RSV testing was performed according to local clinical practice and was not governed by a uniform cross-centre screening protocol. Testing intensity and clinical thresholds may therefore have differed between centres. The total number of HCT recipients tested for RSV was not uniformly available. Consequently, PCR-confirmed cases relative to the transplant population are reported as observed case burden rather than standardised incidence, and cross-centre comparisons should be interpreted cautiously.

At SG, respiratory samples were analysed by the Regional Public Health Laboratory Kennemerland using a multiplex RT-PCR assay (RespiFinder 2Smart, PathoFinder B.V., Maastricht, the Netherlands), which detects 20 viral and four bacterial targets, including human metapneumovirus (HMPV) and human bocavirus (HBoV) [19]. Point-of-care testing in the emergency department was performed using the GeneXpert system (Cepheid, USA) for rapid detection of influenza virus and RSV.

At EMC, diagnostic testing was performed in accordance with institutional laboratory protocols (Jeroen van Kampen, personal communication).

At WDG respiratory specimens were processed by PathCare Laboratories using two RT-PCR platforms: the BioFire® FilmArray® Respiratory Panel (bioMérieux, Durham, NC, USA), targeting 23 viral and four bacterial pathogens, and the Alinity m Resp-4-Plex assay (Abbott Molecular, Des Plaines, Illinois, USA) for point-of-care testing.

Specimen types included nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates or bronchoalveolar lavage (BAL) samples.

2.3.2 Data collection

Data were retrieved manually from the medical records by three researchers (TN, MG and SVB) using harmonised study definitions. Collected variables comprised demographics, body mass index (BMI), HCT comorbidity index (HCT-CI), underlying diagnosis, transplant and donor type, time between HCT and RSV diagnosis, treatment, hospital and ICU admission, length of stay, and in-hospital mortality. Respiratory viral co-infection was defined as detection of an additional respiratory viral pathogen during the RSV episode that was considered clinically relevant by the treating physician rather than incidental co-detection. HIV was reported separately as a concurrent infection. Bacterial and fungal cultures, and CMV or other herpesvirus reactivations were not systematically captured across all centres and were not included in the co-infection analysis. No formal inter-rater reliability analysis was performed. Immunosuppressant use was limited to systemic immunosuppressants, and antibiotic treatment included selective decontamination of the digestive tract.

2.3.3 Data analysis

Observed RSV case burden was estimated overall and per centre as the number of PCR-confirmed RSV cases divided by the total number of transplants, and as detected cases per 100 person-years. These measures used all transplants as the denominator because the number tested for RSV was not uniformly available. Exposure time was approximated from year of transplant to 2025 for recipients without detected RSV, and to the date of RSV diagnosis for recipients with RSV, because complete individual mortality and follow-up data were unavailable.

Categorical variables were presented as counts and percentages with 95% confidence intervals (CIs), calculated using the Wilson method. Exact Poisson 95% CIs were calculated for detected case rates per 100 person-years.

Continuous variables were summarised as means with standard deviations or medians with interquartile ranges, depending on their distribution. Given the small centre-specific sample sizes, analyses were descriptive and no multivariable modelling was performed. Data analysis was carried out using Statistical Analysis System (SAS).

2.4 RESULTS

The observed burden of RSV infection among HCT recipients across the three participating centres between 2015 and 2024 is summarised in Table 1. At EMC, 65 PCR-confirmed RSV cases were identified among 848 HCTs (7.7%; 95% CI 6.1–9.7). SG identified nine cases among a transplant referral population of 150 (6.0%; 95% CI 3.2–11.0). At WDG, eight cases were identified among 191 HCTs (4.2%; 95% CI 2.1–8.0); one of these eight RSV cases occurred in an allogeneic HCT recipient and seven in autologous HCT recipients. Detected case rates per 100 person-years were similar numerically, but the absence of uniform testing denominators precluded direct incidence comparisons.

Table 1. Observed RSV case burden across transplant centres.

	EMC	SG	WDG
Number of transplants (2015–2024)	848	150	191
Exposure (person-years)	4,267	729	554
RSV cases	65	9	8
RSV cases (as % of transplants)	65/848; 7.7% (95% CI 6.1–9.7)	9/150; 6.0% (95% CI 3.2–11.0)	8/191; 4.2% (95% CI 2.1–8.0)
RSV cases (per 100 person-years)	1.5 (95% CI 1.2–1.9)	1.2 (95% CI 0.6–2.3)	1.4 (95% CI 0.6–2.8)

Percentages are calculated using all transplants as the denominator. Proportion CIs use the Wilson method; rate CIs use the exact Poisson method.

The median age of HCT recipients with RSV infection ranged from 50 years at EMC, to 61 years at SG and 55 years at WDG, with a male predominance at EMC and SG (Table 2). Underlying diagnoses reflected major differences in transplant case mix: AML/MDS predominated at EMC (46%), whereas multiple myeloma was most frequent at SG (56%) and WDG (75%). Most WDG patients had an HCT-CI of 0 (75%; 95% CI 40.9–92.9), indicating a lower measured comorbidity burden than among the Dutch cas

Table 2. Demographics and comorbidity indexes of HCT recipients. IQR=interquartile range, SD=standard deviation.

	EMC	SG	WDG
RSV cases	65	9	8
Age (years) median (IQR)	50 (37–64)	61 (59–67)	55 (40–65)
Sex (male)	36/65; 55% (43–67)	8/9; 89% (57–98)	4/8; 50% (22–78)
BMI (kg/m^2) mean (SD)	26 (6)	26 (3)	25 (5)
Diagnosis			
AML/MDS	30/65; 46% (35–58)	1/9; 11% (2–44)	1/8; 13% (2–47)
MM	2/65; 3% (1–11)	5/9; 56% (27–81)	6/8; 75% (41–93)
Lymphoma*	9/65; 14% (8–24)	3/9; 33% (12–65)	1/8; 13% (2–47)
Myelofibrosis	5/65; 8% (3–17)	0/9; 0% (0–30)	0/8; 0% (0–32)
ALL	13/65; 20% (12–31)	0/9; 0% (0–30)	0/8; 0% (0–32)
Other**	6/65; 9% (4–19)	0/9; 0% (0–30)	0/8; 0% (0–32)
HCT CI			
0	26/65; 40% (29–52)	2/9; 22% (6–55)	6/8; 75% (41–93)
1	23/65; 35% (25–48)	5/9; 56% (27–81)	1/8; 13% (2–47)
2 or higher	16/65; 25% (16–36)	2/9; 22% (6–55)	1/8; 13% (2–47)

Categorical values are n/N; % (95% CI). CIs use the Wilson method.

*Lymphoma (Hodgkin's and non-Hodgkin's lymphoma). **Other conditions (aplastic anaemia, chronic myeloid leukaemia, mixed phenotype acute leukaemia and chronic lymphocytic leukaemia).

Figure 1 shows the distribution of RSV cases using the same transplant and donor categories as Figure 2 and Table 4. At EMC, all RSV cases occurred after allogeneic HCT; matched unrelated donor HCT was the largest category, consistent with local transplant activity. At SG and WDG, seven of nine and seven of eight RSV cases, respectively, occurred after autologous HCT. Because transplant-type-specific testing denominators were unavailable, these distributions describe the case mix and should not be interpreted as transplant-type-specific incidence.

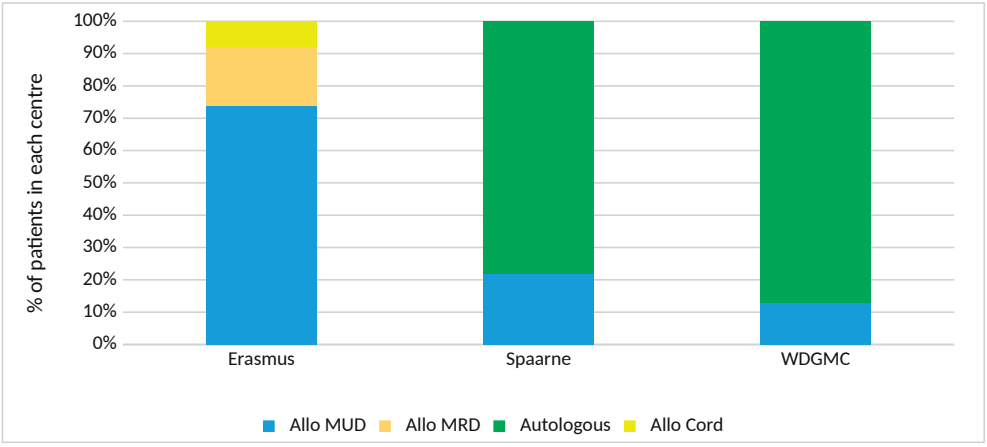


Figure 1. Distribution of RSV cases by transplant type across different transplant centres.

The median time from HCT to RSV detection was 8.6 months (interquartile range (IQR) 4.6–14.9) at EMC, 11.3 months (IQR 2.9–29.7) at SG, and 2.1 months (IQR -0.4–13.1) at WDG (Table 3). The negative lower bound at WDG reflects one patient who tested RSV-positive 0.4 months before HCT while admitted in preparation for transplantation; this patient was retained as a peri-transplant case. Most infections occurred during the local winter (63–83%). Respiratory viral co-infections were identified in 16–33% of cases, most commonly rhinovirus and influenza. No HMPV co-infections were documented in the available cohort data. At SG, where RespiFinder included HBoV, no HBoV co-detection was recorded. CMV and other herpesvirus reactivations were not systematically available for cross-centre analysis. HIV infection was present in one WDG patient and is reported as a concurrent infection rather than a respiratory viral co-infection.

Table 3. Timing, seasonality and concurrent infections.

	EMC	SG	WDG
RSV cases	65	9	8
Time to RSV infection (months): median (IQR)	8.6 (4.6–14.9)	11.3 (2.9–29.7)	2.1 (-0.4–13.1)
RSV in winter months	54/65; 83% (72–90)	7/9; 78% (45–94)	5/8; 63% (31–86)
Respiratory viral co-infection			
Rhinovirus	4/65; 6% (2–15)	1/9; 11% (2–44)	1/8; 13% (2–47)
Influenza	2/65; 3% (1–11)	2/9; 22% (6–55)	1/8; 13% (2–47)
Parainfluenza	3/65; 5% (2–13)	0/9; 0% (0–30)	0/8; 0% (0–32)
Adenovirus	1/65; 2% (0–8)	0/9; 0% (0–30)	1/8; 13% (2–47)
Concurrent HIV infection	0/65; 0% (0–6)	0/9; 0% (0–30)	1/8; 13% (2–47)

Values are n/N; % (95% Wilson CI). CMV and other herpesvirus reactivations were not systematically captured.

Treatment practices differed markedly between centres (Table 4). At EMC, 25% received ribavirin plus IVIG, and 1% received IVIG alone; neither SG nor WDG routinely used ribavirin or IVIG. Route and dose of ribavirin were not consistently available in the harmonised dataset and could therefore not be reported reliably. Antibiotic therapy was recorded in 43% of EMC, 22% of SG and 13% of WDG cases.

Table 4. Treatment approaches and RSV infection outcomes per transplant type.

	EMC	SG	WDG	
RSV cases	65	9	8	
Treatment:				
Oseltamivir	7/65; 11% (5–21)	0/9; 0% (0–30)	0/8; 0% (0–32)	
IVIG only	1/65; 2% (0–8)	0/9; 0% (0–30)	0/8; 0% (0–32)	
Ribavirin + IVIG	16/65; 25% (16–36)	0/9; 0% (0–30)	0/8; 0% (0–32)	
Antibiotics	28/65; 43% (32–55)	2/9; 22% (6–55)	1/8; 13% (2–47)	
Outcomes:				
Admission to hospital	28/65; 43% (32–55)	5/9; 56% (27–81)	2/8; 25% (7–59)	
Admission to ICU	2/65; 3% (1–11)	2/9; 22% (6–55)	1/8; 13% (2–47)	
Hospital LOS (censored for death):				
n (hospitalised)	28	5	2	
median LOS (days)	8 (5–28)	3 (1–49)	not estimable	
In-hospital mortality	2/65; 3% (1–11)	1/9; 11% (2–44)	1/8; 13% (2–47)	
HCT type vs. in-hospital mortality				Overall
Allo MUD	1/48	0/2	1/1	2/51; 4% (95% CI 1–13)
Allo MRD	1/12			1/12; 8% (95% CI 2–35)
Autologous		1/7	0/7	1/14; 7% (95% CI 1–32)
Allo Cord	0/5			0/5; 0% (95% CI 0–43)

Treatment and outcome proportions are n/N; % (95% CI). Cis use the Wilson method. Ribavirin route and dose were not consistently available.

SG had the highest observed hospitalisation rate (5/9; 56%; 95% CI 26.7–81.1) and ICU admission rate (2/9; 22%; 95% CI 6.3–54.7), and the second highest in-hospital mortality (1/9; 11%; 95% CI 2.0–43.5). Corresponding rates at EMC were 43%, 3% and 3%, and at WDG 25%, 13% and 13%, respectively. Median hospital length of stay was eight days (IQR 5–28) at EMC and three days (IQR 1–49) at

SG (length of stay was not estimable at WDG because only two patients were hospitalised). When stratified by transplant type, in-hospital mortality was 7% after autologous and 4% after allogeneic HCT overall, although event numbers were small.

Figure 2 depicts the temporal distribution of all 82 RSV detections relative to HCT, using transplant categories consistent with Figure 1 and Table 4. Overall, 65% of detections occurred within the first 15 months and 43% between 0 and 9 months after HCT, after which the number of detected cases declined. The pattern identifies an early period of increased vulnerability but does not represent a transplant-type-specific incidence curve because testing and denominator data were unavailable.

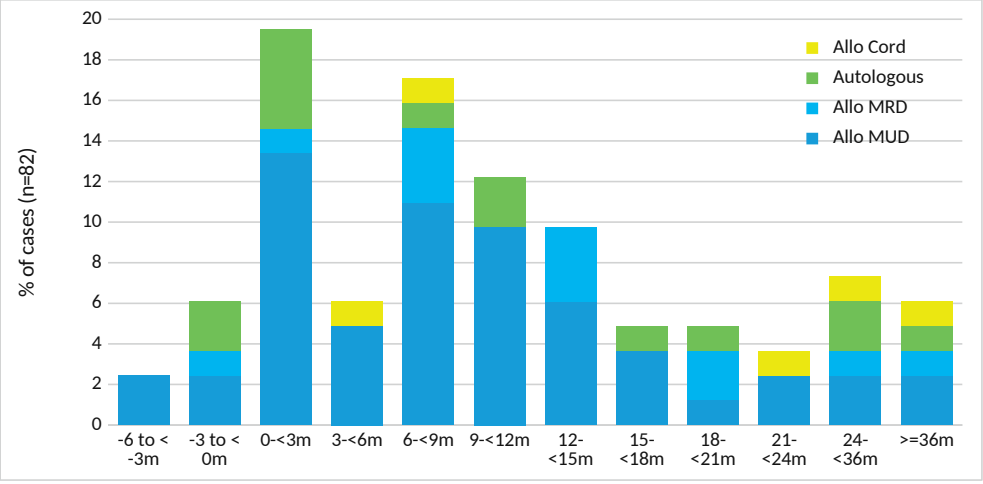


Figure 2. Temporal distribution of RSV detections relative to haematopoietic cell transplantation.

2.5 DISCUSSION

This multicentre retrospective study provides one of the few comparative descriptions of PCR-confirmed RSV infection in HCT care pathways across Europe and Africa and, to our knowledge, the first HCT-specific RSV cohort reported from sub-Saharan Africa. Across the three centres, observed case proportions ranged from 4.2% to 7.7%, corresponding to 1.2–1.5 detected cases per 100 person-years. These estimates align numerically with international cohorts [21–23], but they should not be interpreted as directly comparable incidence estimates. RSV testing was clinician-driven, the number of HCT recipients tested was unavailable, referral pathways differed, and person-time could not be censored for mortality.

Most RSV detections occurred within 15 months of HCT, with 43% occurring between 0 and 9 months, when immune reconstitution is often incomplete. This pattern is consistent with previous reports of early clustering of community-acquired respiratory viral infections after HCT [24,25] and supports targeted surveillance and prevention during the early post-transplant period. Formal risk stratification by transplant type or underlying diagnosis was not feasible because of sparse centre-specific events, strong confounding between centre and transplant type, and unavailable transplant-type-specific testing denominators.

The preventive landscape for RSV is evolving rapidly. Nirsevimab, a long-acting monoclonal antibody providing season-long protection, has shown greater than 75% efficacy in preventing hospitalisation in infants [26] and may represent a future preventive option for selected high-risk HCT recipients, if efficacy and cost-effectiveness can be confirmed in adults. Similarly, prefusion F-protein vaccines (Arexvy, Abrysvo, mRESVIA) have demonstrated promising immunogenicity and safety in transplant recipients beyond six to 12 months post-HCT [27,28]. Pre-transplant vaccination may also be attractive, as vaccination during immune competence could provide more durable antibody responses before transplantation [29,30]. These approaches warrant dedicated evaluation in transplant-specific prospective studies.

In-hospital mortality (3–13%) was lower than or comparable with previously reported rates [31], although the absence of an RSV-negative HCT comparison cohort prevents attribution of hospitalisation, ICU admission or mortality specifically to RSV. SG had the highest hospitalisation and ICU admission proportions and the second-highest mortality, but these percentages were based on only nine cases. Its role as a post-transplant referral centre, differences in admission thresholds and case mix may have influenced these estimates. Conversely, WDG cases had a lower measured comorbidity burden, with 75% having HCT-CI 0, which may limit generalisability of the WDG outcomes to HCT populations with greater comorbidity. Ribavirin-based treatment was used only at EMC, likely reflecting local practice and its predominantly allogeneic case mix. Route and dose were not consistently available, so treatment variation cannot be interpreted as evidence of comparative efficacy.

Respiratory viral co-infections occurred in 16–33% of cases, highlighting diagnostic complexity and the effect of non-uniform multiplex assays on ascertainment. No HMPV co-infections were documented and no HBoV was recorded at SG, where it was included in the RespiFinder panel. CMV and other herpesvirus reactivations were not systematically collected and could not

be assessed. The study period also included the COVID-19 pandemic, during which non-pharmaceutical interventions markedly reduced and displaced RSV circulation in both Europe and South Africa [36,37]. Differences in timing and intensity of national measures may therefore have affected case detection and apparent seasonality across centres. Because annual testing denominators and event numbers were insufficient, we could not reliably separate pre-pandemic, pandemic and post-pandemic trends; the winter predominance reported across the full decade should be interpreted accordingly.

Strengths include the decade-long, multicontinental design and inclusion of a South African HCT centre, addressing an important evidence gap. Limitations include the retrospective design, modest centre-specific sample sizes, clinician-driven testing, absence of uniform testing denominators, incomplete mortality censoring, and differences in referral pathways, assays, patient mix and treatment practice. The absence of RSV-negative HCT recipients precluded assessment of RSV-attributable outcomes or risk factors for infection. A formal Immunodeficiency Scoring Index could not be calculated consistently because the required variables at the time of RSV diagnosis were incomplete. Ribavirin route and dose, CMV and other herpesvirus reactivations, and long-term pulmonary outcomes were not systematically captured. Prospective studies should use standardised testing and follow-up, include RSV-negative comparators, apply validated risk scores, and evaluate late pulmonary sequelae such as bronchiolitis obliterans.

2.6 CONCLUSION

PCR-confirmed RSV infection represented a relatively small observed case burden in these HCT populations and was associated with generally favourable short-term outcomes, although clinician-driven testing and the absence of a RSV-negative comparison cohort limit direct incidence and causal interpretation. Most detections occurred early after HCT, supporting focused evaluation of prevention during this period. Treatment practices varied substantially, while the optimal role of ribavirin and adjunctive therapies remains uncertain. This cohort provides a contemporary baseline for prospective studies using standardised surveillance, risk stratification and long-term pulmonary follow-up across diverse healthcare settings.

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3

Bloodstream Infections and Colonisation in Haematopoietic Stem Cell Transplant Recipients at a South African Centre

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Introduction: Bacterial bloodstream infections (BSIs) are a serious complication after haematopoietic stem cell transplantation (HCT), especially when caused by multidrug-resistant (MDR) bacteria. However, data on BSIs post-HCT from centres in sub-Saharan Africa are limited. This study aims to describe the incidence, etiology and outcomes of BSIs, including those caused by carbapenem-resistant *Enterobacterales* (CRE), in both autologous and allogenic HCT recipients in South Africa. Furthermore, this study examines the incidence and clinical impact of colonisation with CRE.

Methods: A retrospective analysis was performed on clinical data from HCT recipients, transplanted between January 2018 and December 2023 at Groote Schuur Hospital (GSH) and Red Cross War Memorial Children's Hospital, the academic hospitals of the University of Cape Town. Data were extracted from the transplant unit electronic and paper patient records, Hematology Patient Registry and the National Health Laboratory Service electronic database. Surveillance cultures were taken upon admission and subsequently repeated weekly for GSH patients.

Results: In total, 270 HCT recipients were included: 145 underwent autologous HCT, and 124 an allogenic HCT. The most common indication for autologous HCT was multiple myeloma (52%), while acute myeloid leukemia was the most frequent for allogenic HCT (34%). The overall incidence of BSIs was 35%, with a higher rate observed in allogeneic HCT recipients (48%) compared to autologous HCT recipients (23%). Gram-negative bacteria were the most common pathogens, and CRE were responsible for 9% of BSIs. Carbapenem-resistant *Klebsiella pneumonia* spp. was the most common CRE found in BSI and rectal surveillance cultures. Surveillance cultures with CRE were detected in 19% of the HCT recipients during admission to the transplant unit, with oxacillinase-48 (and variants) the most prevalent carbapenemase. Colonisation by CRE was an independent risk factor for developing BSIs. Within 100 days post-HCT, 25 HCT recipients (9%) died, of whom nine (36%) had a BSI in the last seven days of life. Bivariate analysis revealed that BSIs significantly reduced overall survival in both autologous and allogenic HCT recipients, with a further decrease in survival when the BSI was caused by MDR bacteria.

Conclusion: BSIs are a frequent and severe complication among HCT recipients in South Africa, particularly in those receiving allogenic HCT. Colonisation with CRE significantly increases the risk of BSIs, underscoring the need for vigilant infection control and targeted antimicrobial strategies in this vulnerable population.

3.2 INTRODUCTION

HCT forms an important therapeutic modality for various hematologic diseases. HCT recipients are susceptible to infections throughout the transplant procedure, with BSIs appearing as a major complication, particularly during the neutropenic pre-engraftment phase [1,2,3,4]. BSIs significantly raise the risk of patient mortality and extend hospital stays [1]. Moreover, the consequences are worse when these infections are caused by MDR bacteria, which have limited or no therapeutic options available [5,6].

Various MDR bacteria are known to contribute to infections in patients, including MDR *Enterobacterales*, MDR *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MRSA). Extended-spectrum beta-lactamase-producing *Enterobacterales* exhibit a high global prevalence in HCT patients [1,6]. In recent years, there has been an increasing incidence of CRE, mainly attributed to the dissemination of enzymes capable of hydrolyzing carbapenems and other beta-lactam antibiotics [7]. Several types of carbapenemases have been identified, with regional variations in incidence [6]. For example, in the USA, Brazil and Southern European countries, *K. pneumoniae* carbapenemase (KPC) is the most common type. In contrast, New Delhi metallo-β-lactamase (NDM) is predominant in countries like India and Mexico, while oxacillinase-48 (OXA-48) is most frequently detected in Russia, Turkey and certain Eastern European countries [8,9]. Previous studies conducted in South African hospitals have identified OXA-48 and NDM as the most detected carbapenemases in CRE infections [10,11].

Although BSIs in HCT recipients have been studied previously, data from sub-Saharan Africa are limited. Mapping underlying pathogens in this group may help in making appropriate and efficient antimicrobial therapy choices and prevention strategies. In addition, patients in sub-Saharan Africa, such as South Africa, have a different profile in terms of comorbidities compared to other countries, due to high rates of human immunodeficiency virus and tuberculosis infections [12]. This study aims to describe the incidence, etiology and clinical course of BSIs up to 100 days post-HCT, with a specific focus on infections caused by CRE and the role of colonisation with CRE.

3.3 METHODS

3.3.1 Study design

In this retrospective cohort study, we systematically analysed all episodes of positive rectal surveillance cultures and BSI in patients who had undergone HCT at the Department of Hematology at GSH or Red Cross War Memorial Children's Hospital, both academic hospitals of the UCT, South Africa. Hematological malignancies were categorised into diagnostic subsets. Severe aplastic anemia (SAA) and Fanconi anemia were grouped together as bone marrow failure syndromes (BMFS). Patients with hemoglobinopathies and primary immune deficiencies were excluded due to their low prevalence in the cohort.

The observation period for BSI lasted from seven days before transplantation up to 100 days after transplantation. The inclusion criteria encompassed all autologous and allogeneic HCTs performed between January 2018 and December 2023, including second and third HCTs. All study participants provided informed consent for the storage and use of their medical records for research purposes. Demographic and clinical data were retrieved from multiple sources, including the electronic Hematology Patient Registry hosted on REDCap, the electronic database of the National Health Laboratory Service, and paper patient records. The study was approved by UCT's Human Research Ethics Committee and adheres to South African Good Clinical Practice Guidelines (Human Research Ethics Committee reference number: 973/2023. Date of approval: 19-12-2023).

3.3.2 Definitions and MDR pathogens

In this study, BSIs were defined as the isolation of a bacterial pathogen from a blood culture. Notably, BSIs with commensal skin bacteria were considered significant only when present in at least two blood cultures obtained at different time points or if the clinical picture was highly suspicious, such as the presence of fever (temperature spikes, rigors, sweating) or signs of catheter-related infection, including pain, swelling, tenderness, redness or purulent exudate at the catheter site. BSIs were further classified into Gram-positive, Gram-negative or polymicrobial categories, the latter identified when two or more pathogens were isolated. Polymicrobial cultures or isolation of the same pathogen within 14 days were counted as one episode. Neutropenia was defined as an absolute neutrophil count below 0.5×10^9/L, while neutrophil engraftment was defined as being achieved on the first day of three consecutive days, with an absolute neutrophil count exceeding 0.5×10^9/L. BSI-related mortality was

identified if a patient died within seven days after a BSI occurrence, with no distinct alternative cause of death identified.

3.3.3 Conditioning regimens and graft-versus-host disease prophylaxis

Autologous HCT was performed for patients with multiple myeloma and in most patients with Hodgkin's and non-Hodgkin's lymphomas who required a transplant. Allogeneic HCT was performed for patients with acute leukemia (AML and ALL), BMFS, chronic myeloid leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, myelodysplastic/myeloproliferative neoplasms and myelodysplastic syndromes. Conditioning regimens were predominantly myeloablative, except for BMFS cases. In multiple myeloma, high-dose melphalan was the most used regimen. For auto-HCT, conditioning typically included a combination of busulfan, melphalan and cyclophosphamide. For allo-HCT, conditioning included combinations such as busulfan, melphalan and fludarabine; cyclophosphamide with fludarabine for SAA; or cyclophosphamide combined with total body irradiation. The selection of graft-versus-host disease (GVHD) prophylaxis depended on the underlying diagnosis and the specific type of allogeneic HCT. A combination of ex vivo alemtuzumab ("Campath-in-the-bag") and a calcineurin inhibitor was mostly administered as GvHD prophylaxis in cases with BMFS and transplantations with matched related donors (MRD). Additional T cell depletion with antithymocyte globulin was used in matched unrelated donors (MUD), Fanconi anemia and SAA patients with a haploidentical donor. In cases with haploidentical donors, a regimen comprising a calcineurin inhibitor, post-transplant cyclophosphamide and mycophenolate mofetil was used.

3.3.4 General management of HCT patients and microbiological surveillance cultures

HCT patients were admitted to single rooms with HEPA filters in a specialised hematologic high-care unit and routinely managed under contact isolation protocols. Upon admission to GSH, nasopharyngeal swabs were obtained for MRSA surveillance screening and weekly rectal swabs were performed to screen for CRE. All surveillance swabs collected 30 days before HCT and up to 100 days after HCT at GSH were included in this study. Routine screening for vancomycin-resistant enterococci (VRE) was not included due to low regional incidence. Due to the absence of routine weekly rectal swab collection at Red Cross War Memorial Children's Hospital, these samples were not included in the analysis. A central venous catheter (Hickman line) was inserted on admission and replaced if catheter-related complications occurred. Patients received antibiotic and antifungal prophylaxis at the onset

of severe neutropenia. Prophylactic treatment included oral ciprofloxacin 500 mg twice daily and fluconazole 200 mg twice daily (or weight-adjusted dosing for pediatric patients), administered until engraftment. Following engraftment, cotrimoxazole 480 mg once daily was prescribed orally for *Pneumocystis jirovecii* pneumonia prophylaxis. Voriconazole prophylaxis was used for patients with prior evidence of invasive aspergillosis. In cases of fever, characterised by an axillary temperature surpassing 37.7 degrees Celsius, a comprehensive physical and laboratory examination was performed, accompanied by the collection of at least one blood culture. Antibiotic initiation was guided by clinical judgment, neutrophil count and prior (surveillance) cultures. Neutropenic fever lacking a discernible non-infectious cause prompted the initiation of broad-spectrum intravenous antibiotics, which would initially be piperacillin-tazobactam in combination with amikacin in patients admitted longer than 48 hours, to cover for common nosocomial infections encountered as per the local antibiogram. In patients with suspected serious BSIs and known colonisation with a CRE, meropenem in combination with amikacin were started immediately. Antibiotic therapy was subsequently adjusted based on the pathogens cultured and their sensitivity profiles, as well as the clinical response to empiric therapy.

Blood cultures were incubated using BACTAlert (bioMérieux) system and positive flagged bottles were Gram-stained and inoculated onto appropriate agar plates. Surveillance cultures were inoculated onto chromID MRSA agar and chromID CARBA SMART agar plates. Collected isolates were identified using the Vitek 2 (bioMérieux, l'Étoile, France). Routine susceptibility testing was performed using Vitek 2 and interpreted following the Clinical and Laboratory Standards Institute guidelines [13]. To identify CRE, Etest strips (bioMérieux, l'Étoile, France) were used to determine minimum inhibitory concentrations of meropenem, ertapenem and imipenem. Lateral flow assay (LFA) was performed to identify specific carbapenemase enzymes (Coris O.K.N.V.I RESIST 5, CORIS Bioconcept, Gembloux, Belgium). This LFA detects five carbapenemase enzymes: KPC, NDM, OXA-48 (including its variants), Verona Integron-encoded Metallo-beta-lactamase and Imipenemas. CRE were defined as *Enterobacterales* that are resistant to any carbapenem or are confirmed to produce a carbapenemase by LFA.

3.3.5 Statistical analysis

Categorical variables are reported as numbers and percentages. Continuous variables are reported as means with their standard deviations (SD). Gray's test was used to calculate the cumulative incidence (CI) of BSI with death and second HCT without prior BSI as competing events. In the case of multiple BSIs

post-transplantation, only the first episode was taken into account. Overall survival (OS) was estimated using the Kaplan–Meier method, with survival differences assessed using the log-rank test. Surviving patients who did not undergo a second HCT were censored at day 100 post-transplantation. Associations between individual variables and the presence of a BSI were assessed using chi-square tests. Fisher's exact test was applied when any cell had an expected count of less than five. All *P* values were two-sided and considered statistically significant if lower than 0.05. R (4.4.0) was used to analyse the data. Graphs were created using GraphPad (version 10.2.3).

3.4 RESULTS

3.4.1 Baseline information

Between 2018 and 2023, 254 patients underwent a total of 275 HCTs, with 88% receiving their first transplant (Table 1). Five patients were excluded from the study due to the low prevalence of their underlying diagnosis. Auto-HCT patients (*N* = 145, 54%) had a mean age of 45 (SD 15), while allo-HCT recipients (*N* = 125, 46%) were younger with a mean age of 25 (SD 16). Human immunodeficiency virus comorbidity was present in 4% of cases. Multiple myeloma was the leading indication for auto-HCT (52%) and AML for allo-HCT (34%). Hematopoietic stem cells were derived from peripheral blood, as per institutional practice, in 269 transplants. Among allo-HCT, 51% received grafts from MRD, 34% from haploidentical donors and 14% from MUD. Of the pediatric patients (< 18 years), 54 out of 66 received allo-HCT, with BMFS (41%) and AML (32%) being the most common indications (Table S1).

Table 1. Baseline characteristics stratified by type of HCT.

	Total	**Autologous HCT**	**Allogenic HCT**
	***N* = 270**	***N* = 145 (54%)**	***N* = 125 (46%)**
Baseline variables			
Gender, *n* (%)			
Female	103 (38%)	57 (39%)	46 (37%)
Male	167 (62%)	88 (61%)	79 (63%)
Age at HCT, mean [SD]	36 [19]	45 [15]	25 [16]
HIV status			
Negative	240 (90%)	130 (90%)	110 (88%)
Positive	10 (4%)	9 (6%)	1 (0.8%)
Missing	20 (7%)	6 (4%)	14 (11%)

Table 1. Continued

	Total	Autologous HCT	Allogenic HCT
Hematological diagnosis			
Acute myelogenous leukemia (AML)	48 (18%)	5 (3%)	43 (34%)
Precursor lymphoid neoplasms (=ALL)	21 (8%)	2 (1%)	19 (15%)
Non-Hodgkin's lymphoma	30 (11%)	19 (13%)	11 (9%)
Hodgkin's lymphoma	45 (17%)	43 (30%)	2 (2%)
Multiple myeloma	76 (28%)	76 (52%)	0 (0%)
Chronic myeloid leukemia (CML)	2 (1%)	0 (0%)	2 (2%)
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)	3 (1%)	0 (0%)	3 (2%)
Myelodysplastic syndrome (MDS)	8 (3%)	0 (0%)	8 (6%)
Bone marrow failure syndromes (BMFS)	37 (14%)	0 (0%)	37 (30%)
Type of allogeneic donor			
MRD		NA	64 (51%)
MUD		NA	18 (14%)
Haploidentical		NA	43 (34%)
HCT			
First HCT	238 (88%)	124 (86%)	114 (91%)
Second HCT	31 (12%)	20 (14%)	11 (9%)
Third HCT	1 (0.4%)	1 (0.7%)	0 (0%)
Disease status at transplant			
Complete remission – first	91 (34%)	39 (27%)	52 (42%)
Complete remission – multiple	52 (19%)	26 (18%)	26 (21%)
Partial remission*	81 (30%)	80 (55%)	1 (1%)
No prior treatment (indicated/needed)	46 (17%)	0 (0%)	46 (37%)
Stem cell source			
Peripheral stem cells	269 (100%)	145 (100%)	124 (99%)
Cord blood	1 (0.4%)	0 (0%)	1 (0.8%)
Karnofsky performance score pre-HCT			
100 (ECOG 0)	179 (66%)	90 (62%)	89 (71%)
80–90 (ECOG 1)	88 (33%)	54 (37%)	34 (27%)
40–50 (ECOG 3)	1 (0.4%)	0 (0%)	1 (1%)
20–30 (ECOG 4)	2 (0.7%)	1 (0.7%)	1 (1%)
Conditioning regimen			
Myeloablative	240 (89%)	145 (100%)	95 (76%)
Reduced intensity	30 (11%)	0 (0%)	30 (24%)
TBI			
Yes	44 (16%)	3 (2%)	41 (33%)
No	226 (84%)	142 (98%)	84 (67%)

* Partial remission in auto-HCT: 71 patients with multiple myeloma, five patients with Hodgkin's lymphoma and four patients with non-Hodgkin's lymphoma. Partial remission in allo-HCT: one

patient with MDS/MPN.

HCT indicates hematopoietic stem cell transplantation; IQR, interquartile range; MRD, matched related donor; MUD, matched unrelated donor; ECOG, Eastern Cooperative OncologyGroup (performance score); TBI, total body irradiation.

The mean time to neutrophil engraftment was 18 days (SD 8) (Table 2). Ten patients (4%) failed to achieve engraftment; of these, eight died during the 100-day follow-up period, one received a second transplant at day 42 after the initial HCT, and the remaining patient was alive at day 100 but in poor clinical condition. Acute GVHD occurred in 20% of allo-HCT patients (8% grade I and 12% grade II to IV). By day 100, 90% of patients were alive, while 9% had died, with a mean follow-up of 48 days.

Table 2. Outcome characteristics at day 100 post-HCT stratified by type of HCT.

Empty Cell	Total	Autologous HCT	Allogenic HCT
Empty Cell	*N* = 270	*N* = 145	*N* = 125
Outcome variables			
Neutrophil engraftment			
Yes	260 (96%)	143 (99%)	117 (94%)
No	10 (4%)	2 (1%)	8 (6%)
Day of neutrophil engraftment, mean (SD)	18 [8]	17 [6]	19 [9]
At least 1 BSI episode (%)	94 (35%)	34 (23%)	60 (48%)
1 BSI episode	65	31	34
2 BSI episodes	18	2	16
3 BSI episodes	8	0	8
4 BSI episodes	2	0	2
5 BSI episodes	1	1	0
Acute GvHD			
None		NA	100 (80%)
Mild (I)		NA	10 (8%)
Severe (II–IV)		NA	15 (12%)
Karnofsky performance score day 100 post-HCT			
100–Normal (ECOG 0)	181 (67%)	108 (75%)	73 (58%)
80–90 (ECOG 1)	42 (16%)	21 (15%)	21 (17%)
60–70 (ECOG 2)	3 (1%)	2 (1%)	1 (0.8%)
40–50 (ECOG 3)	5 (2%)	1 (0.7%)	4 (3%)
20–30 (ECOG 4)	3 (1%)	1 (0.7%)	2 (1.6%)
Not evaluated	36 (13%)	12 (8%)	24 (19%)

Table 2. Continued

Empty Cell	Total	Autologous HCT	Allogenic HCT
Outcome day 100			
Alive	244 (90%)	138 (95%)	106 (85%)
Second HCT before day 100	1 (0.4%)	0 (0%)	1 (0.8%)
Dead	25 (9%)	7 (5%)	18 (14%)

HCT indicates hematopoietic stem cell transplantation; IQR, interquartile range; BSI, blood steam infection; GvHD, graft-versus-host-disease; ECOG, Eastern Cooperative Oncology Group (performance score).

3.4.2 Incidence and etiology of BSI

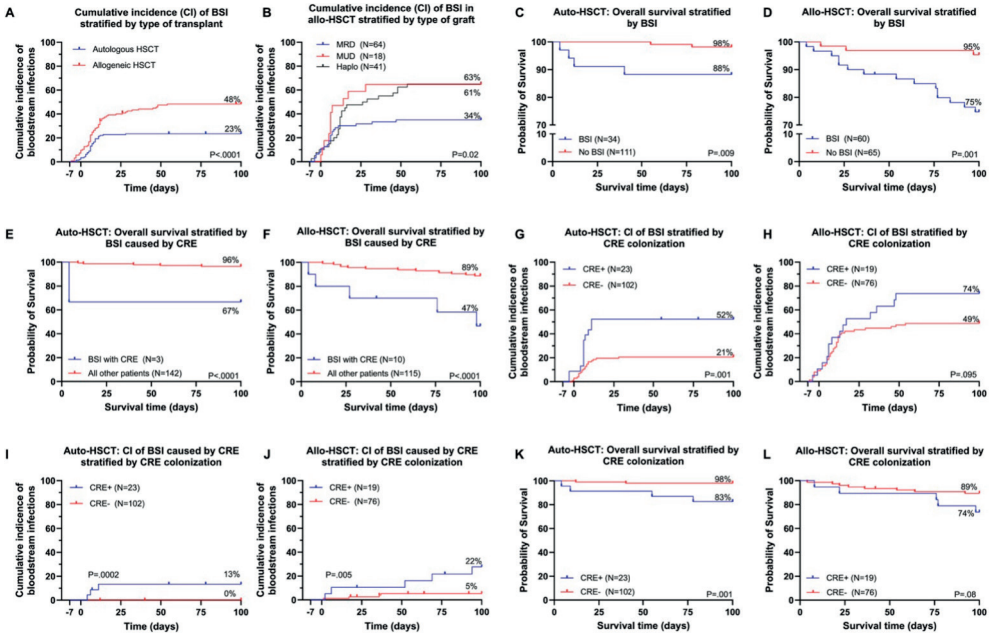
In total, 94 patients (35%) were diagnosed with a bacterial BSI. Of these, 34 received auto-HCT and 60 patients received allo-HCT (Table 2), with 43 cases occurring in adult patients and 17 in pediatric patients (Table S1). Among the 94 patients with a BSI, 29 (31%) had more than one episode. The median time to the first BSI was 10 days post-HCT (SD 12). By day 100 post-transplant, the CI of BSIs was significantly lower in auto-HCT recipients (23%) compared to allo-HCT recipients (48%) (P < .001) (Figure 1A). Within the allo-HCT group, stratification by donor-type demonstrated the highest CI of BSI in haploidentical transplants (63%), followed by MUD transplants (61%) and MRD transplants (34%) (Figure 1B). OS for auto-HCT recipients was 88% in patients diagnosed with a BSI and 98% in those without a BSI (P = .009) (Figure 1C). In the allo-HCT group, OS was 75% when diagnosed with a BSI, compared to 95% for those without a BSI (P = .001) (Figure 1D).

Gram-negative bacteria (GNB) were isolated in 53% of the BSIs of the auto-HCT and 56% of the BSI of the allo-HCT recipients. Gram-positive bacteria (GPB) were isolated in 35% and 39%, and polymicrobial cultures were found in 13% and 5%, respectively (Table 3). Among auto-HCT recipients with BSIs, the most frequently isolated GNB were *K. pneumoniae* spp. (*pneumoniae* and *ozaenae)* (N = 8, 38%), *Escherichia coli* (N = 4, 19%) and Stenotrophomonas maltophilia (N = 4, 19%). The most frequently isolated GPB in this group were coagulase-negative staphylococci (N = 5, 36%), S. aureus (N = 3, 21%) and Enterococcus faecium (N = 3, 21%). Among allo-HCT recipients with BSIs, the most frequently isolated GNB were: *K. pneumoniae* spp. (N = 30, 55%), *E. coli* (N = 8, 15%) and Acinetobacter spp. (N = 4, 7%). The most frequently isolated GPB in this group were *E. faecium* (N = 16, 42%), coagulase-negative staphylococci (N = 9, 24%) and *S. aureus* (N = 6, 16%).

CREs were identified in three BSIs among auto-HCT (8%) and in 10 BSIs among allo-HCT recipients (10%) (Table 3). Of the 10 CRE BSIs in allo-HCT recipients, five were diagnosed more than 30 days post-HCT. In three of these

Figure 1. Incidence and impact of BSI and CRE colonisation in HCT recipients.

BSI indicates bloodstream infections; CRE, carbapenem-resistant Enterobacterales; HCT, hematopoietic stem cell transplant; MDR, multidrug-resistant.



(A) Cumulative incidence (CI) of BSI stratified by type of transplant. Autologous HCT are represented by the blue line, allogenic HCT are represented by the red line. (B) Cumulative incidence of BSI in allogeneic HCT recipients stratified by type of graft. MRD are represented by the blue line, MUD are represented by the red line and haploidentical donor (haplo) are represented by the green line. (C and D) Overall survival in autologous (C) and allogeneic (D) HCT recipients stratified by presence of BSI. Patients with at least one BSI are indicated by the blue line, and those without one BSI are indicated by the red line. (E and F) Overall survival in autologous (E) and allogeneic (F) HCT recipients stratified by BSI caused by CRE. The colour codes are consistent, with blue lines indicating BSI caused by CRE presence and red lines indicating BSI caused by non-CRE presence or BSI absence. (G and H) Cumulative incidence of BSI in autologous (G) and allogeneic (H) HCT recipients stratified by CRE colonisation (rectal swabs). CRE colonisation is represented by the blue line, while no CRE colonisation is represented by the red line. (I and J) Cumulative incidence of BSI caused by CRE in autologous (I) and allogeneic (J) HCT recipients stratified by CRE colonisation (rectal swabs). Blue lines represent CRE+ patients and red lines represent CRE- patients. (K and L) Overall survival in autologous (K) and allogeneic (L) HCT recipients stratified by CRE colonisation status (rectal swabs). All x-axis present time in days, with day 0 marking the day of transplant. Days prior to day 0 represent the pre-transplant period, while days after day 0 represent the post-transplant period. Gray's test was used to calculate the cumulative incidence of BSI, with death and second HCT (without BSI) as competing events in patients who has not yet had a BSI. In case of multiple BSI post-transplantation, only the first episode was taken into account. Overall survival was estimated using the Kaplan–Meier method, with survival difference assessed using the log-rank test.

cases, the clinical course was complicated by grade IV GvHD. All 13 CREs isolated from blood cultures were *K. pneumoniae.* Among these, nine CREs harboured OXA-48 (or its variants), one harboured both OXA-48 and NDM carbapenemases, and no carbapenemase was detected in the remaining three. Additionally, extended-spectrum beta-lactamases were found in 27 of the 38 other (non-CRE) *Enterobacterales* (*K. pneumonia, E. coli,* and Enterobacter cloacae*complex)*. The presence of a BSI caused by a CRE significantly reduced OS rates compared to the presence of a BSI caused by non-CRE pathogens or the absence of BSI. In autologous HCT (auto-HCT) recipients, survival decreased from 96% to 67% ($P < .002$) (Figure 1E), while in allogeneic HCT (allo-HCT) recipients, it dropped from 89% to 47% ($P < .0001$) (Figure 1F).

Table 3. Etiology of BSI diagnosed seven days pre-HCT and 100 days post-HCT stratified by type of transplantation.

	Total *N* = 138	**Auto-HCT *N* = 40**	**Allo-HCT *N* = 98**
Gram-positive bacteria (%)	52 (38%)	14 (35%)	38 (39%)
Enterococcus faecium	19	3	16
Staphylococcus aureus [MRSA]	9 [3]	3 [1]	6 [2]
Coagulase-negative staphylococci	14	5	9
Viridans streptococci	7	2	5
Enterococcus spp.	1	1	-
Granulicatella adiacens	1		1
Mycobacterium tuberculosis	1		1
Gram-negative bacteria (%)	76 (55%)	21 (53%)	55 (56%)
Klebsiella pneumonia spp.	38	8	30
CRE[*]	13	3	10
ESBL (excl. CRE)	21	5	16
Wild type	4	0	4
Escherichia coli	12	4	8
ESBL	5	1	4
Wild type	7	3	4
Acinetobacter spp. [CR-GNB]	7 [4]	3 [1]	4 [3]
Stenotrophomonas maltophilia	7	4	3
Pseudomonas aeruginosa [CR-GNB]	2 [1]	1	1 [1]
Ralstonia insidiosa	1		1
Serratia marcescens [CR-GNB]	2 [2]		2 [2]
Burkholderia cepacia	1		1
Enterobacter cloacae complex [ESBL]	1 [0]		1 [0]
Salmonella spp. (non-Typhi)	1		1
Campylobacter spp.	2	1	1
Other non-fermentative bacteria[†]	2		2

Table 3. Continued

	Total *N* = 138	Auto-HCT *N* = 40	Allo-HCT *N* = 98
Polymicrobial‡	10 (7%)	5 (13%)	5 (5%)

[CR-GNB] indicates number of infections with carbapenem-resistant Gram-negative bacteria other than CREs; [ESBL], number of infections with extended-spectrum beta-lactamase-producing Enterobacterales; MRSA, Methicillin-resistant Staphylococcus aureus; CRE, carbapenem-resistant Enterobacterales.

• Carbapenemases were detected in 10 out of 13 BSI with carbapenem-resistant Klebsiella pneumonia spp. Nine of these harboured OXA-48 (or its variants) and one harboured both OXA-48 and NDM carbapenemases. No carbapenemase was detected in the remaining three cases.

†Other non-fermentative: Delftia acidovorans, and non-fermentative not further specified.

‡ Causes of polymicrobial cultures for auto-HCT: Acinetobacterbaumannii complex

+ Enterococcus faecalis, Enterobacter aerogene + Escherichia coli + Enterococcus faecium, Enterococcus faecium + Pseudomonas aeruginosa, Staphylococcus haemolyticus + Enterococcus faecalis, Viridans streptococcus + Streptococcus mitis. Causes of polymicrobial cultures for allo-HCT: Enterococcus faecium + Stenotrophomonas maltophilia + Staphylococcus epidermidis, Escherichia coli + Enterococcus faecalis, Escherichia coli + Klebsiella pneumoniae, Serratia marcescens + Klebsiella pneumoniae, Pseudomonas putida + Delftia acidovorans.

3.4.3 Surveillance cultures

A total of 125 auto-HCT recipients were tested for gut colonisation by a CRE at least once at and/or during admission to the hematologic high-care unit of GSH. Of these, 23 (18%) tested positive (Table 4). Among allo-HCT recipients, 95 were tested at GHS, with 19 (20%) testing positive. *K. pneumoniae* was the most prevalent CRE found in the surveillance cultures, identified in 20 out of 23 auto-HCT recipients, and 15 out of 19 allo-HCT recipients (Table S2). The most prevalent carbapenemase was OXA-48, which was found in 17 out of 23 auto-HCT recipients and 13 out of 19 allo-HCT recipients. The percentage of CRE-positive rectal swabs increased with the length of hospital stay, rising from 4% before HCT (8 out of 211 swabs) to 18% in patients tested between days 30 and 100 post-HCT (7 out of 38 swabs).

Table 4. Surveillance cultures.

	Total *N* = 246	Auto-HCT *N* = 143	Allo-HCT *N* = 103
Total CRE rectal surveillance cultures			
Performed	220	125	95
Positive (% of performed)	42 (19%)	23 (18%)	19 (20%)
Before HCT (30 days)			
Performed	211	119	92
Positive (% of performed)	8 (4%)	3 (3%)	5 (5%)

Table 4. Continued

	Total *N* = 246	Auto-HCT *N* = 143	Allo-HCT *N* = 103
Day 0–30			
Performed	188	109	79
Positive (% of performed)	27 (14%)	17 (16%)	10 (13%)
Day 30–100			
Performed	38	7	31
Positive (% of performed)	7 (18%)	3 (43%)	4 (13%)
Carbapenemase			
OXA-48	30	17	13
NDM	1		1
OXA-48 + NDM	3	3	
Absent/unknown	8	3	5

Routine screening took place only at GSH, and not at Red Cross Children's Hospital. Therefore, the numbers of performed surveillance cultures do not correspond to the total number of patients. On admission, rectal swabs were obtained for CRE surveillance screening and was theoretically repeated weekly after admission. Testing was also done for the carbapenemases VIM (Verona Integron-Encoded Metallo-beta-lactamase) and KPC (Klebsiella pneumonia carbapenemase), but they were not detected in our cohort.

CRE indicates carbapenem-resistant Enterobacterales; OXA-48, oxacillinase-48 and variants; NDM, New Delhi metallo-β-lactamase.

Higher CI rates of BSI were observed in patients with positive rectal surveillance cultures compared to those without CRE in surveillance cultures: 52% versus 21% in auto-HCT recipients (P = .001), and 74% versus 49% in allo-HCT recipients (P = .095) (Figure 1 G and H). The CI of BSI caused by a CRE was significantly higher in both auto-HCT and allo-HCT recipients who tested positive for CRE colonisation: 12% versus 0% in auto-HCT recipients (P = .0002), and 22% versus 5% in allo-HCT recipients (P = .005) (Figure 1 I and J). All three auto-HCT recipients who developed a BSI with a CRE had a positive rectal surveillance culture detected prior to or on the same day as the infection. Additionally, five out of 10 allo-HCT recipients were known to have a CRE in their rectal surveillance culture before a CRE BSI was diagnosed.

The OS rate was significantly lower in auto-HCT recipients with CRE colonisation (83%) compared to those without (98%) (P = .001) (Figure 1 K). In allo-HCT recipients, OS in patients with CRE colonisation was 74%, compared to 89% in non-colonised patients (P = .08) (Figure 1 L). Colonisation with MRSA was found in one out of 101 (1%) auto-HCT recipients tested, and in two out of 74 (3%) of allo-HCT recipients tested. One of these allo-HCT recipients developed two BSIs with MRSA.

3.4.4 Risk factors, 100-day survival and survival after BSI with CRE

Univariate analyses were conducted to examine risk factors for bacterial BSI. Auto-HCT patients diagnosed with non-Hodgkin's lymphoma and those with CRE colonisation exhibited a significantly higher risk of BSI (Table 5) (*P* = .0005, and *P* = .003, respectively). For allo-HCT recipients, older age, a diagnosis of myelodysplastic syndromes, and CRE colonisation were significantly associated with an increased risk of BSI (Table 5) (*P* = .001, *P* = .028 and *P* = .007, respectively). The rate of BSI varied by donor source, with the highest rates observed in the haploidentical donors (63%), followed by MUDs (61%), and the lowest in MRDs (34%) (*P* = .008).

Table 5. Associations between individual variables and the presence of a bloodstream infection.

	Auto-HCT			Allo-HCT		
	No BSI *N* = 111	**BSI *N* = 34**	**P**	**No BSI *N* = 65**	**BSI *N* = 60**	**P**
Gender, n (%) (male)	65	23	.45[A]	43	36	.58[A]
Age at HCT (years)			.45[B]			.001[B]
0–17	8	4		37	17	
18–49	52	14		25	31	
> 50	51	16		3	12	
Hematological diagnosis						
Acute myelogenous leukemia (AML)	5	0	.59[B]	23	20	.85[B]
Precursor lymphoid neoplasms (=ALL)	1	1	.42[B]	13	6	.14[B]
Non-Hodgkin's lymphoma	8	11	.0005[B]	6	5	1.0[B]
Hodgkin's lymphoma	33	10	1.0[B]	2	0	.50[B]
Multiple myeloma	65	12	.03[B]			
Chronic myeloid leukemia (CML)				1	1	1.0[B]
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)				1	2	.61[B]
Myelodysplastic syndrome (MDS)				1	7	.028[B]
Bone marrow failure syndromes (BMFS)				18	19	.70[B]
Type of allogenic HCT						.008[A]
MRD				42	22	
MUD				7	11	
Haploidentical				16	27	
Disease status			.32[A]			.31[B]
Complete remission – first	28	11		29	23	
Complete remission – multiple	18	8		16	10	

Table 5. Continued

	Auto-HCT			Allo-HCT		
	No BSI *N* = 111	**BSI *N* = 34**	**P**	**No BSI *N* = 65**	**BSI *N* = 60**	**P**
Partial remission	65	15		0	1	
No prior treatment (indicated/ needed)				20	26	
HIV status (positive)	5	4		0	1	.46[B]
Karnofsky performance score pre-HCT (high)	108	32	.14[B]	59	53	.67[B]
Rapid engraftment (≤16 days) (yes versus no)	54	22	.10[A]	34	38	.21[A]
Total body irradiation (yes versus no)	3	0	1.0[B]	23	18	.57[B]
CRE colonisation (positive)	11	12	.003[A]	5	14	.007[A]
Graft-versus-host disease (grade II–IV versus grade I)				6	9	.41[B]

Associations between individual variables and the presence of a bloodstream infection were assessed using chi-square tests (A). Fisher's exact test (B) was applied when any cell had an expected count of less than five. P-values below 0.05 are shown in bold.

BSI indicates bloodstream infection; HCT, hematopoietic stem cell transplantation; MRD, matched related donor; MUD, matched unrelated donor; (performance score); CRE, carbapenem-resistant Enterobacterales.

25 HCT recipients died in the first 100 days post-transplant, with 20 (80%) having experienced a BSI during the study period (Table 2). Of these, nine patients (36%) developed a BSI in the last seven days before their death (three out of seven in auto-HCT and six out of 18 in allo-HCT). Among the 13 patients who developed a BSI with a CRE, six died within 10 days (one out of three auto-HCT and five out of 10 allo-HCT). The remaining patients either reached the 100-day endpoint (*N* = 6) or the day of their second transplant (*N* = 1).

3.5 DISCUSSION

In this retrospective study, we evaluated the incidence, etiology and outcomes of a BSI in 270 HCT who were managed at the academic hospitals of UCT, South Africa. The overall incidence of BSIs in the first 100 days after HCT was 35%. A substantial proportion of these BSIs (9%) were caused by a CRE. Among patients with BSIs caused by CRE, 46% (six out of 13) died within 10 days, a significantly higher mortality rate compared to the rest of the cohort. In both allo- and auto-HCT recipients, colonisation with a CRE increased the risk of BSI and negatively impacted 100-day survival.

The overall incidence of BSIs was higher in allo-HCT recipients compared to auto-HCT recipients (48% versus 23%). This difference is consistent with previous studies attributing increased susceptibility to BSIs in allo-HCT patients and can be explained by mucosal injury caused by the preparative regimen for transplant, GvHD and more intense immunosuppressive treatment post-HCT to prevent or treat GvHD [3,14,15]. The higher incidence of BSI in haploidentical and MUD transplants compared to MRD emphasizes the added risk posed by more intense immunosuppressive approaches and higher rates of GvHD when using these donor types.

Just over half of the BSI in both auto-HCT and allo-HCT recipients were caused by GNB, with *K. pneumoniae* and *E. coli* being the most frequently isolated pathogens. This aligns with the global trend of an increasing GNB-to-GPB ratio [1,16,17,18]. This shift can be partially attributed to a decrease in BSI caused by coagulase-negative staphylococci, likely due to improved intravascular catheter management [1,16,17]. Additionally, the global rise of MDR GNB, including CRE, complicates effective treatment and leads to increased nosocomial infections with GNB in hospital settings [1]. In our cohort, 17% of the BSI caused by GNB were CRE. Although this observation is alarming, it is in line with results from other regions. In 2021, eight out of 45 countries in the WHO European Region reported carbapenem resistance rate of *K. pneumoniae* of 50% or higher in invasive isolates [19]. Consistent with the literature, having a BSI with a CRE significantly worsened OS, underscoring the severity of these infections [15,20,21].

In half of the allo-HCT recipients who developed a BSI with a CRE, prior CRE colonisation (detected in rectal swabs) was known. Furthermore, CRE colonisation was associated with a significantly higher risk of BSI (caused by any pathogen) post-HCT. This finding is consistent with previous studies, where colonisation with MDR bacteria was also identified as a risk factor [5,14,22,23,24]. Approximately 20% of the tested patients in our cohort were colonised with a CRE (18% auto-HCT, 20% allo-HCT), slightly higher compared to the 14% reported in a Brazilian study that included 405 HCT patients from 2015 to 2021. In our study, *K. pneumoniae* was the most prevalent CRE found in rectal cultures, and the primary detected carbapenamase was OXA-48 (and variants). This pattern is consistent with other studies on carbapenemases in South Africa [10,11]. While the Brazilian study found exclusively KPC-carbapenemases in HCT patients, this highlights regional variation in carbapenemase distribution [5].

Most HCT patients colonised by a CRE did not develop a CRE BSI. In fact, most CRE colonisation cultures turned positive later during the hospital stay.

Nevertheless, routine surveillance cultures remain important for identifying colonised patients, as these cultures can effectively guide antimicrobial decisions and trigger source control measures. Nosocomial spread of resistant strains is likely a significant contributing factor [9,25]. Since patients in this cohort were already being treated in single rooms with HEPA filters under contact isolation protocols, this poses challenges in preventing nosocomial transmission. Additionally, chromosomal mutations that lead to resistant strains and their selection due to antibiotic use during a complicated treatment may also play a role [7].

In total, 5% of the auto-HCT recipients and 14% of the allo-HCT recipients died within the first 100 days post-HCT. This aligns with a large Italian multicentre survey study that reported mortality rates of 3% in auto-HCT and 14% in allo-HCT [15]. Due to the small sample size in this study (and the resulting small number of deaths), it was not possible to analyse generalisable independent risk factors for mortality. However, other studies have identified several factors associated with mortality, including male gender, the presence of a BSI caused by CRE, a haploidentical donor type, acute GVHD grade III/IV, higher disease risk scores, and colonisation with CRE bacteria [15,20,22,26,27,28].

This study fills a gap in the literature by providing data from sub-Saharan Africa, where information on BSI incidence and pathogen profiles in HCT patients has been limited. However, this study also has limitations. The small number of patients per transplant type reduced the study's power, hindering analyses such as multivariate regression analysis to account for confounders like GvHD and non-bacterial infections. Furthermore, the retrospective nature and single-centre design of this study may limit the generalisability of the findings. This generalisability is further complicated by the unique demographics of our cohort, particularly in terms of age. The median age of allo-HCT recipients in this study was 21 years, more than 20 years younger than the median age reported in other (non-African) studies [27,28,29]. This difference can be attributed to more organised pediatric hematological care in the region and a generally lower life expectancy [30]. Lastly, there was no detailed study performed regarding antibiotic use, which prevented an analysis of the impact of surveillance cultures on antibiotic treatment.

In conclusion, this retrospective cohort study highlights the significant burden of BSIs among HCT recipients in the academic hospitals of UCT, particularly in the context of CRE. These findings underscore the importance of infection control measures, antimicrobial stewardship and targeted interventions to manage CRE colonisation and infection in HCT recipients.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to improve readability and language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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4

Clinician-reported Respiratory Viral Testing and Intended Management of Community-acquired Respiratory Viral Infections in Haematology and Haematopoietic Cell Transplantation – An international exploratory survey

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Background: Community-acquired respiratory viruses (CARVs) cause significant morbidity and mortality in patients with haematological malignancies and those undergoing haematopoietic cell transplantation (HCT). Despite this, clinical management remains variable. This study evaluated clinicians' awareness, diagnostic practices and treatment approaches to major respiratory viruses in this high-risk population.

Methods: An international electronic survey was conducted among haematology, oncology, intensive care and emergency medicine physicians. Four clinical vignettes, covering human metapneumovirus (HMPV), respiratory syncytial virus (RSV), human parainfluenza virus (HPIV) and influenza A virus (IAV), assessed diagnostic strategies, treatment decisions, and approaches to antiviral and immunoprophylactic interventions. Emphasis was placed on respiratory panel selection, testing practices and the influence of results on clinical decision-making.

Results: Ninety-eight clinicians from 26 countries responded. Diagnostic approaches, including testing indications and panel selection, varied substantially among respondents. For HMPV and HPIV, most clinicians recognised the absence of specific antivirals or vaccines, with management largely limited to supportive care, immunoglobulin replacement and infection control. RSV practices were more heterogeneous, with one-third reporting ribavirin use and limited use of monoclonal antibodies or vaccine-based prophylaxis. In contrast, IAV management was more consistent and guideline-aligned, with widespread use of vaccination, antiviral therapy and post-exposure prophylaxis.

Conclusion: Clinicians caring for patients with haematological malignancies or undergoing HCT reported substantial variation in respiratory viral testing infrastructure and in how results informed management. Priorities are more equitable access to rapid diagnostics and stronger linkage of results to virus- and risk-specific pathways. IAV testing was most consistently linked to established treatment and prevention, RSV showed the greatest therapeutic and preventive heterogeneity, and HMPV/HPIV testing primarily informed infection control, monitoring and antimicrobial stewardship.

4.2 INTRODUCTION

Respiratory viral infections are a major cause of morbidity and mortality in immunocompromised patients, particularly those with haematological malignancies and recipients of haematopoietic cell transplantation (HCT) [1]. CARVs, including RSV, IAV, HMPV, HPIV and seasonal coronaviruses, may cause severe disease in this population and can progress rapidly from upper to lower respiratory tract infection [1–3].

The introduction of multiplex polymerase chain reaction (PCR) platforms and point-of-care testing (POCT) has improved the detection of respiratory viruses in clinical practice. However, availability, turnaround time, testing indications and diagnostic panel selection vary substantially between centres. These differences may influence both the likelihood of identifying a viral pathogen and the way in which results are applied in clinical decision-making.

Importantly, the clinical consequence of a positive result differs by virus. IAV detection usually leads to clear antiviral treatment and post-exposure prophylaxis decisions. RSV may prompt risk stratification and selected use of antiviral or immunoprophylactic strategies, although practice remains heterogeneous and high-quality evidence is limited [4,5]. In contrast, detection of HMPV or HPIV more commonly informs infection-control measures, monitoring and supportive care, given the lack of widely available targeted therapies.

Given these uncertainties, we aimed to assess current awareness, testing practices and management approaches for major CARVs in patients with haematological malignancies, including HCT and non-HCT populations. Using a case-based international questionnaire distributed to haematology, intensive care and emergency medicine physicians, we sought to capture real-world variation in diagnostic strategies, panel selection and the extent to which diagnostic results influence clinical management in this high-risk population.

4.3 MATERIALS AND METHODS

We developed a questionnaire based on real-life clinical cases involving patients with haematological malignancies and post-HCT recipients who tested PCR-positive for CARVs. All patient information was anonymised. The questionnaire was independently reviewed by three experts in survey methodology, clinical haematology and intensive care medicine for clarity, relevance, interpretability

and clinical plausibility, with revisions incorporated before dissemination. As an exploratory, case-based assessment of self-reported clinical practice, it did not undergo formal psychometric validation or quantitative content-validity assessment. The final questionnaire is provided in Supplementary File 1.

Using convenience sampling, the questionnaire was distributed electronically by email and WhatsApp to clinical haematologists, intensive care specialists and emergency medicine physicians, including trainees, between 1 September and 31 October 2025. Recruitment targeted approximately 100 clinicians with relevant experience in managing immunocompromised patients with respiratory viral infections. Invitations were distributed through professional societies and clinical networks until this target was reached; by closure of recruitment, 650 individual invitations had been distributed. The mailing list was compiled through the European Society for Blood and Marrow Transplantation (EBMT) Trainee Committee and the South African Stem Cell Transplant Society (SASCETS), with additional contacts including heads of bone marrow transplant units in Australia, the United Kingdom, the United States, Brazil and several African countries, including Algeria, Tanzania and Kenya. Intensive care and emergency medicine specialists were approached predominantly in the Netherlands and European Union. Institution identifiers were not collected to preserve respondent anonymity. Participation was voluntary, and participants were informed before beginning the questionnaire that completion and submission constituted informed consent for participation and use of the survey data for research purposes.

The questionnaire assessed respiratory viral testing strategies, including indications for testing, diagnostic panel selection and responses to positive results, with particular emphasis on how diagnostic confirmation influenced management, including antiviral and immunoglobulin use. Responses were analysed descriptively. Exploratory subgroup analyses examined six prespecified outcomes: use of a standardised respiratory viral testing strategy, reported availability of POCT at respondents' institutions, use of an immunodeficiency score, testing for HMPV in the clinical vignette, consideration of systemic ribavirin for RSV, and recommendation of oseltamivir for post-exposure prophylaxis. Outcomes were compared across clinical specialty, World Bank income group and world region. Missing responses were excluded from item-specific denominators. Between-group comparisons used two-sided Fisher–Freeman–Halton exact tests with Monte Carlo approximation (200,000 samples), with Holm adjustment across the six outcomes within each grouping. Given the small subgroup sizes and non-probability sampling design, these analyses were considered exploratory. Respondent-level transplant-

practice status was not collected; therefore, transplant-versus-non-transplant comparisons were not performed.

The study was approved by the Ethics Committee of Spaarne Gasthuis Hospital on 7 July 2025 (approval number 2025.0091).

During preparation of this manuscript, the authors used ChatGPT and Paperpal Preflight to improve readability and language. The authors subsequently reviewed and edited the content as required and take full responsibility for the final publication.

4.4 RESULTS

4.4.1 Characteristics of survey respondents (N = 98)

A total of 98 clinicians completed the questionnaire (98/650; 15.1% response proportion). Clinical haematology specialists or trainees comprised 64/98 (65.3%) respondents. Respondents represented 26 identifiable countries across six world regions; 63/98 (64.3%) were based in Europe and 19/98 (19.4%) in Africa, with smaller representation from South America (6/98, 6.1%), North America (4/98, 4.1%), Asia (3/98, 3.1%) and Oceania (2/98, 2.0%); country was missing for one respondent (1.0%). Using World Bank fiscal year 2026 classifications [6], 69/98 (70.4%) respondents were from high-income countries, 27/98 (27.6%) from upper-middle-income countries, and 1/98 (1.0%) from a lower-middle-income country; no respondents were from low-income countries and one country response (1.0%) could not be classified. Median reported clinical experience was 15 years (interquartile range, 8.75–25; 96/98 responses). Participant characteristics are summarised in Table S1, with country-level counts in Table S2 in the supplementary materials.

4.4.2 Diagnostic testing strategies

Standardised testing protocols were reported by 65/98 (66.3%) respondents, while 55/98 (56.1%) reported POCT availability at their institutions; only 4/95 (4.2%) respondents to the risk-score item reported routine use of the Basel framework or MD Anderson Immunodeficiency Scoring Index (ISI) [3,7] (Figure 1).

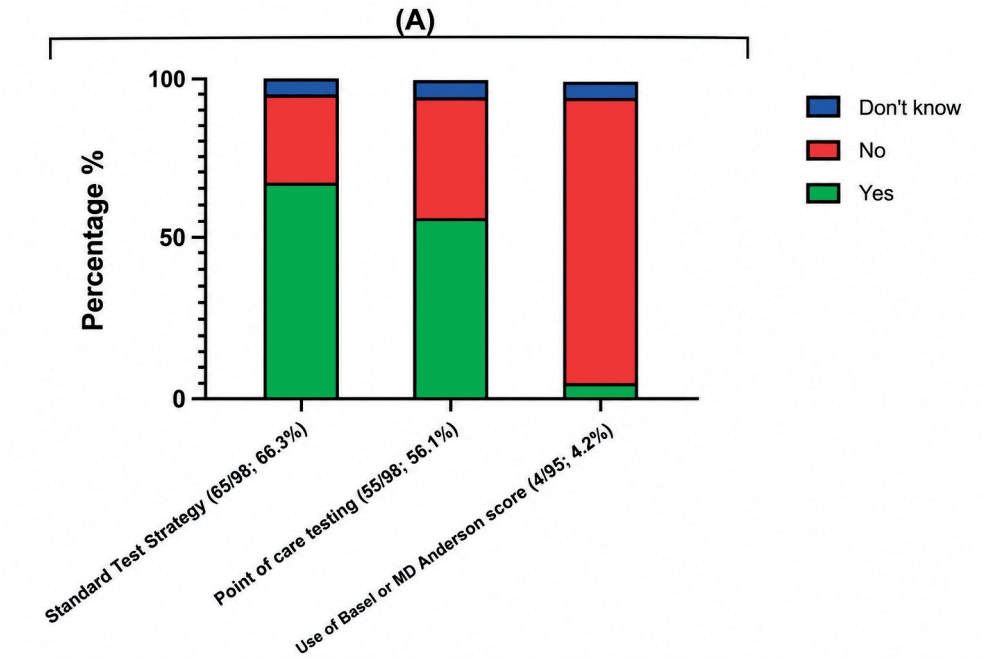


Figure 1. Respiratory viral testing strategies and use of immunodeficiency scoring systems. Bars show reported use of a standardised testing strategy, reported availability of point-of-care testing at respondents' institutions, and use of the Basel framework or MD Anderson Immunodeficiency Scoring Index (ISI); uncertain and missing responses are shown where applicable.

4.4.3 Case 1 – HMPV

A 55-year-old female presented to the emergency department with progressive dyspnoea. Her history included hypertension, acute myeloid leukaemia (AML) treated with allogeneic HCT, and ongoing immunosuppression for graft-versus-host disease.

Most respondents (76.5%) indicated that they would test the patient for HMPV, while 23.5% would not. Nearly all (91.8%) recognised the absence of targeted antiviral therapy or prophylaxis. Management was predominantly supportive, including supportive care (31.3%) and intravenous immunoglobulin (IVIG) for hypogammaglobulinaemia (32.7%). Following a positive HMPV result, 26/96 (27.1%) respondents would discontinue empiric antibiotics, whereas 65/96 (67.7%) would continue them and 5/96 (5.2%) were uncertain (Figure 2).

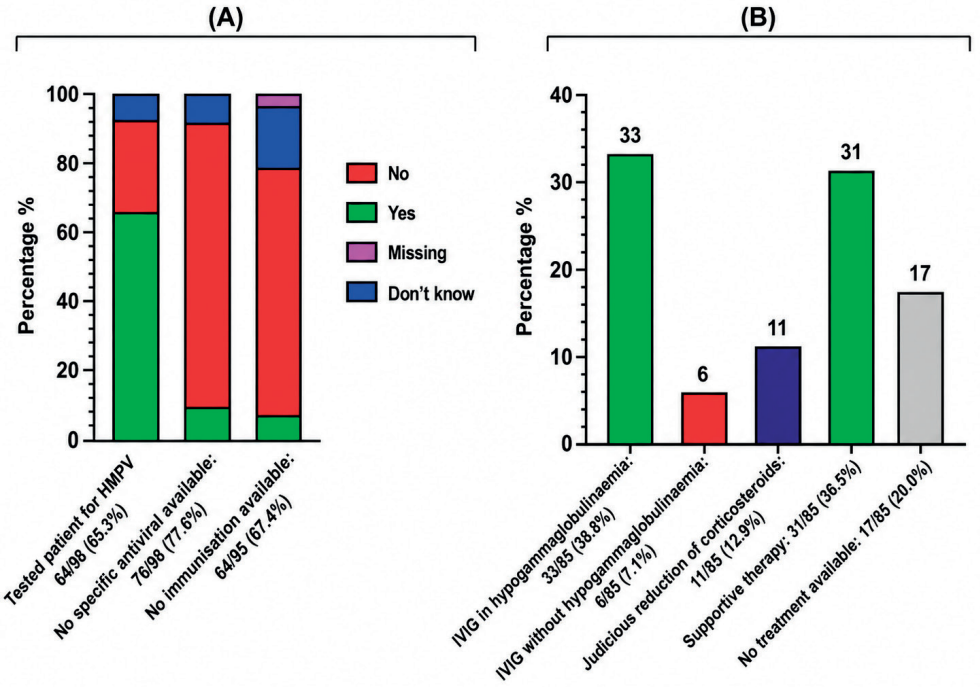


Figure 2. Human metapneumovirus (HMPV) testing, antiviral/immunisation availability and management responses. (A) Testing and reported availability of HMPV-specific antiviral therapy and immunisation. (B) Multiple-response management options. Percentages use item-specific denominators.

4.4.4 Case 2 – RSV

You see a 62-year-old male patient, history of AML, now two years post allogeneic HCT, for routine follow-up in your outpatient clinic. He mentions complaints of headaches, exertional dyspnoea and a persistent heavy cough. He is an active smoker.

RSV-related prevention and treatment practices varied substantially. RSV vaccination was reported as available by 39.8% of respondents, although only a minority indicated that vaccination was recommended for haematology patients based on perceived evidence in this population. Respondents largely reported passive immunisation as unavailable, with availability mainly restricted to children younger than two years. Ribavirin practice was heterogeneous: most respondents would consider systemic ribavirin based on immunodeficiency risk scoring, while fewer would use it irrespective of risk score or in clinically well patients with mild symptoms. IVIG use also varied, with responses influenced by hypogammaglobulinaemia, risk of progression to lower respiratory tract infection, perceived lack of evidence and reported local availability (Figure 3).

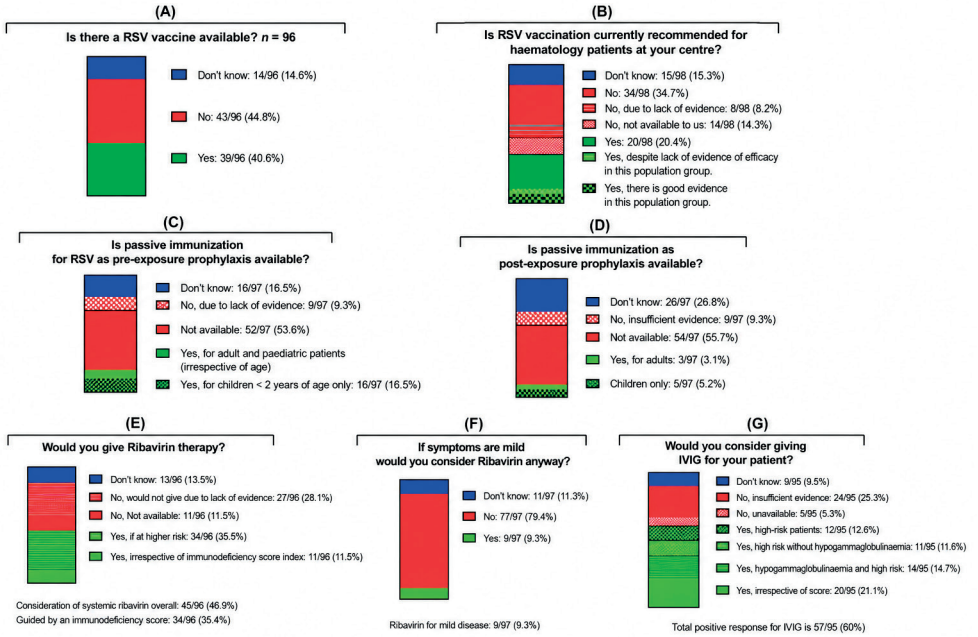


Figure 3. Respiratory syncytial virus (RSV) prevention and treatment practices. Panels show reported RSV vaccine availability/recommendation, passive immunisation, systemic ribavirin and intravenous immunoglobulin (IVIG) responses for the vignette. Percentages use item-specific denominators.

4.4.5 Case 3 – HPIV

You see a 79-year-old male with multiple myeloma on lenalidomide, daratumumab, dexamethasone therapy (two pathological fractures) presenting with 10-day history of increasing fatigue, muscle aches, shortness of breath, no cough, but febrile (38.7° C) in the emergency department (COVID-19 test: negative). Chest X-ray: Possible pneumonia left basally. He tests positive for HPIV.

Most respondents recognised that no active or passive immunisation existed for HPIV (67.3%) and that no specific antiviral therapy was available (73.5%). Management was mainly supportive (74.7%), with 61.1% emphasising infection control precautions. Half (50.5%) would use IVIG for hypogammaglobulinaemia and 48.4% would taper corticosteroids to facilitate immune recovery (Figure 4).

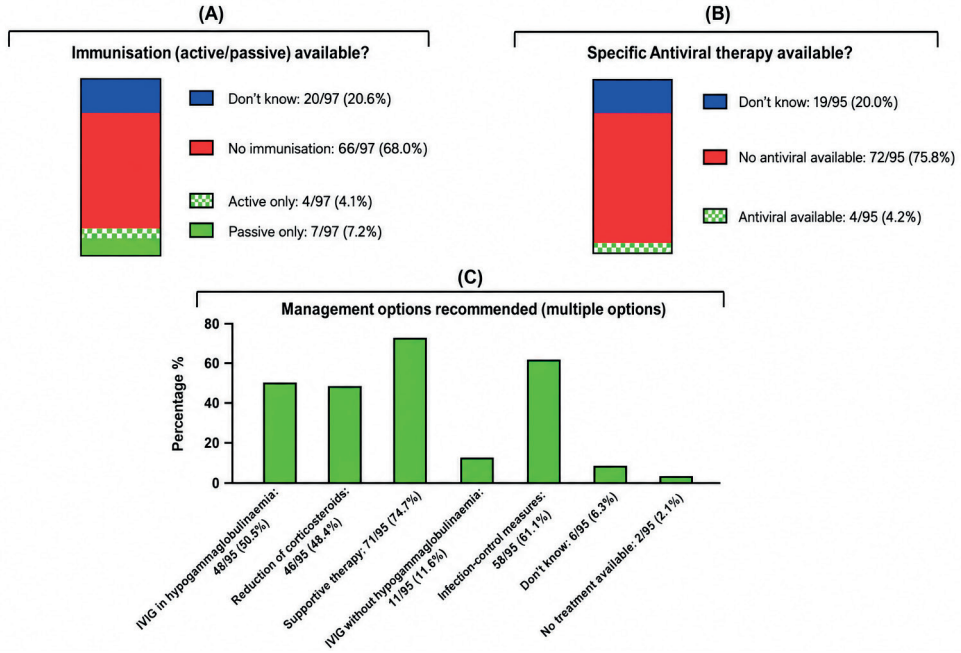


Figure 4. Human parainfluenza virus (HPIV) immunisation/antiviral availability and management. (A) Reported immunisation availability; (B) reported specific antiviral availability; (C) multiple-response management options.

4.4.6 Case 4 – IAV

A 75-year-old female presented to the emergency department following an out-of-hospital cardiac arrest, presumed secondary to hypoxia after progressive respiratory deterioration. Her medical history included breast cancer (lumpectomy 2008), peripheral arterial disease, chronic obstructive pulmonary disease (Global Initiative for Chronic Obstructive Lung Disease stage III) and newly diagnosed myelodysplastic syndrome. She tested positive for IAV.

Active IAV vaccination was reported as available by 54% of respondents, while 14% reported access to both active and passive immunisation, 9% to passive immunisation alone, and 13.3% had no immunisation options; a further 9% were unsure. Almost all respondents (89%) reported access to antiviral therapy, predominantly oseltamivir, and 70.3% routinely recommended oseltamivir for post-exposure prophylaxis. Management of persistent PCR positivity was more variable: 55% would extend treatment to 10 days, 23% would discontinue therapy, 15% would add baloxavir, and 8% would double the oseltamivir dose (Figure 5).

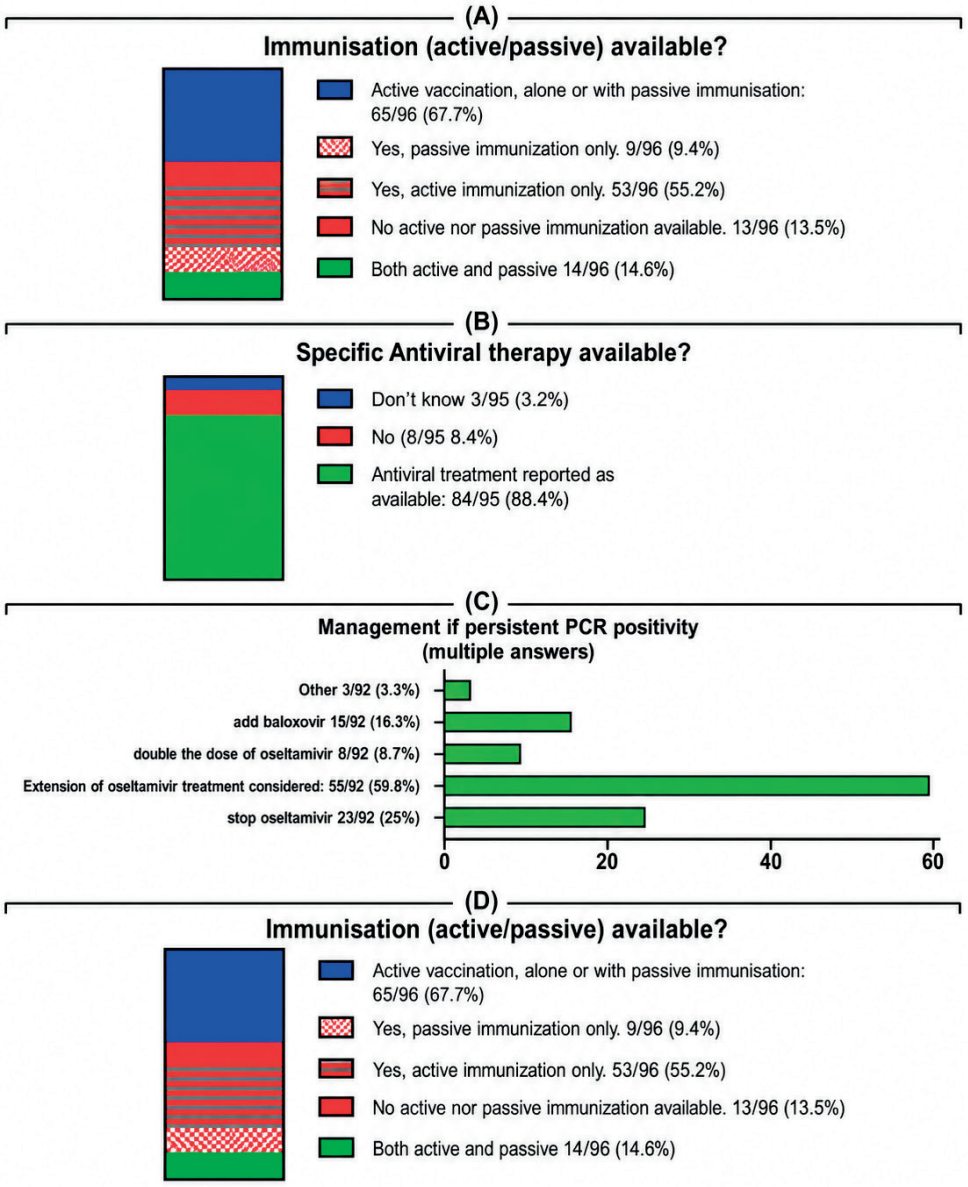


Figure 5. Influenza A virus (IAV) immunisation, antiviral access, post-exposure prophylaxis, and management of persistent polymerase chain reaction (PCR) positivity. Panel D permits multiple responses; percentages use the item-specific denominator.

4.4.8 Comparison of key finding across all four viral scenarios

Management differed across viral scenarios. HMPV and HPIV were characterised mainly by limited immunisation and antiviral options, with greater reliance on supportive care and infection-control measures. In contrast, IAV had

the greatest reported availability of vaccination and antiviral therapy, while RSV management showed more variable use of vaccination, passive prophylaxis, and ribavirin (Table 1).

Table 1. Comparison of the four viral scenarios.

Virus	Testing/prevention	Antiviral therapy	Key management responses
HMPV	Test for HMPV: 64/98 (65.3%) No immunisation: 64/95 (67.4%)	No specific antiviral: 76/98 (77.6%)	IVIG if hypogammaglobulinaemic: 33/85 (38.8%) Supportive therapy: 31/85 (36.5%)
RSV	RSV-positive case Reported active vaccine availability: 39/96 (40.6%) Adult passive prophylaxis rare: pre 4/97 (4.1%); post 3/97 (3.1%)	Systemic ribavirin: 45/96 (46.9%) Risk-score guided: 34/96 (35.4%)	IVIG considered: 57/95 (60.0%) Ribavirin for mild disease: 9/97 (9.3%)
HPIV	HPIV-positive case No immunisation: 66/97 (68.0%)	No specific antiviral: 72/95 (75.8%)	Supportive therapy: 71/95 (74.7%) Infection control: 58/95 (61.1%) IVIG if hypogammaglobulinaemic: 48/95 (50.5%)
Influenza A	Influenza A-positive case Reported vaccination availability: 65/96 (67.7%)	Reported specific antiviral availability: 84/95 (88.4%)	Oseltamivir post-exposure prophylaxis: 68/96 (70.8%) Persistent PCR positivity: extend oseltamivir 55/92 (59.8%)

Note: Data are n/N (%), using item-specific valid-response denominators. Multiple-response items may exceed 100%. Scenarios used analogous rather than identical survey items. Abbreviations: HMPV, human metapneumovirus; RSV, respiratory syncytial virus; HPIV, human parainfluenza virus; IVIG, intravenous immunoglobulin; PCR, polymerase chain reaction.

4.5 DISCUSSION

This international survey shows that clinician-reported testing and management practices for CARVs in haematology and HCT are heterogeneous. The most clinically relevant pattern was virus-specific actionability: IAV results generally mapped to established prevention and antiviral pathways, RSV results prompted variable risk-adapted decisions, whereas HMPV and HPIV results mainly informed supportive care and infection control. The four-virus comparison therefore suggests that the value of a diagnostic panel depends not

only on pathogen detection but also on whether a positive result is linked to a defined management pathway.

That distinction was also evident in antimicrobial stewardship. For HMPV, only 26/96 (27.1%) respondents would discontinue empiric antibiotics after a positive result, whereas 65/96 (67.7%) would continue them. Thus, viral detection alone did not reliably translate into antibiotic de-escalation in this high-risk population, possibly because clinicians remain concerned about bacterial co-infection and clinical deterioration. Rapid multiplex PCR has been associated with faster diagnosis and, in some adult cohorts, reduced antibiotic exposure [8], but our findings suggest that these benefits require explicit stewardship pathways rather than testing alone.

Clinician-reported differences in diagnostic infrastructure were substantial and were reinforced by the exploratory subgroup analysis. Respondents in high-income countries more often reported standardised testing strategies than those in upper-middle-income countries (75.4% vs. 44.4%; adjusted $p = 0.024$), and more often reported POCT availability at their institutions (69.6% vs. 22.2%; adjusted $p < 0.001$). Reported POCT availability at respondents' institutions also differed by world region (adjusted $p = 0.009$), including 68.3% in Europe versus 26.3% in Africa. These findings suggest resource-linked differences in access to rapid diagnostics, but small strata and non-probability sampling preclude causal or globally representative conclusions. They nevertheless support harmonised diagnostic principles that can be adapted to local laboratory and isolation capacity.

Risk stratification showed a similar implementation gap. Only 4/95 (4.2%) respondents reported routine use of the MD Anderson ISI or Basel framework [3,7], yet 34/96 (35.4%) would consider systemic ribavirin when an immunodeficiency score indicated high risk in the RSV vignette. This discordance suggests greater conceptual acceptance of risk-adapted treatment than routine use of formal scoring. Given the limited-quality evidence for ribavirin and heterogeneity in practice [4], current guidance from the 10th European Conference on Infections in Leukaemia (ECIL-10) supports risk-adapted management rather than indiscriminate treatment [5]. More consistent use of validated risk frameworks may improve comparability of treatment decisions and future studies.

For HMPV and HPIV, respondents largely recognised the absence of established pathogen-specific therapy and relied on supportive care, infection-control measures and immune optimisation, consistent with current guidance [5,9].

RSV occupied an intermediate position: only about 40% of respondents reported vaccine availability at their institutions, adult passive prophylaxis was rarely reported, and ribavirin and IVIG use varied. Adult RSV vaccines have shown efficacy in older adults [10,11], but evidence specific to haematological malignancy and HCT remains limited, a gap also emphasised by ECIL-10 [5]. This helps explain why expanding prevention options have not yet produced uniform practice.

IAV was the most standardised scenario: almost 89% of respondents reported antiviral access and 70.3% recommended oseltamivir post-exposure prophylaxis. Access to active vaccination was reported by roughly two-thirds of respondents when active-only or combined active/passive availability options are considered, yet persistent PCR positivity still generated divergent treatment choices. This pattern is compatible with guidance supporting seasonal inactivated vaccination and early antiviral therapy in immunocompromised patients [5], while highlighting uncertainty in prolonged infection and repeat-testing scenarios.

Interpretation should account for the convenience-sampling strategy, 15.1% response proportion, and marked geographic and economic imbalance, which introduce potential selection and non-response bias. Respondents were concentrated in Europe and high- and upper-middle-income countries, with no representation from low-income countries, and may have had greater interest or expertise in respiratory viral infections than non-respondents. Several subgroup strata were small, and institution type, transplant-centre volume, annual patient load, and respondent-level transplant practice were not collected. As institution identifiers were also unavailable, multiple respondents may have originated from the same institution. Furthermore, responses reflect stated management of clinical vignettes rather than observed care or patient outcomes. Accordingly, the findings represent clinician-reported practices among surveyed participants; subgroup analyses are exploratory and should not be generalised as prevalence estimates of international practice.

In conclusion, these exploratory findings identify two linked areas that may warrant further attention: improving access to rapid diagnostics and strengthening the linkage between test results and virus- and risk-specific management pathways. Among surveyed clinicians, IAV showed the most consistent reported treatment and prevention approaches and the clearest diagnostic actionability, whereas RSV showed greater therapeutic and preventive heterogeneity. Testing for HMPV and HPIV was reported primarily to guide infection-control measures, clinical monitoring, and antimicrobial

stewardship. These findings reflect clinician-reported intended practices and should not be interpreted as estimates of international practice or observed clinical outcomes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, File 1: Questionnaire and Table S1, S2 and S3.

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Institutional Review Board Statement: This study was reviewed and approved by the Ethics Committee of Spaarne Gasthuis Hospital, Haarlem/Hoofddorp, the Netherlands (approval number: 2025.0091; approval date: 7 July 2025). The clinical cases used in the questionnaire were derived from real patient cases and were carefully reviewed prior to inclusion to ensure that no traceable or identifiable elements were present. The full questionnaire is provided in supplementary file 1.

Informed Consent Statement: The study was conducted as an anonymous questionnaire among medical specialists. Participation was voluntary, and participants were informed before beginning the questionnaire that completion and submission constituted informed consent for participation and use of the survey data for research purposes. No patient-identifiable information was collected.

The study did not use exact ages; potentially identifying factors were deleted or adjusted. Given the anonymous nature of the survey and the use of fully de-identified clinical vignettes, the requirement for individual patient informed consent was waived by the ethics committee.

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S. van Lelyveld has received fees from Pfizer, GSK and Moderna, unrelated to the present research and paid to his institution.

All other authors declare that they have no conflicts of interest.

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Part III: Interplay between Pathogenesis of Infectious Diseases and the Haematological System

5

Thrombocytopenia in Virus Infections – A review

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5.1 Abstract

Thrombocytopenia, which signifies a low platelet count usually below 150 × 109/L, is a common finding following or during many viral infections. In clinical medicine, mild thrombocytopenia, combined with lymphopenia in a patient with signs and symptoms of an infectious disease, raises the suspicion of a viral infection. This phenomenon is classically attributed to platelet consumption due to inflammation-induced coagulation, sequestration from the circulation by phagocytosis and hypersplenism, and impaired platelet production due to defective megakaryopoiesis or cytokine-induced myelosuppression. All these mechanisms, while plausible and supported by substantial evidence, regard platelets as passive bystanders during viral infection. However, platelets are increasingly recognised as active players in the (antiviral) immune response and have been shown to interact with cells of the innate and adaptive immune system, as well as directly with viruses. These findings can be of interest both for understanding the pathogenesis of viral infectious diseases and predicting outcome. In this review, we will summarise and discuss the literature currently available on various mechanisms within the relationship between thrombocytopenia and virus infections.

5.2 INTRODUCTION

In patients presenting to care with signs or symptoms of infectious disease, a full blood count is part of a routine diagnostic evaluation. Mild thrombocytopenia, often combined with lymphocytopenia, is typical of most acute viral infections, but neither are sufficiently sensitive nor specific to reliably distinguish viral from bacterial or parasitic pathogens. Except for viral hemorrhagic fevers and rare cases of severe disseminated viral infections, virus-induced thrombocytopenia does not lead to significant bleeding, rarely requires platelet transfusions, and is therefore easily dismissed as clinically irrelevant. However, when the relationship between platelets and viral infection is studied more closely and in larger study populations, important findings emerge which shed light on previously unrecognised aspects of viral diseases. The incidence of thromboembolic complications is elevated in individuals during and after influenza virus infection, for example, a relation which may not be apparent to physicians diagnosing and treating influenza-like illness [1]. Platelet counts during peak symptomatic disease have also been found to be a marker of disease severity in certain viral infections, [2–19] or can serve as a first clue towards diagnosing chronic viral infections [20–23]. These phenomena are typically not explained by changes in platelet quantity, but rather by the effects of viral infections on platelet function.

Platelets are small, anucleate cells that circulate in the blood for approximately seven to 10 days after being formed. Their main physiological role is hemostasis, forming blood clots (thrombi) to safeguard vascular integrity. Platelets originate from megakaryocytes, which are giant polyploid cells residing in the bone marrow that have themselves formed from hematopoietic stem cells. Megakaryocytes develop proplatelets that bud off numerous platelets into the blood stream, after endoplasmic maturation [24]. In individuals with normal bone marrow function, platelets circulate at levels between 150 to 450 × 10^9/L [25]. Megakaryopoiesis is stimulated by a number of cytokines, with Stromal Derived Factor 1 (SDF-1), Granulocyte-Monocyte Colony Stimulating Factor (GM-CSF), Interleukins (IL-3, IL-6 and IL-11), Fibroblast Growth Factor 4 (FGF-4) and thrombopoietin (TPO) being the most important [26]. Whereas TPO plays a crucial role in maintenance of hematopoietic stem cells [27], most of these cytokines are proinflammatory and induce rapid maturation and activation of leukocytes, as well as stimulating megakaryopoiesis, which illustrates how platelet production is affected by inflammatory processes.

Conversely, platelets also affect the inflammatory response to viral infection and can even internalise several viruses directly. In response to infection, platelets interact with leukocytes and vascular endothelial cells before activating and

secreting soluble prothrombotic and inflammatory mediators stored within granules [28]. Despite not having a nucleus, platelets do contain some RNA and maintain a limited ability for protein translation, enabling some regulation of this response [29], but also potentially supporting replication of some RNA viruses [30].

In this review, all of the literature on the relationship between platelets and viral infectious diseases published between 2010 and late 2020 has been systematically assessed and summarised in a narrative format, classified per virus category.

5.2.1 Search strategy

The online database from the National Center of Biotechnology Information, PubMed, was queried on 3 August 2020 using the search term available in the supplemental information section included with this paper. This search was repeated on 21 December 2020, in order to include the latest publications on the currently pandemic Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Additional filters were applied to restrict results to original research papers published during the previous 10 years, related to humans and with full text available. Individual papers were assessed by the first author, based on title and abstract for relevance, originality and quality, and sorted based on virus species or virus family. The findings of the papers that are relevant for the subject matter of this review were presented in a narrative format within the following subgroups: ''General topics'', ''Arboviruses'', ''Blood-borne viruses'', ''Rodent-borne viruses'', ''Gastrointestinal viruses'', ''Herpesviruses'' and ''Respiratory tract viruses''. Additional references were added to provide more context when necessary.

5.3 RESULTS

The search yielded 413 papers. The results of the author classification process are summarised in Figure 1.

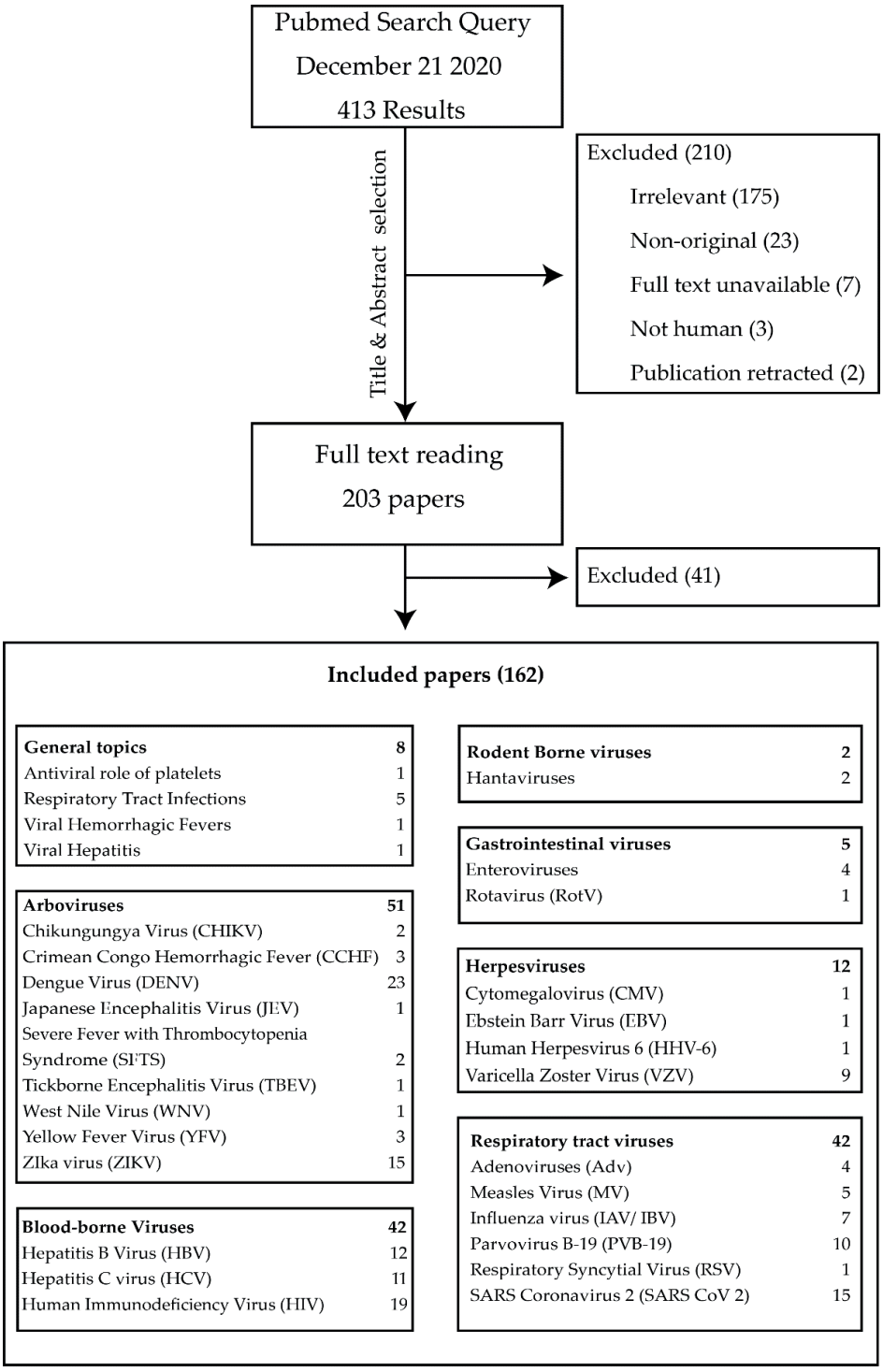


Figure 1. Flow diagram of PubMed search results.

5.3.1 Platelets and viral infections: general principles

An overview of the mechanisms that contribute to thrombocytopenia in the main viral infections discussed in this review, including references, can be found in Table 1.

5.3.1.1 Aggregation

Platelet agglutination or adhesion to leukocytes is often found in patients with systemic inflammatory diseases, including viral infections. Standard automated hematology analysers are often unable to accurately detect leukocyte-bound platelets or platelet aggregates, leading to a false finding of a reduced platelet count (pseudo-thrombocytopenia). This can also be caused by drawing blood into tubes containing EDTA, the most common anticoagulant used for complete blood counts. If pseudo-thrombocytopenia is suspected, performing a manual peripheral blood smear or repeating the platelet count in using different anticoagulants, can help avoid unnecessary diagnostic procedures or transfusions [31]. Isolated thrombocytopenia should prompt investigation into chronic viral infections, such as hepatitis B, C and HIV, whereas leukocyte abnormalities and rise in other infection biomarkers, raises suspicions of an acute viral illness [32].

5.3.1.2 Impaired Hematopoiesis

One of the most common accepted etiologies underlying virus-induced thrombocytopenia is the one where viruses can directly infect bone marrow stromal cells and hematopoietic stem cells leading to defective hematopoiesis and thrombocytopenia [33]. Furthermore, decreased platelet production can be the result of changed cytokine profiles, during infection, leading to lower TPO production in the liver, reducing megakaryopoiesis [34,35]. Finally, viruses can infect and replicate in megakaryocytes while other viruses modulate megakaryocyte function or decrease the expression of Myeloproliferative Leukemia Protein (c-MPL), the receptor for TPO, leading megakaryocyte destruction and subsequent lowered platelet production [36–38].

5.3.1.3 Sequestration and intravascular destruction

Platelet destruction can occur via direct interaction of platelets with viruses. This interaction occurs via a range of receptors including Toll-Like Receptors (TLRs), integrins (GPIIb/IIIa) and c-type lectins (CLEC), that interact with the different viruses leading to platelet activation, degranulation and clearance in liver and spleen [39–41]. Upon viral infection the host defence generally induces a systemic inflammatory response, which leads to platelet activation and subsequent clearance [42]. Furthermore, platelets can bind to neutrophils, forming platelet-neutrophil aggregates, which in turn triggers the phagocytosis

of platelets [43,44]. Additionally, many viruses can activate the coagulation system by induction of tissue factor leading to thrombin generation and platelet activation with subsequent platelet clearance via protease activating receptor (PAR) signalling. PARs are present on platelets, leucocytes and endothelial cells which modulate the innate immune responses [45]. Platelets can bind immunoglobulins (attached to viruses) via Fc-gamma-RII receptors leading to platelet activation, aggregation and clearance [46,47], while immunoglobulins produced by B-lymphocytes that target viruses can cross-react with platelet surface integrins (GPIIb/IIIa or GPIb-IX-V) leading to immune thrombocytopenia (ITP) [48]. In depth, more virus specific mechanisms will be discussed in the specific virus groups.

5.3.1.4 Platelet expression of Pattern Recognition Receptors

Pattern Recognition Receptors (PRRs) such as TLRs and CLECs [49] or messenger ribonucleic acids (mRNAs) can identify pathogen associated molecular patterns (PAMPs) from viruses and many are expressed by platelets [44]. This direct interaction of a virus or its genome with PRR can lead to platelet activation and subsequent release of chemokines. This enhances endothelial cell signalling, leucocyte migration and direct interaction and activation of leucocytes [50]. These complex interactions may have both an immune protective mechanism [44], or be injurious to the host.

5.3.1.5 Platelets can induce inflammation and secrete antimicrobial proteins

Activated platelets undergo degranulation and release numerous inflammatory mediators, cytokines and chemokines stored in granules. Three types of granules exist: α granules, dense granules and lysosomal granules. These granules contain different molecules that can exert pro-thrombotic and immune effects leading to direct and indirect interactions with different pro-inflammatory immune cells, causing a local or systemic inflammatory milieu [51]. In addition, the α-granules can secrete platelet microbicidal peptides (PMPs) that have direct anti-viral effects, for example, it has previously been shown that synthetic PMPs have strong viricidal effects against vaccinia virus [52].

5.3.1.6 Platelets act as Antigen Presenting Cells

Antigen Presenting Cells (APCs) require MHC-I molecules to present antigen to CD8+ T cells. There is evidence now that platelets and megakaryocytes contain all the MHC-I and co-stimulatory molecules necessary for antigen presentation, including the entire proteome [53,54]. In addition, it has been shown that platelets can successfully present ovalbumin and malarial antigens to activate CD8+ T cells [55].

5.3.2 Arboviruses

Arthropod-borne viruses (arboviruses) are viruses that are transmitted to humans through arthropods, mainly mosquitoes and ticks. Some of these arboviruses, especially flaviviruses, potentially cause hemorrhagic fever. In this section, we will discuss the most prevalent arboviruses as well as potentially emerging arboviruses.

5.3.2.1 Dengue virus

With an estimated 390 million annual infections, dengue virus (DENV) is the most prevalent arbovirus worldwide [56]. Thrombocytopenia is a hallmark of severe DENV infection and platelet levels are lower in DENV-infected patients compared to other febrile illnesses [57]. Severe thrombocytopenia < 20×10^9/L occurs frequently in hospitalised dengue patients, and is associated with prolonged admission, plasma leakage and the presence of clinical warning signs [58,59]. Severe DENV infections with severe thrombocytopenia, hemorrhage and plasma leakage occur more often in secondary DENV infection compared to primary DENV infection, due to antibody-dependent enhancement [60–62]. Below, the many mechanisms for thrombocytopenia in DENV disease that have been identified will be discussed.

Platelets can directly be activated by circulating DENV particles and by immune factors released during the acute phase of DENV infection. This activation induces upregulation of platelet adhesion molecules on the surface of vascular endothelial cells, causing more indirect platelet activation [63,64]. Activated platelets undergo degranulation, attach themselves to the vascular wall and form thrombi, effectively removing them from circulation, which results in thrombocytopenia. Despite this activation, platelets from dengue patients are hyporesponsive to procoagulant stimuli in aggregometry assays, which is likely the result of exhaustion [65]. This illustrates thrombocytopenia and platelet dysfunction go hand in hand during DENV infection. The DENV NS1 protein interacts with both TLR 4 and TLR 2, expressed by platelets, leading to platelet activation, aggregation, adherence to endothelial cells and phagocytosis by macrophages [66]. DENV can also activate platelets via CLEC2, that in turn stimulate macrophages and neutrophils via CLEC5A and TLR 2. Activated neutrophils subsequently form Neutrophil Extracellular Traps (NETs), providing a scaffold for prothrombotic factors, such as platelets, red blood cells and molecules involved in both the intrinsic and extrinsic coagulation pathways. NETs also activate platelets via TLR-4, creating a positive feedback loop between NETosis and platelet activation. These mechanisms contribute to thrombocytopenia, bleeding, vascular leakage and lethality in mouse models of dengue hemorrhagic shock syndrome [66,67]. The formation of platelet-

monocyte aggregates in DENV infection has also been demonstrated and correlates with thrombocytopenia and clinical signs of vascular leakage [68,69]. These platelets alter the monocyte's innate immune response, by inducing production of IL-10 [68] and inhibiting the production of interferon α [70]. Serotonin enhances DENV-mediated platelet activation and is released by perivascular mast cells in a mouse model of DENV infection, leading to thrombocytopenia, suggesting serotonin-blocking drugs might be beneficial [71]. Platelet activating factor (PAF) is another inducer of platelet aggregation and vascular leakage elevated in plasma of acute dengue patients. A recent small phase II randomised placebo-controlled clinical trial showed that Rupatadine, a licensed antihistamine with PAF receptor blocking activity, is safe in acute dengue patients, but not clinically beneficial, with no effect on nadir platelet counts [72].

In response to DENV infection, the immune system also depletes platelets through direct cytotoxic effects. This occurs through complement factor C3 binding to the platelet surface and opsonisation of platelets by DENV NS1 specific IgG, leading to subsequent phagocytosis by macrophages [73,74]. The observation that patients with severe DENV infection have increased afucosylated IgG1 antibodies and that these antibodies can cross-react with platelet antigens, further supports the notion that platelet-NS1 cross-reactive antibodies contribute to the depletion of platelets during severe DENV infection [75]. Infusion of anti-NS1 IgG cannot elicit thrombocytopenia in mouse models. However, it does enhance thrombocytopenia when administered during DENV infection. This could indicate that platelet binding and destruction is mediated by IgG bound NS1 dimers, rather than a direct interaction between cross-reactive IgG and platelets. This could have implications for vaccine design [76,77]. Platelets upregulate their expression of Human Leukocyte Antigen (HLA) class I molecules in the presence of DENV, suggesting a possible role in viral antigen presentation and T-cell-mediated cytotoxicity as a mechanism for thrombocytopenia [64].

A third mechanism is direct infection of platelets and megakaryocytes by DENV, leading to viral replication, cell lysis and impaired production of platelets. DENV infects platelets by binding DC-SIGN (CD209) and heparan sulfate proteoglycan (HSP) on the platelet surface and indirectly via Fc receptor FcϒR2A (CD32), after binding of antibodies to the virus particle [30]. The presence of DENV specific antibodies is not required for platelet infection [78]. Especially during the viremic stage and in those patients with more severe dengue, less DC-SIGN and FcϒR2A expression is detected on platelets when compared to patients suffering from other febrile illnesses. The relation between this phenomenon

with thrombocytopenia has not been explored, however, and it is unclear whether the presence of DENV particles in the blood could interfere with the binding of the detection antibodies. There could also be a survival advantage of platelets with low DC-SIGN and FCγR2A expression [79]. DENV productively infects a megakaryoblast-like cell line, using Glycoprotein Ib (GPIb) and was found circulating megakaryocyte-like cells in Rhesus Macaques [80]. This suggests that DENV can infect mature megakaryocytes and impair platelet production and survival through replication, although this has not been confirmed in vivo [81].

5.3.2.2 Chikungunya virus

Despite sharing many clinical characteristics with DENV, thrombocytopenia has only been occasionally described in patients infected with the Chikungunya virus (CHIKV) [82]. In a cross-sectional study of arbovirus infections in Pakistan, thrombocytopenia was observed in 18% of CHIKV infections, compared to 74% in DENV. Furthermore, thrombocytosis was significantly associated with CHIKV infection (OR 2.2) [83].

5.3.2.3 Crimean Congo hemorrhagic fever

Crimean Congo hemorrhagic fever (CCHF) is a tick-borne virus from the Bunyaviridae family that is associated with pronounced thrombocytopenia, accompanied by bleeding complications [84]. CCHF cases with bleeding have lower platelet counts and slightly raised platelet distribution width compared to those who do not bleed [85]. A lower platelet lymphocyte ratio (PLR) upon presentation to hospital care is predictive of adverse clinical outcomes in CCHF patients [6].

5.3.2.4 Japanese encephalitis virus

This vaccine-preventable flavivirus has a wide distribution in eastern Asia. Although central nervous system infection has a substantial mortality rate of 20–30%, most cases are either asymptomatic or mild, without clinically overt signs of encephalitis [86]. A recent prospective study performed in hospitalised patients with dengue-negative febrile illness in Indonesia found that 6% had serological evidence of recent Japanese encephalitis virus (JEV) infection. Thrombocytopenia was common during the acute phase of illness in these non-encephalitic cases (69%) but did not occur as frequently as dengue cases from the same study (92%) [87].

5.3.2.5 Severe fever with Thrombocytopenia Syndrome

Severe fever with Thrombocytopenia Syndrome (SFTS) is an emerging infectious disease caused by a recently discovered Bunya virus that, as the name

suggests, is associated with profound thrombocytopenia [88]. DC-SIGN, which is expressed by human platelets, was identified as a receptor for Bunya viruses, but no studies have shown virus entry or replication of SFTS in platelets [89]. A recent detailed study in Chinese SFTS patients revealed a severity-dependent depletion of the essential amino acid arginine due to arginase released by granulocytic Myeloid-derived suppressor cells (gMDSCs), which are recruited during the acute stage of the disease. The resulting lack of both arginine and its metabolite, nitric oxide, are believed to disinhibit platelet activation, leading to platelet aggregation, destruction and thrombocytopenia. A randomised, non-controlled clinical trial was performed where arginine supplementation with best supportive care was compared with supportive care alone. Whilst not demonstrating a statistically significant survival benefit, platelet counts returned to normal more rapidly in the treatment arm [90].

5.3.2.6 Tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV) is a flavivirus that is transmitted through bites of ticks and is prevalent in Europe and northeastern Asia [91]. After initial viremia, the central nervous system may become infected, which can result in severe neurological damage and occasionally death. Like most other viruses that can enter the bloodstream, TBEV causes thrombocytopenia, albeit generally mild and not typically associated with bleeding. A case–control study in patients with suspected and confirmed central nervous system infections, reported a decreased mean platelet count in TBE cases versus neuroborreliosis cases. However, the mean platelet count (173.8×10^9/L) remained above the lower limit of normal in the TBE group. In addition, platelet counts correlated positively with concentrations in serum of IL-23, a cytokine secreted by dendritic cells, which is believed to stimulate essential host-defence mechanisms against viruses [92]. This could indicate a role for IL-23 in stimulating megakaryopoiesis during TBEV infection.

5.3.3.7 Viral hemorrhagic fevers

The extreme containment precautions required to study the most highly pathogenic viral hemorrhagic fevers (VHFs), such as Ebola virus, Marburg virus and Lassa virus, makes studying the interactions of live virus with human platelets an expensive and labour-intensive endeavour. Only a small number of laboratories worldwide are equipped with biosafety level 4 facilities and only two are located on the African continent, where most VHF infections occur [93]. Lymphocytic Choriomeningitis Virus (LCMV) is an arenavirus, which has sporadically caused severe Lassa-virus-like illness in humans. Its reservoir host is mice, who only develop very mild disease. Experimental infections of mice with LCMV, after platelet depletion treatment, however, result in a more severe VHF

with uncontrolled viral replication and dissemination, similar to that observed in humans. The platelet-depleted mice had impaired LCMV specific CD8+ T cell responses. Severe disease and mortality only occurred in mice whose platelet reduction treatment was initiated shortly before LCMV infection. This may suggest that the innate antiviral response against LCMV requires platelets. However, Interferon α and β production appeared to be unaffected by platelet depletion treatment, indicating this part of the innate immune response had remained intact. Further examination of the spleen in LCMV-infected mice who underwent platelet depletion demonstrated extensive disruption of the splenic architecture and cellular necrosis, which could be the common mechanism of both the defective innate and T cell responses. These experiments provided novel insights in the role of platelets in controlling VHF through protection of splenic vascular integrity and the importance of this organ in mounting sufficient cellular and innate immune responses to eliminate the virus [94].

5.3.3.8 West Nile virus

West Nile virus (WNV) is a neurotropic flavivirus that mainly causes asymptomatic infections, while severe complications such as meningitis or encephalitis can occur, but are rare. WNV infections with hemorrhagic complications have sporadically been reported but severe thrombocytopenia and hemorrhage typically do not occur during WNV infection [95]. A case of WNV transmission through a platelet transfusion unit from a donor that tested negative on whole blood on the day of transfusion has been described, raising the possibility that the virus may concentrate in platelets, although the platelet unit itself was never tested [96].

5.3.3.9 Yellow fever virus

Yellow fever virus (YFV) is a flavivirus that can be transmitted through Aedes mosquitoes and other vectors and is endemic in Africa and the Americas, but not in Asia [97]. Even though there is an effective vaccine against YFV, annually several tens of thousands of persons die of YFV infection in Africa [98]. Disseminated yellow fever is characterised by hepatitis and hepatic failure, with resulting thrombocytopenia, deficiencies in plasma coagulation factors, prolonged activated Partial Thromboplastin Time (aPTT) and an elevated International Normalized Ratio (INR) [99,100]. A Brazilian retrospective cohort study of patients suffering severe YFV infections showed severe hemorrhagic complications, mostly from the gastrointestinal tract. Despite this, only mild thrombocytopenia was present, with a median platelet count of 74×10^9/L, indicating platelet consumption as a result of hemorrhage, rather than thrombocytopenia-induced bleeding [100]. This is supported by the presence of ischemic and hemorrhagic microvascular pathology upon fundoscopic

examination of hospitalised yellow fever patients, which correlate with the degree of thrombocytopenia present, disease severity and markers for renal and hepatic disease [101].

5.3.3.10 Zika virus

Like DENV, Zika virus (ZIKV) is a flavivirus that is mainly transmitted through Aedes mosquitoes. In 2015/2016, there was a large outbreak of ZIKV in the Americas, which led to an increase in the incidence of Guillain-Barré syndrome in adults and congenital abnormalities in newborns [102]. Seroprevalence studies indicate that, depending on the country and location, up to 60% of inhabitants got infected with ZIKV during this outbreak [103–105]. A large prospective cohort study of ZIKV infected patients in Puerto Rico identified thrombocytopenia (defined in this study as a platelet count below 100×10^9/L) in 1.2% of confirmed cases. Only 25% of those had platelet counts below 20×10^9/L and 16% had etiologies other than ZIKV infection [106]. Platelet counts below 150×10^9/L are present in a small minority of ZIKV infections, although profound thrombocytopenia has been described in fulminant cases, associated with bleeding, liver failure and other coagulation disorders [107,108]. Most ZIKV patients have platelet counts between 150 and 300×10^9/L [109–111]. Thrombocytopenia on first diagnosis was significantly associated with higher odds of hospitalisation in a case series study of US veterans with laboratory-confirmed or presumptive ZIKV infection (OR 6.4, 95% CI 4.0–10.1) [3]. Suspected diagnosis of de novo Immune Thrombocytopenic Purpura (ITP) has been reported in patients presenting with severe thrombocytopenia during ZIKV convalescence, who responded to treatment with corticosteroids, intravenous immunoglobulin (IVIG), or both [112–114]. Exacerbation of pre-existing ITP during the acute phase of ZIKV disease has also been described [115]. ZIKV infects monocytes, macrophages and dendritic cells, but is unable to propagate in megakaryocyte-differentiated human hematopoietic stem cells and platelets [116]. Thrombocytopenia presents more often in DENV infections compared to ZIKV and can be used to distinguish the two otherwise similar diseases to a limited extent [117–119].

5.3.3 Blood-borne viruses

5.3.3.1 Hepatitis B and C

Chronic hepatitis B (HBV) and C (HBC) virus infections are associated with hepatic cirrhosis, portal hypertension, liver failure and hepatocellular carcinoma (HCC). Thrombocytopenia is a predictor of such adverse outcomes, especially when combined in clinical scoring systems with other parameters such as age, gamma-glutamyl transpeptidase, Alanine and Aspartate aminotransferase

[7–10,13–15,17,19,120–122]. However, in individuals that have already developed HCC, thrombocytosis carries a negative prognosis rather than thrombocytopenia. Elevated platelet counts, when combined in a ratio with lymphocyte counts (PLR), is a biomarker of malignant inflammation and elevation is predictive of a poor prognosis in patients with HCC [123,124]. However, this marker does not appear to have much added value when tumour size and histological parameters are known, or in patients with either no cirrhosis or very severe cirrhosis [11,125].

A likely explanation for the lower platelet counts observed in patients with hepatic cirrhosis, including those with a non-viral etiology, is portal hypertension leading to increased sequestration of platelets and red blood cells in the liver and spleen [126].

Another factor more specific to HBV and HCV infection could be increased platelet activation and consumption due to chronic inflammatory processes caused by these viruses. Mean platelet volume (MPV), a biomarker of platelet activation, was found to be increased in patients with chronic hepatitis B infection, compared to controls with no documented HBV infection [127]. Chronic HBV infection is also associated with a low platelet response to clopidogrel, which could be due to platelet activation by the virus or changes in drug metabolism caused by liver disease [128].

HBV and hepatitis delta (HDV) co-infection also appears to lower platelet counts compared to HBV mono-infection, independently of the severity of liver disease, though the mechanism is unknown [129].

In hepatitis C infection, intravascular destruction of platelets is likely also mediated by anti-platelet antibodies and ITP related to HCV has been reported in the literature [130]. In HCV-infected patients, platelet auto-antibodies were more commonly identified as a cause in mild thrombocytopenia (platelet count 126–149 $\times 10^9$/L), whereas decreased megakaryopoiesis was a more prominent contributing factor in more severely thrombocytopenic patients (platelet count $< 100 \times 10^9$/L) [131].

Since both hepatitis B and C are blood-borne pathogens, a direct interaction between these viruses and platelets is possible. Hepatitis C virus RNA appears more stable when incubated with platelets than with platelet-free plasma, although there is no evidence of replication [132]. Instead, platelets may shield the virus from complement, interferons, cytokines, antibodies or other antiviral factors present in serum, perhaps through internalisation. There have

been reported cases of HCV infected patients with much higher viral loads in the platelet compartment compared to plasma [130]. One study found that while HCV viral load was higher in plasma compared to platelets in patients before interferon/Ribavirin treatment, some patients had detectable RNA in platelets after treatment, but not in serum, resulting in relapse [133]. This could indicate that platelets and their megakaryocyte precursors can provide a site for therapeutic drug and immune evasion, either perhaps due to diminished response to interferons or a relative impermeability for nucleoside analogues or both, while extrahepatic replication is of minor importance during untreated HCV infection. The therapeutic relevance of this finding today is questionable, however, given the high curation rate that can be achieved using the novel direct antiviral agents that have become available. The effect of direct interactions between HCV and platelets on platelet lifespan remains to be established.

5.3.3.2 Human immunodeficiency virus

Thrombocytopenia is a common finding in human immunodeficiency virus (HIV) infection and HIV testing is part of a routine evaluation for unexplained thrombocytopenia. Screening individuals with thrombocytopenia and other so-called indicator conditions, has been shown to be a cost-effective and more efficient compared to universal screening [20–22]. HIV as the underlying cause for otherwise unexplained thrombocytopenia is frequently missed [23]. Decline of platelet count over time in HIV patients has been associated with development of dementia and reduced grey matter volume on MRI scans, although univariate and multivariate analyses were not entirely consistent [134]. Several mechanisms for thrombocytopenia in HIV-infected individuals have been proposed and these will be discussed in more detail below.

Chronically infected HIV patients, even when adequately suppressed with combination antiretroviral therapy (c-ART), have a substantially increased risk of developing cardiovascular disease and deep venous thrombosis compared to HIV-negative individuals. Platelet activation is believed to be a major driving factor behind this phenomenon. Untreated HIV-positive individuals have elevated platelet activation markers in plasma compared to matched healthy controls, which positively correlates with viral load and negatively with CD4+ T cell counts. In addition, platelets from HIV patients express more oxidative stress-related proteins [135]. Studies investigating platelet morphology (mean platelet volume (MPV), platelet distribution width (PDW)) in HIV patients, report contradictory results [136,137]. Platelets can bind HIV-1 virus through DC-SIGN and serve as a carrier, protecting virions in the circulation from antiviral factors through formation of RBC-platelet aggregates [138]. Platelets may also become activated after binding to HIV and release platelet factor 4 (PF4/CXCL4), from

their α granules, which has been shown to inhibit cell attachment and entry of several HIV-1 strains through binding of its main envelope glycoprotein, GP-120 [139]. PF4 and other α-granule cytokines such as CCL5 likely also contribute to a chronic proinflammatory state in HIV patients. Interestingly, HIV patients on c-ART who become infected with DENV, were found to have milder disease and their platelets released less proinflammatory PF4 and CCL5 from α granules, likely resulting from previous HIV-associated depletion [140].

Very recently, platelets from long-term virologically suppressed HIV-infected individuals were shown to contain infectious HIV-1, demonstrating its ability to infect platelets and remain viable. Both viral RNA and proviral DNA of HIV was detected in bone marrow megakaryocytes from these same individuals, indicating platelets may already be infected upon formation. Platelets phagocytosis by macrophages was shown to lead to productive infection in this cell type [141]. The effects of HIV-1 infection on the activation status or lifespan of platelets were not investigated, meaning the role this mechanism plays in thrombocytopenia in people living with HIV remains unclear.

Another factor contributing to the prothrombotic state of HIV patients is the induction of platelet-monocyte aggregates (PMA) by the virus, which causes mutual activation of these two cell types [142,143]. Monocytes expressing CD16 (nonclassical and intermediate monocytes) appear to be primarily involved in the formation of PMA [144]. Electron microscopy (EM) studies of PMA in HIV-infected individuals show a maximum of four simultaneously attached platelets per monocyte [145]. However, a single monocyte is likely capable of transiently interacting with many more platelets during its lifetime, leading to platelet activation and cumulative loss over time. The formation of PMA is mediated by binding of P-selectin on the platelet surface with P-Selectin Glycoprotein Ligand-1 (PSGL-1) on the monocyte surface. This leads to monocyte activation, including secretion of proinflammatory cytokines and tissue factor (TF), which initiates the extrinsic coagulation pathway. Monocytes may be able to downregulate PSGL-1 expression in response to platelet binding, but this has not been definitively established [143]. HIV transactivating factor (Tat) interacts with platelet integrin β3 and CCL3, resulting in the secretion of soluble CD40 ligand (sCD40L), a platelet activator [146]. Subsequently, activated platelets initiate the formation of PMA [144,145]. The CD40L released, also increases B cell activation and secretion of immunoglobulins in vitro and in vivo in mouse models, which has been associated with ITP in non-HIV-infected individuals, and could play a similar role in HIV-positive individuals [147]. Platelets isolated from HIV patients secrete significantly more sCD40L in response to stimulation compared to healthy controls. The same effect was seen for other

proinflammatory molecules, such as CCL5 and P-selectin expression. Patients who are not on c-ART can also have diminished responses, however, which is attributed to exhaustion [148].

Overall, this evidence suggests that HIV infection sensitises platelets to procoagulant and proinflammatory stimuli, which in response release more procoagulant and proinflammatory factors and induce monocytes to do the same. This creates a positive feedback loop underlying chronic inflammation and risk of thromboembolic complication. Although initiation of c-ART normalises both platelet and monocyte activation markers in plasma within 12 weeks [149], these studies using isolated platelets and monocytes demonstrate that virological suppression seems unable to completely disrupt this inflammation-coagulation cycle present in people living with HIV.

5.3.4 Rodent-borne viruses

5.3.4.1 Hantavirus

Hemorrhagic fever and renal syndrome (HFRS) caused by Eurasian hantaviruses is associated with severity-dependent thrombocytopenia. The clinical characteristics of the disease include both hemorrhagic and thrombotic complications. This suggests that platelet consumption plays a significant role in the pathogenesis of thrombocytopenia. A longitudinal study of 35 hospitalized HFRS patients with Puumala virus (PUUV) infection revealed a biphasic pattern in platelet counts over time. This started with marked thrombocytopenia upon first presentation to the clinic, followed by a rise to just below the upper limit of normal approximately two weeks after symptom onset, and subsequent normalisation of platelet counts. Elevated plasma TPO levels, reticulated platelet counts and MPV (each peaking at the platelet count nadir) suggest a compensatory myeloproliferative response to an acute loss of platelets within the circulation during early infection. Ex vivo expression of platelet surface activation markers was higher during the acute disease stage compared to follow up, although the opposite was found for in vitro stimulation assays, indicating platelet exhaustion. Finally, patients who had signs of Disseminated Intravascular Coagulation (DIC) or thrombosis during the disease had higher plasma platelet activation markers, such as soluble P-selectin and soluble Glycoprotein IV (GP IV) compared to those who had not [150]. Another longitudinal study measured Von Willebrand factor, Fibrinogen, fibronectin and A Disintegrin. Additionally, Metalloproteinase with a ThromboSpondin type 1 domain (ADAMTS13) concentrations in plasma of PUUV-infected patients during the acute and recovery phase. These factors where indeed elevated during acute disease when compared to recovery.

Thrombocytopenia was present in 15 out of the 19 patients studied, but the exact numbers and how they correlate with the markers measured in plasma is not described [151]. Furthermore, during the acute phase of PUUV infection platelet aggregation appears impaired, especially when induced with thrombin, when tested on impedance aggregometry. Platelet adhesive mechanisms on collagen are intact, despite thrombocytopenia, while thrombopoiesis is active [152]. Potential mechanisms explaining the decrease in platelet count based on In vitro data include decreased production due to bone marrow invasion and megakaryocyte infection [153] and binding of platelets to infected endothelial cells [154].

5.3.5 Gastrointestinal tract viruses

5.3.5.1 Enteroviruses

Since the near-eradication of poliomyelitis, severe enterovirus infections have become rare, typically causing mild cold-like illness in children and hand, foot and mouth disease. Coxsackievirus infections occasionally cause myocarditis in adults, and enterovirus 68 has recently been implicated in episodes of acute flaccid myelitis in children. Thrombocytopenia has been described in neonatal cases of coxsackievirus B3 (CoxV B3) infections, both in Japan, with one case being related to secondary Hemophagocytic Lymphohistiocytosis (HLH) triggered by CoxV B13 [155,156]. A cross-sectional study of neonatal cases with severe enteroviral infection in Japan demonstrated significantly decreased platelet and WBC counts in Human Parechovirus 3 infections compared to RSV infected controls. In addition, the Human-Parachovirus-3-infected patients showed elevated plasma ferritin and lactate dehydrogenase (LDH) levels, both compared to the RSV controls and infants with other enterovirus infections. This may suggest an HLH-like illness secondary to this viral infection [157]. Human platelets express the Coxsackie-Adeno Receptor (CAR), aCoxVB3 cell entry receptor. Whether this mechanism plays a role remains unclear, in particular as the virus appears to be unable to replicate in platelets in vitro. However, platelets become activated after incubation with CoxVB3, increasing their expression of P-selectin and showing signs of apoptosis (i.e. increased phospatidylserine (PS) expression) [158].

5.3.5.2 Rotavirus

Viral gastroenteritis is a major cause of child mortality in the developing world and a significant burden on the healthcare system in developed countries [159]. Outbreaks of norovirus and rotavirus (RotV) frequently occur in childcare institutions and nursing homes, where those most vulnerable to dehydration are affected. These infections are typically limited to the gastrointestinal epithelium

and rarely cause severe systemic disease with involvement of multiple organs or severe inflammation. It is therefore not surprising that thrombocytopenia is not a dominant clinical feature of viral gastroenteritis. Mean platelet counts in children with rotavirus gastroenteritis are reported within the normal range and not diverging from children with other viral causes of gastroenteritis [160]. It is important to recognise both concentration and dilution effects of intravascular fluid shifts when assessing platelet counts or other blood cell counts in patients with conditions such as severe gastroenteritis or other critical illnesses.

5.3.6 Herpesviruses

5.3.6.1 Cytomegalovirus

Cytomegalovirus (CMV) is present in nearly all humans and remains latent for life after primary infection, which is usually asymptomatic in immunocompetent hosts. In contrast, severe immune suppression can lead to reactivation in later life leading most notably to CMV-mediated enterocolitis, hepatitis, retitinis or encephalitis [161]. However, very little is known about platelet counts during primary infection in immunocompetent hosts because CMV infection comes to the attention of clinicians only when these rare complications develop. In vitro, CMV has been shown to interact with platelets through TLR-2. However, rather than directly resulting in platelet aggregation, these activated platelets produce a proinflammatory response, form aggregates with leukocytes and increase their adhesion to human vascular endothelial cells (HUVEC). Thus, in this model of CMV infection, platelets act as an intermediary between the virus and circulating immune cells [39].

5.3.6.2 Epstein Barr virus

Like all other human herpesviruses, infection with Epstein Barr virus (EBV) occurs in the majority of the population at an early age. This leads frequently to a mild but sometimes protracted viral symptomatic episode called infectious mononucleosis, which is followed by lifelong latency. Occasionally reactivation occurs in immunocompromised hosts, most commonly in organ transplant recipients. Recently, primary EBV infection has been associated with a variety of autoimmune diseases, whereas latent EBV infection and reactivation plays a role in the pathogenesis of Hodgkin's lymphoma and B and T cell lymphomas, through poorly understood mechanisms. EBV infection is also a well-described trigger for secondary Hemophagocytic Lymphohistiocytosis (HLH) [162].

In the case of EBV reactivation, the relation with lymphoproliferative disorders is important to keep in mind when evaluating a patient with thrombocytopenia, especially when other cell-lineages are involved.

In cases of primary EBV infection, thrombocytopenia and hemolytic anaemia are occasionally also found and have been associated with the presence of platelet and erythrocyte auto-antibodies. Typical of primary EBV infection is the production of heterophile antibodies by naïve B cells that have become infected with latent-phase EBV [162]. Some of these antibodies may be autoreactive and bind to platelets, leading to their destruction. Due to EBV's restriction to human hosts, well-established animal models to study viral-platelet interactions in vivo do not exist. However, experimental infections with the related murine gammaherpesvirus 68 (γHV68) produce a mononucleosis-like illness in mice. This shows a significantly reduced platelet count during the early latent replication phase (nadir 17 days post infection). In this model, thrombocytopenia was found to be the result of antibodies induced by the infection and depended on viral latency, supporting the notion that polyclonal antibodies produced by latently infected B cells include autoantibodies against platelets [163]. This mechanism appears to be unique to EBV infection and is separate from auto-antibodies induced by another viral infection, which is believed to be the result of molecular mimicry between viral and self-antigens.

5.3.6.3 Human herpesvirus 6

Human herpesvirus 6 (HHV-6) causes a near-universal childhood illness, exanthema subitum, before entering its latent stage. Reactivation is rare and generally only occurs during profound immunosuppression, such as during allogenic hematopoietic stem cell transplantation. In this population, HHV-6 reactivation (defined as a positive PCR on blood samples) was significantly associated with delayed platelet engraftment and the development of graft versus host disease (GVHD) [164].

5.3.6.4 Varicella Zostervirus

Primary Varicella Zostervirus (VZV) infection almost universally presents itself as a self-limiting childhood illness, with more significant sequalae emerging later in life, ranging from common herpes zoster to rare cases of severe disseminated disease. The latter is typically only found in immunocompromised hosts, although not exclusively [165]. Typical Herpes Zoster manifests itself as a vesicular cutaneous eruption restricted to one dermatome and is most frequently seen in the elderly and patients who received chemotherapy for solid or hematological malignancies. Herpes Zoster can have long-term sequalae, such as post-herpetic neuralgia, but does not cause systemic disease and patients usually have normal platelet counts [166]. Profound thrombocytopenia has been described in reports of disseminated VZV infection, combined with DIC, hemorrhaging, ischemic strokes, ileus, abdominal pain, hepatitis, meningoencephalitis and vasculitis [167–170]. The vasculitis is believed to

be caused by VZV infection of the arterial walls themselves and can be found in arteries in various organs, including smaller cerebral arteries, where it is associated with stroke [171]. Case series describing VZV-related strokes report elevated platelet activation markers, such as PF-4 and α-thromboglobulin levels in some patients [172]. Splenomegaly with associated hypersplenism is a common feature of systemic herpesvirus infections, which contributes to thrombocytopenia and sometimes leads to splenic rupture [173]. The differential diagnosis of thrombocytopenia during a VZV infection is broad, because of comorbidity-related immune suppression, and includes immune thrombocytopenia, drug-induced thrombocytopenia [174] and bone marrow dysfunction, particularly if other lineages are affected. First presentation or relapse of ITP has been reported during primary VZV infection in adulthood [175,176]. A platelet count <200 × 10^9/L was found to be predictive of a poor outcome in patients suffering from Ramsey Hunt Syndrome [2]. A large prospective cohort study identified thrombocytopenia as an independent risk factor for ICU admission in hospitalised children with VZV infection, although the rate of underlying hematological comorbidities and bacterial coinfections was high, suggesting VZV was not the sole cause of this phenomenon [177].

5.3.7 Respiratory tract infections

A platelet count close to the lower reference limit is a common finding in more severe viral respiratory tract infections [178]. Data on platelet counts in mild respiratory infections are scarce, possibly due to the fact that these cases typically do not present to care and blood counts are rarely performed. Interestingly, the literature reports thrombocytosis in infants hospitalised with respiratory tract infections, especially RSV and rhinoviruses, with platelet counts decreasing with age [179–181]. Platelet counts in patients with acute exacerbations of heart failure who tested positive for respiratory viruses by PCR did not differ significantly from those who tested negative [182]. The lungs have recently been found to host resident megakaryocytes, which contribute to platelet production [183]. Investigating the interactions between respiratory viruses and platelets could be key to understanding the high rate of thromboembolic complications that arise during viral acute respiratory distress syndrome (ARDS).

5.3.7.1 Adenoviruses

Among viruses causing mild upper respiratory tract infection, adenoviruses (Adv) appear to be most studied in relation to platelets. Coxsackie and adenovirus receptor (CAR) is the receptor adenoviruses use for cellular attachment. Expression of this receptor has been reported in healthy human platelets, albeit at a very low frequency (3.5%) [184]. In vitro studies, where platelet-rich plasma

was incubated with very high concentrations of human adenovirus 3 and 5, showed a moderate increase in platelet aggregation and platelet activation marker expression, with uptake of adenovirus 5 by platelets demonstrated using EM [185,186]. Since natural infection in humans is unlikely to expose platelets to the high viral titres used in these incubation experiments, the clinical relevance for this finding is mostly related to the potential future use of adenoviruses for gene therapy purposes. Indeed, cancer patients experimentally treated intravenously with oncolytic adenovirus where serially sampled to determine relative abundance of viral DNA in various blood cell populations. Although very little platelet-associated virus was found in vivo, in vitro experiments where whole blood was incubated with the studied adenoviruses revealed a large proportion of virus bound to platelets. Given the thrombocytopenia observed during adenovirus-based treatments, this discrepancy could be the result of a survival disadvantage of adenovirus bound platelets in the circulation [187].

5.3.7.2 Influenza virus

Influenza virus (IAV/IBV) infection is associated with a severity-dependent thrombocytopenia. Pediatric outpatients with confirmed IAV or IBV infection showed slightly, though significantly, lower mean platelet counts compared to asymptomatic controls. Children with influenza-like illness who were IAV and IBV PCR negative had platelet counts in between the confirmed positive and healthy groups, and platelet counts could not reliably distinguish between influenza positive and negative children [188]. In adults, severe influenza infection is accompanied by an increased risk of pulmonary thromboembolisms and cardiovascular events (Sellers, 2017 #1) suggesting platelet activation occurs during infection. Whole-blood transcriptome studies have found gene expression signatures in patients during H1N1 infection that are associated with a poor response to antiplatelet agents. Conversely, patients undergoing coronary catheterisation that had a gene expression signature associated with viral infection, were more likely to have a confirmed myocardial infarction compared to those that did not express this signature [189]. Pathogenic H3N2 and H1N1 strains are capable of infecting pulmonary vascular endothelial cells, which increases platelet adhesion to both infected and nearby uninfected cells through interaction between endothelial fibronectin and platelet integrins [190]. Various influenza A strains cause thrombocytopenia in experimentally infected ferrets, with highly pathogenic strains (H5N1) showing a stronger decrease compared to moderate (H1N1) or mildly pathogenic (H3N2) strains. In addition, these viruses are capable of directly infecting platelets in vivo through binding of sialic acids on glycans on their cell surface. EM imaging has demonstrated the ability of platelets to phagocytose influenza virus particles. This infection of platelets results in their activation, aggregation and subsequent clearance

from the circulation. Interestingly, desialylation of platelet glycans by viral neuraminidase is hypothesised to reduce the lifespan of affected platelets through increased hepatic clearance [191]. Influenza virus can also interact with platelets through TLR7, which leads to the formation of platelet-neutrophil aggregates and neutrophil NETosis, through complement (C3) secreted by the platelets [192]. Immune-complexes of antibodies against influenza virus are also capable of activating platelets through an interaction with the Fc-αIIA receptor present on the platelet surface, leading to thrombocytopenia in a humanised mouse model. These findings, combined with reported influenza vaccine induced ITP, point to a link between influenza-virus-specific adaptive immunity and thrombocytopenia [193–195].

5.3.7.3 Measles virus

Likely the most contagious virus known to affect humans, the measles virus (MV) first infects the respiratory tract and subsequently spreads to lymphoid organs, infecting lymphocytes, including memory B and T cells [196]. A highly effective vaccine has been available for several decades, yet immunisation programs have not been able to reach sufficient coverage to eradicate the disease, leading to sporadic outbreaks [197]. Studies published in the past 10 years describing natural infection in adults report mild leukocytopenia and thrombocytopenia as a frequent finding, occasionally with minor bleeding complications, but no thromboembolisms [198–200]. A link between Subacute Sclerosing Panencephalitis (SSPE), a late complication of measles caused by persistence of MV in the brain, and ITP has been proposed, based on the co-occurrence of both extremely rare diseases in 3 pediatric cases [201]. This is further supported by an increased incidence of ITP after MV vaccination, where platelet binding anti-MV (and anti-rubella) IgG and IgM was demonstrated [202].

5.3.7.4 Parvovirus B19

While best known as a mild, self-limiting childhood illness (fifth disease), parvovirus B19 (PVB-19) can occasionally cause more severe disease, especially during pregnancy, resulting in hydrops fetalis. Due to its tropism for erythroid progenitor cells and megakaryocytes, fetal PVB-19 infection causes severe anaemia and thrombocytopenia, requiring intrauterine transfusion (IUT) of platelets and erythrocytes in some cases [203,204]. While most severe and best described in fetal infections, PVB-19 can also cause thrombocytopenia, anaemia, leukopenia or pancytopenia in children and adults [205–208]. A retrospective cohort study reports PVB19 infection in children undergoing chemo- and radiation therapy for non-hematological malignancies increases the risk of thrombocytopenia and transfusion of blood products [209]. A

similar study comparing malignant and non-malignant hematological disease in a pediatric population found that PVB-19 DNA positivity was not associated with a higher risk of transfusion, but the number of platelet transfusion units administered per patient was over three-fold higher in PVB-19 DNA positive patients [210]. PVB-19 is also able to infect myocardial tissue, leading to clinical myocarditis and dilated cardiomyopathy. This raises the question whether PVB-19 is also capable of infecting vascular endothelium and cause vasculitis and platelet adhesion to infected vessel walls. A case–control study exploring differences in microparticle (MP) profiles in the peripheral circulation of patients with myocarditis caused by PVB-19 versus other causes, found significant increases in apoptotic endothelial, platelet and leukocyte-derived MPs in PVB-19 mediated disease. This suggests that, in addition to impaired hematopoiesis, PVB also causes platelet destruction and vascular damage [211]. In vitro studies suggest PVB-19 nonstructural protein 1 (NS1) causes endothelial activation, upregulation of adhesion molecules and an increase in platelet and monocyte binding [212].

5.3.7.5 Respiratory syncytial virus

Severe respiratory infections with respiratory syncytial virus (RSV) occur mainly in children, the immunocompromised and those with underlying pulmonary disease [213]. In contrast to other respiratory infections, thrombocytosis rather than thrombocytopenia appears to be a common phenomenon found during acute RSV disease [181]. In vitro experiments demonstrate a reduction of monocyte RSV infection when platelets are added to the culture, possibly by binding and internalisation of RSV. Platelets increase surface P-selectin expression in the process, but why this would lead to thrombocytosis rather than thrombocytopenia is unclear [214].

5.3.7.6 SARS Coronavirus 2

The literature cited in this part of the review was updated shortly before submission to include the high volume of scientific work that has been published on SARS Coronavirus 2 (SARS-CoV-2, COVID-19), which has caused a pandemic of severe pneumonia of historical proportions. Besides bilateral pneumonia, critical COVID-19 cases are characterised by multi-organ disease [215], and a remarkably high incidence of pulmonary embolisms [216,217]. Several mechanisms involving hypercoagulability and inflammation interact resulting in thrombotic phenomena both in the microvasculature and in the larger, mostly pulmonary blood vessels [218].

In fact, upon autopsy these embolisms were found to be mainly composed of platelets, fibrinogen and neutrophils [219–221]. Another typical finding

during autopsy of deceased COVID-19 patients is the presence of widespread microvascular thrombosis in both pulmonary and extrapulmonary vessels, including in patients without true thromboembolisms, indicating a systemic prothrombotic state [220].

A low to low-normal platelet count is present during peak symptomatic illness, with increased MPV and PDW, and expression of surface activation markers [222,223]. However, one study identified subpopulations of platelets with a downregulated phenotype, which were highly enriched in severe, but not in intermediate COVID-19 cases, suggesting exhaustion of circulating platelets [220]. The total platelet population from these patients still showed hyperresponsiveness to procoagulant stimuli in vitro, likely driven by a hyperactive minority that was also present. This hyperresponsiveness was also found in other studies [224]. Some clinical studies report that thrombocytopenia is associated with increased mortality [4,5], whereas other do not [215,225]. This discrepancy might depend on disease severity, comorbidities or the type of care provided. For example, a well-defined cohort of mechanically ventilated critically ill patients showed that daily platelet concentrations were not associated with intensive care unit survival [215]. However, this observation does not exclude a role for platelet (dys)function in immunothrombosis. Other coagulation-related markers during acute illness show strongly elevated levels of D-dimers and fibrinogen degradation product (FDP), normal to slightly prolonged PT, APTT, elevated plasma viscosity and coagulability, and normal to mildly increased INR [5,12,222,223]. Whether these markers have diagnostic or prognostic value requires investigation and might differ along the course of infection depending on disease severity, comorbidities and type of care provided. Platelet counts appear to rise slowly over the course of the disease, which coincides with a sharp peak in IL-6, suggesting this cytokine may play a role in the thrombopoietic response [220]. Plasma TPO levels are elevated in severe COVID-19 patients, but gene expression of its receptor c-MPL is decreased, suggesting desensitisation of the bone marrow as an additional mechanism for thrombocytopenia in COVID-19. When thrombocytopenia is present, it is often accompanied by relative deficiencies in other myeloid and lymphoid cell lineages [226,227], which could indicate bone marrow displacement caused by a proliferative response to hyperinflammation, either as a toxic effect of cytokines to progenitor cells in the bone marrow or a result of homing to inflamed tissues and extravasation.

Considerable work within a relatively short time span has been done unravelling the mechanisms through which SARS-CoV-2 infection causes platelet activation. One study shows that platelet activation in severe COVID-19 is

associated with detectable viral RNA in blood. Furthermore, the viral spike protein enhanced platelet activation, aggregation, thrombus formation and degranulation in vitro and in a mouse model. This effect was only seen when the full spike protein or its ACE-2 binding S1 subdomain were used, not the S2 domain. This suggests that ACE-2 signalling mediates this platelet activation. Further analysis of intracellular messaging points towards involvement of the Mitogen-Activated Protein Kinase (MAPK) signalling pathway. The same study confirmed expression of ACE-2 in human platelets using immunofluorescence [223]. However, another study did not detect any ACE-2 mRNA or protein expression in COVID-19 patients by RNA-seq, qPCR or Western blot [224]. To date, no study has demonstrated SARS-CoV-2 internalisation by platelets.

It is also clear that platelets influence the host immune response to SARS-CoV-2. Platelet gene expression profiles in severe and critically ill COVID-19 patients showed shared pathways with sepsis and influenza H1N1 infection. These show related antigen presentation and immune regulation, including differential expression of interferon-induced transmembrane protein 3, which has antiviral properties [224]. The formation of platelet-leukocyte aggregates was also found, with neutrophil-platelet aggregates correlating with the severity of lung injury and leading to the formation of NETs [220,221,224,228,229]. Similar to observations in HIV and DENV, platelet-monocyte complexes are formed in severe COVID-19 patients via platelet P-selectin, which results in overexpression of tissue factor on the monocyte surface, the key initiator of the extrinsic coagulation pathway [230].

As with many other viral infections, reports have been published of cases of ITP associated with COVID-19 infection, including one case of Evans syndrome [231]. Another case report illustrates the importance of performing a peripheral blood smear in COVID-19 patients with severe thrombocytopenia to exclude EDTA dependent pseudo-thrombocytopenia [232].

5.4. CONCLUSION

The topic of thrombocytopenia in viral infectious diseases has been actively studied for many decades, with the last 10 years yielding many new insights. A scientific field combining the disciplines of virology, hematology and increasingly immunology is revealing a complex system of interactions between various viruses, the coagulation cascade, and the innate and adaptive immune system. Increasingly, platelets are regarded as part of the immune system, in addition to being capable of forming blood clots. The rapidly changing world of

viruses ensures that this field is constantly forced to adapt to new outbreaks and is therefore equally dynamic. The current COVID-19 pandemic has brought platelet-virus interactions to the forefront, with many publications addressing this topic being available within a year after the SARS-CoV-2 virus first emerged.

The absence of research on the "classical" hemorrhagic fevers, such as the Ebola, Lassa and Marburg viruses, has been notable however, despite two large outbreaks of Ebola occurring in the last decade. The high level of biological containment required to safely study these viruses, combined with the extremely resource-limited settings in which these outbreaks occurred make doing research into these viral diseases extremely challenging. Nonetheless, significant progress has been made in preventive and therapeutic interventions for Ebola virus, with the successful trials of several vaccines [233], antiviral drugs and monoclonal antibodies [234].

DENV, another viral infection disproportionately affecting people in resource limited settings, was the virus we found most publications about in relation to platelets in the past decade. This is not surprising, given the considerable role platelets play in the pathophysiology of severe disease and the enormous public health burden associated with the virus. Despite the considerable knowledge gained, this has so far not been translated into clinically effective interventions. However, we did find several studies investigating therapeutics aimed at modifying platelet function in DENV infection, which will hopefully bear fruit in the coming decade.

Looking in detail at the interactions between viral infections and platelets revealed several common pathways connecting inflammation and platelet activation, which has been termed "Immunothrombosis". This is a term which has not yet been clearly defined as a clinical or pathological entity and has some overlapping features with DIC, with the main clinical difference being the absence of significant bleeding. COVID-19 may provide us the opportunity to increase our overall understanding of thrombocytopenia in viral infections and perhaps to study a new dimension of immunothrombosis which could be translated to other viral infections. It is especially important to gain more understanding about which interventions could aid in reducing the morbidity and mortality related to immunothrombosis. As platelet dysfunction is often accompanied by an increased risk of both bleeding and thrombosis, approaching this issue with conventional anticoagulants often involves having to choose the lesser of two evils. Immunomodulation therapy is a rapidly evolving field, with many newly available therapeutics, most of which have not yet been trialed in viral infectious diseases. This approach warrants further study, but

here caution is also advisable, given the possibility that some mechanisms involved in immunothrombosis are required in the antiviral response in the host.

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Table 1. Overview of mechanisms contributing to thrombocytopenia in a selection of major viral infections.

Virus	Platelet Binding (Receptors)	Platelet Activation (Receptors, Markers)	Platelet Infection (Receptors)	Platelet Replication	Vascular Endothelial Disruption	Impairs Platelet Production	Associated with Hemorrhage	Associated with Thrombo-embolism	Platelet Sequestration	PLA Formation	Autoimmunity
DENV	DC-SIGN, HSP, FcγR2A, GPIb, CLEC2, CLEC5A [66,67,81]	Receptors: CLEC2, CLEC5A, TLR 2/4, 5HT2 [66,67,71] Markers: P-Selectin, Integrin α2b, PF4, CCL5, PS, CD40L, CD63, GP1b, GPIIb/IIIa, microparticle release, aggregation [63–67,70,73]	DC-SIGN, HSP, FcγR2A (Ig bound virus) [30]	Yes [30]	Plasma leakage [59,67]	Infects megakaryocyte-like cells [80,81]	With endothelial and platelet dysfunction [56–58]	Rare [235]	Phagocytosis [73,76]	PMA, PNA, inducing NETosis [68,69]	Immune complexes [75]
ZIKV	U	U	No [116]	No [116]	Infects vascular endothelial cells in vitro [236]	U	In severe cases [107,108]	Rare [237]	U	U	ITP [112–114]
YFV	U	U	U	U	Fundoscopic abnormalities [101]	U	Hepatic failure and deficiency of plasma coagulation factors [99,100] Cirrhosis associated varices (HBV/HCV)	Microvascular thrombosis [101]	U	U	U
HBV	U	Markers: Morphological changes [127] resistance to antiplatelet agents [128]	U	U	Polyarteritis nodosa (rare) [238]	Impaired hepatic TPO production [131,239]		Increased risk of VTE [240] Portal vein thrombosis in cirrhosis [241] Risk of ischemic cardiovascular disease elevated for HCV only [242,243]	Portal hypertension and hypersplenism [239]	U	U
HCV	Likely, Mechanisms unknown. [130, 132,133]	U	Greater stability of HCV in platelets [130,132], persistence in platelets during treatment [133]	No [132]	Endothelial activation [244], capillarisation of liver sinusoidal endothelial cells [245], (non) cryoglobulinemic vasculitis [246]					U	ITP [130]

Table 1. Continued

Virus	Platelet Binding (Receptors)	Platelet Activation (Receptors, Markers)	Platelet Infection (Receptors)	Platelet Replication	Vascular Endothelial Disruption	Impairs Platelet Production	Associated with Hemorrhage	Associated with Thrombo-embolism	Platelet Sequestration	PLA Formation	Autoimmunity
HIV	DC-SIGN, [138] GPIIIa, CCL-3 [146]	Receptors: CXCL4, CCR3, GPIIIa Markers: PF-4, CCL5, P-selectin, sCD40L, CCL5, Conflicting reports on morphological changes, oxidative stress (135–137,139]	Yes, via megakaryo-cyte precursors [141]	No	HIV-associated vasculopathy [247], infects arterial smooth muscle cells [248]	Infection and impairment of hema-topoietic progenitor cells [249]	No	Increased risk of VTE [250], myocardial in-farction [251] and cerebrovascular disease [247]	Hyper-splenism [252]	PMA, increasing monocyte TF expres-sion [144,145]	ITP [253], HLH [254]
IAV/ IBV	Likely NA binding of glycans [191]	Receptors: Glycans, TLR7, [44] Immune complexes via Fc-ϒIIA receptor [193] Markers: P-selectin [191], CD40L, C3, CD63 [192] Resistance to antiplatelet agents [189]	Phago-cytosis [191]	U	Infection of pulmonary vascular endothelial cells [190]	U	No	VTE, myocardial infarction, ischemic cerebrovascular accidents [1]	U	PNA, inducing NETosis [192]	ITP [194,195]

Table 1. Continued

Virus	Platelet Binding (Receptors)	Platelet Activation (Receptors, Markers)	Platelet Infection (Receptors)	Platelet Replication	Vascular Endothelial Disruption	Impairs Platelet Production	Associated with Hemorrhage	Associated with Thrombo-embolism	Platelet Sequestration	PLA Formation	Autoimmunity
SARS CoV 2	ACE2, conflicting evidence on platelet expression [223,224]	Receptors: ACE2, TLR4, CLEC2, CXCR-4 Markers: P-selectin, GPIb, GP IIa GPIIIb, GPIIb/IIIa, CD40L, CD63, morphological changes, aggregation, degranulation [220,222–224]	U	U	U	Decreased cGMP expression [224]	No	Microvascular thrombosis and VTE [216–219]	U	PMA increasing monocyte TF expression, PNA inducing NETosis [221,230]	ITP, Evans Syndrome [231]

U = unknown

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6

Increased Monocyte Tissue Factor Expression and End-tidal to Arterial Carbon Dioxide Gradient are Associated with Occurrence of Pulmonary Embolism in COVID-19 Patients Admitted to the Intensive Care Unit

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6.1 Abstract

Purpose: Pulmonary emboli (PE) contribute substantially to coronavirus-disease-2019-related mortality and morbidity. Immune cell-mediated hyperinflammation drives the procoagulant state in coronavirus disease 2019 (COVID-19) patients, resulting in immunothrombosis. To study the role of peripheral blood mononuclear cells (PBMC) in the procoagulant state of COVID-19 patients, we performed a functional bioassay and related outcomes to the occurrence of PE. Secondary aims were to relate this functional assay to plasma D-dimer levels, ventilation perfusion mismatch and TF expression on monocyte subsets.

Methods: PBMC from an ICU biobank were obtained from 20 patients with a computed tomography angiograph (CTA) proven PE and compared to 15 COVID-19 controls without a proven PE. Functional procoagulant properties of PBMC were measured using a modified fibrin generation time (MC-FGT) assay. Tissue factor (TF) expression on monocyte subsets was measured by flow cytometry. Additional clinical data were obtained from patient records including end-tidal to arterial carbon dioxide gradient.

Results: MC-FGT levels were highest in the samples taken closest to the PE detection, similar to the end-tidal to arterial carbon dioxide gradient ($ETCO_2$–$PaCO_2$), a measurement to quantify ventilation-perfusion mismatch. In patients without proven PE, peak MC-FGT relates to an increase in end-tidal to arterial carbon dioxide gradient. We identified non-classical, CD16 positive monocytes as the subset with increased TF expression.

Conclusion: We show that the procoagulant state of PBMC could aid in early detection of PE in COVID-19 ICU patients. Combined with end-tidal to $ETCO_2$–$PaCO_2$ gradient, these tests could improve early detection of PE on the ICU.

6.2 INTRODUCTION

COVID-19 results from infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The clinical spectrum ranges from mild, flu-like symptoms to severe viral pneumonia in a minority of cases, which may result in acute respiratory distress syndrome (ARDS). ARDS due to COVID-19 is characterised by a high risk of venous thromboembolism (VTE), particularly pulmonary embolism (PE), in critically ill patients [1,2]. Severe COVID-19 is associated with a hyperinflammatory innate immune response to SARS-CoV-2, including dysregulated monocyte and neutrophil activation, which may fail to adequately limit viral replication while contributing to tissue injury [3]. Once severe illness has developed, treatment is mainly supportive, complemented by appropriately timed immunomodulatory treatment, management of superinfections, and thromboprophylaxis or therapeutic anticoagulation where clinically indicated.

Thrombotic complications contribute substantially to COVID-19-related morbidity and mortality and are associated with endothelial dysfunction and a hypercoagulable state induced by a complex, multifactorial process collectively termed "immunothrombosis" [4]. In this process, SARS-CoV-2 infection and infection-induced tissue injury trigger an innate immune response involving activated neutrophils and monocytes, including the formation of neutrophil extracellular traps and the release of proinflammatory and prothrombotic mediators, thereby promoting a hypercoagulable state [3,5–7]. Tissue factor (TF) has been identified as one of the key prothrombotic factors contributing to COVID-19-associated immunothrombosis [8–10]. When activated, TF may be expressed on the cell membrane of circulating monocytes and other activated cells or released in extracellular vesicles [8–10]. TF subsequently binds factor VII/VIIa, initiating the extrinsic coagulation pathway [10]. The procoagulant effects of monocyte-associated TF can be assessed functionally using plasma-based cellular coagulation assays [11,12]. In the present study, peripheral blood mononuclear cells (PBMCs) were suspended in plasma and calcium ions were added to initiate coagulation, with the time to clot formation measured as the fibrin generation time (FGT).

Timely recognition and treatment of PE are vital to improving clinical outcomes. Plasma D-dimer is a highly sensitive marker used primarily to help exclude PE in patients with an appropriate pre-test probability. D-dimer is produced following plasmin-mediated degradation of cross-linked fibrin and therefore reflects activation of both coagulation and fibrinolysis. According to the YEARS diagnostic algorithm, PE can be considered excluded in patients with

no YEARS criteria when the D-dimer concentration is <1,000 ng/mL, whereas a threshold of <500 ng/mL applies when one or more YEARS criteria are present [13]. However, D-dimer concentrations are frequently markedly elevated in patients with severe COVID-19, thereby reducing the specificity of D-dimer for PE in this population [14]. Studies in hospitalised patients with COVID-19 have therefore investigated empiric therapeutic-dose anticoagulation using elevated D-dimer concentrations and other clinical risk criteria for patient selection. Although therapeutic-dose heparin showed benefit in selected non-critically ill patients, this benefit was not demonstrated in patients requiring ICU-level care [15]. In critically ill patients in whom objective imaging for PE is not feasible, a presumptive diagnosis may be based on clinical features such as otherwise unexplained worsening hypoxia, hypotension or tachycardia and other indicators of thrombotic risk [16]. However, hypoxia and hypotension frequently occur in critically ill patients for reasons unrelated to PE, while sedation and analgesia may obscure symptoms such as chest pain. Furthermore, elevated D-dimer concentrations correlate with COVID-19 disease severity even in the absence of proven PE, further limiting their diagnostic specificity for PE in critically ill patients [14,16].

Currently used methods for diagnosing PE predominantly identify the downstream consequences of thrombosis, namely the presence of pulmonary arterial thrombi and their resulting physiological effects. The aim of this study was therefore to determine whether an assay measuring the procoagulant properties of PBMCs, which may contribute to immunothrombosis in COVID-19, could show potential for earlier detection of PE in critically ill patients. FGT was compared with findings from computed tomography angiography and the arterial-to-end-tidal carbon dioxide gradient ($PaCO_2$–$ETCO_2$). An increased $PaCO_2$–$ETCO_2$ gradient reflects increased physiological dead space and ventilation/perfusion mismatch and has been investigated as an adjunctive marker for PE [17,18].

6.3 METHODS

6.3.1 Study design and population

We performed longitudinal measurements of functional prothrombotic properties of PBMCs in an ICU cohort of COVID-19 patients with and without proven PE (Figure 5-1). Clinical data and samples used in this study originate from a longitudinal biorepository study (CIUM) of patients with ARDS and sepsis in the ICU of Erasmus Medical Center, the Netherlands. After written informed consent, PBMC from critically ill COVID-19 patients from the first COVID-19

wave in the Netherlands were used, collected from March through to May 2020. For patients with a documented PE, a monocyte fibrin generation test (MC-FGT) result before and after the diagnosis of PE were compared. In addition, a case-control analysis of peak MC-FGT values of COVID-19 patients who suffered PE, compared to patients with no documented PE was performed. During the first COVID-19 wave in the Netherlands, EDTA anticoagulated whole blood was obtained for the isolation of plasma and PBMC on a weekly basis for up to four weeks, or until the patient was discharged from the ICU. For patients who were not conscious at the time of inclusion, deferred proxy consent was obtained, followed up with a retrospective opt-out procedure after recovery. Studies involving human participants were conducted in accordance with institutional guidelines and the declaration of Helsinki. The CIUM study protocol was approved by the institutional review board of Erasmus Medical Center (MEC-2017-417 and MEC-2020-0222). Healthy control PBMC were isolated from buffy coats, ordered from the Dutch blood transfusion service (Sanquin).

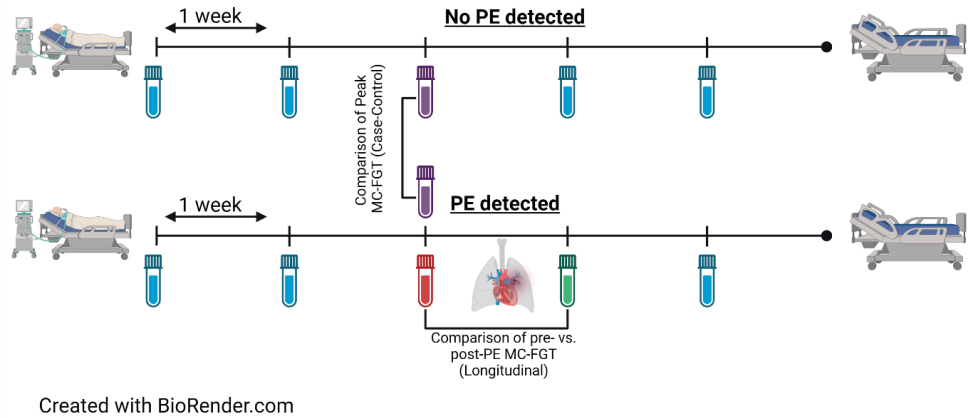


Figure 1. Study design.

6.3.2 Fibrin Generation Time assay on PBMC (MC-FGT)

For this assay, we used an adapted version of a previously described protocol [302]. Blood was drawn into tubes containing EDTA for preparation of plasma and PBMC. PBMC were isolated using histopaque gradient medium, frozen in freezing medium (90% FBS, 10% DMSO) at -80° C overnight and subsequently stored in Liquid Nitrogen until use. After thawing, PBMC were resuspended in ice-cold RPMI medium, centrifuged at 400 g for 10 minutes, resuspended in prewarmed R10 medium and counted. Subsequently, cells were washed twice in assay buffer: 25 nM HEPES, 137 mM NaCl, 0.1% BSA in deionized water at pH 7.4. Pooled citrated plasma from healthy donors was prewarmed to 37° C and plated in 96 well flat-bottomed cell culture plates (80 ul/well). Subsequently,

75 ul of PBMC suspended in assay buffer ($2x10^6$ cells/mL) were added in duplicate to sample wells. Recombinant tissue factor (rTF; Innovin, Siemens Healthineers, Marburg, Germany) was used as positive control and to generate a standard curve by two-fold serial dilution in assay buffer (range 244–1525 pg/mL). PBMC from healthy donors were used as negative controls and stimulated using LPS, which is known to increase TF expression, for use as positive controls. Subsequently, 75 µl of assay buffer with 38 mM calcium chloride was added to the wells containing plasma with TF or PBMC, initiating the extrinsic coagulation cascade. A plate reader (Infinite F200, Tecan) was used to measure increasing absorption at 450 nm in each well in 30-second intervals, while keeping the plate at a stable temperature of 37° C. OD values were plotted over time in minutes using a nonlinear regression curve to calculate the time until half of maximum OD was reached ($T_{1/2}$). Subsequently, the values obtained from the sample wells containing PBMCs were interpolated on the standard curve, to yield results in rTF equivalent concentrations.

6.3.3 Flow cytometry

Monocytes were isolated from PBMC using negative selection by magnetic activated cell sorting (MACS, Human Pan Monocyte Isolation Kit, Miltenyi Biotech) according to the manufacturer's instructions. Monocyte were stained anti-$CD14^{BV605}$ (Clone M5E2, BD, 1:100), anti-$CD16^{AF700}$ (Clone 3G8, Biolegend, 1:100) and anti-TF^{AF647} (Clone #323519, R&D systems, 1:150) for 15 minutes, washed twice and resuspended in FACS buffer. Samples were measured on a BD FACSLyric.

6.3.4 Statistics

Statistical comparisons between the PE and non-PE groups were performed using independent samples T tests (after confirmation of a normal distribution, or after log transformation) for continuous variables and chi-square tests for binominal variable, or Fisher's exact test when appropriate. For longitudinal analyses of MC-FGT, D-dimer and $ETCO_2$–$PaCO_2$ gradients, paired t tests were used, after log-transformation in the case of the MC-FGT. For case-control analyses, Mann-Whitey U tests were used. We made use of GraphPad Prism version 9.0.0 for Windows, (GraphPad Software, San Diego, California USA, www.graphpad.com).

6.4 RESULTS

6.4.1 Patients

To assess the performance of the MC-FGT in COVID-19 ICU patients, PBMC were collected from 35 ICU patients, admitted during the first wave of COVID-19 in the Netherlands. Patient baseline characteristics are shown in Table 1. Detailed information about individual patients in the non-PE group is presented in Table 2. Both groups were similar regarding disease severity at the time of admission (SOFA score), comorbidities (CCI), age, sex and BMI. Of the 35 COVID-19 patients, 20 had a CTA-proven PE. CTA was performed because of a clinical suspicion of PE. The remaining 15 patients either had a negative CTA during their hospitalisation (n = 5) or did not have a CTA performed (n = 10). Two patients did not undergo CTA because they had an existing indication for therapeutic anticoagulation (Atrial Fibrillation with an elevated CHA2DS2-VASc score), which was continued. The remaining eight subjects were not suspected of having PE or VTE but were treated with prophylactic- or intermediate-dose low molecular weight heparin (LMWH), at the discretion of the intensive care specialist.

Table 1. Patient characteristics.

Characteristic	No CTA or CTA negative (n = 15)			CTA proven PE (N = 20)			P	All (n = 35)		
Age (Median, $IQR_{min-max}$)	62	54	70	66	63	73	0.165	65	59	72
Female (N, %)	4	27		4	20		0.700	8	8	23
BMI (Median, $IQR_{min-max}$)	30	24	33	27	25	30	0.260	28	25	31
Days in ICU (Median, $IQR_{min-max}$)	17	13	29	23	16	31	0.095	21	15	29
Days on ventilator (Median, $IQR_{min-max}$)	17	6	28	20	12	30	0.479	18	11	28
Comorbidities N, %										
Cardiovascular	1	7		4	27		-	5	5	14
Hypertension	1	7		4	27		-	5	5	14
Vascular disease	2	13		1	7		-	3	3	9
Diabetes	2	13		5	33		-	7	7	20
Neurological disease	2	13		0	0		-	2	2	6
Malignancy	3	20		1	7		-	4	4	11
Pulmonary disease	4	27		3	20		-	7	7	20
CCI (Median, $IQR_{min-max}$)	3	1	4	2	2	3	0.585	2	2	3
SOFA (Median, $IQR_{min-max}$)	6	5	8	6	5	8	0.525	6	5	8

Table 2. Clinical characteristics of COVID-19 patients without a CTA-proven PE, including anticoagulant treatment, MC-FGT and $ETCO_2$–$PaCO_2$ gradient results.

Id	Age	Sex	CTA	Anticoagulant	MC-FGT						$PaCO_2$	$ETCO_2$	$PaCO_2$	$ETCO^2$	Description
			Y/N Day Result		Peak	Nadir	At peak D-dimer	At nadir D-dimer	At peak PaCO2/ ETCO2	At nadir PaCO2/ ETCO2	D0	D0	At nadir MC-FGT	At nadir MC-FGT	
1	59	F	No	Nadroparin 5700 IU day 0-3 Therapeutic dose from day 3 onward	51	10	2.25	4.12	2.3	1.8	5.6	3.3	6.5	4.7	Prone ventilation. Deceased.
2	74	F	No	Pre-hospital VKA. therapeutic Nadroparin during ICU stay		14		2.82		1.1			5.6	4.5	No CTA because of existing indication for therapeutic anticoagulation. Recovered.
3	53	M	No	Nadroparin 5700 IU bid	112	6	4.03	2.71	2.1	0.8	6.9	4.8	6.20	5.4	No aXa levels measured. Recovered.
4	62	F	No	Nadroparin 5700 IU bid		10		1.57		1.4			5.6	4.2	cT4NxM0 IIIb NSCLC.
5	61	M	Day 0 No PE	Nadroparin 5700 IU bid	77	3			1.5	0.1	7.5	6	4.8	4.7	CTA report: suboptimal contrast. tr. Pulmonalis 31mm RV/ LV ratio 1.2 no central PE. Plain CT 12 weeks later: normal a. pulmonalis.

Table 2. Continued

Id	Age	Sex	CTA	Anticoagulant	MC-FGT						$PaCO_2$	$ETCO_2$	$PaCO_2$	$ETCO^2$	Description
			Y/N Day Result		Peak	Nadir	At peak D-dimer	At nadir D-dimer	At peak PaCO2/ ETCO2	At nadir PaCO2/ ETCO2	D0	D0	At nadir MC-FGT	At nadir MC-FGT	
6	77	M	No	Nadroparin 5700 IU od; day 14 Dabigatran	37		0.74		1.7		6.1	4.4			Thrombophlebitis upper extremities. DOAC started after AF de novo with high CHA_2DS_2-VASc. Deceased.
7	66	M	No	Nadroparin 5700 IU od	66	4	4.62	1.42	1.1	0.5	5.1	4	4.3	3.8	2x 72 hr prone position
8	61	M	No	Nadroparin 5700 IU od		9		0.66		0.6			5.00	4.4	Pre-existing pulmonary sarcoidosis. Recovered.
9	54	M	No	Nadroparin 5700 IU od; 1-5 bid		9		2.6		0.7			6	5.3	2x prone position. control CT complete recovery; first PBMC sample day 7.
10	29	M	Day 8 No PE	Nadroparin 5700 IU bid	79		2.29								No mechanical ventilation. 2017 CML treated with Allo-SCT.
11	40	M	Plain thorax CT	Nadroparin 5700 IU bid	89	3	6.6	1.6	0.7	0.1	5.4	4.7	5.80	5.70	72 hr. prone ventilation during peak FGT. Recovered.

Table 2. Continued

Id	Age	Sex	CTA	Anticoagulant	MC-FGT						$PaCO_2$	$ETCO_2$	$PaCO_2$	$ETCO^2$	Description
			Y/N Day Result		Peak	Nadir	At peak D-dimer	At nadir D-dimer	At peak PaCO2/ ETCO2	At nadir PaCO2/ ETCO2	D0	D0	At nadir MC-FGT	At nadir MC-FGT	
12	75	M	Plain thorax CT	Nadroparin 5700 IU bid	117		2.5								No ET-CO_2 measurement recorded. Hemoptysis reported on day 5.
13	70	F	Day 0 No PE	Nadroparin 5700 IU od		4		2.95		1.7			6.40	4.7	
14	64	M	Day 0 No PE	DOAC prehospital; therapeutic UFH during ICU stay	59				2		7.5	5.5			anti Xa during FGT 0.25.
15	66	M	Day 0 No PE	Nadroparin 5700 IU	-	0	-	5.5		2.0	5.5	3.5			Follow up: massive PE; deceased; no samples available after 02-04.

The occurrence of PE could not be ruled out in some cases during high MC-TGT results. Allo-SCT = Allogeneic

6.4.2 MC-FGT, D-dimer and $PaCO_2$–$ETCO_2$ gradient in COVID-19 patients with proven PE

From patients diagnosed with PE, sample collection time relative to diagnosis was categorised as: 1. timepoint available closest to the positive CTA confirming PE (sample drawn within five days before positive CTA); 2. timepoint available before the positive CTA confirming PE (more than five days); and 3. ≥48 hours after the CTA confirming PE. As controls, we analysed MC-FGT in PBMCs from four healthy individuals. Clinical data from timepoints matching the PBMC collection dates were extracted from patient records, including D-dimer levels and $PaCO_2$–$ETCO_2$ gradients.

D-dimer levels (Figure 2A) were highly elevated (>1 µg/mL) in 18/19 (95%) patients who suffered PE at all available timepoints (one patient had missing D-dimer data; n = 20). D-dimer levels within 48 hours before detection of PE (mean 7.2, SD 5.3) did not differ significantly from D-dimer levels >5 days before detection of PE or ≥48 hours after PE (p = 0.07).

When comparing the $PaCO_2$–$ETCO_2$ gradient (Figure 2B), a significant difference was observed (p = 0.0003), with a mean gradient of 2.0 kPa (±1.2) closest to PE versus 0.9 kPa (±0.7) more than five days before PE. This gradient also decreased significantly to 1.4 kPa (±1.2) ≥48 hours after treatment with anticoagulants (p = 0.04).

When performing the same analysis on the MC-FGT assay results, a similar effect was observed, with the highest values measured at the closest timepoint before PE (Figure 2C). These values were significantly higher compared with ≥48 hours after PE (p = 0.02) and compared with healthy controls (p = 0.003). For the earliest timepoint (>5 days before PE), only four paired samples were available. All were either higher than the ≥48-hour post-PE timepoint or lower than the closest pre-PE timepoint, but the difference was not statistically significant.

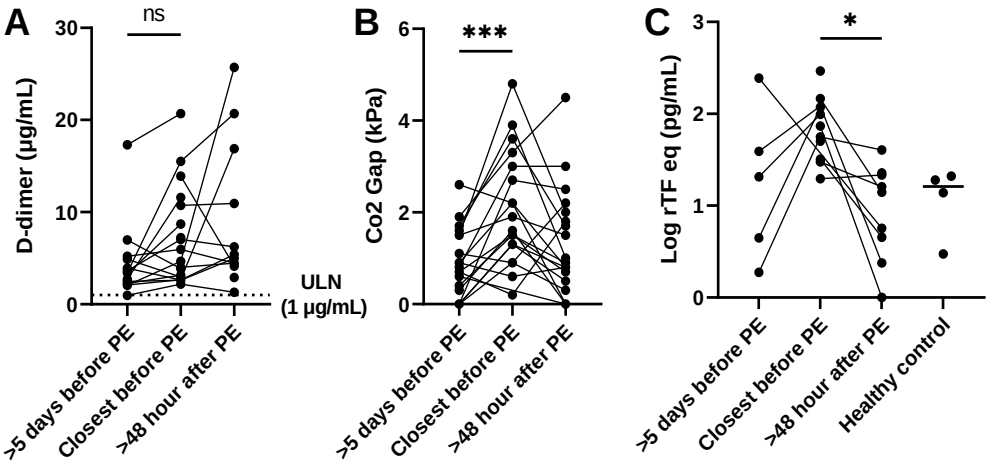


Figure 2. D-dimer, ETCO2−PaCO2 gradient and MC-FGT in COVID-19 patients with CTA-proven PE.PE.

A. 19 patients with computed tomography angiography proven pulmonary embolism show a statistically nonsignificant increase in D-dimer closest to the detection of PE compared to a sample taken before PE, with D-dimer remaining elevated after the start of treatment. B. Closest before detection of PE, a significant increase of the $ETCO_2$–$PaCO_2$ gradient is observed, with a subsequent decrease after initiation of anticoagulant treatment. C. MC-FGT levels significantly higher closely before PE compared to > 48 hours after and compared to healthy controls (line indicates the median). Black dots are individual values, with connecting lines between values originating from the same patients.

ULN = Upper Limit of Normal, NS = nonsignificant. * = P < 0.05, *** = P < 0.0001.

6.4.3 MC-FGT, D-dimer and $PaCO_2$–$ETCO_2$ gradient in COVID-19 patients with or without a CTA-proven PE

Subsequently, a case-control analysis was performed between patients with and without a CTA-proven PE. Because of the observed increase in MC-FGT in the longitudinal analysis of patients with proven PE, peak observed MC-FGT values in both groups were used for comparison.

Median peak MC-FGT in patients with proven PE was 109 pg/mL (range 17–292), versus 51 pg/mL in patients without a proven PE (range 0–117; p = 0.02), whereas the highest MC-FGT in healthy controls was 21 pg/mL (range 3–21). Despite this statistically significant difference, several patients without a proven PE showed MC-FGT levels higher than those observed in healthy controls (Figure 3).

This raised the possibility that these patients were in a similar procoagulant state to the proven-PE group, potentially reflecting undiagnosed PE or pulmonary microvascular thrombosis not detected by CTA. To investigate this possibility further, an additional analysis was performed comparing ventilation/perfusion mismatch, assessed using the $PaCO_2$–$ETCO_2$ gradient, in patients without

CTA-proven PE at timepoints at which MC-FGT was above that of healthy controls (>20 pg/mL) versus below or equal to this threshold (≤20 pg mL).

This revealed a significantly higher $PaCO_2$–$ETCO_2$ gradient at timepoints with elevated MC-FGT (Figure 4; p = 0.04), suggesting greater ventilation/perfusion mismatch, which can have multiple causes, including PE. Individual MC-FGT values and their corresponding $PaCO_2$–$ETCO_2$ gradients used for this analysis are listed in Table 2.

Patient details provide possible explanations for increased MC-FGT results. For instance, patient 6 suffered from thrombophlebitis. In specific cases (patients 1, 3, 5 and 12), CTA was not performed or was suboptimal. Of particular interest is patient 15. For this patient, only a sample drawn at inclusion was available for MC-FGT testing, which yielded a low result. A negative CT scan was also available on that day. Follow-up sampling was not performed; however, this patient subsequently died following a CTA-proven massive PE. Of particular interest is the high $PaCO_2$–$ETCO_2$ gradient already present early after ICU admission (Table 2).

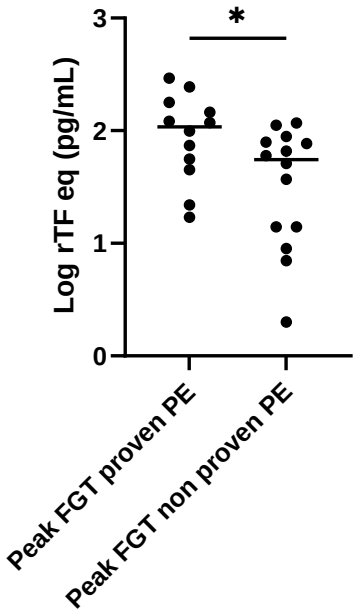


Figure 3. Peak MC-FGT results in patients without a proven PE compared to those without a proven PE.

* = P < 0.05. PE = pulmonary embolism; MC-FGT = modified fibrin generation time.

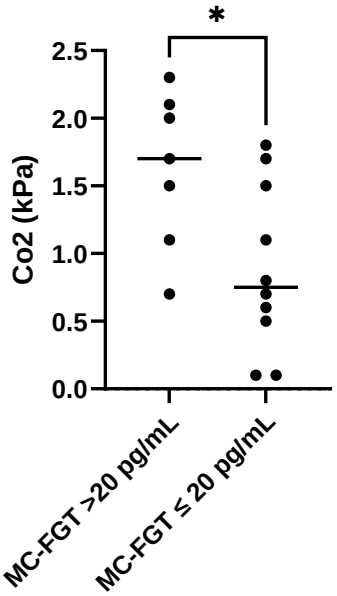


Figure 4. End-tidal CO_2–$PaCO_2$ gradient taken concurrently with elevated versus non-elevated MC-FGT measurements from patients without a CTA-proven PE.
** = P < 0.05*

6.4.4 TF expression in monocyte subsets of COVID-19 patients in the ICU

It has previously been shown that TF expressed by monocytes is elevated in PBMCs from patients with COVID-19 [8]. These were specifically monocytes with high CD16 expression, comprising the non-classical and intermediate subtypes. To determine whether TF measured immunocytochemically correlated with the functional coagulant properties measured using MC-FGT, cell-surface staining was performed on monocytes from COVID-19 ICU patients for CD14, CD16 and TF.

Classical, non-classical and intermediate monocyte subsets were gated using CD14 and CD16 markers (Figure 5A,B). A longitudinal analysis of monocyte subsets demonstrated increased proportions of non-classical and intermediate monocytes at the closest timepoint before PE, which normalised at the post-PE timepoints (Figure 5C). Patients without a proven PE also had increased proportions of these monocytes at timepoints corresponding to both peak and nadir MC-FGT results (Figure 5C).

Patients with proven PE and those without proven PE had similar average levels of CD16-expressing monocytes; however, patients with proven PE had a greater

proportion of non-classical monocytes, whereas intermediate monocytes were overrepresented in patients without proven PE (Figure 5D).

There was no positive correlation between immunocytochemically measured TF or CD16 expression on the monocyte cell surface and functional coagulability measured using the MC-FGT assay (Pearson's R^2 = 0.02 and 0.06, respectively). However, there was highly significant co-expression of TF and CD16 in monocytes (Figure 5E).

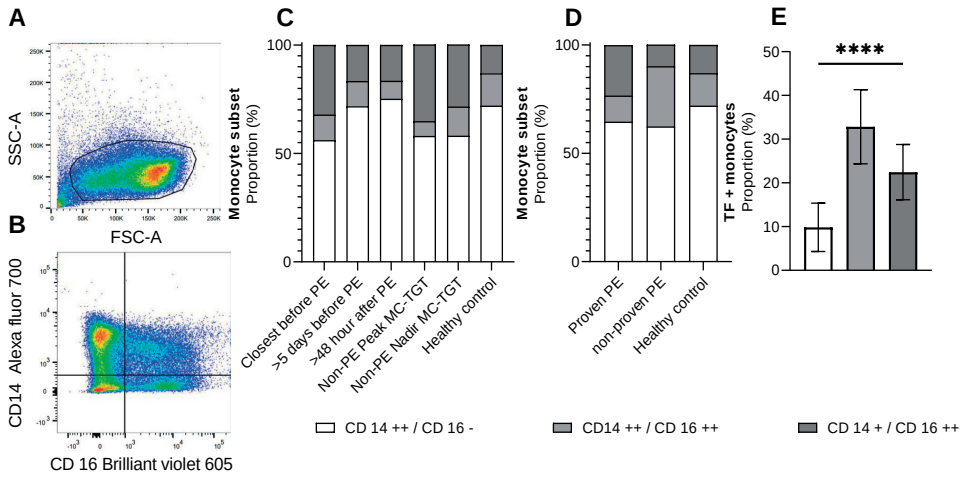


Figure 5. Relative tissue factor expression in classical (CD14++/CD16-), intermediate (CD14++/CD16++) and non-classical (CD14+/CD16++) monocyte subsets, determined by flow cytometry using cell-surface staining of monocytes isolated from COVID-19 ICU patients' monocytes.

A. Forward-sideward scatter plot of MACS-separated monocytes of a healthy donor. B. Monocyte subset gating on the same healthy donor. C. monocyte subset distribution in longitudinal analysis groups. D. Average monocyte subset distribution across all timepoints of patients with proven PE, versus non-proven PE. E. Percentage of TF-expressing monocytes in monocyte subsets of COVID-19 ICU patients.

6.5 DISCUSSION

In this study, we investigated whether a functional coagulation assay performed on PBMCs could be used to measure procoagulant changes in patients with COVID-19 that may contribute to immunothrombosis. D-dimer levels were highly elevated in all subjects and at all timepoints and therefore did not discriminate between patients with and without PE. Longitudinal analysis of COVID-19 ICU patients who developed PE during the course of their disease demonstrated an increase in MC-FGT values shortly before the diagnosis was

made radiographically, suggesting that this assay may have future potential as an early diagnostic tool.

Furthermore, when CTA is not possible, a combination of MC-FGT and the PaCOα–ETCOα gradient could potentially assist in identifying patients in whom therapeutic anticoagulation should be considered at an earlier stage. Peak MC-FGT results were higher in patients who developed PE compared with those without a diagnosis of PE during their ICU stay, although MC-FGT levels were elevated above healthy-control levels in both groups.

There is a distinct possibility that PE was missed in some patients in this cohort for several reasons: 1. obtaining CTA scans in mechanically ventilated patients is not always feasible because of the risks associated with transportation, particularly in patients ventilated in the prone position; 2. a negative CTA for PE does not exclude the possibility of PE occurring subsequently; and 3. pulmonary microvascular thrombosis may not have been detected by CTA in patients classified as not having PE.

We attempted to address this by correlating MC-FGT with the PaCOα–ETCOα gradient, which is related to ventilation/perfusion mismatch, a hallmark of PE. However, an elevated PaCOα–ETCOα gradient may also occur because of atelectasis or other causes of ventilation/perfusion mismatch [15]. Therefore, combining the PaCOα–ETCOα gradient with an assay assessing the patient's procoagulant state could potentially provide additional diagnostic information.

It is unclear what the relative contribution of different cell types is to the procoagulant effect observed in the MC-FGT assay, although previous work suggests that monocytes and neutrophilic granulocytes are major contributors [4]. We found a dominant presence of CD16+ monocyte subsets, which have previously been implicated in tissue factor-mediated procoagulant changes [4]. We were unable to demonstrate a positive correlation between MC-FGT and tissue factor expression on these monocyte subsets. This may be because the TF antibody used binds both active and inactive forms of TF, while TF released in microvesicles is not detected by staining of the cell membrane.

In addition to expressing TF, neutrophils undergo NETosis during thrombus formation, which is also likely to contribute to pulmonary thrombosis. Previous work has demonstrated high levels of neutrophil extracellular traps (NETs) in patients with severe COVID-19, including patients from this cohort [16,17]. Very little is known about the extent to which these cell types regulate immunothrombotic processes through expression of inhibitory proteins

such as tissue factor pathway inhibitor (TFPI) [18]. A more mechanistic approach is required to determine the precise role of monocytes in pulmonary immunothrombosis.

Due to the limited sample size, we were unable to use the MC-FGT assay at the scale required to evaluate its utility as a potential diagnostic tool. A prospective cohort study in ICU patients with severe infectious diseases who are at risk of developing immunothrombotic pathology could be used to evaluate novel approaches to diagnosing PE, including functional cell-based coagulation assays such as MC-FGT. The use of fresh PBMCs, combined with a simplified isolation protocol such as erythrocyte lysis or simple centrifugation and resuspension in buffer, could make this assay more feasible for routine diagnostic use in the future.

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7

HIV-associated and Idiopathic-acquired Haemophilia A – A single-centre case series from Cape Town, South Africa

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7.1 Abstract

Acquired haemophilia A is a rare coagulation disorder, which can lead to life-threatening haemorrhages if not identified and treated promptly. It is characterised by the presence of autoantibodies (inhibitors) to factor VIII. Acquired haemophilia A associated with HIV is a rare but well-described phenomenon, with limited directions to its management. We comparatively describe four patients – two with HIV and two without – that presented with unusual bleeding episodes, with a prolonged activated partial thromboplastin time, secondary to factor VIII inhibitors. An empiric observation is that the patients with acquired haemophilia A associated with HIV had higher antibody titres at presentation, that required more prolonged immunosuppressive therapy to induce remission.

7.2 INTRODUCTION

Acquired haemophilia A (AHA) is a rare coagulation disorder with a reported incidence of 1.20–1.48 million/year [1,2]. The condition is characterised by the production of autoantibodies to factor VIII (FVIII), leading to inhibition of FVIII. Early detection is essential, as most patients develop severe haemorrhage leading to considerable morbidity and mortality [3].

The most commonly associated conditions are other autoimmune diseases, malignancies, pregnancy, specific pathogens (e.g. hepatitis B and C, human herpes virus 8) and drug exposure (including antibiotics, clopidogrel and interferon), although in approximately 50% of patients, the appearance of these autoantibodies is idiopathic [3,4].

Acquired FVIII inhibitors associated with human immunodeficiency virus (HIV) infection, either with or without hepatitis C virus (HCV) co-infection, have been described by several case reports in the literature [5–10]. South Africa has the largest HIV epidemic in the world, with a prevalence of 18.9% [11]. The rare association of AHA with HIV infection is therefore relatively more prevalent in South African patients and experience in how to treat them, essential.

We present here a case series of the two HIV-positive patients, as well as the two HIV-negative patients, with AHA diagnosed and treated by the haematology unit at Groote Schuur Hospital in Cape Town, South Africa, from 2011-2019.

What was known about this topic?

AHA is a rare bleeding disorder caused by the formation of FVIII inhibitors, which are autoantibodies against FVIII. If left untreated, the condition can lead to life-threatening haemorrhage. About half of all cases are idiopathic. The remaining cases are associated with autoimmune diseases, pregnancy, malignancies, infections and certain medications. Several case reports of HIV-associated AHA have been published.

What does this add?

The HIV-associated AHA patients in this sample presented with a higher antibody titre and required a longer time on treatment to induce remission, compared with the idiopathic AHA patients. This observation correlates with other reports of HIV-associated AHA in the literature. Due to the rarity of this condition, recording and registration of cases is of great importance, to improve understanding and future patient care.

South African patients and experience in how to treat them, essential.

We present here a case series of the two HIV-positive patients, as well as the two HIV-negative patients, with AHA diagnosed and treated by the haematology unit at Groote Schuur Hospital in Cape Town, South Africa, from 2011–2019.

7.3 CASES

We suspected a diagnosis of AHA in all patients when a prolonged activated partial thromboplastin time (aPTT), which did not correct with mixing studies, was observed. Subsequently, we found decreased FVIII activity and presence of FVIII inhibitors, confirming the diagnosis. Platelet count and prothrombin time (PT) were characteristically normal in all patients.

There is no formalised treatment protocol for AHA in South Africa and management is determined by local expertise, dependent on both availability and cost of drugs. Once haemostasis is achieved, the focus of therapy is inhibitor eradication. In our setting we endeavour to achieve immunosuppression with corticosteroids and an alkylator agent such as cyclophosphamide. As the public health sector in South Africa does have significant resource constraints and administration of biologic immune modulators (e.g. rituximab), as well as bypassing agents, are generally reserved for refractory cases, due to their high cost. See table 1 for an overview of all patients.

7.3.1 HIV-positive patients

Patient 1

Patient 1 was a 43-year-old female, diagnosed with HIV and started on antiretroviral drugs (ARVs; stavudine, lamivudine, and efavirenz) two years before presentation. She was also receiving rifampicin, ethionamide, ethambutol and pyrazinamide for isoniazid-resistant pulmonary tuberculosis, diagnosed two months prior to presenting. At presentation, her CD4 count was 118 cells/μl and HIV viral load (VL) was undetectable.

She presented with bilateral knee haemarthrosis and easy bruising with a duration of two weeks. Initial laboratory investigations showed aPTT of 140.1 seconds (s), which did not correct on mixing studies, and haemoglobin (Hb) of 3 g/dl. Subsequently, we observed a FVIII inhibitor titre of 218 BU/ml and FVIII levels of 0 IU/dl.

Table 1. Patient characteristics and outcomes.

Patient	Age/ Sex	Year of Dx	Ass. illness	Symptoms	aPTT (seconds)	Min. Hb (g/dl)	FVIII (IU/ dl)	FVIII Inhibitor (BU/ml) at diagnosis	Bypassing agent needed (FEIBA)	Treatment	Time to absence of FVIII Inhibitor (months from starting Rx)
1	43/F	2011	HIV	Knee haemarthrosis, easy bruising	140.1	3	0	218	No	Steroids, cyclophosphamide, chlorambucil	9 (off all treatment after 15 months)
2	45/F	2015	HIV/ IRIS	Haematuria, muscle haematomas, easy bruising	150.3	5.9	0	629.5 (peaked at 1,106)	Yes	Steroids, cyclophosphamide, rituximab	7 (off all treatment after 4.5 years)
3	50/M	2018	None	Neck haematoma, malaena stool	172.5	7.1	2.9	172.65	Yes	Steroids, cyclophosphamide	1 (off all treatment after 6 months)
4	84/F	2019	None	Neck haematoma	117.4	10.7	0.8	33.67	No	Steroids, cyclophosphamide	2 (currently on steroids and cyclophosphamide; 9 weeks on Rx)

aPPT = activated partial thromboplastin time; BU = Bethesda units; Dx = diagnosis; F = female; FEIBA = factor eight inhibitor bypassing activity; FVIII = factor VIII; HIV = human immunodeficiency virus; IRIS = immune reconstitution inflammatory syndrome; M = male; Rx = treatment

Testing for antinuclear antibody (ANA), anti-double stranded DNA antibodies (anti-dsDNA), lupus anticoagulant and hepatitis B and C were all negative.

She received red cell transfusions and was given methylprednisolone intravenously (IV) at 500 mg/day for four days, which was then changed to oral prednisone at 1.5 mg/kg.

The patient experienced no further bleeds, but a month later was given cyclophosphamide (1 g IV weekly x 2 doses) as her aPTT, although much improved, was still prolonged and she still had a high FVIII inhibitor titre (47.7s and 54.56 BU/ml, respectively).

One week later, her aPTT had increased again to 60 s; thus, chlorambucil at a dose of 6 mg daily was commenced.

Over the following months, corticosteroids were tapered and aPTT and FVIII inhibitor levels followed a downward trend. Nine months after commencing immunosuppressive therapy, inhibitors were absent and corticosteroids were stopped. All signs of bleeding, including the haemarthrosis, had fully resolved. Chlorambucil was continued for a year in total. The patient is seen for follow-up annually and has had no relapse to date – eight years after achieving remission.

Patient 2

Patient 2 was a 45-year-old female who had been diagnosed with HIV and started on ARVs (tenofovir, emtricitabine and efavirenz) two weeks prior to presenting. Her CD4 count at presentation was 144 cells/µl and HIV VL was undetectable at presentation.

She presented with a several-day history of painless haematuria, easy bruising and large haematomas to the lower limbs. Initial laboratory investigations showed aPTT of 150.3 s, which did not correct on mixing studies, and Hb of 5.9 g/dl. Subsequently, we observed a FVIII inhibitor titre of 629.5 BU/ml and FVIII levels of 0 IU/dl. ANA, anti-dsDNA, lupus anticoagulant and viral hepatitis studies were negative. We also identified tenofovir-induced nephropathy, which resolved when ARVs were changed to stavudine, lamivudine and efavirenz.

Patient 2 received blood transfusions and one dose of methylprednisolone 125 mg IV, followed by prednisone at 1.5 mg/kg. Symptoms began resolving and aPTT improved until one week later, when her steroid dose was weaned to 1 mg/kg and she experienced a re-bleed and prolongation of aPPT to above 150 s. This resolved when the original steroid dose was resumed.

Patient 2 experienced a re-bleed into her thigh muscle requiring a blood transfusion six weeks later, and cyclophosphamide 100 mg daily was added to her treatment. At this point, the FVIII inhibitor level had increased to 1103.6 BU/ml. She also required two doses (50 U/kg each) of FEIBA (Factor Eight Inhibitor Bypassing Activity), due to failure of the haematoma to resolve.

One month later, another attempt to wean her steroid and cyclophosphamide doses induced bilateral buttock haematomas, requiring multiple blood transfusions and, over the course of four days, five doses of FEIBA (50 U/kg each). At this stage, due to failure to wean immunosuppressive therapy as well as the need for repeated costly blood transfusions and use of bypassing agent, rituximab was given (375 mg/m2 per week for four doses). Several days after receiving the first dose of rituximab, the buttock haematoma worsened and she received eight more doses of FEIBA at 25-50 U/kg over the course of 12 days. The patient was discharged and an attempt was made to taper the steroid dose again.

The patient suffered one more re-bleed a month later, a thigh haematoma requiring one dose of FEIBA (50 U/kg). However, aPTT continued to improve and eventually, seven months after diagnosis and two months after rituximab administration, FVIII inhibitors were absent with aPTT and FVIII levels fully normalised.

Immunosuppressive therapy dosage was tapered and, four-and-a-half years after diagnosis, discontinued. FVIII inhibitors did recur asymptomatically at low titres (1.48 and 0.49 BU/ml) on two occasions: six months after receiving rituximab at 1.48 BU/ml (at the time, the patient was on prednisone 0.5 mg/kg and the inhibitors resolved when the dose was increased to 0.6 mg/kg for three weeks); and again 11 months after rituximab (the patient was on prednisone 0.5 mg/kg; this time the inhibitors resolved without any dose adjustment two weeks later).

7.3.2 HIV-negative patients

Patient 3

Patient 3 was a 50-year-old male taking enalapril, hydrochlorothiazide and amlodipine for hypertension for more than 10 years.

He presented with a several-day history of a midline neck haematoma and melena stool. aPTT was found to be 172.5 s and Hb 7.1 g/dl. FVIII inhibitors were present at 172.5 BU/ml and FVIII levels were 2.9 IU/dl. ANA, anti-dsDNA

antibodies, anti-cardiolipin antibodies, HIV, and hepatitis B and C testing were all negative. Lupus anticoagulant was weakly positive: patient/control ratio 1.28 (normal <1.2).

The patient received FEIBA 50 U/kg daily for three days and the neck haematoma stabilised (the airway was never compromised) and melena resolved. Tranexamic acid was also given (1 g IV three times daily). He was started on prednisone 1.5 mg/kg and cyclophosphamide 100 mg daily.

His aPTT decreased to 39 s the following week and he was discharged on the above medication. An absent inhibitor screen was found a month after initiating treatment and his immunosuppressive medication was tapered over the following six months, with no recurrence of symptoms.

Patient 4

Patient 4 was an 84-year-old female with a long history of hypertension, dyslipidaemia, chronic obstructive pulmonary disease (COPD) and hypothyroidism of unknown aetiology. Her chronic medication was amlodipine, furosemide and simvastatin, inhaled salbutamol, budesonide and levothyroxine.

She presented with a six-week history of a sub-lingual haematoma that had spread to the anterior neck but was fortunately not compromising the airway. aPTT was 117.4 s and Hb 10.7 g/dl. FVIII inhibitors were present at 33.67 BU/ml and FVIII levels were 0.8 IU/dl. No other testing was done for autoimmunity.

She was started on methylprednisolone 80 mg IV daily and the haematoma soon began improving. This was changed to prednisone 1 mg/kg and cyclophosphamide 100 mg daily, one week later.

The haematoma resolved with no recurrence of symptoms and FVIII inhibitors were absent two months after initiating therapy. The patient is still on immunosuppression therapy nine weeks after being discharged, with dosage being tapered.

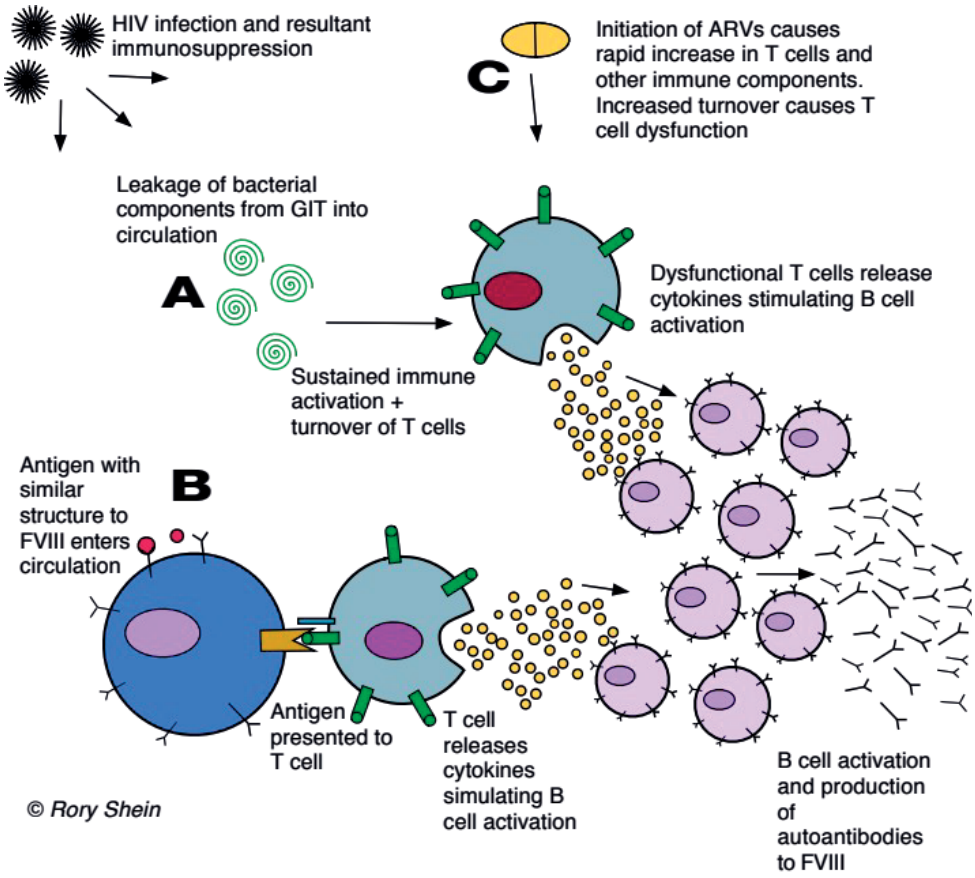


Figure 1. Schematic representation of possible mechanisms for the production of FVIII inhibitor in HIV (refer to text for explanation of pathways A, B and C).

ARVs = antiretroviral drugs; FVIII = Factor VIII; GIT = gastrointestinal tract; HIV = human immunodeficiency virus

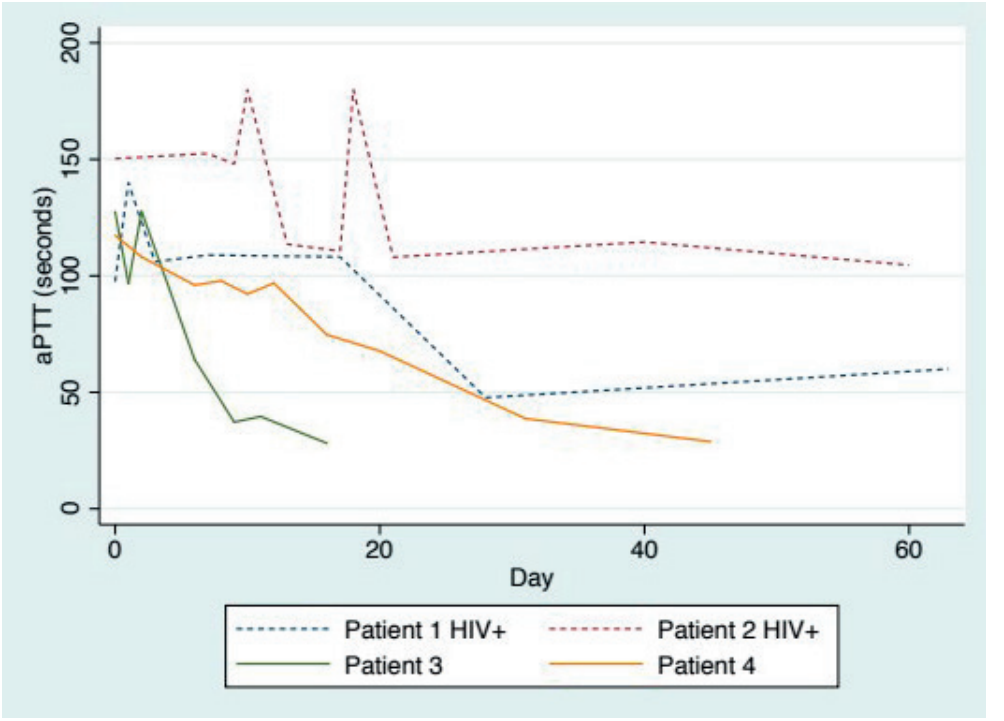


Figure 2. aPTT versus time of the HIV-positive (patient 1 and 2) and HIV-negative patients (patient 3 and 4).

aPTT = Activated partial thromboplastin time; HIV = human immunodeficiency virus

Table 2. Comparison of patients in this series with reported literature.

Study	Associated conditions/ medications	Factor VIII Inhibitor Titre at presentation (BU/ml)	FVIII activity
A) HIV-positive patients in present study and case reports of HIV-positive AHA patients			
Present study	**HIV on ARVs**		
Patient 1 (HIV+)		218	0 IU/dL
Patient 2 (HIV+)		1,106 (peak level)	0 IU/dL
Shweiber, et al. (2005) [5]	HIV, HCV. On ARVs, IFN-α	> 20	2%
Paul, et al. (2007) [6]	HIV, HCV. On ARVs, IFN-α	800	NR
Migliore, et al. (2009) [7]	HIV, on ARVs	278	<1IU/dL
Zeichner, et al. (2013) [8]	HIV, HCV, not on ARVs	230,4	<1% (80–150)
Rattanathammethee, et al. (2014) [9]	HIV on ARVs	140	0,7% (60–150)

Table 2. Continued

Study	Associated conditions/ medications	Factor VIII Inhibitor Titre at presentation (BU/ml)	FVIII activity
Rivoisy, et al. (2014) [10]	**HIV not on ARVs**		
Patient 1		11	<1%
Patient 2		160	<1%
B) HIV-negative patients in present study and registry-based studies of AHA from all causes			
Present study Patient 3 (HIV-)	None	172.65	2.9 IU/dl
Patient 4 (HIV-)		33.67	0.8 IU/dl
Collins, et al. (2007) [2] n = 154	All causes	Median: 7.2 (Range: 1.4–219) *(fatal bleeds only)*	Median: 4 IU/dl (Range: 1–12) *(fatal bleeds only)*
Knoebel, et al. (2012) [4] n = 501	All causes	Median: 12.8 (IQR: 4.3–42.4)	Median: 2 U/dl (IQR: 1–5)

A) HIV-positive AHA patients in present study compared with previously reported cases.

B) Non-HIV-infected AHA patients in present study compared with two registry-based studies of AHA from all causes.

IFN = Interferon; IQR = interquartile range; HCV = Hepatitis C virus; HIV = human immunodeficiency virus; NR = not r

7.4 DISCUSSION

The precise mechanism for the appearance of FVIII inhibitors, usually a population of polyclonal IgG autoantibodies directed to FVIII12, is not known.

In HIV, the breakdown of T cell immune regulation and subsequent immune dysfunction allows for the development of autoimmunity, and numerous autoimmune conditions are associated with the disease. Various mechanisms of how this occurs have been postulated, including (figure 1):

A. Long-term, sustained immune activation (due to leakage of bacterial components through the gut mucosa), caused by a dramatic decline in CD4+ T lymphocytes at this site seen early in HIV infection [13], causing increased turnover and dysfunction of non-infected T cells. This leads to abnormal production of cytokines, causing the activation of B cells and increased production of immunoglobulins [14].

B. Molecular mimicry of a self-antigen from an infectious agent, where T or B cells that react to pathogen-derived peptides cross react with host self-peptides [9].
C. The immune dysfunction seen in patients with immune reconstitution inflammatory syndrome (IRIS) recently started on ARVs. This has been well described as a cause for autoimmune thyroid disease [15,16].

The development of AHA in patient 2 could have been due to IRIS, as she had started ARVs two weeks prior to developing FVIII inhibitors and had experienced a rapid decline in viral load during that time.

Most patients with AHA experience soft tissue, muscle and mucosal (gastrointestinal tract, epistaxis, urinary tract) bleeds, while bleeding into the joints is rare (as opposed to the bleeding pattern in congenital haemophilia) [3]. Interestingly, this was not the case with patient 1, whose primary sites of bleeding were both knee joints.

The HIV-positive patients in this series had a significantly higher FVIII inhibitor titre and lower FVIII level than the HIV-negative patients, and had a much longer and more complicated course of treatment to induce their remission (see figure 2). This reflects existing data that shows that higher inhibitor titre and lower FVIII at diagnosis are associated with a longer time to remission [17]. This correlates with other case reports of HIV-infected AHA patients, as well as with British and European multicentre registry-based studies of patients with AHA from all causes (see table 2). Inhibitors also developed at a younger age in the HIV-positive patients. All four patients in this case series survived and responded to immunosuppressive treatment.

As mentioned above, treatment of AHA patients in the SA public health sector is especially challenging due to resource constraints. Biologic immune modulators need special motivation and FEIBA is reserved for patients with non-resolving bleeds. As can be seen from patient 2 above, the delay in administering rituximab led to slower resolution of symptoms and the need to use more FEIBA, ironically resulting in far more treatment costs than if it had been given sooner. This case series will serve to better inform the "balancing act" necessary to ensure efficient resource allocation while also optimising individual patient care.

7.5 CONCLUSION

AHA is a rare autoimmune condition, and in our setting, associated with HIV infection in two of four patients. Early detection, as well as prompt initiation of treatment is essential, and for one patient in our series, timid initial use of scarce resources may have led to higher eventual costs. All patients survived without permanent morbidity. In this small cohort, the association with HIV infection seemed to portend a longer pathway to remission. This observation also correlates with other cases of HIV-associated AHA reported in the literature. Due to the rarity of the disease, it is vital to record and register cases to improve understanding and optimise future patient care.

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Part IV: Treatment Considerations of Haematologic Malignancies in a High-infectious Disease Setting

8

Predictors of Poor Haematopoietic Stem Cell Mobilisation in Patients with Haematological Malignancies at a South African Centre

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8.1 Abstract

Background: Autologous stem cell transplant (ASCT) is an established consolidation strategy in the treatment of haematological malignancies, however, poor mobilisation (PM) can contribute to patient morbidity and high resource utilisation. Identifying the incidence, risk factors for PM and engraftment outcomes are important goals in our resource-limited setting.

Methods*:* We retrospectively analysed patients with haematological malignancies that consecutively underwent ASCT at Groote Schuur Hospital, Cape Town, South Africa, from January 2013 to January 2019.

Results*:* 146 patients – majority with multiple myeloma (MM) (41.8%), F:M = 1:2, underwent leukapheresis with median age of 32 years (range 9–66 years). PM occurred in 25/146 (17%), mobilisation failure (MF) in 3/146 (2%) and super mobilisation (SMs) in 99/146 (68%), respectively. Risk factors for PM were diagnosis of acute leukaemia (RR = 25, 95% CI 3.4–183, p = 0.002) and Hodgkin's lymphoma (RR = 19, 95% CI 2.6–142, p = 0.004); low white cell count (WCC) at harvest (WCC < 9 × 109/L (RR = 4.3, 95% CI 2.3 – 8.3, p < 0.0001) and two versus one line of prior therapy (RR = 3.1, 95% CI 1.45 – 6.7, p = 0.0037). Median days to neutrophil and platelet engraftment were 14 days (95% CI 14–15 days) and 16 days (95% CI 15–16 days), respectively.

Conclusion*:* PM occurred in 17% of a contemporary South African ASCT cohort, albeit with a low MF rate (2%). There was surprisingly high rate (68%) of SMs, possibly reflective of superfluous mobilisation strategy in MM patients. We identified predictive factors for PM that will lead to enhanced cost-effective use of plerixafor**.**

8.2 INTRODUCTION

Peripheral blood haematopoietic stem cells (PBSCs) are used to restore haematopoiesis more rapidly after high-dose chemotherapy in treatment of multiple myeloma (MM) [1], non-Hodgkin's lymphoma (NHL) [2], Hodgkin's lymphoma (HL) [3] and good- or intermediate-risk acute myeloid leukaemia (AML) [23,30]. Mobilisation of haematopoietic stem cells (HSC) from the bone marrow to the peripheral blood can be performed by two methods: administration of granulocyte-colony stimulating factor (G-CSF) alone or by combining chemotherapy with G-CSF administration, both followed by leukapheresis [4].

It is well established that engraftment rates are influenced by the CD34+ stem cell dose infused and that a minimum dose of 2 × 106 CD34+ cells/kg has been shown to be safe for successful haematopoietic recovery [5,6], which in turn has a direct impact on the duration of hospital stay and blood product utilisation. However, some patients fail to harvest an adequate amount of CD34+ cells after mobilisation [5,7] ("poor mobilisation" [PM]) leading to a transplant delay or cancellation, and increased healthcare resource utilisation. It is widely accepted that the peripheral blood CD34+ cell count (PBCD34) prior to apheresis is the most robust predictor of stem cell mobilisation [8,9]. However, several studies have also identified some factors associated with PM which include: the amount of prior myelotoxic chemotherapy and radiotherapy [10–12], advanced age [10,11, 13], diabetes mellitus [14], bone marrow involvement at diagnosis [5,15] and thrombocytopenia [13,16,17].

Identifying these factors can candidates for strategies such as the pre-emptive administration of plerixafor [18] – a molecule that inhibits the binding of chemokine receptor-4 with stromal-cell-derived factor-1, resulting in the enhanced release of CD34+ cells in the peripheral blood. Current guidelines recommend the pre-emptive use of plerixafor based on PBCD34 cell count monitoring, as it appears to prevent mobilisation failure (MF) and may avoid unnecessary use of plerixafor [19], which is very expensive. "Super mobilisers" (SM) are patients that mobilize more CD34+ cells (> 8–10 × 106/kg) that is required for a single or tandem autologous haematopoietic stem cell transplant depending on the specific disease [20]. Infusing > 8 × 10^6/kg CD34+ cells and whether it has a positive impact on patient outcome is currently not known [21]. Therefore, avoiding SM can also lead to more optimal use of the apheresis procedures and save costs.

At our high-volume public transplant unit, identifying the burden of PM and SM, the risk factors that influence PM in our context and the engraftment kinetics will lead to more optimal use of the costly apheresis procedure and plerixafor use – important goals in our resource-limited setting.

Our objective was to determine these factors. Therefore, we performed a retrospective review of all patients with haematological malignancies that underwent PBSC mobilisation and collection over a seven-year period.

8.3 MATERIALS AND METHODS

8.3.1 Patients

Between January 2013 and January 2019, 146 patients diagnosed with MM (risk unknown), 19 with NHL (four Burkitt lymphoma, five diffuse large B cell five mantle cell lymphoma, two peripheral T-cell lymphoma, one plasmablastic lymphoma and one primary CNS lymphoma) and 32 with HL. Thirty-three patients had acute leukaemia (AL)/lymphoblastic lymphoma (LL), 4 favourable risk-acute myeloid leukaemia (AML), according to ELN classification [22], nine intermediate risk AMLs without a suitable allogeneic donor, three acute promyelocytic leukaemias (APL) in 2nd CR and seven T-cell LLs planned for autologous haematopoietic stem cell transplant (ASCT) underwent PBSC mobilisation and leukapheresis in the haematology apheresis unit at Groote Schuur Hospital (GSH) affiliated to the University of Cape Town.

For the patients with favourable and intermediate risk AML and without a sibling donor available, as per our local protocol, we consolidate with ASCT as we (Novitsky et al.) have previously shown comparable survival rates between recipients of allogeneic versus autologous stem cell transplant. Written informed consent for the PBSC procedure and data collection was obtained from each donor prior to commencement of the procedure. Medical records of all patients (for baseline characteristics and apheresis information) were collected retrospectively for analysis.

This retrospective cohort study was approved by the University of Cape Town Human Research Ethics Committee (HREC number 078/2017).

8.3.2 Mobilisation procedure and collection

All patients planned for ASCT followed the GSH Haematology unit protocol for the mobilisation and collection of PBSCs. Donors received Etoposide 1000 mg/m2 IV for two consecutive days (day one and two of the protocol) in the outpatient

department. G-CSF was commenced at 10 ug/kg daily subcutaneously from day five up to day 15; a split dose regimen of 300 ug twice daily (300 ug Filgrastim vials were used). Patients would be followed up on days nine and 11 to check their blood counts and renal function, assess the patient clinically and to ensure compliance with G-CSF administration. On days 14 and 15 a PBCD34 count was obtained to assess the likelihood of a successful CD34 collection. Patients with a PBCD34 < 10 cells/uL were continued on G-CSF for up to four days depending on the PBCD34 and WCC response. If the PBCD34 reached a plateau despite ongoing G-CSF administration, apheresis was abandoned. Apheresis was performed utilising a continuous flow apheresis system (Spectra Optia, Terumo BCT) through a percutaneous femoral venous catheter.

8.3.3 Enumeration of peripheral blood CD34+ cells

Multiparametric flowcytometry using monoclonal antibodies CD45 (FITC, IOTest, Beckman-Coulter) and CD34 (PE, IOTest, Beckman-Coulter) was performed to immunophenotype circulating CD34+ cells in the peripheral blood. An amount of 200,000 cells were acquired for analysis using a Fc500 flowcytometer (Beckman-Coulter) and CXP software (Beckman-Coulter). The ISHAGE protocol was followed to determine the CD34+ population of cells while dual platform analysis was utilised for CD34+ cell quantification.

8.3.4 Definition of poor mobilisers

Patients that achieved a PBCD34 count of <20 cells/ul at maximal stimulation were defined as PMs. Furthermore, PMs were subdivided: patients with PBCD34 counts between 11 and 19 cells/ul were defined as ‘‘borderline PMs’’, patients with counts between six and 10 cells/ul were defined as ‘‘relative PMs’’, and patients with a PBCD34 <5 cells/ul were defined as ‘‘absolute PMs’’ [12].

8.3.5 Definition of super-mobilisers [31]

Patients in whom a total of > 8 × _106/kg CD34+ cells were collected in the final apheresis bag.

8.3.6 Definition of neutrophil and platelet engraftment [26]

Neutrophil engraftment is defined as the first date when absolute neutrophil count (ANC) of ≥ 0.5 × 10^9/L was achieved for three consecutive days.

Platelet engraftment was defined as the first date of three consecutive days that platelets are ≥ 20 × 10^9/L without platelet transfusions in the last seven consecutive days.

8.3.7 Data analysis

The association between the PBCD34 count and CD34 yield in the apheresis product was evaluated by the Spearman rank correlation. Donor characteristics included in the binomial regression analysis were age, gender, weight, body mass index (BMI), haematological diagnosis and pre-mobilisation blood cell parameters i.e. haemoglobin, platelet count, absolute neutrophil count, absolute monocyte count, absolute lymphocyte count and total white cell counts. In addition, the mid-mobilisation (day nine of mobilisation) blood cell parameters and blood cell parameters on day of apheresis and previous lines of chemotherapy were also analysed. Age and weight were categorised into approximate quartiles. The relative risk (RR) of each variable on PM was determined using binomial regression. Categories with $N < 15$ were not included in analyses as no reliable inference can be made based on such small groups. Donor characteristics/variable significant at $p < 0.15$ were combined into a multivariable model, after examining each pair of variables for possible confounding using the chi-square test (or Fisher's exact test for 2 × 2 tables). A value of Cramer's V (or the phi coefficient for Fisher's exact test) > 0.50 was regarded as too strong an association to include both variables in a multivariable model. Non-significant variables were sequentially removed from the multivariable model. The association between PM and final CD34 harvest, was determined by the Wilcoxon Rank Sum test (since the data did not meet the assumptions of the independent samples t-test) and Fisher's exact test, respectively. The association between PM and days to neutrophil engraftment and days to platelet engraftment was determined by Cox Proportional Hazards regression, with censoring for death. Statistical analysis was carried out using SAS version 9.4 for Windows. A 0.05 p-value for significance level was used. Graphs were created with the software GraphPad Prism Version 8.0.2. and Excel.

8.4 RESULTS

8.4.1 Patient characteristics

Patient and disease characteristics are summarised in Table 1. Between January 2013 and January 2019, 146 consecutive patients were scheduled to undergo leukapheresis for ASCT: median patient age was 45 years (range, 9–66 years), the majority (61%) were male and 67.8% received only one previous line of therapy. Disease groups that underwent ASCT were MM (41.8%), lymphoma (39.7%) and AL (18.5%). Most MM patients (41.8%) achieved a VGPR before transplant, while 28.1% were in CR1 and 30.1% in > CR1. HIV infection affected 3.4% of the entire cohort. The majority (56.8%) had not received radiotherapy prior to ASCT.

Table 1. Patient characteristics.

Variable	Category	n	(%)
Age	9–29y	40	(27.4)
	30–45y	35	(24.0)
	46–55y	45	(30.8)
	56y+	26	(17.8)
Gender	F	57	(39)
	M	89	(61)
Diagnosis	MM	61	(41.8)
	HL/NHL/T-LL	58	(39.7)
	AML	27	(18.5)
Lines of therapy	1	99	(67.8)
	2	26	(17.8)
	> 3	21	(14.4)
Previous radiotherapy	Yes	7	(4.8)
	No	83	(56.8)
	Missing	56	(38.4)
HIV infection	Yes	5	(3.4)
	No	141	(96.6)
Disease status at SCT	CR1	41	(28.1)
	> CR1	44	(30.1)
	VGPR	61	(41.8)

Table 1. Continued

Blood cell parameters on collection day	Median	Range
WBC x 10^9/L	24	(2.83–106.7)
Neutrophils x 10^9/L	14.9	(0.00–69.6)
Lymphocytes x 10^9/L	1.94	(0.39–20.27)
Monocytes x 10^9/L	2.11	(0.01–28.74)
Haemoglobin, g/dL	9.55	(7.00–15.2)
Platelets x 10^9/L	94	(16.00–339)

Abbreviations: F, female; M, male; MM, multiple myeloma; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; T-LL, T-lymphoblastic lymphoma; AML, acute myeloid leukaemia (includes 3 APLs); CR, complete remission; VGPR, very good partial response; HIV, human immunodeficiency virus.

8.4.2 Utility of peripheral blood CD34 (PBCD34) to predict CD34 yield

Figure 1 A illustrates a significantly good correlation between the PBCD34 count on day of apheresis and the amount of CD34+ cells in the final apheresis bag.

Figure 1 B shows that patients who achieved a PBCD34 count > = 20 cells/uL at maximal stimulation could reliably predict a single day apheresis in our cohort.

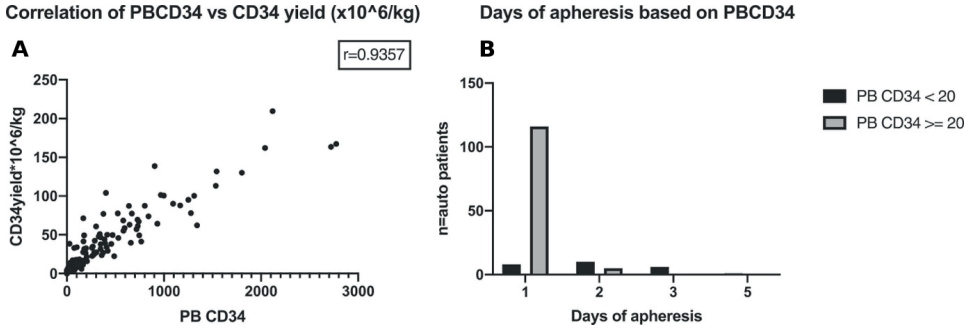


Figure 1. A correlation of CD34 yield with peripheral blood stem cell CD34 count and correlation of peripheral blood CD34 count with days of apheresis.

8.4.3 Incidence and clinical characteristics of PMs

Twenty-five patients were PMs (17.1%): 14 (9.6%) absolute PMs, two (1.4%) relatively PMs and nine (6.2%) borderline PMs. The absolute and relative PMs were young patients with median ages of 27 (range, 16–58) and 39 years (range, 27–51), respectively. For both the absolute and relative PMs, the median previous lines of therapy were two (range, 1–3). The borderline PMs had a median age of 32 (range, 19–53) and a median of one line of therapy prior to ASCT. In all three groups of PMs, the majority were females with the diagnoses of HL and AML. See Table 2.

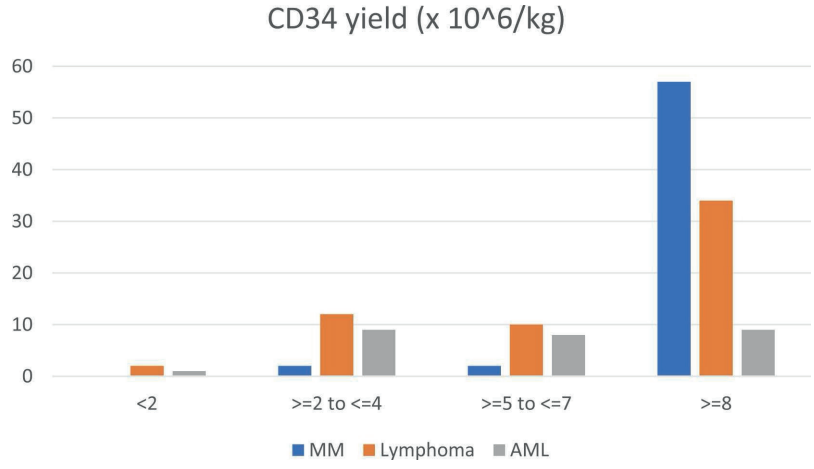
Table 2. Characteristics of poor mobilisers.

Poor mobilisers/ Diagnosis	N = 25 (17%)	Days of apheresis (Med, range)	Minimum target (2 x 10^6/kg CD34) achieved	Sex	Age (med, range)	Prev lines of therapy (med, range)
Absolute PM (<5 PBCD34 cells/ul) - HL = 6 - AML = 5 - NHL = 2 - MM = 1	N = 14 (9.6)	2 (1–5)	Yes = 11 No = 3	F = 10 M = 4	27 (16–58)	2 (1–3)
Relative PM (6–10 PBCD34 cells/ul) - AML = 1 - HL = 1	N = 2 (1.4)	2	Yes = 2 No = 0	F = 1 M = 1	39 (27–51)	2 (2–3)
Borderline PM (11–19 PBCD34 cells/ul) - AML = 5 - HL = 3 - NHL = 1	N = 9 (6.2)	1 (1–3)	Yes = 9 No = 0	F = 4 M = 5	32 (19–53)	1 (1–3)

8.4.4 Spread of super-mobilisers

68% of patients experienced a high CD34 yield (>= 8 CD34 cells x 10^6/kg) in the final apheresis product ("super-mobilisers"). Most of these patients had multiple myeloma (57%), while 34% had lymphoma (both NHL and HL) and 9% had AML (Figure 2).

Figure 2. CD34 yield across different disease groups.



8.4.5 Univariate analysis

The incidence of PM was associated with a diagnosis of AL (AL vs. MM, RR = 25, 95% CI 3.4–183) and HL (HL vs. MM, RR = 19, 95% CI 2.6–142). On day of collection, PM was associated with a lower platelet count (platelets < 90 vs. platelets > = 90, RR = 8.9, 95% CI 2.8–28), lower absolute neutrophil count (< 7.5 vs. > = 7.5, RR = 2.7, 95% CI 1.35–5.4), lower monocyte count (< 0.6 vs. > = 0.6, RR = 2.4, 95% CI 1.05–5.3) and lower total white cell count (< 9 vs. > = 9, RR = 4.3, 95% CI 2.3–8.3). PM was significantly associated with two lines of therapy versus one line (RR = 3.1, 95% CI 1.45–6.7). No significant differences were found for baseline and mid-harvest blood cell parameters.

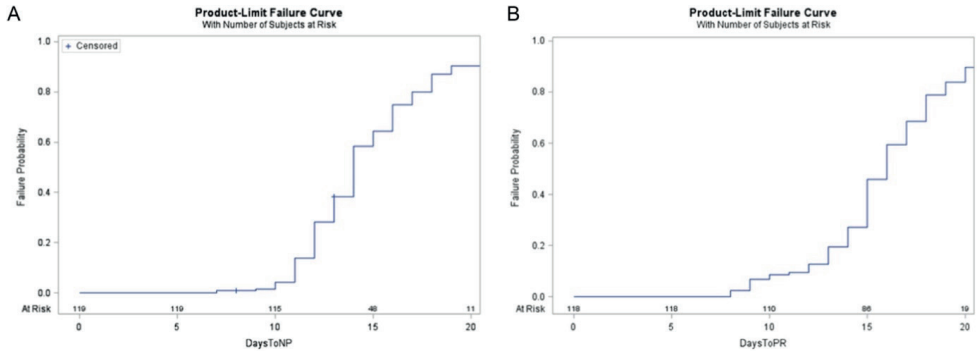
8.4.6 Multivariable analysis

In addition to the variables identified in the univariate analysis, age, mid-harvest haemoglobin, mid-harvest platelet count, mid-harvest absolute monocyte count and the at-harvest haemoglobin were significant at $p < 0.15$ and were thus candidate variables for a multivariable model for PM. Before embarking on the multivariable model, we checked pairs of candidate variables for possible confounding. Eleven variables and 16 parameters were to be estimated, since sample size considerations dictate that n = 25 cases with PM allowed for the estimation of only two parameters. Therefore, we could not fit a multivariable model and instead we considered the effects of each of the significant variables, as shown in the univariate analysis.

8.4.7 Cumulative incidence of neutrophil and platelet engraftment

The median number of days to neutrophil engraftment was 14 days (range, 4–34 days) whereas the median number of days to platelet recovery was 16 days (range, 8–54 days). There were no significant differences in neutrophil ($p = 0.15$) and platelet engraftment ($p = 0.70$) when comparing PM with non-PMs. (Figure 3).

Figure 3. Cumulative incidence of neutrophil (left) and platelets engraftment (right).



8.5 DISCUSSION

In this cohort of patients, we observed a PM rate of 17%, a low rate of MF (2%) and a high rate of super-mobilisers (68%). A diagnosis of AL and HL with low blood cell parameters on day of harvest were identified as risk factors for PM. We show a surprisingly high rate of super-mobilisers in this cohort (68%), which predominantly consisted of MM patients (57%). This can be explained by the exclusion of thalidomide or lenalidomide in our remission induction therapy and the use of high dose etoposide (1000 mg/m2 × 2 days) during CM – this implies an over-effective CM strategy. We should be mindful that high dose etoposide can lead to harmful side-effects like mucositis, hepatic dysfunction and prolonged cytopenias with subsequent life-threatening complications like neutropenic sepsis. Song et al. has previously shown that a lower dose etoposide of 375 mg/m2 during CM for MM to be safe and effective [23]. Therefore, the use of lower dose etoposide should be explored for our MM patients while the higher dose etoposide is appropriate for the lymphoma and AL disease groups.

We confirm the utility of the PBCD34 counts in our cohort, as there is a good correlation between the PBCD34 and CD34 yield. Additionally, if a patient achieved a PBCD34 count > 20 cells/ul at maximal stimulation with CM that they will mostly harvest successfully in a single day – an important goal in our context. On univariate analysis we identified that a diagnosis of AL and HL to be associated with PM. As per our local protocol, favourable and intermediate risk AML patients (without suitably matched HLA donors) receive two cycles of "7 + 3" chemotherapy (seven days cytarabine with three days of daunorubicin) followed by ASCT, as we have previously shown similar outcomes in both autologous and allogeneic stem cell transplant recipients in this context [24]. Shin et al. has previously shown that the stem cell yield linearly decreases after

each consolidation chemotherapy in patients with AML [25] and we confirm this observation. For the patients with HL, the majority had advanced stage disease at diagnosis and were heavily pre-treated prior to referral for ASCT. Both factors are known to be associated with PM [10–12]. Other risk factors identified on univariate analysis were low blood cell parameters at harvest, specifically total white cell count (WCC) < 9, absolute neutrophil count < 7.5, monocyte count < 0.6 and platelet count < 90. This might reflect poor stem cell reserve prior to start of CM leading to a delayed recovery, possibly bone marrow involvement at diagnosis and/or non-compliance with G-CSF during mobilisation. It has previously been shown that there is a poor correlation between total WCC and CD34 yields, making it a less reliable marker to assess start of apheresis [26,27]. If the total WCC is low, the PBCD34 is likely to be low leading some to suggest that PBCD34 should be measured when the WCC exceeds a certain threshold [26,28,29]. Our data show that the probability of achieving an acceptable PBCD34 for successful mobilisation is only achieved when WCC is > 9. This will lead to fewer unnecessary PBCD34 measurements at lower WCCs and therefore save costs.

The three patients that failed harvest (2%) in this series could arguably have benefited from "on demand" plerixafor [18]. Based on the current data we can now identify patients at high risk of MF for which we can apply for its use, specifically for patients with AL or HL with PBCD34 counts at maximal stimulation of < 5 cells/ul. This will lead to more efficient use of this expensive drug while preventing patient morbidity. The engraftment rates for both neutrophils and platelets were good with no significant differences found between the PM and non-PMs. Therefore, we confirm that patients receiving a minimal CD34 dose of 2 × 10^6/kg engraft well in our cohort. Limitations of our study include its retrospective nature and heterogenous sample. However, the homogeneity of the clinical staff in our transplant unit, CM regimen, uniformity of our CD34 apheresis sessions (using the same cell separator) and CD34 enumeration adds additional value.

This is the first retrospective study done on stem cell mobilisation from the only public sector teaching hospital with its own designated apheresis unit in South Africa. In conclusion, we describe a PM rate of 17%, a low MF rate of 2% and a surprisingly high rate of super-mobilisers (68%) reflecting an over-effective CM strategy, especially in MM patients. We confirm the utility of the PBCD34 count and a target of > 20 cells/ul post CM will mostly lead to a single day apheresis. Finally, we identified risk factors for PM that will lead to more rational use of plerixafor.

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9

Is Haplo-identical Haematopoietic Stem Cell Transplantation (HaploHCT) using Post-Transplantation Cyclophosphamide (PTCY) feasible in sub-Saharan Africa?

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Background: Identifying a suitable volunteer-unrelated donor (UD) in South Africa is challenging due to the highly diverse ethnic groups and mixed-race populations in this region. Haplo-identical Haematopoietic Cell Transplantation (HaploHCT) is thus an attractive procedure for patients with high-risk haematological malignancies.

Study design: We retrospectively analysed the outcome of 134 patients with haematological malignancies who received unmanipulated haploHCT with Post-Transplant Cyclophosphamide at two high-volume HCT centres between 2014 and 2019. We assessed overall survival (OS), disease-free survival (DFS), non-relapse mortality (NRM), relapse incidence (RI) and incidence of acute graft-versus-host disease (GVHD).

Results: Median recipient and donor age was 44 years (range, 15–73) and 36 years (range, 9–68), respectively. Acute myeloid leukaemia or myelodysplastic syndrome (AML/MDS) and acute lymphoblastic leukaemia (ALL) were the most common indications for haploHCT (61.2%). The EBMT risk score was $\geq$ 5 in 44 patients (32.8%), and 77 patients (57.4%) received myeloablative conditioning regimens. The majority of patients (57.4%) received gender-matched transplants and peripheral blood stem cells (PBSC) (70.9%) as cell source. Sixteen patients (11.9%) had an incongruent CMV serostatus at transplant. Median follow up was 10.8 months (range 0.36–70.8).

OS at one and three years was 56% (95% CI 47–64) and 37% (95% CI 28–47), respectively. DFS at one and three years was 47% (95% CI 38–55) and 32% (95% CI 24–41), respectively. The 100-day and three-year cumulative incidence of NRM was 18% (95% CI 11–25) and 41% (95% CI 32–50), respectively, whereas the one- and three-year cumulative relapse incidence (RI) was 16% (95% CI 11–24%) and 21% (95% CI 14–29), respectively. The one-year OS for AML/MDS was 55% (95% CI 40–67) versus 41% (95% CI 21–60) for ALL. Forty-five patients (41.7%) developed a GVHD by day 100; of these, 80% had grades I & II disease.

In multivariable analysis, older donor age was an independent risk factor for lower DFS. RI was higher for diagnoses other than acute leukaemia/MDS (RR = 2.62; 95% CI 1.12–6.15; p = 0.027), decreased for PBSC versus BM (RR = 0.43; 95% CI 0.19–0.95; p = 0.038) and decreased for offspring donors (RR = 0.25; 95% CI 0.09–0.67; p = 0.006).

Conclusion: These data support the feasibility of haploHCT and suggest that unmanipulated haploHCT utilising a younger parent or offspring donor are viable options for adults with acute leukaemia and MDS who lack a suitable RD or UD in sub-Saharan Africa.

9.2 INTRODUCTION

Allogeneic haematopoietic stem cell transplantation (HCT) from an HLA-matched related donor (RD) or matched unrelated donor (UD) is a curative option for many high-risk haematological malignancies [1]. Finding a fully HLA- matched family donor occurs in about one-third of patients. Other donor sources available are matched unrelated donors, mismatched unrelated donors, haploidentical donors, and single or double umbilical cord blood units. Haploidential HCT (HaploHCT) has historically been associated with high rates of graft-versus-host disease (GVHD) and graft failure leading to high non-relapse mortality [2,3]. However, using the post-transplant cyclophosphamide (PTCY) approach [4], haploHCT has become more effective, limiting GVHD and treatment-related mortality and leading to its increasing adoption [5]. In addition, a recent meta-analysis confirms its equivalent all-cause mortality and relapse incidence for acute leukaemia in comparison to unrelated donor transplants [6]. HaploHCT is therefore an attractive approach in patients that require an urgent allograft.

The South African Bone Marrow Registry (SABMR) for recruitment of local volunteer-unrelated stem cell (SC) donors, had 73,027 locally registered donors in 2017, of whom the majority were of Caucasian origin. However, the majority of the South African population consists of other ethnic groups and mixed-race backgrounds [7]. Additionally, the potential donor pool is negatively impacted by the high seroprevalence of human immunodeficiency virus (13.1%) in South Africa [8]. The HLA alleles in people of African descent are genetically highly diverse [9]. Therefore, finding a matched related, local unrelated and even international unrelated donor for the South African patient of African or mixed-race descent, is exceptionally challenging. If an international matched UD can be identified, it is often prohibitively expensive, requiring a high-tier private medical insurance to fund the transplant, a benefit that is affordable to only 17% of South Africans [10]. Identification and recruitment of a suitable unrelated donor can also lead to a delay in receiving a time-sensitive transplant. HaploHCT is therefore a potentially meaningful therapeutic intervention for patients in South Africa with high- risk haematological malignancies.

HaploHCT was implemented in 2014 at the two largest public (Groote Schuur Hospital) and private (Pretoria East Netcare Hospital) transplant centres in South Africa. We describe our first five years of experience, focusing on the efficacy and outcome of haploHCT. Our objective was to review all patients with high-risk haematological malignancies that underwent T-cell-replete haploHCT with PTCY. In order to determine the feasibility of this procedure we assessed

the OS, DFS, non-relapse mortality (NRM),T RI, incidence of acute GVHD and the risk factors that influenced these outcomes.

9.3 MATERIALS AND METHODS

9.3.1 Patients

Consecutive patients undergoing haploHCT at Pretoria East Netcare Hospital, and the public-academic centre at Groote Schuur Hospital, University of Cape Town (UCT), from January 2014 to December 2019 were included in this retrospective cohort study. Patients were $\geq$ 15 years of age, with high-risk haematological malignancies undergoing first haploHCT (previous autologous and allogeneic transplants permitted), family donor with $\geq$ 2 HLA mismatches and no matched unrelated donor. Peripheral blood (PB) or bone marrow (BM) or both were used as SC source and GVHD prophylaxis was performed with PTCY [28] without ex vivo T cell depletion. The UCT research ethics committee approved this study (HREC REF: 592/2018) and patient data were captured in a REDCap® database.

9.3.2 Statistical analysis and definitions

The primary aim was to determine patient characteristics, DFS and OS of the cohort. Secondary aims were to determine RI, NRM and the incidence of acute GVHD by day 100 after transplant. Pre-transplantation disease status was evaluated before HCT, which included a bone marrow aspiration and biopsy with flow cytometry and cytogenetics for patients with leukaemia, or computed tomography and/or a positron emission tomography scan for patients with lymphoma. Morphological complete remission (CR) of leukaemia was defined as <5% blasts on BM biopsy and absence of active extramedullary disease. Patients not in morphologic CR were considered to have active disease. Untreated myelodysplastic syndrome (MDS) patients without response to therapy prior to transplant were considered to have active disease regardless of blast count. DFS was defined as time to death or relapse/progression, whichever came first. OS was defined as time to death from all causes. NRM was defined as death without evidence of relapse or progression. Neutrophil engraftment was defined as the first day of three consecutive days when absolute neutrophil count was >= 0.5 x 10^9/L, while platelet engraftment was defined as the first day of three consecutive days when platelet count was > 20 x 10^9/L without a platelet transfusion in the past seven days. Acute GVHD was graded according to the modified Seattle Glucksberg criteria [29]. DFS and OS were estimated by the Kaplan-Meier method [30]. Cumulative incidence (CI) functions were used to estimate engraftment, RI and NRM [31]. The competing risks were specified as

follows: death for engraftment, death for RI and relapse for NRM. The incidence of acute GVHD was estimated for those who had not died. Univariate analyses were performed for OS and DFS using the Cox Proportional Hazards model, while risk factors for RI, NRM and engraftment were analysed using competing-risks Cox Proportional Hazards regression analysis with Gray's test [32,33,34]. The following variables were assessed in the univariate analysis: disease type, disease status at transplant (CR1/2 vs. > CR2), SC source (BM vs. PB vs. PB + BM), intensity of conditioning regimen (myeloablative [MAC] vs. reduced intensity [RIC]), recipient age at transplant (categorised into quartiles), donor age (categorised into quartiles), recipient/donor gender mismatch, recipient/donor cytomegalovirus (CMV) status, donor family relationship, number of previous autologous, matched related or matched unrelated transplantations, and transplant centre. Categories with less than 15 patients overall were not included in analyses. Study variables identified in the univariate analysis that were significant at $p < 0.20$ were combined into the multivariable model, after examining each pair of variables for possible confounding using the chi-squared test (or Fisher's exact test for 2 x 2 tables). A value of Cramer's V (or the phi coefficient for Fisher's exact test) > 0.50 was regarded as too strong an association to include both variables in the multivariable model. Non-significant variables were sequentially removed from the multivariable model. The association between cell source and acute GVHD was determined by Fisher's exact test. Statistical analysis was carried out using SAS version 9.4 for Windows. A 5% significance level was used.

9.4 Results

9.4.1 Patient characteristics

Patient, donor and transplant characteristics are summarised in Table 1. The median recipient age was 44 years (range, 15–73) and the median donor age was 36 years (range, 9–68). AML, MDS and ALL were the most common indications for transplant, comprising 61.2% of the cohort. The EBMT risk score [35] was used to assign disease risk which included 27 patients (20.1%) with a score of less than 2, 63 patients (47%) with a score between 2–4, and 44 patients (32.8%) with a score >= 5. Therefore, 79.8% of patients had high risk disease. Seventy-seven patients (57.4%) received myeloablative conditioning regimens. The majority of patients (57.4%) received a gender-matched donor transplant and peripheral blood stem cells (70.9%) as cell source. Sixteen patients (11.9%) had an incongruent CMV serostatus with their donor at transplant. Median follow up of surviving patients was 10.9 months (range 0.36–70.8).

Table 1. Patient, donor and transplant characteristics. (N = 134)

Characteristic	Value
Age at transplant, median (range), years	44 (15–73)
Patient gender, male, n (%)	83 (62)
Disease, n (%)	
AML and MDS	60 (44.8)
- CR1/CR2	42
- > CR2	18
B and T-ALL	22 (16.4)
- CR1/CR2	18
- > CR2	4
"Other" diagnoses included	
HL	9 (6.7)
- CR1/CR2	3
- > CR2	6
MM	8 (6.0)
- CR	3
- PR/VGPR	5
CML	6 (4.5)
- 2nd CP	2
- AP	3
- BP	1
NHL[a]	17 (12.7)
- CR1/CR2	10
- > CR2	7

Table 1. Continued

Characteristic	Value
CLL	4 (3.0)
- CR1/CR2	1
- > CR2	3
Therapy related myeloid neoplasm	3 (2.2)
- PR	2
- Active disease	1
CMML	3 (2.3)
- Active disease	3
MPN (myelofibrosis)	1 (0.8)
- Active disease	1
Blastic plasmacytoid dendritic cell tumour	1 (0.8)
- CR1	1
EBMT risk score	
1–2	27 (20.1)
2–4	63 (47.0)
>= 5	44 (32.8)
Conditioning	
- MAC-busulphan based	46 (34.3)
- MAC-TBI based	31 (23.1)
- RIC-TBI based	57 (42.6)
Donor age, median (range), y	36 (9–68)
Donor gender, male, n (%)	78 (58)
Donor to recipient gender mismatch	
- Female to male	31 (23.1)
- Male to female	26 (19.4)
- Matched	77 (57.5)
Stem cell source	
- PBSC	95 (70.9)
- BM	36 (26.9)
- PBSC and BM	3 (2.2)
Recipient/donor CMV serostatus	
- Neg/neg	7 (5.2)
- Neg/pos	9 (6.7)
- Pos/neg	7 (5.2)
- Pos/pos	77 (57.5)
- Missing	34 (25.4)
Donor relationship	
- Son	46 (34.3)
- Mother	25 (18.7)

Table 1. Continued

Characteristic	Value
- Brother	24 (17.9)
- Sister	17 (12.7)
- Daughter	14 (10.5)
- Father	5 (3.7)
- Other family member male/female	3 (2.3)
Number of previous transplants	
- 0	110 (82.1)
- 1	19 (14.2)
- 2	5 (3.7)
Centre	
- Groote Schuur Hospital	24 (17.9)
- Pretoria East Netcare	110 (82.1)
Acute GVHD	
- Yes	45 (41.7)
- No	63 (58.3)
Acute GVHD grade	
- I	17 (37.8)
- II	19 (42.2)
- III	6 (13.3)
- IV	3 (6.7)

Abbreviations: AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukaemia; HL, Hodgkin's lymphoma; MM, multiple myeloma; CML, chronic myeloid leukaemia; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukaemia; CMML, chronic myelomonocytic leukaemia; MPN, myeloproliferative neoplasm; PBSC, peripheral blood stem cells; BM, bone marrow; CMV, cytomegalovirus; CR, complete remission; PR, partial remission; VGPR, very good partial response; CP, chronic phase; AP, accelerated phase; BP, blastic phase; MAC, myeloablative chemotherapy; RIC, reduced intensity conditioning; TBI, total body irradiation a NHL includes high grade B cell, low grade B cell and T cell not otherwise specified.

9.4.2 Engraftment

Neutrophils and platelets recovered at a median of 20 (95% CI, 19–21 days) and 29 days (95% CI, 27–33 days), respectively. The cumulative incidence of neutrophil and platelet recovery at day 28 was 87% (95% CI, 80–92%) and 42% (95% CI, 32–51%), respectively, and overall were 90% (95% CI, 83–94%) and 82% (95% CI, 74–88%). Thirteen patients had primary graft failure (9.7%), of whom only one survived. For both neutrophil and platelet engraftment, there was a significantly faster neutrophil (HR = 1.74; 95% CI 1.18–2.59; p = 0.006) and platelet recovery (HR = 1.56; 95% CI 1.03–2.37; p = 0.038) when PBSC were used as cell source in comparison with bone marrow (supplementary table 1).

9.4.3 Disease-free survival and overall survival

The probability of DFS at one- and three-years was 47% (95% CI 38–55) and 32% (95% CI 24–41), respectively, for the entire cohort (Figure 2A). In univariate analysis, lower DFS was associated with older donor age (46–68y vs. 9–25y) (HR 1.90; 95% CI 1.02–3.5; p = 0.43) and remained an independent risk factor in multivariable analysis.

The probability of OS at one and three years was 56% (95% CI 47–64) and 37% (95% CI 28–47), respectively, for the entire cohort (Figure 2B). In univariate analysis no significant risk factors could be identified for OS.

The one- and three-year OS of the ALL group was 41% (95% CI 21–60) and 21% (95% CI 6–42), respectively. In comparison, the one- and three-year OS of the AML/MDS group was 55% (95% CI 41–67) and 43% (95% CI 29–56), respectively, and the one- and three-year OS of the "other" diagnostic group (predominantly lymphoma) was 65% (95% CI 50–76) and 40% (95% CI 26–54), respectively (Figure 2C). There was a trend towards better OS for the AML/MDS group versus the ALL group (p = 0.067).

The one- and three-year DFS of the ALL group was 32% (95% CI 14–51) and 24% (95% CI 8–45), respectively. The one- and three-year DFS of the AML/MDS group was 54% (95% CI 40–65) and 42% (95% CI 28–55), respectively, and the one- and three-year DFS of the other diagnostic group were 46% (95% CI 32–59) and 27% (95% CI 14–41), respectively (Figure 2D). The DFS of the AML/MDS group was marginally significantly better versus the ALL group (p = 0.053).

9.4.4 Non-relapse mortality

The CI of NRM at day 100 and at three years was 18% (95% CI 11–25%) and 41% (95% CI 32–50%), respectively (Figure 3). Table 2 summarises causes and contributory causes of death.

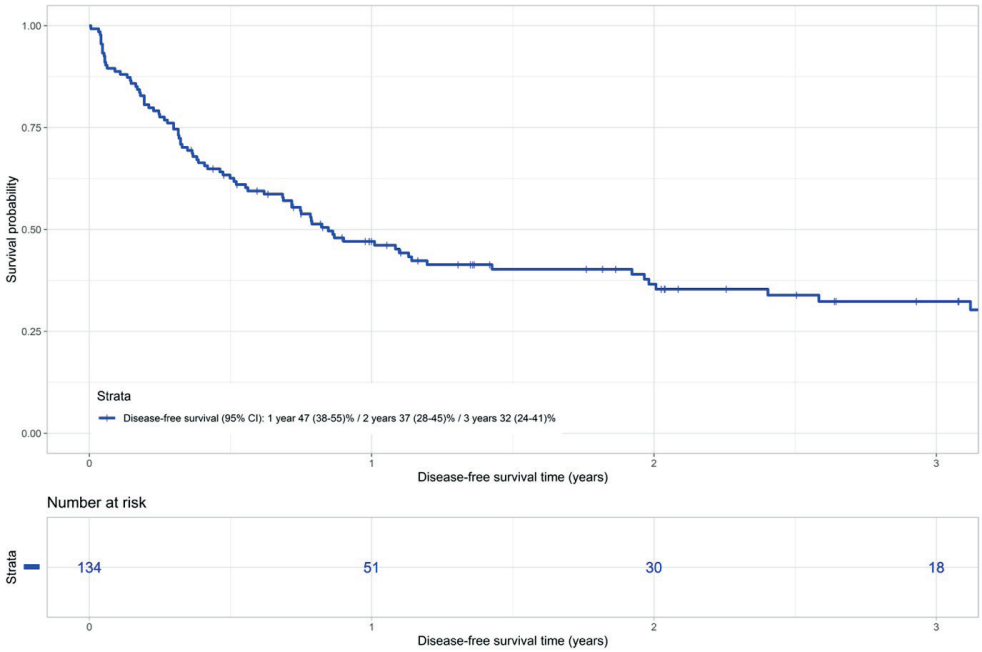


Figure 2A. Disease-free survival of whole cohort of patients.

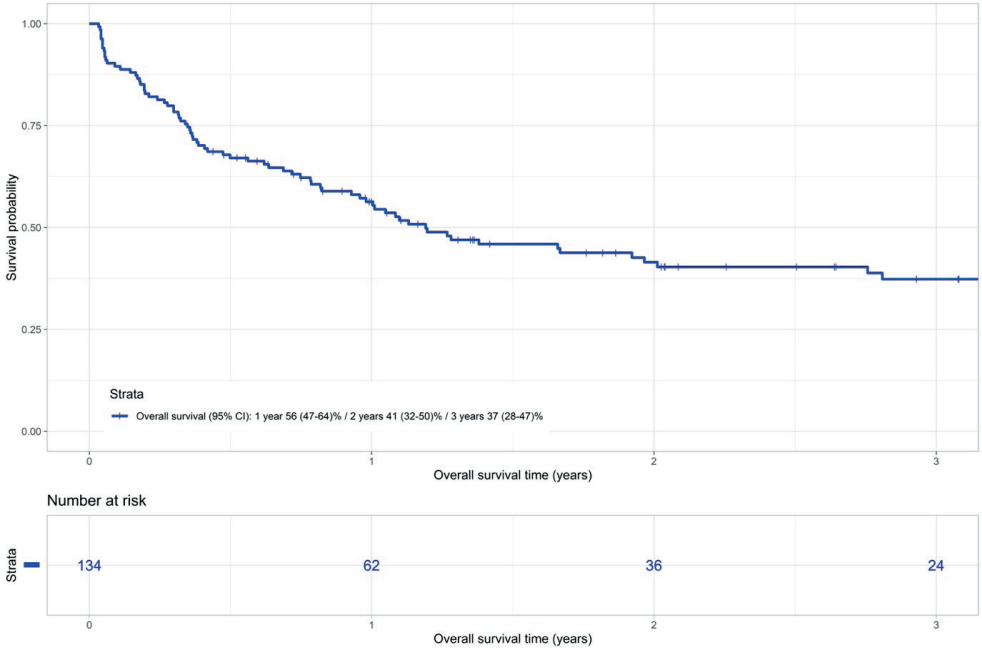


Figure 2B. Overall survival of whole cohort of patients.

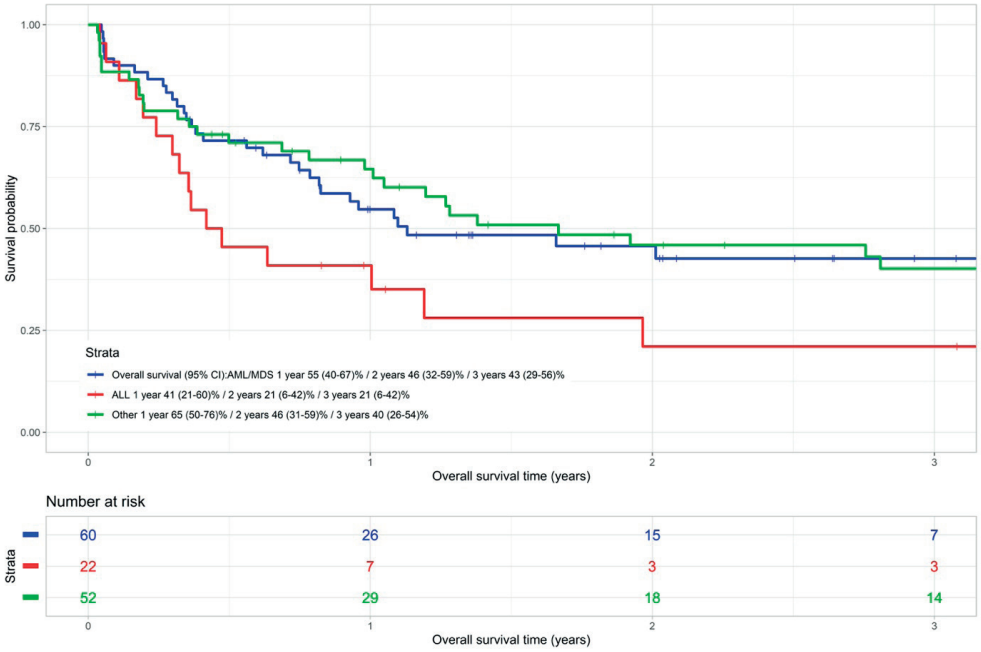


Figure 2C. Overall survival categorised by disease type (P = 0.067).

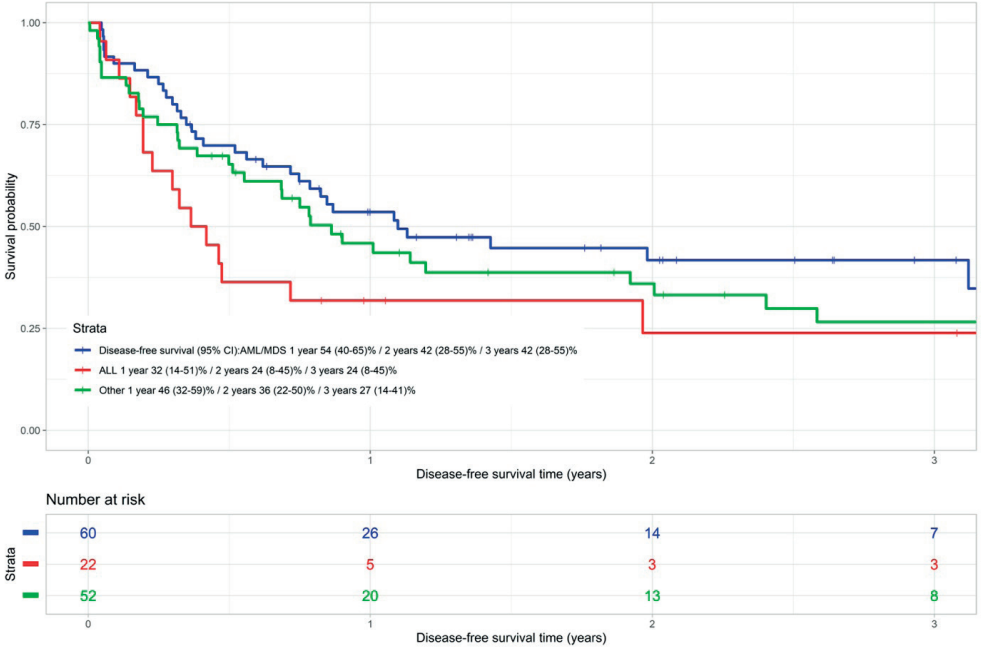


Figure 2D. Disease-free survival categorised by disease type (P = 0.053).

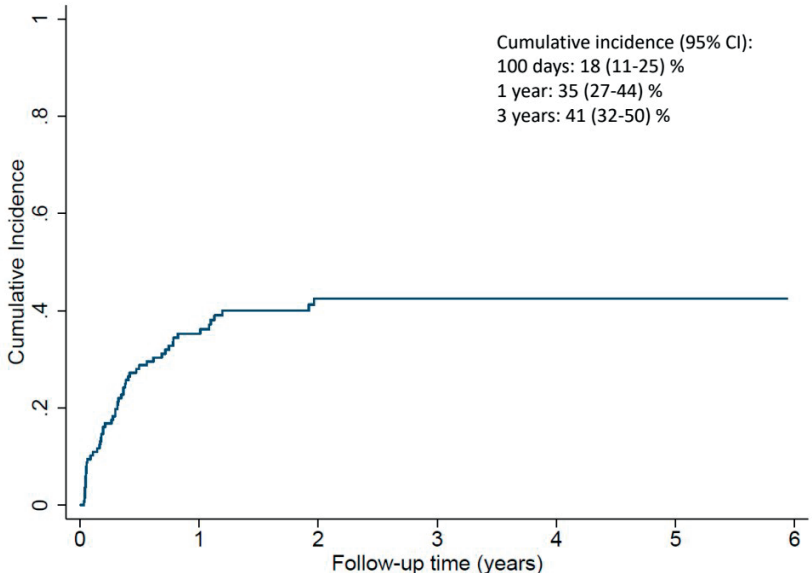


Figure 3. Cumulative incidence of non-relapse mortality.

Table 2. Causes and contributory causes of death.

Characteristic	Value
Cause of death, n (%)	76 (100)
- HCT related cause*	53 (69.7)
- Relapse or progression	20 (26.3)
- Unknown	3 (3.9)
Contributary cause of death, n (%)	76
- Bacterial infection	31 (40.8)
- Graft failure/poor graft function	23 (30.3)
- Viral infection	22 (28.9)
- Fungal infection	20 (26.3)
- GVHD	19 (25.0)
- Multiple organ failure	13 (17.1)
- Pulmonary toxicity	5 (6.6)
- Renal failure	5 (6.6)
- Cardiac toxicity	3 (3.9)

*Death due to a complication of transplant.

9.4.5 Relapse incidence (RI)

The CI of relapse at one and three years from transplant was 16% (95% CI 11–24%) and 21% (95% CI 14–29%), respectively (Figure 4). In total, 29 patients relapsed: eight AML (five in CR1/CR2 and three in CR3+), five ALL (all five in CR1/CR2) and 16 from other diagnostic groups (predominantly lymphoma) (five in CR1/CR2, while the remaining 11 were in CR3+).

In multivariable analysis, RI was higher for "other" diagnoses (predominantly lymphoma) versus AML/MDS (HR = 2.62; 95% CI 1.12–6.15), decreased for PBSC versus BM (RR = 0.43; 95% CI 0.19–0.95; p = 0.038) and decreased for offspring donors (RR = 0.25; 95% CI 0.09–0.67; p = 0.006). See Table 3.

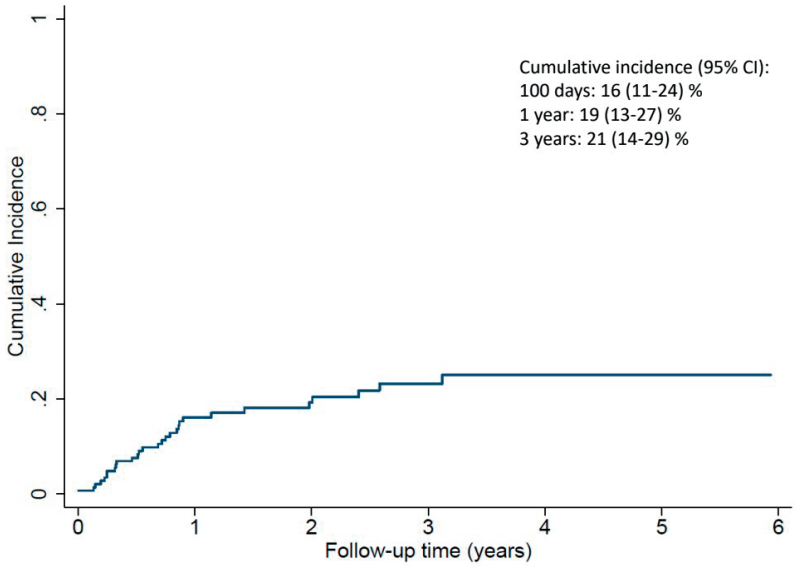


Figure 4. Cumulative incidence of relapse.

Table 3. Multivariable regression model: disease-free survival and relapse incidence.

Characteristic	Disease-free survival			Relapse incidence		
	HR	95% CI	p-value	HR	95%CI	p-value
Donor age						
46–68 years	1.9	1.02–3.53	0.043	-	-	-
36–45 years	1.4	0.7–2.8	0.34	-	-	-
26–35 years	1.16	0.6–2.28	0.66	-	-	-
9–25 years	1.0	reference	-	-	-	-
Diagnostic group						
Other*	-	-	-	2.62	1.12–6.15	0.027

Table 3. Continued.

Characteristic	Disease-free survival			Relapse incidence		
ALL group	-	-	-	1.51	0.38–5.96	0.56
AML/MDS group	-	-	-	1.0	reference	-
Stem cell source						
PBSC	-	-	-	0.43	0.19–0.95	0.038
BM	-	-	-	1.0	reference	-
Donor relation						
Offspring	-	-	-	0.25	0.09–0.67	0.006
Sibling	-	-	-	0.39	1.15–1.01	0.053
Parent	-	-	-	1.0	reference	-

*predominantly lymphoma

9.4.6 Graft-versus-host disease

Forty-five patients (41.7%) developed acute GVHD before day 100 – the majority developed grade I (37.8%) and grade II (42.2%) disease, with six patients (13.3%) and three patients (6.7%) developing grade III and IV disease, respectively. We analysed if there was an association between the cell source and development of acute GVHD, however, no significant association could be found (supplementary table 2).

9.5 DISCUSSION

This is the first analysis of the South African experience with T-cell-replete haploHCT in adults with haematological malignancies. Our study supports the feasibility and safety of haploHCT and suggests that unmanipulated haploHCT is a viable option, especially for patients with acute leukaemia and MDS. Finding a RD or UD in South Africa is challenging for three reasons: firstly, this region of the world faces an ongoing HIV epidemic, limiting our local volunteer donor pool; secondly, the local population is genetically highly diverse; and finally, the costs associated with finding and recruiting an international UD is prohibitive for most patients. The applicability of HaploHCT, as compared to unrelated donor transplantation, is consistent with the imperative to provide affordable and equitable access to stem cell transplantation for all patients in South Africa, irrespective of socioeconomic status. HaploHCT is therefore an attractive option for some patients with high-risk haematological malignancies.

We observed a three-year probability of OS and DFS of 37% and 32%, respectively, for the entire cohort. The outcome of the AML/MDS sub-group

approximates that of other international transplant centres [12,13], with a three-year OS and DFS of 43% and 42%, respectively. However, the results of the ALL group appear disappointing compared to other publications [6,14], with a three-year OS and DFS of only 21% and 24%, respectively. The heterogeneity of minimal residual disease (MRD) assessment, the lack of MRD standardisation, unavailability of novel ALL drugs, and distinct disease biology in this population can be possible explanations for this observation.

In multivariable analysis, we were unable to identify any risk factors associated with OS, whereas increased donor age was associated with inferior DFS. Similarly, Canaani et al. [16] observed the importance of donor age > 40 years leading to inferior leukaemia-free survival and OS when recipient age is > 40 years in patients with acute leukaemia. In contrast, McCurdy [25] and Solomon et al. [26] have reported that donor age was not associated with inferior patient outcomes. These conflicting results notwithstanding, our data suggest that donor age should be taken into account when deciding on a potential donor for a patient if more than one donor is available. Other advantages of using younger donors are the lower probability of clonal haematopoiesis in younger individuals and better CD34 stem cell yields [36].

The three-year probability of relapse was 21% for the entire cohort, 12% for AML/MDS, 23% for ALL and 31% for the "other" group, mostly comprising of advanced lymphomas. For this last group a higher relapse rate was not unexpected, and was in keeping with other studies [6,12].

The use of PBSC as cell source led to significantly faster rates of neutrophil and platelet engraftment [17], but had no significant impact on rates of acute GVHD (supplementary table 2). However, this must be interpreted with caution as comparative groups are small, with insufficient statistical power. This is in contrast to Ruggeri [18] and the meta-analyses of Durer [19] and Yu et al. [20], which have shown a higher likelihood of acute GVHD, with no impact on OS and RI, with the use of PBSC. A limitation of our study is the missing data on the specific date of diagnosis of acute and chronic GVHD, preventing us from calculating the cumulative incidence of acute and chronic GVHD.

The favourable association of offspring donors with a lower RI in our study has also been observed by Solomon et al. [26]. In addition, they showed a worse OS with the use of parental donors versus sibling or offspring donors using the PTCY method. McCurdy et al. [25] has also recently shown that recipients of haploHCT from parental donors was associated with increased risk of graft failure, while no differences were seen with sibling or offspring donors. These

observations together with our data, suggest that the use of offspring or sibling donors is preferred above parental donors. Finally, most studies argue in favour for the use of offspring or sibling donors, and our data confirm this observation.

The 100-day and three-year NRM in our cohort was 18% and 41%, respectively. The former rate is similar, but the latter rate higher than the rates recently reported by Piemontese et al. [12] (three-year NRM 28%), yet lower than historical haploHCT reports [2] (55–67%). The only risk factor identified on univariate analysis for NRM was older recipient age (> 57 years), an expected conventional risk factor for NRM. Half of our cohort was > 44 years of age and the majority (79.8%) had high EBMT risk scores; both these factors can contribute to the relatively high three-year NRM. As the cohort that we describe represents our early experience of haploHCT with PTCY in Southern Africa, this could contribute to the high NRM and we expect NRM to improve over time as our experience with this procedure matures. Our data can now serve as a comparator for future studies of haploHCT, and to assist clinicians and patients to make evidence-informed clinical decisions. Whether myeloablative conditioning, CMV incongruence, gender-mismatched transplants and older maternal donors contributed to the relatively high NRM in our cohort remains unanswered and needs further investigation.

We also observed a relatively high rate of graft failure and poor graft function (23 patients; 17.2% of whole cohort) as compared to recent reports [12,27]. Further analysis is needed to understand the contribution of donor specific antibodies, desensitisation techniques and cell source on engraftment in order to create tailored preventative strategies.

As with any retrospective analysis, our results should be interpreted cautiously owing to inherent biases in data collection, presence of missing data, the variations in clinical practice among the public and private transplant centres and the heterogeneity of this group of haematological malignancies. However, consecutive patients that received a haploHCT at the two centres were included in this analysis, removing potential selection bias.

We conclude that our data confirms the feasibility of unmanipulated haploHCT in South Africa and suggests that utilising younger parental or offspring donors are valid options for adults with acute leukaemia and MDS lacking a related or unrelated donor.

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10

Variations in Blood Transfusion Practices Among South African Haematology Providers – A survey of haemato-oncology and haematopoietic stem cell transplant prescribers

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Background: Patients with haematological malignancies and haematopoietic stem cell transplant (HCT) recipients are major consumers of blood products. In South Africa (SA), both medical oncologists and clinical haematologists (haematology providers) manage these patients. We hypothesised that there may be varied blood transfusion practices between these two specialty groups.

Objectives: To compare red cell (RBC) and platelet transfusion practices among haematology providers in South Africa, focusing on transfusion thresholds, blood product modifications, and differences between clinical haematologists and medical oncologists.

Methods: We conducted an online REDCap® survey among SA haematology providers, including clinical haematologists (and trainees), medical oncologists (and trainees), paediatric oncologists/haematologists (and trainees) and medical officers managing haemato-oncology patients.

Results: The survey response rate was 51% (79/155), of which 58 (73%) responses were complete and eligible for analysis. RBC and prophylactic platelet transfusion thresholds were generally similar across haematology providers. However, significant variation was observed in platelet thresholds for central venous catheter insertion, lumbar puncture and major surgery. Marked inconsistency was also noted in clinicians' understanding of when blood product irradiation is indicated.

Conclusion: This study confirms a variation in blood transfusion practices and blood product modification requests between haematology providers. This emphasises the need for targeted education and national standardisation efforts to optimise transfusion practice and ensure patient safety.

10.2 INTRODUCTION

Patients with aggressive haematological malignancies receive intensive chemotherapy, often followed by either autologous or allogeneic haematopoietic stem cell transplantation (HCT). During these treatment modalities there is substantial bone marrow suppression during which RBC and platelet transfusions are required to prevent symptomatic anaemia and thrombocytopenia-related bleeding. Nevertheless, transfusion support is costly and can negatively impact the patient: transmission of blood-borne diseases, transfusion reactions (e.g. febrile reaction, transfusion-associated circulatory overload, transfusion-related acute lung injury, allergic reactions), alloimmunisation, immune-modulation, transfusion-associated graft-versus-host disease (TA-GVHD), secondary iron overload and potentially lethal transfusion errors [1,2].

Globally there has been a paradigm shift towards restrictive transfusion practices, supported by evidence that restrictive RBC transfusion strategies are safe (and sometimes superior), especially in surgical and critical care settings [3,4,5]. Patient blood management (PBM) is globally defined as a patient-centred, systematic, evidence-based approach to improve patient outcomes by managing and preserving a patient's own blood, while promoting patient safety and empowerment [6,7], and the use of restrictive strategies.

Despite the heavy transfusion requirements in the haematology-oncology/HCT setting, very few trials have investigated restrictive versus liberal RBC transfusion thresholds in patients undergoing intensive chemotherapy, as highlighted by a recent Cochrane review [8]. A systematic review and meta-analysis done 2017 [9], which included 15 trials (2,636 patients), showed no negative impact on clinical outcomes for a restrictive approach in haemato-oncology patients. These findings were supported by the TRIST trial [10], the largest randomised controlled trial to date to evaluate restrictive versus liberal transfusion thresholds in patients undergoing HCT. This trial favoured a restrictive strategy as no significant differences were found in clinical outcomes or patient-reported quality of life.

RBC and platelet modifications are mostly requested in haemato-oncology patients which includes leuco-reduction and/or irradiation – the leuco-depletion decreases the risk of HLA alloimmunisation and platelet refractoriness, febrile non-haemolytic transfusion reactions, bacterial infections and reperfusion injury in cardiac patients [11,12,13,14,15]. The irradiation mainly prevents TA-GVHD [16] which, although rare, is invariably fatal. Both modifications can be specifically

requested from the blood bank, but these modifications add complexity and cost and therefore should be done for the correct indications.

There is currently limited published data on transfusion practice and modification requests, specific to haemato-oncology/HCT patients in SA, where patients with haematological malignancies are typically treated either by medical oncologists or clinical haematologists. Compared to medical oncologists, clinical haematologists receive training in transfusion medicine as part of their standard curriculum. Based on this difference in training, we hypothesised that there would be variations in blood transfusion practices and blood product modification requests between these two subspecialties and their respective trainees. This information will help us to harmonise transfusion and modification practices to establish best care recommendations specifically for our haemato-oncology patients.

To address this question, we performed an online survey of haematology providers (clinical haematologists, medical oncologists, paediatric oncologists and haematologists and their respective trainees) in SA.

10.3 MATERIALS AND METHODS

10.3.1 Study setting and participants

A cross-sectional study was conducted using a questionnaire distributed between July 2022 and June 2023 via an online REDCap® survey (23 questions related to blood transfusion practices). The survey link was circulated through WhatsApp and email to haematology providers who care for patients with haematological malignancies, recipients of HCT and patients with chronic anaemias such as myelodysplastic syndrome (MDS), haemoglobinopathies, including thalassaemia major and sickle cell disease, and severe aplastic anaemia. Eligible participants included registered clinical haematologists, medical oncologists, paediatric oncologists/

haematologists and their trainees, as well as medical officers and haematopathology registrars working within haematology departments, who prescribe red cells and platelets. Clinical haematologists in training are required to complete a minimum of three months in a blood transfusion unit as part of their specialisation, whereas oncology trainees do not undergo this training. Participants were unable to proceed with the survey if they did not regularly prescribe these products within the haemato-oncology setting.

10.3.2 Statistical analysis

Data were captured using REDCap® – a secure electronic data capture tool [17]. A descriptive analysis of the survey responses and a comparison of the survey responses between the different clinician groups were performed. We divided the clinician groups into three categories: clinical haematologists, medical oncologists and "other" which comprised medical officers (a qualified medical doctor not in training) and general medicine registrars (a medical doctor part of internal medicine training program in SA) rotating in oncology and haematology, and haemato-pathologists.

The chi-square test was used to assess relationships between survey questions and clinician groups. Fisher's exact test was applied to 2 x 2 tables or when the assumptions for the chi-square test were not met. Data analysis was carried out using SAS version 9.4 for Windows. A 5% significance level was applied.

10.3.3 Ethical consideration

Research ethics approval was obtained through the University of Cape Town Human Research Ethics Committee (HREC REF: 340/2022). Participation was voluntary. Participants were not asked to provide their names or any other personal identifying information. The participant was only allowed to access the survey questions once the consent was obtained.

10.4 RESULTS

Of 155 requests, 79 survey responses were received, corresponding to an overall response rate of 51%. Of these, 19 responses were non-qualifiers, and two further responses were incomplete and were omitted. Thus, 58 responses remained for further analysis. The characteristics of survey respondents are shown in Table 1.

Table 1. Characteristics of survey respondents. (n = 58)

	N (%)
Haematology	
Clinical Haematologist	21 (36)
Paediatric Clinical Haematologist	1 (2)
Registrar/Fellow in Clinical Haematology	3 (5)
Medical Oncology	
Medical Oncologist	9 (16)
Paediatric Oncologist	11 (19)
Registrar/Fellow in Medical Oncology	3 (5)
Registrar/Fellow in Paediatric Oncology	2 (3)
Other	**8 (14)**
Medical Officer	4
Haemato-pathologist	2
Registrar in haemato-pathology	1
Internal medicine registrar	1

Across all clinical scenarios (chemotherapy, haematopoietic stem cell transplantation and chronic anaemia), participants predominantly adopted a restrictive red cell transfusion strategy, with most selecting haemoglobin thresholds between 6.0–6.9 g/dL (Figure 1) [8]. Only a small minority reported using thresholds below 6 g/dL or above 8 g/dL.

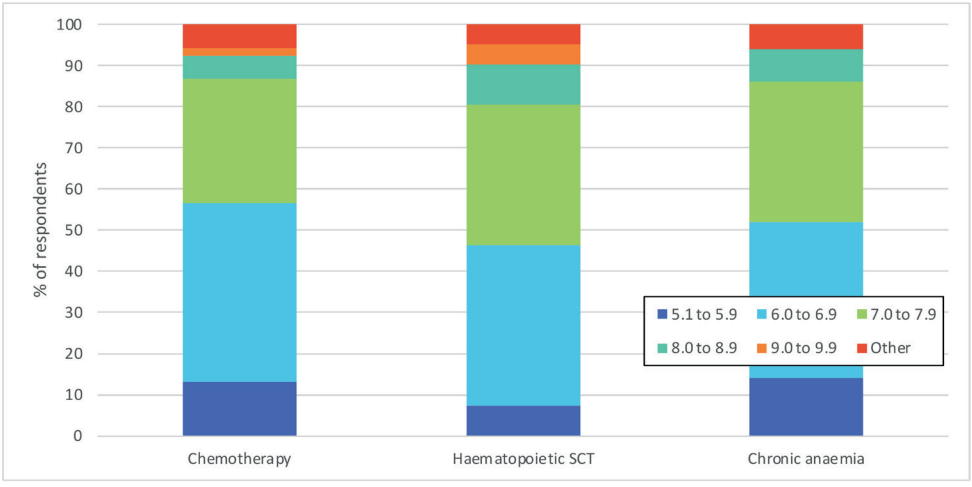


Figure 1. Haemoglobin thresholds (g/dL) for red cell transfusion for patients receiving chemotherapy, haematopoietic transplant and chronic anaemia.

Clinical haematologists were more likely to choose decreased cardio-pulmonary reserve as a reason to transfuse more than one unit of red cells, compared with medical oncologists and the "other" group (p = 0.036) (Figure 2). Medico-legal concerns were reported mainly by the "other" group (p = 0.017), although these were ranked lower than other clinical factors influencing transfusion decisions.

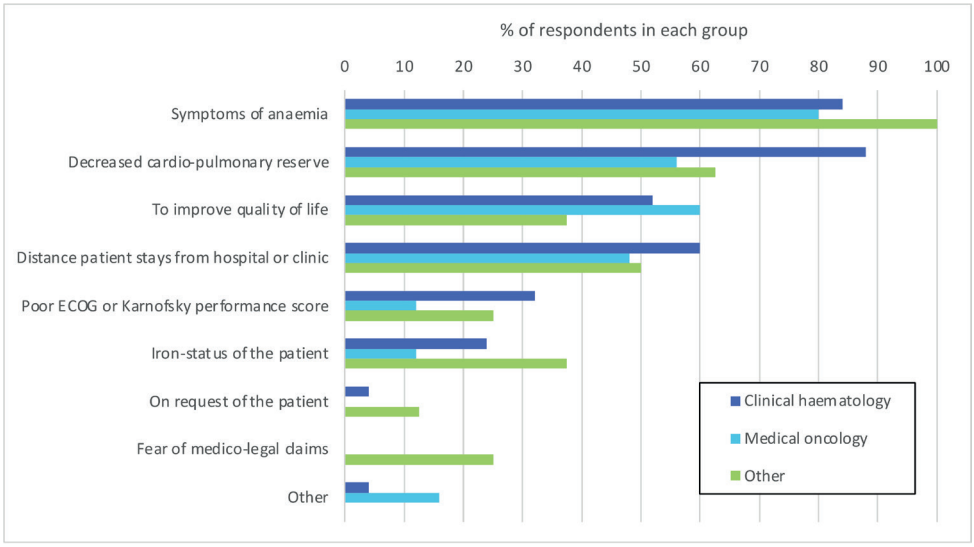


Figure 2. Patient-specific factors that influenced the decision to transfuse more than one red blood cell unit.

In patients without fever or infection, most clinical haematologists (68%) and medical oncologists (56%) selected a transfusion threshold of <10 × 10^9/L, compared with 38% of the "other" group (p = 0.45; Figure 3). In the context of fever or infection, clinical haematologists (92%) and medical oncologists (72%) predominantly used a threshold of <20 × 10^9/L whereas only half of the "other" group did so (p = 0.18). These findings indicate overall adherence to standard prophylactic platelet thresholds, with modest inter-group variation.

In fig. 4, the number of platelet units ordered per transfusion episode (prophylaxis versus therapy) is shown. Most respondents across all provider groups selected one platelet unit for both prophylactic transfusions.

In the prophylaxis group, 92% of clinical haematologists, 100% of medical oncologists and 88% of the "other" group reported transfusing a single platelet unit (p = 0.20).

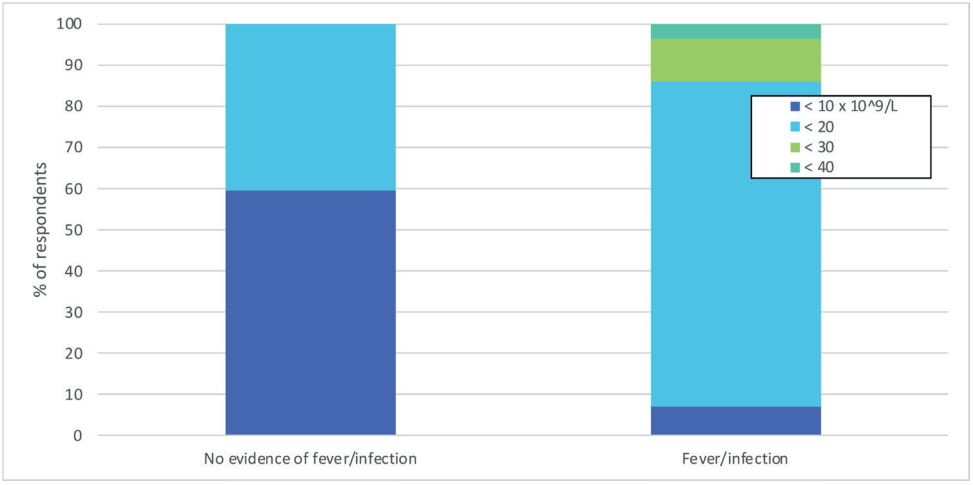


Figure 3. Prophylactic platelet transfusion thresholds.

For therapeutic platelet transfusions, 92% of clinical haematologists and 96% of medical oncologists, but only 50% of the "other" group selected one unit (p = 0.0013). Three respondents in the "other" group reported transfusing more than two units, and one reported using more than three units per episode.

Preferred prophylactic platelet transfusion thresholds prior to various invasive procedures are shown in Figure 4.

For central venous catheter (CVC) placement, most clinical haematologists and those from the "other" group selected a platelet threshold of > 20 × 10^9/L (74% and 100%, respectively). In contrast, only 3/18 (17%) of medical oncologists selected this threshold, with the majority (13/18; 72%) opting for >50 × 10^9/L (p = 0.0032). Notably, international guidelines [19] recommend a platelet threshold of >10 × 10^9/L for CVC insertion at compressible sites.

For lumbar puncture (LP), most clinical haematologists and medical oncologists selected a platelet threshold of >50 ×10^9/L (71% and 75%, respectively), Most recent guidelines suggest transfusing platelets when < 20 x 10^9/L [19].

For major surgery, clinical haematologists mostly selected >50 ×10^9/L (61%), whereas oncologists tended to select >100 × 10^9/L (72%) (p = 0.0060). The vast majority in all groups correctly selected >100 × 10^9/L for neurosurgery and retinal surgery. For liver biopsy, clinical haematologists and the "other" group predominantly selected >50 × 10^9/L, while medical oncologists were equally divided between >50 × 10^9/L and >100 × 10^9/L.

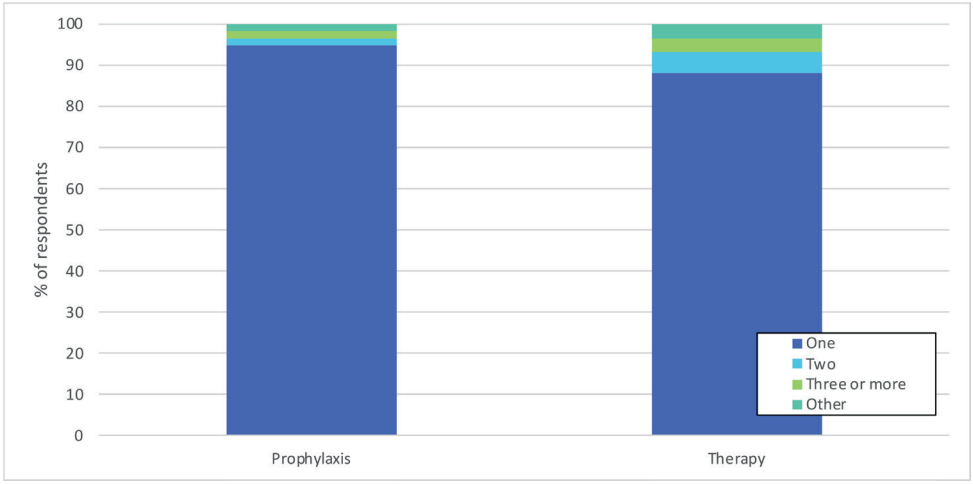


Figure 4. Number of platelet units ordered per transfusion episode (prophylaxis versus therapy). Prophylactic platelet transfusion is given to prevent bleeding in a patient who is not actively bleeding. Therapeutic transfusion is to transfuse when patient is actively bleeding.

There were no significant differences in platelet transfusion modification requests across provider groups, and overall practice aligned well with current guideline recommendations [16] (see Figure 5). In this analysis, the haematological conditions refer specifically to patients with haematological malignancies receiving chemotherapy.

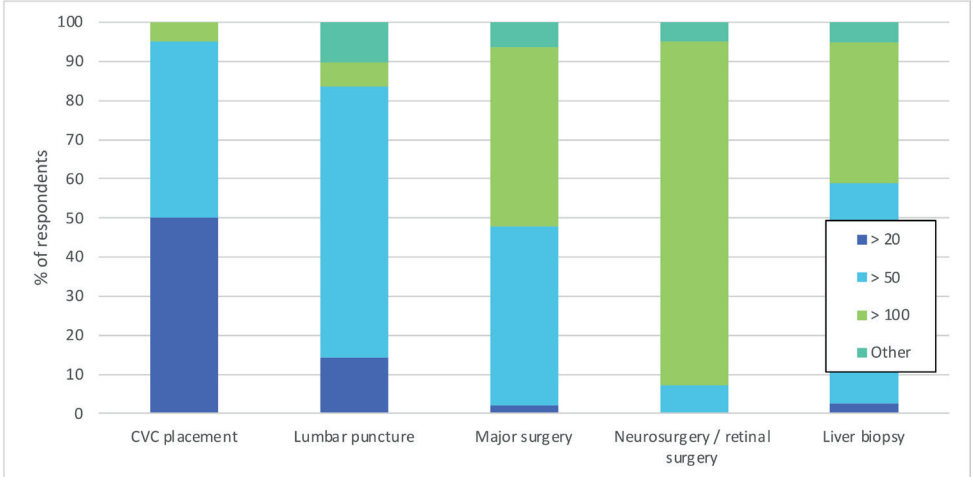


Figure 5. Prophylactic platelet transfusion thresholds during procedures.

Significant differences were noted regarding indications for irradiation of blood products between the three groups of haematology providers as highlighted

(Figure 7). Most clinical haematologists would irradiate blood products for the correct indications according to international guidelines in comparison to the medical oncologists and "other" group, except for patients receiving chemotherapy for Hodgkins's lymphoma where all three groups would, in contrast to the recent guidelines, not irradiate blood products.

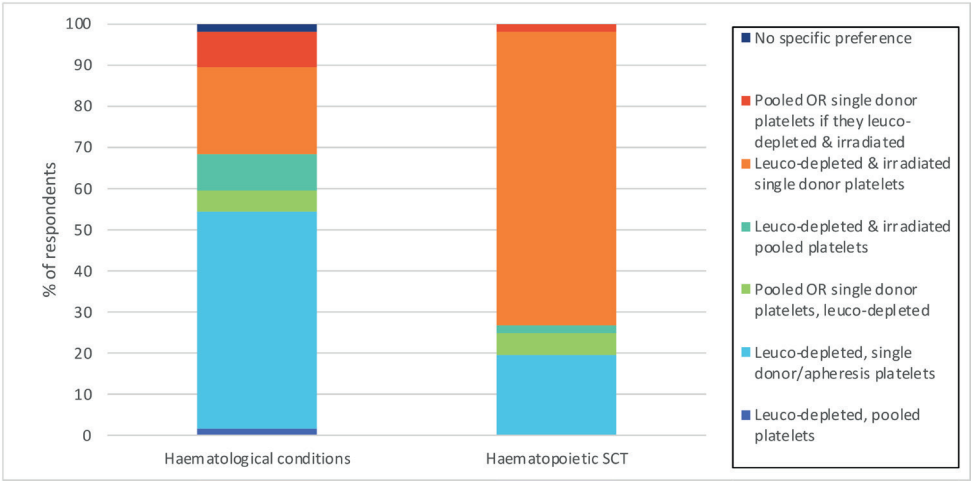


Figure 6. Platelet product modification requests for haematological conditions versus transplant recipients.

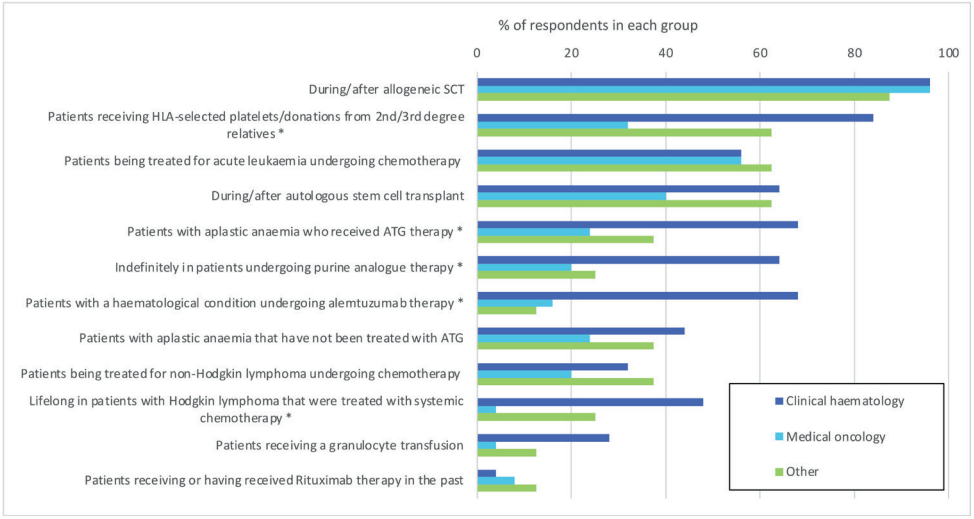


Figure 7. Different clinical scenarios when irradiated blood products would be requested.

SCT = stem cell transplant, HLA = human leucocyte antigen, ATG = anti-thymocyte globulin, SAA = severe aplastic anemia

10.5 DISCUSSION

This national survey of haematology providers in South Africa highlights notable variations in transfusion practices, particularly in two domains: platelet transfusion thresholds before procedures and requests for blood product modifications, such as irradiation.

RBC transfusion thresholds provide insight into clinicians' overarching transfusion philosophies, whether they favour liberal or restrictive approaches. Most SA haematology providers in this study selected a restrictive haemoglobin trigger of 6.0–6.9 g/dL, with no significant differences between provider groups. Although our survey did not formally assess knowledge of PBM, this uniformity may suggest baseline awareness of PBM principles among specialists and trainees. This pattern aligns with international trends, where studies from Europe and North America demonstrate increasing adoption of restrictive transfusion strategies, consistent with evolving PBM guidelines recommending haemoglobin thresholds ≤7 g/dL for clinically stable patients [3,8].

Several patient-specific factors influenced RBC transfusion decision-making. Clinical haematologists more often prioritised cardiopulmonary reserve, while the "other" group, which included haematopathologists, internal medicine practitioners and trainees, expressed greater concern about medico-legal implications. These differences likely reflect variation in clinical exposure to transfusion scenarios and differing levels of experience with complex decision-making.

Haematology providers are recognised globally as among the highest users of platelet transfusions [21,21]. Our findings reveal significant variation in platelet transfusion behaviours across provider groups. Although prophylactic platelet transfusion thresholds were broadly similar, even in the presence of fever or infection, the "other" group was significantly more likely to transfuse more than one platelet unit per episode. This practice diverges from both local and international recommendations, which typically advise transfusing a single apheresis unit in most clinical scenarios [8,18]. In SA, such variation carries economic consequences, with a single apheresis unit from the South African National Blood Service (SANBS) costing approximately R15,311.91 in 2025 [22]. Importantly, transfusing two units does not proportionally increase clinical benefit, underscoring the need for improved adherence to cost-effective, evidence-based protocols.

We also observed wide variation in platelet transfusion thresholds prior to invasive procedures. Clinical haematologists were more likely to align with guideline-recommended thresholds, possibly reflecting greater formal training in transfusion medicine and practical experience. Similar international patterns have been documented; for example, a large UK audit demonstrated significantly lower compliance with guideline-recommended platelet thresholds among non-haematology specialties compared with haematology teams [23].

Regarding blood product modifications, especially irradiation, we identified significant differences between provider groups. Although leucodepletion requests did not differ, clinical haematologists were substantially more likely to request irradiation for appropriate indications, consistent with current guidelines. Since irradiation remains a key measure in preventing transfusion-associated graft-versus-host disease (TA-GVHD), deviations from recommended practice are clinically relevant. Internationally, comparable knowledge gaps have been reported, with studies from the UK and the Netherlands describing underutilisation or inappropriate use of irradiation among non-haematology services [24,25]. These findings highlight the need for targeted education on transfusion safety principles.

As with most survey-based research, our 51% response rate introduces the possibility of response bias. Nevertheless, representation across public and private sectors, multiple specialties and varying levels of training enhances generalisability within the SA haematology context. Study limitations include selection bias inherent to convenience sampling, potential recall bias due to self-reporting, and reduced statistical power in subgroup analyses. Additionally, institutional-level variations could not be fully accounted for. Finally, because this was an observational survey, we were not able to link reported practices to patient outcomes. Evaluating clinical outcomes in relation to transfusion decisions will be the focus of a future study to provide a more robust evidence base for local practice.

Despite these limitations, our findings reveal important knowledge and practice gaps. Variation in platelet transfusion thresholds and inconsistencies in blood product modification requests point to opportunities for improving standardisation. Regular CPD activities on transfusion medicine and PBM, combined with audit-and-feedback strategies, could help harmonise practice across provider groups. Integrating decision-support tools into electronic ordering systems may further improve guideline adherence. Strengthening collaboration among haematologists and oncologists through national forums and research initiatives could also support shared learning, consensus-building

and ultimately contribute to more consistent practice and improved patient outcomes.

10.6 CONCLUSION

This national survey demonstrates considerable variation in transfusion practices among SA haematology providers, particularly in platelet transfusion thresholds and indications for blood product modifications. These findings highlight the need for greater standardisation and targeted education efforts to optimise transfusion practice, save costs and ensure patient safety.

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Conflicts of Interest: None

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Part V: Summary and Discussion

11

Summary of thesis

SUMMARY OF THESIS

This thesis presents a comprehensive evaluation of infectious, haematological, immunological and transplantation-related complications affecting patients with haematological malignancies and those undergoing haematopoietic stem cell transplantation (HCT), with a strong focus on the Southern African context and direct comparison to international practice. Central to this work is the bidirectional interplay between virology and haematology: viral pathogens contribute directly to haematological pathology through immune-mediated and immunothrombotic mechanisms, while patients with haematological diseases are uniquely vulnerable to severe viral infections due to underlying and treatment-related immunosuppression. The thesis was designed to address three broad aims:

First aim

To determine how infectious disease burden influences outcomes among haematology patients undergoing transplantation in South Africa compared with international centres.

In Part II, respiratory viral infections, including respiratory syncytial virus (RSV), were shown to occur infrequently and to be associated with relatively low morbidity and mortality across geographically and economically diverse HCT centres, including the Wits Donald Gordon Medical Centre in South Africa and collaborating centres in the Netherlands. These findings indicate that universal pre-transplant RSV prophylaxis might be unnecessary for most patients. Instead, prevention strategies should be risk-adapted and targeted toward clearly defined high-risk subgroups to maximise clinical benefit while preserving healthcare resources.

Although diagnostic awareness of community-acquired respiratory viruses among clinicians is high, substantial variation in treatment approaches, prevention strategies and testing policies was observed. This heterogeneity reflects the absence of harmonised international guidelines, as well as rapidly evolving evidence related to emerging antiviral agents and vaccines. Collectively, these findings highlight the pressing need for guideline alignment, improved clinician education and optimisation of testing strategies across centres locally and internationally.

Bloodstream infections emerged as a major contributor to morbidity and disease severity among South African HCT recipients, particularly in the allogeneic transplant setting. Colonisation with carbapenem-resistant

Enterobacteriaceae (CRE) was identified as a strong independent risk factor for subsequent bloodstream infection, underscoring the critical importance of robust infection-prevention measures, enhanced antimicrobial stewardship and the development of targeted prophylactic or pre-emptive strategies in this highly vulnerable population.

Second aim

To investigate how viral infections drive haematological pathology through immune-mediated and immunothrombotic mechanisms.

In Part III, the complex interplay between viral infections, immune dysregulation, thrombocytopenia and coagulation abnormalities was explored. Through a comprehensive literature review, thrombocytopenia was demonstrated not to be merely a passive consequence of viral illness, but rather a manifestation of dynamic virus–platelet interactions involving both innate and adaptive immune responses. Platelets increasingly appear to function as active mediators of antiviral defence and immune signalling, reframing their role in viral pathogenesis and supporting their potential utility as biomarkers of disease severity and clinical outcomes.

In critically ill patients with COVID-19, identification of a procoagulant phenotype among peripheral blood mononuclear cells, combined with assessment of the end-tidal to arterial carbon dioxide gradient ($ETCO_2$–$PaCO_2$), showed promise as a non-invasive approach for early detection of pulmonary embolism in the intensive care environment. This strategy may facilitate earlier diagnosis and therapeutic intervention when conventional imaging is delayed or impractical, potentially improving outcomes in critically ill patients.

Within the spectrum of rare bleeding disorders, acquired haemophilia A (AHA) was characterised as a potentially life-threatening but treatable condition. In this cohort, patients with HIV-associated AHA presented with markedly higher inhibitor titres and required more prolonged immunosuppressive therapy to attain remission. Early recognition and prompt initiation of treatment were associated with excellent survival outcomes, whereas delayed diagnosis or limited initial resource utilisation increased morbidity and overall management costs. These findings reinforce the importance of maintaining a high index of suspicion regarding AHA and establishing formal registries to strengthen clinical understanding and optimise future management pathways for this rare disorder.

Third aim

To evaluate how key treatment processes, namely haematopoietic stem cell mobilisation, donor selection via haploidentical transplantation strategies, and transfusion practices can be optimised to improve patient outcomes and resource utilisation in South Africa.

In Part IV, contemporary transplant practices and supportive-care strategies were evaluated within the South African setting. Poor mobilisation of haematopoietic stem cells occurred in 17% of patients undergoing autologous transplantation, however, true mobilisation failure remained uncommon at 2%. A notably high rate of super mobilisation (68%) was observed, particularly among patients with multiple myeloma, suggesting unnecessarily aggressive upfront mobilisation strategies. These findings directly informed clinical practice, leading to the adoption of lower-dose etoposide mobilisation protocols and updated standard operating procedures across both public and private transplant centres. Predictive factors for poor mobilisation were identified, enabling refined risk stratification and more cost-effective use of plerixafor for rescue mobilisation. Selective deployment of rescue agents allows optimisation of limited therapeutic resources while maintaining favourable patient outcomes.

The feasibility and efficacy of haploidentical transplantation were also demonstrated in South Africa, representing the first study of its kind reported from sub-Saharan Africa. Use of unmanipulated haploidentical grafts, particularly from younger parent or offspring donors, yielded effective transplant outcomes in adults with acute leukaemia or myelodysplastic syndrome lacking matched related or unrelated donors, although non-relapse mortality remained high. While the underlying contributors to this elevated non-relapse mortality remain incompletely defined and warrant further investigation, the data establishes a critical baseline for future programme development, quality-improvement initiatives and outcome optimisation strategies. Overall, haploidentical transplantation is confirmed as a highly valuable approach for expanding access to curative therapy in regions with constrained donor availability.

Finally, marked national variation in blood transfusion practices and blood-product modification requests was identified among South African haematology providers. This variability reflects inconsistent education, heterogeneous institutional protocols and limited standardisation. The findings support the urgent need for focused clinician education initiatives, including expanded training in patient blood management principles and increased exposure to transfusion medicine during oncology training, and for the development of

standardised national transfusion guidelines. Such measures are essential to improve patient safety, promote consistent evidence-based practice, optimise utilisation of blood products, reduce healthcare costs and ultimately strengthen overall health-system efficiency.

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Discussion

This thesis provides a comprehensive evaluation of the infectious, immunological and clinical challenges affecting patients with haematological malignancies and those undergoing haematopoietic stem cell transplantation (HCT) in South Africa, interpreted within an international comparative framework. Through a series of complementary studies, it examines the epidemiology and management of community-acquired respiratory and bloodstream infections; the immune–haematological mechanisms underlying thrombocytopenia and coagulopathy; and practice patterns related to stem cell mobilisation, haploidentical HCT and transfusion practices in haematology patients. Collectively, the findings demonstrate that although diagnostic vigilance and overall clinical standards are high across settings, substantial heterogeneity persists in management strategies and resource utilisation. These inconsistencies highlight important opportunities to improve patient outcomes through evidence-based standardisation, risk-stratified interventions and pragmatic, locally adapted innovations tailored to resource-constrained healthcare environments such as South Africa.

In Part II, community-acquired respiratory viral infections (CARVs), particularly the incidence of respiratory syncytial virus (RSV), were evaluated across three haematopoietic stem cell transplant centres representing distinct economic and healthcare contexts in South Africa and the Netherlands. RSV incidence was lower than anticipated and associated morbidity and mortality were relatively modest, findings consistent with contemporary international series in HCT populations [1–4]. These results do not support routine universal prophylaxis in HCT populations. Rather, they favour targeted, risk-adapted prevention strategies focused on patients at highest risk for progression to severe lower respiratory tract disease, as reflected in current management recommendations and risk stratification models [5–9]. Most infections clustered within the first 15 months post-transplant and demonstrated clear seasonal predominance, reflecting recognised periods of immune vulnerability and delayed immune reconstitution following transplantation [10–12]. Co-infections with other respiratory viruses were frequent, consistent with reported viral co-detection rates in transplant recipients, and reinforced the need for comprehensive molecular surveillance strategies rather than pathogen-specific diagnostic testing alone [2,13,14].

Using an exploratory questionnaire-based study, we demonstrated that despite good diagnostic awareness of CARVs among clinicians (haematologists, ICU specialists and emergency medicine physicians), considerable variability exists in testing policies and therapeutic interventions, particularly with respect to ribavirin and intravenous immunoglobulin (IVIG) use during RSV

infection. Similar heterogeneity in diagnostic and management practices across transplant centres has been widely reported, reflecting differences in access to diagnostics, evolving evidence and institutional protocols [2,15]. These inconsistencies have historically been compounded by the absence of harmonised, resource-sensitive international guidelines and by uncertainty regarding the optimal use of antiviral or immunomodulatory therapies [5,6,16]. Since completion of this work and hence improved awareness, alignment with updated ECIL-10 recommendations has supported the refinement of local protocols at Wits Donald Gordon Medical Centre, illustrating how local guideline development can translate emerging evidence into practical improvements in clinical standardisation and patient care [9].

Bacterial bloodstream infections (BSIs) emerged as persistent, high-impact complications, particularly among recipients of allogeneic transplants at our high-volume public-sector haematopoietic stem cell transplant centre. Gram-negative pathogens predominated, with carbapenem-resistant *Enterobacterales* (CRE), notably *Klebsiella pneumoniae* harbouring OXA-48-type carbapenemases, identified as major contributors to early post-transplant morbidity and mortality in our cohort. These observations are consistent with international experiences reported by Mikulska et al., Gudiol et al., Ustun et al. and Lin et al. [17–20]. Pre-transplant CRE colonisation was a strong independent predictor of subsequent invasive infection, highlighting the critical role of active surveillance cultures in identifying patients at highest risk for bacteraemia and adverse outcomes, as similarly demonstrated by Korula et al., Mendes et al. and Patriarca et al. [21–23]. Collectively, these findings highlight the importance of stringent infection-prevention and control measures, robust antimicrobial stewardship programmes and systematic surveillance strategies within transplant units to mitigate the burden of multidrug-resistant Gram-negative infections [23–25]. As our data was derived exclusively from a large public-sector transplant programme, future studies should incorporate comparable analyses across private transplant centres to define sector-wide incidence rates and antimicrobial resistance patterns, and to assess the generalisability of these findings within the broader South African context.

In Part III, mechanistic insights were integrated with the haematological complications that present clinically during viral infections. We performed a narrative review of thrombocytopenia in viral illness, a complication traditionally attributed to platelet consumption, hypersplenism or impaired marrow production [26,27]. However, emerging evidence shows that platelets are active immune effectors rather than passive markers of disease [28–33]. Platelet involvement in viral recognition, cytokine signalling and propagation, and direct

viral clearance, including interactions with infected megakaryocytes in marrow and pulmonary niches, highlights a complex immune–haematological axis that contributes both to host defence and to pathological inflammation [34–41]. These insights suggest potential avenues for the development of platelet-based biomarkers of disease severity and outcome, as well as more refined immunomodulatory approaches to viral infection in immunocompromised patients [42,43].

The investigation of immunothrombosis in COVID-19 further supported this integrated immune–coagulation paradigm. COVID-19 is now recognised as a disorder of immune-driven thromboinflammation characterised by monocyte and neutrophil activation, endothelial dysfunction, platelet–monocyte aggregate formation and tissue factor up-regulation, all contributing to the heightened thrombotic risk observed in severe disease [44–49]. Procoagulant activation of peripheral blood mononuclear cells, demonstrated using functional fibrin generation time assays, emerged in our work as a potential biomarker of thrombotic risk, congruent with studies demonstrating circulating tissue-factor-bearing extracellular vesicles and immune-cell transcriptional reprogramming toward a pro-coagulant phenotype [48–51]. When combined with established non-invasive physiological measures, including end-tidal–arterial CO_2 gradients and D-dimer testing, this approach may provide a feasible strategy for earlier identification of pulmonary embolism in intensive care settings, where conventional imaging is often delayed or impractical [52–55]. These findings reinforce the contemporary shift toward conceptualising COVID-19 as an immune-mediated thromboinflammatory syndrome rather than isolated viral pneumonia, with important implications for translational risk stratification and early, individualised anticoagulation strategies guided by emerging clinical trial and consensus guidelines [44,45,49,56–58].

The exploration of acquired haemophilia A (AHA), particularly in the setting of HIV infection, further emphasised the influence of immune dysregulation in haematological disease. Patients with HIV-associated AHA have been reported to present with higher inhibitor titres, more severe factor VIII depletion, and to require prolonged immunosuppressive therapy to achieve remission when compared with non-HIV-associated AHA cohorts [60–64]. These clinical patterns are thought to reflect HIV-associated immune activation, microbial translocation, cytokine imbalance and immune reconstitution dynamics, potentially involving molecular mimicry or immune reconstitution inflammatory syndrome (IRIS) phenomena [65–71]. Access to optimal therapy, including rituximab and bypassing agents, remains limited within the South African public healthcare sector, contributing to delays in achieving haemostasis and

increased cumulative treatment costs. Nevertheless, data from international registries and cohort studies indicate that early recognition and prompt, decisive immunosuppressive intervention are associated with excellent survival outcomes [63,64,72], emphasising the importance of heightened clinician awareness and supporting the establishment of formal AHA registries (locally and internationally) to consolidate collective experience and guide future management of this rare condition.

Part IV addressed practical challenges related to stem cell mobilisation, Haplo HCT in our context with restricted donor availability, and transfusion support practices, particularly in patients with haematological malignancies and receiving HCT.

Although poor haematopoietic stem cell mobilisation occurred in approximately 17% of patients undergoing autologous transplantation, outright mobilisation failure was uncommon, consistent with published international experience [73–77]. In contrast, the unexpectedly high rates of super-mobilisation observed in our cohort suggest overtreatment driven by high-dose etoposide-based chemo-mobilisation regimens, which are known to be associated with increased toxicity and healthcare resource utilisation, including hospitalisation for neutropenic sepsis, intensive care admissions, and higher red cell and platelet transfusion requirements [78–81]. Identification of predictive markers for poor mobilisation performance, particularly peripheral blood CD34+ cell counts, white cell recovery kinetics and platelet parameters, now allows for more individualised, risk-adapted mobilisation strategies, enabling selective use of plerixafor in patients at genuine high risk of mobilisation failure [82–89]. This precision-based approach improves cost-effectiveness without compromising stem-cell yield or transplant outcomes, a critical consideration in the South African public healthcare setting where infectious comorbidities are common and resource constraints are substantial [90–92].

We continue to employ chemo-mobilisation strategies in South Africa, partly to achieve cytoreduction for multiple myeloma and lymphoma in an environment where access to modern induction agents remains limited. Even within the private sector, many patients do not have access to newer therapies such as daratumumab, teclistamab or carfilzomib, with coverage largely restricted to only the highest medical aid tiers. Public-sector patients faced even more restricted therapeutic options, with access to agents such as lenalidomide only becoming available around 2018. In this context, chemo-mobilisation represents a pragmatic compromise between disease control and transplant feasibility,

although ongoing refinement toward lower-toxicity, risk-adapted mobilisation remains a priority [73,88,89,91].

A major contribution of this thesis is the first comprehensive national evaluation of haploidentical haematopoietic stem cell transplantation (haplo-HCT) in adults. In a donor-limited context characterised by high HLA genetic diversity and a high prevalence of HIV, haploidentical transplantation using post-transplant cyclophosphamide (PTCy) was demonstrated to be both feasible and effective. Survival outcomes for patients with acute myeloid leukaemia and myelodysplastic syndrome were comparable to international benchmarks, supporting haplo-HCT as a viable strategy for expanding donor availability in sub-Saharan Africa [93–99]. Donor age emerged as a key determinant of disease-free survival, emphasising the importance of optimised donor selection algorithms [103–106]. Although non-relapse mortality remained higher than that reported in some contemporary series, this likely reflects the advanced disease burden at presentation, limited access to novel therapies such as blinatumomab, and the high comorbidity profile of the South African transplant population [95,107]. Overall, haplo-HCT represents a scalable and cost-effective approach to increasing equitable access to potentially curative cellular therapy across the region.

All transplant centres in South Africa currently predominantly employ alemtuzumab (“Campath-in-the-bag”) for ex vivo T-cell depletion as graft-versus-host disease (GVHD) prophylaxis in matched related and matched unrelated donor transplants. However, results from the CIBMTR ACCESS trial and subsequent analyses have demonstrated favourable outcomes for PTCy-based GVHD prophylaxis in mismatched unrelated donor transplantation, with acceptable rates of GVHD, non-relapse mortality, and overall survival. These data support PTCy as an effective and potentially lower-toxicity alternative that could substantially expand donor access for patients without fully matched donors, and may represent an even more suitable strategy for the South African population [108–111]. This needs to be explored in the future.

A national survey demonstrates substantial variation in transfusion practices among South African haematology providers, particularly with respect to platelet transfusion thresholds and the indications for blood product modification, despite broadly consistent approaches to red cell and baseline platelet transfusion support [112–115]. Discrepancies were especially evident in platelet thresholds applied for invasive procedures and in irradiation practices, reflecting ongoing knowledge gaps and incomplete integration of patient blood management (PBM) principles into routine haematology care

[116–121]. These findings highlight an urgent need for greater standardisation of transfusion protocols, alongside targeted educational initiatives, to promote evidence-based practice, enhance patient safety and optimise utilisation of scarce blood products within financially constrained healthcare systems [112,115,116,122]. The implementation of continuous professional development programmes, electronic clinical decision-support tools, and nationally endorsed transfusion guidelines represents feasible and impactful strategies to improve practice consistency and cost-effectiveness in the South African transfusion environment [118–120,123,124].

Synthesis and broader implications

Collectively, the findings of this thesis provide an integrated overview of the infectious, immunological and systems-level challenges faced by patients with haematological malignancies and those undergoing haematopoietic stem cell transplantation (HCT) in South Africa. Across multiple complementary studies, a consistent theme emerges: while clinical expertise and diagnostic vigilance are generally high, significant heterogeneity persists in management strategies and resource utilisation, driven largely by variations in access to diagnostics, therapeutics and standardised protocols. This variability represents both a challenge and an opportunity, highlighting critical gaps in care delivery while identifying pragmatic pathways for optimisation through evidence-based standardisation, risk stratification and context-specific innovation.

Infectious complications remain a dominant driver of morbidity post-transplant. The analysis of community-acquired respiratory viral infections (CARVs) demonstrates that severe outcomes such as RSV-related mortality occur less frequently than historically reported, supporting contemporary risk-adapted prevention strategies rather than universal prophylaxis. Clinician awareness of viral diagnostics is strong, however, substantial variability in testing algorithms and therapeutic responses, particularly the use of ribavirin and IVIG in RSV infections, reflects evolving evidence and previously fragmented guideline frameworks. Alignment with updated international recommendations has already begun to drive meaningful improvements in local protocol harmonisation, highlighting the importance of dynamic guideline integration tailored to regional healthcare capabilities.

Bacterial bloodstream infections, particularly those caused by multidrug-resistant gram-negative organisms, remain a persistent high-impact complication, especially in the early post-transplant period. The strong association between pre-transplant carbapenem-resistant *Enterobacterales* colonisation and subsequent invasive infection emphasises the central role

of surveillance cultures, antimicrobial stewardship and rigorous infection-prevention strategies. These findings extend international observations into the South African public-sector context and underscore the need for coordinated, sector-wide surveillance to accurately define resistance patterns and guide empirical therapy across transplant centres.

The immunological components of this thesis further bridge mechanistic insight with clinical consequence. Recognition of platelets as integral immune effectors refines long-standing paradigms of viral-associated thrombocytopenia and supports the development of platelet-based biomarkers for disease severity and outcome prediction. In COVID-19, the demonstration of immune-driven thromboinflammation, with measurable procoagulant activation of mononuclear cells and tissue factor signalling, provides translational relevance for early risk stratification strategies and the rational application of therapeutic anticoagulation in high-risk patients. These observations reinforce the broader concept that immune dysregulation across infectious contexts, not viral cytotoxicity alone, drives clinically meaningful haemostatic complications.

The exploration of acquired haemophilia A (AHA) in HIV-infected individuals further illustrates how perturbed immune homeostasis translates directly into complex haemorrhagic disease. Higher inhibitor titres, prolonged treatment courses and restricted access to optimal therapies within the public sector contribute to increased morbidity and treatment cost. Nevertheless, international evidence confirms that survival outcomes remain excellent when AHA is recognised early and treated aggressively. This supports increased efforts toward clinician education, formalised case registries, and improved access to immunosuppressive and haemostatic agents.

Operational challenges within transplantation practice, particularly in stem cell mobilisation and donor access, expose some of the most resource-sensitive trade-offs facing South African transplant programmes. While mobilisation failure rates remain low, the unexpectedly high incidence of super-mobilisation suggests overtreatment driven by cytotoxic chemo-mobilisation regimens, resulting in avoidable toxicity and inpatient resource consumption. The identification of predictive strategies, especially peripheral CD34+ counts and early haematologic recovery indices, enables personalised mobilisation pathways that preserve transplant success while improving cost-effective resource utilisation. The continued necessity of chemo-mobilisation reflects persistent inequities in access to novel induction therapies, both within the public sector and among the majority of privately insured patients, wherein advanced biologics remain financially inaccessible.

One of the most significant contributions of this thesis lies in the national evaluation of haploidentical transplantation, demonstrating that post-transplant cyclophosphamide delivers outcomes comparable to international standards, despite operating within a donor-limited environment shaped by high HLA diversity and HIV prevalence. The validation of haplo-HCT as a scalable strategy offers a clear pathway to expand transplant access throughout the region. Furthermore, emerging data from the CIBMTR ACCESS trial support the adoption of PTCy-based prophylaxis for mismatched unrelated donor transplants as a potentially lower-toxicity alternative to alemtuzumab-based regimens. Such approaches may further enhance donor availability while limiting treatment-related complications, an outcome of particular importance for high-comorbidity populations in resource-constrained systems.

Finally, the national transfusion survey highlights substantial variability in platelet transfusion thresholds and blood product modification practices, even where red blood cell transfusion approaches are largely consistent. Gaps in adherence to patient blood management principles and irradiation guidelines expose avoidable safety risks and inefficiencies within transfusion services. These findings support the urgent implementation of standardised national protocols, continuous professional education, and electronic clinical decision-support tools aligned with international PBM frameworks. Such interventions represent cost-effective mechanisms to improve safety and reduce wastage of blood resources.

This body of work demonstrates that improving outcomes for patients with haematological malignancies and transplant recipients in South Africa does not depend solely on expanding access to high-cost biomedical interventions. Rather, meaningful improvements can be achieved through coordinated standardisation of care processes, systematic surveillance, personalised risk stratification, and the adaptation of internationally validated strategies to local realities. Collectively, these findings provide a framework for strengthening transplant and haemato-oncology services both within South Africa and across other low- and middle-income healthcare systems confronting similar clinical and structural challenges.

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13

Supplementary materials

13.1. SUPPLEMENTARY MATERIAL FOR BLOOD STREAM INFECTIONS TUDY

Table S1: Baseline variables, outcome variables and etiology of BSI of allogeneic HCT recipients in pediatric patients versus adult patients.

Table S2: Presence of bacteria in surveillance cultures detecting carbapenem-resistant gram-negative bacteria.

Supplementary table 1: Baseline variables, outcome variables and etiology of BSI of allogeneic HCT recipients in pediatric patients versus adult patients

	<18 years	≥18 years
	N = 54	N = 71
Baseline variables		
Hematological diagnosis (%)		
Acute Myelogenous Leukaemia (AML)	17 (32%)	26 (37%)
Precursor Lymphoid Neoplasms (=ALL)	10 (19%)	9 (13%)
Non-Hodgkin's Lymphoma	3 (6%)	8 (11%)
Hodgkin's Lymphoma	0 (0%)	2 (3%)
Chronic Myeloid Leukaemia (CML)	0 (0%)	2 (3%)
Myelodysplastic/Myeloproliferative neoplasms (MDS/MPN)	1 (2%)	2 (3%)
Myelodysplastic syndrome (MDS)	1 (2%)	7 (10%)
Bone marrow failure syndromes (BMFS)	22 (41%)	15 (21%)
Outcome variables		
Neutrophil engraftment (%)		
Yes	48 (89%)	69 (92%)
No	6 (11%)	2 (3%)
Day of neutrophil engraftment, median (IQR)	18.0 [10, 69]	18.0 [11, 76]
At least 1 BSI (%)	17 (31%)	43 (61%)
BSI caused by CRE (% of those with at least 1 BSI)	3 (18%)	7 (16%)
Outcome day 100		
Alive	45 (83%)	61 (86%)
Dead	8 (15%)	10 (14%)
HCT 2	1 (2%)	0 (0%)
Etiology of BSI		
Total BSI's	27	71
Gram positive bacteria	10 (37%)	28 (39%)
Enterococcus faecium	4	12
Staphylococcus aureus [MRSA]	2	4 [2]
Coagulase-negative Staphylococci	2	7
Viridans streptococci	1	4
Granulicatella adiacens	1	0
Mycobacterium tuberculosis	0	1
Gram negative bacteria	17 (63%)	38 (54%)
Klebsiella pneumonia spp [CR-GNB]	6 []	24 [7]
Escherichia coli	4	4
Acinetobacter spp. [CR-GNB]	0	4 [3]
Stenotrophomonas maltophilia	3	0
Pseudomonas aeruginosa	0	1
Ralstonia insidiosa	0	1

Supplementary table 1: Continued

	<18 years	≥18 years
	N = 54	N = 71
Serratia marcescens [CR-GNB]	1 [1]	1 [1]
Burkholderia cepacia	0	1
Enterobacter cloacae complex	1	0
Salmonella spp. (non-Typhi)	1	0
Campylobacter spp.	0	1
Other non-fermentative bacteria*	1	1
Polymicrobial BSI	0 (0%)	5 (7%)

Other non-fermentative: Delftia acidovorans, and non-fermentative not further specified. HCT = hematopoietic stem cell transplantation, IQR = interquartile range, MRSA = Methicillin-resistant *Staphylococcus aureus,* CR-GNB = Carbapenem resistant gram-negative bacteria.

Supplementary table 2: Presence of bacteria in surveillance cultures detecting carbapenem-resistant gram-negative bacteria

Name	Auto-HCT (N = 23)	Allo-HCT (N = 19)	Total (N = 42)
Klebsiella pneumoniae spp	20	15	35
Escherichia coli	3	4	7
Enterobacter cloacae complex	0	1	1
Citrobacter freundii	1	0	1

Two surveillance cultures (1 in auto-HCT, 1 in allo-HCT) detected two different carbapenem-resistant pathogens (both E. coli and K. pneumoniae). Therefore, totals in this table are higher compared to Table 4.

13.2. QUESTIONNAIRE FOR CARVS IN HAEMATOLOGY AND TRANSPLANT PATIENTS

Testing policies for Haematology patients: Respiratory Infections and Virus knowledge across multiple centres (THRIVE-study).

Dear clinician,

We are doing research about the different testing policies of community acquired respiratory viruses (CARVs) specifically in haematology patients (with haematological malignancies receiving treatment and/or bone marrow transplant) across different centres in the world and the knowledge base of its management.

The purpose of this study is to better understand the heterogeneity in testing policies across the different centres and differences in the knowledge base among clinicians.

Your participation in this study can contribute to improve the testing policies and education of clinicians to improve management of vulnerable haematology patients that acquire CARVs.

The study will be conducted in the form of an online digital questionnaire. See link below.

Participation is entirely voluntary and no identifying information will be collected.

This study has been approved by the Human Research Ethics Committee of Spaarne Gasthuis Hospital, the Netherlands. The principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

If you have any concern over the way the study is being conducted, please contact the Supervisor Dr Marco Goeijenbier, who may be contacted on telephone number +31232240000, or by e-mail on m.goeijenbier@spaarnegasthuis.nl.

To obtain any additional information regarding this study please contact Dr Justin du Toit at justin@bonemarrowtransplant.co.za or +27799081872.

By filling out the questionnaire, you automatically consent to participating in this study.

General questions:

1. If haematology patients (with haematological malignancies receiving treatment and/or bone marrow transplant) who present themselves with respiratory complaints at the emergency department, does your hospital run a standard test strategy (i.e. are all patients tested for the same pathogens)
   ☐ Yes
   ☐ No
   ☐ Don't know

2. Is there the availability of point-of-care (POC) testing for these patients (test performed in the emergency department with results within 3 hours)?
   ☐ Yes
   ☐ No
   ☐ Don't know
   (if Q2 = yes)

3. Which pathogens are included in the POC test?
   ☐ RSV
   ☐ SARS-CoV-2
   ☐ Influenza
   ☐ other, namely
   ☐ Don't know
   (if Q2 = yes)

4. If POC is negative, do you test further for other respiratory pathogens?
   ☐ Yes
   ☐ No
   ☐ Don't know
   (If Q4 = yes)

5. What would you include in your respiratory panel if you decide to perform molecular diagnostics after the point-of-care test?
   ☐ SARS-CoV-2
   ☐ Influenza
   ☐ Respiratory Syncytial Virus (RSV)
   ☐ Human Metapneumovirus (HMPV)
   ☐ Parainfluenza viruses
   ☐ Bocavirus
   ☐ Rhinovirus
   ☐ Mycoplasma pneumoniae
   ☐ Adenovirus

☐ Legionella pneumophila
☐ Chlamydophila pneumoniae
☐ Bordetella pertussis
☐ Other, namely...
☐ All of the above
☐ Don't know

6. For haematology patients with respiratory complaints (with haematological malignancies undergoing treatment and/or bone marrow transplant), do you perform an immunodeficiency score (Basel or MD Anderson scoring system) to judge progression of disease?
   ☐ Yes
   ☐ No
   ☐ Don't know

We would now like to present you some real live cases to better understand the heterogeneity in testing policies across the different centre.

Case 1 – Human Metapneumovirus (HMPV)

A 55-year-old female presents to the emergency department with progressive dyspnea. Her medical history includes hypertension, a history of Acute Myeloid Leukemia treated with an allogeneic stem cell transplantation, and chronic immunosuppression for graft-versus-host disease (GVHD). This patient tests positive for Human Metapneumovirus (HMPV).

Questions

7. Based on your centres testing policy, would you have tested this patient for HMPV?
   ☐ Yes
   ☐ No
   ☐ Don't know

8. Is specific antiviral therapy for prophylaxis or treatment available for HMPV in your medical centre?
   ☐ Yes
   ☐ No
   ☐ Don't know

9. Is active or passive immunisation for HMPV available currently?
   ☐ Yes
   ☐ No
   ☐ Don't know

10. Which treatment or treatments are recommended by your centre for HMPV currently? Can choose more than one
    - ☐ intravenous immunoglobulin with hypogammaglobulinaemia
    - ☐ intravenous immunoglobulin without hypogammaglobulinaemia (< 4.5g/L) only
    - ☐ judicious evaluation of lowering corticosteroid treatment
    - ☐ administer ribavirin alone without IVIG
    - ☐ administer ribavirin in combination with IVIG

11. Would you discontinue empiric antibiotic therapy based on the positive HMPV result?
    - ☐ Yes
    - ☐ No
    - ☐ Don't know

Case 2 RSV

You see a 62-year-old male patient, past history of AML, now 2 years post allogeneic stem cell transplant, for routine follow-up in your outpatient clinic. He mentions complaints of headache, exertional dyspnea and a persistent heavy cough. He is an active smoker. He tests positive of RSV.

12. Is there a vaccine available against RSV (active immunisation) at your centre?
    - ☐ Yes
    - ☐ No
    - ☐ Don't know

13. Is RSV vaccination currently recommended for haematology patients at your centre?
    - ☐ Yes, despite lack of evidence of efficacy in this population group.
    - ☐ Yes, there is good evidence in this population group.
    - ☐ No, due to lack of evidence.
    - ☐ No, not available to us.
    - ☐ Don't know

14. Is passive immunisation (monoclonal antibodies like Palivizumab) for RSV as pre-exposure prophylaxis available at your centre?
    - ☐ Yes, for adult and paediatric patients (irrespective of age)
    - ☐ Yes, for children < 2 years of age only
    - ☐ No, due to lack of evidence
    - ☐ Not available
    - ☐ Don't know

15. Is passive immunisation (monoclonal antibodies like Palivizumab) for RSV as post-exposure prophylaxis available at your centre?
    - ☐ Yes, for adult and paediatric patients (irrespective of age)
    - ☐ Yes, for children < 2 years of age only
    - ☐ No, due to lack of evidence
    - ☐ Not available
    - ☐ Don't know

16. Would you consider giving systemic ribavirin therapy for the above patient?
    - ☐ Yes, if immunodeficiency score index indicate that patient is at high risk of progression to lower respiratory tract infection.
    - ☐ Yes, irrespective of immunodeficiency score index
    - ☐ No, would not give due to lack of evidence
    - ☐ Not available
    - ☐ Don't know

17. If symptoms are mild and patient is clinically well, would you consider systemic Ribavirin therapy anyway?
    - ☐ Yes
    - ☐ No
    - ☐ Don't know

18. Would you consider giving IVIG for your patient? LRTI = lower respiratory tract infection.
    - ☐ Yes, if immunodeficiency score index indicate that patient at high risk of progression to LRTI (or established LRTI) (not hypogammaglobulinaemic)
    - ☐ Yes, if immunodeficiency score index indicate that patient at high risk of progression to lower respiratory tract infection (or established LRTI) (patient is hypogammaglobulinaemic)
    - ☐ Yes, irrespective of immunodeficiency score index
    - ☐ No, would not give due to lack of evidence
    - ☐ Not available
    - ☐ Don't know

Case 3 Parainfluenza

You see a 79-year-old male with myeloma on Lenalidomide, Daratumumab, Dexamethasone therapy (two pathological fractures) presenting with 10-day history of increasing fatigue, muscle aches, shortness of breath, no cough, but febrile (38.7° C) in emergency department (COVID-19 test: negative). Chest X-ray: Possible pneumonia left basally. He tests positive for human parainfluenza (HPIV) virus.

19. Is there active and/or passive immunisation available against HPIV?
☐ Yes, active immunisation only.
☐ yes, passive immunisation only.
☐ yes, both active and passive immunisation only.
☐ No active nor passive immunisation available.
☐ Don't know

20. Is there a specific antiviral therapy available against HPIV?
☐ Yes
☐ No
☐ Don't know

21. Management options for this patient includes: can choose more than one.
☐ IVIG if patient hypogammaglobulinaemic.
☐ Judicious lowering of corticosteroid treatment.
☐ Supportive therapy
☐ IVIG even if patient is not hypogammaglobulinaemic
☐ No treatment available
☐ Infection control measures as adopted by your centre (good personal hygiene, private rooms, personal protection measures in hospital (gloves, gowning and masks) for healthcare providers and visitors.
☐ Don't know
Other, namely _____

Case 4 Influenza A

You see a 75-year-old female in the emergency department with left-sided breast cancer (2008) - lumpectomy, known with peripheral arterial disease, COPD GOLD III and newly diagnosed Myelodysplastic syndrome (MDS) with Out-of-hospital cardiac arrest, most likely due to hypoxia but suspected respiratory deterioration leading up to event. She tests positive for Influenza A virus (IAV).

22.Is there active and/or passive immunisation available against IAV?
☐ Yes, active immunisation only.
☐ yes, passive immunisation only.
☐ Both active nor passive immunisation available.
☐ No active nor passive immunisation available.
☐ Don't know

23. Is there a specific antiviral therapy available against IAV?
☐ Yes, oseltamivir therapy is available
☐ No

☐ Don't know

24. Is post exposure prophylaxis with Oseltamivir (within 48 hours) recommended for haematology patients?
☐ Yes, recommended
☐ No, not recommended.
☐ Don't know

25. If above patient continues to test positive for IAV on nucleic acid testing (NAT) testing after 7 days with continuing symptoms, would you consider the following: choose more than one.
☐ Stop Oseltamivir and just supportive treatment.
☐ Extend oseltamivir for at least 10 days of therapy.
☐ Double the dose of Oseltamivir to 150mg twice daily.
☐ Consider adding Baloxavir therapy to Oseltamivir.
Other, namely _____

Demographic Information

Age: _____

Medical specialty: _____

- Intensive care specialists or trainee
- Emergency medicine specialist or trainee
- Medical oncology or trainee
- Clinical haematology or trainee
- Paediatric oncologist or trainee
- Paediatric haematology or trainee

Years of experience or study: _____

Country of residence: _____

Thank you for your participation and watch out for a link soon illustrating the research results.

Table S1. Characteristics of survey respondents (N = 98)

Characteristic	Category	n	% of N
Specialty	Clinical haematology or trainee	64	65.3%
	Medical oncology or trainee	8	8.2%
	Intensive care specialist or trainee	7	7.1%
	Emergency medicine specialist or trainee	4	4.1%
	Paediatric oncology or trainee	4	4.1%
	Paediatric haematology or trainee	2	2.0%
	Missing/not reported	9	9.2%
World region	Europe	63	64.3%
	Africa	19	19.4%
	South America	6	6.1%
	North America	4	4.1%
	Asia	3	3.1%
	Oceania	2	2.0%
	Country missing	1	1.0%
World Bank income group	High income	69	70.4%
	Upper-middle income	27	27.6%
	Lower-middle income	1	1.0%
	Low income	0	0.0%
	Country missing/ unclassifiable	1	1.0%
Clinical experience	Median (IQR), years	15 (8.75–25)	96 responses
Country	26 identifiable countries	See Supplementary Table S1	97 country responses

Footnotes: Country names were standardised before assigning world region and World Bank FY2026 income category. Percentages use N=98 unless otherwise stated.

Table S2. Geographic distribution of survey respondents (N = 98)

World region	Participating countries (n)	Respondents, n	Respondents, %
Europe	Belgium (6); Croatia (1); Czech Republic (1); Denmark (1); France (6); Germany (5); Italy (17); Lithuania (1); Netherlands (16); Portugal (1); Serbia (1); Slovakia (1); Spain (2); Sweden (1); Switzerland (1); United Kingdom (2)	63	64.3
Africa	Algeria (1); Ghana (1); South Africa (17)	19	19.4
South America	Argentina (2); Brazil (3); Chile (1)	6	6.1
North America	Canada (1); United States (3)	4	4.1
Asia	Türkiye (3)	3	3.1
Oceania	Australia (2)	2	2.0
Not reported	Country not reported	1	1.0
Total		**98**	**100.0**

Note: Percentages use all 98 analytic respondents as the denominator. One respondent (1.0%) did not report country. Country names were standardised before classification. Türkiye was classified in Asia (Western Asia).

Supplementary table S3. Exploratory subgroup comparisons

S3A. By clinical specialty

Group	n	Standardised test	POCT	Immunodeficiency score	Test HMPV	Any ribavirin	Oseltamivir PEP
Clinical haematology	64	42/64 (65.6%)	34/64 (53.1%)	3/64 (4.7%)	40/64 (62.5%)	29/62 (46.8%)	39/63 (61.9%)
Medical oncology	8	6/8 (75.0%)	5/8 (62.5%)	0/8 (0.0%)	7/7 (100.0%)	6/8 (75.0%)	7/8 (87.5%)
Intensive care	7	4/7 (57.1%)	5/7 (71.4%)	0/6 (0.0%)	5/7 (71.4%)	1/7 (14.3%)	6/7 (85.7%)
Emergency medicine	4	3/4 (75.0%)	3/4 (75.0%)	0/4 0.0%)	1/4 (25.0%)	0/4 (0.0%)	3/4 (75.0%)
Paediatric oncology	4	3/4 (75.0%)	3/4 (75.0%)	0/3 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	4/4 (100.0%)
Paediatric haematology	2	0/2 (0.0%)	0/2 (0.0%)	0/2 (0.0%)	0/2 (0.0%)	1/2 (50.0%)	2/2 (100.0%)
Overall exact p		0.5522	0.5455	1.0000	0.0444	0.0677	0.3724
Holm-adjusted p		1.0000	1.0000	1.0000	0.2663	0.3383	1.0000

S3B. By World Bank income group

Group	n	Standardised test	POCT	Immunodeficiency score	Test HMPV	Any ribavirin	Oseltamivir PEP
High income	69	52/69 (75.4%)	48/69 (69.6%)	4/68 (5.9%)	49/68 (72.1%)	36/69 (52.2%)	50/68 (73.5%)
Upper-middle income	27	12/27 (44.4%)	6/27 (22.2%)	0/26 (0.0%)	14/27 (51.9%)	8/25 (32.0%)	18/27 (66.7%)
Lower-middle income	1	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)
Overall exact p		0.0048	<0.0001	0.5893	0.0532	0.1050	0.2798
Holm-adjusted p		0.0239	0.0003	0.5893	0.2130	0.3151	0.5597

S3C. By world region

Group	n	Standardised test	POCT	Immunodeficiency score	Test HMPV	Any ribavirin	Oseltamivir PEP
Europe	63	47/63 (74.6%)	43/63 (68.3%)	3/62 (4.8%)	43/62 (69.4%)	33/63 (52.4%)	44/62 (71.0%)
Africa	19	6/19 (31.6%)	5/19 (26.3%)	0/18 (0.0%)	7/19 (36.8%)	3/17 (17.6%)	9/19 (47.4%)
Asia	3	2/3 (66.7%)	0/3 (0.0%)	0/3 (0.0%)	2/3 (66.7%)	1/3 (33.3%)	3/3 (100.0%)
North America	4	3/4 (75.0%)	3/4 (75.0%)	1/4 (25.0%)	4/4 (100.0%)	3/4 (75.0%)	4/4 (100.0%)
South America	6	4/6 (66.7%)	2/6 (33.3%)	0/6 (0.0%)	5/6 (83.3%)	4/6 (66.7%)	6/6 (100.0%)
Oceania	2	2/2 (100.0%)	1/2 (50.0%)	0/2 (0.0%)	2/2 (100.0%)	0/2 (0.0%)	2/2 (100.0%)
Overall exact p		0.0117	0.0015	0.3844	0.0399	0.0306	0.0636
Holm-adjusted p		0.0586	0.0091	0.3844	0.1225	0.1225	0.1272

Definition note: "Any ribavirin" includes any affirmative response to Q16 (risk-score guided or irrespective of risk score). "Affirmative" outcomes are compared against all other non-missing response categories. No transplant-versus-non-transplant clinician analysis is possible because transplant-practice status was not collected.

13.3 SUPPLEMENTARY FOR HAPLO-TRANSPLANT STUDY

Supplementary information:

Supplementary table 1. Neutrophil and platelet engraftment with PBSC versus BM as SC source.

		Overall		Platelet engraftment			Neutrophil engraftment		
Charac-teristic	**Category**	**n**	**%**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**
n		134							
Stem cell source	Bone marrow	36	26.9		1	reference		1.00	reference
	PBSC	95	70.9	0.038	1.56	1.03–2.37	0.0060	1.74	1.18–2.59
	BM + PBSC	3	2.2						

Supplementary table 2. Association between acute and chronic GVHD rates and SC source.

		Overall		SC source = BM		SC source = PBSC		p-value for between-group test
Characteristic	**Category**	**n**	**%**	**n**	**%**	**n**	**%**	
Acute GVHD (n = 108 alive at day 100)	no	63	58,3	13	48,1	48	60,8	0,25
	yes	45	41,7	14	51,9	31	39,2	
Chronic GVHD (n = 51 alive and no relapse)	no	31	60,8	4	44,4	26	63,4	0,45
	yes	20	39,2	5	55,6	15	36,6	

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About the author

Justin Rudolph du Toit

MBChB (Pretoria, 2005); FCP(SA) (2013); MSc (Rotterdam, 2015); Cert Clin Haem (Phys) (SA) (2018); MPhil (UCT, 2023)

Born in Krugersdorp, South Africa, on 13 December 1980, Justin du Toit obtained his MBChB degree from the University of Pretoria in 2005 and qualified as a Specialist Physician at the University of Cape Town in 2013. He was subsequently awarded a scholarship to pursue an MSc in Infection and Immunity at the Erasmus Medical Center in Rotterdam, which he completed in 2015. During this period, he undertook two research internships in the Viroscience Laboratory, where he investigated viral–host immune interactions.

Following his return to South Africa, Dr du Toit completed subspecialty training in Clinical Haematology at the University of Cape Town, qualifying in 2018. He was awarded the Mabashan Prize for best student in Clinical Haematology in recognition of his academic performance.

In 2019, Dr du Toit established his private practice at the Wits Donald Gordon Cellular + Immunotherapy Centre, where he serves as Bone Marrow Collection Director and Transplant Physician. The centre achieved JACIE accreditation in 2025, reflecting its adherence to international standards of transplant care.

With a strong commitment to medical education, he founded the first online learning platform in South Africa for senior registrars and physicians interested in bone marrow transplantation. He serves on the European Society for Blood and Marrow Transplantation (EBMT) Training Committee and is a member of the executive committee of the South African Stem Cell Transplant Society.

Dr du Toit commenced his PhD in 2020 under the supervision of Dr Marco Goeijenbier and Professor Eric van Gorp, later joined by Professor Estelle Verburgh and Dr Jurjen Versluis. The collaborative research undertaken during this period culminated in the work presented in this thesis.

He lives in Johannesburg with his husband, Vincent Jones, and their two dogs, Newton and Pluto. Outside of medicine, his interests include fossils (paleontology), history, running, cycling, and travel.

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Abbreviations

ACE	angiotensin converting enzyme
AdV	adenovirus
AHA	acquired haemophilia A
AL	acute leukaemia
AML	acute myeloid leukaemia
ANA	antinuclear antibody
ANC	absolute neutrophil count
anti-dsDNA	anti-double stranded DNA antibodies
APC	Antigen Presenting Cell
APL	acute promyelocytic leukaemias
aPTT	activated partial thromboplastin time
ARDS	Acute Respiratory Distress Syndrome
ASCT	autologous haematopoietic stem cell transplant
ATG	anti-thymocyte globulin
BAL	bronchoalveolar lavage
BM	bone marrow
BMFS	bone marrow failure syndrome
BMI	body mass index
BSI	bloodstream infections
BU	Bethesda units
CAR	Coxsackie-Adeno Receptor
c-ART	combination antiretroviral therapy
CARV	community-acquired respiratory virus
CCHF	Crimean Congo hemorrhagic fever
CCL	Chemokine Ligand
CD	cluster of differentiation
CHIKV	Chikungungya virus
CI	confidence interval
CI	cumulative incidence
CLEC	c-type lectin
CLL	chronic lymphocytic leukaemia
c-MPL	myeloproliferative leukemia protein
CML	chronic myeloid leukemia
CMML	chronic myelomonocytic leukaemia
CMV	cytomegalovirus
COPD	chronic obstructive pulmonary disease
COVID-19	coronavirus-disease-2019

CoxV	coxsackie virus
CR	complete remission
CR-GNB	carbapenem resistant gram-negative bacteria
CRE	carbapenem-resistant *Enterobacterales*
CTA	computed tomography angiograph
CVC	central venous catheter
DC-SIGN	Dendritic Cell-Specific Intercellular Adhesion Molecule-3-Grabbing Non-Integrin
DENV	Dengue virus
DFS	disease-free survival
DIC	Disseminated Intravascular Coagulation
DNA	desoxyribonucleic acid
EBMT	European Society for Blood and Marrow Transplantation
EBV	Ebstein Barr virus
ECIL-10	European Conference on Infections in Leukaemia
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
EM	Electron Microscopy
EMC	Erasmus Medical Centre
FcϒR2A	Fc Gamma Receptor 2a
FDP	Fibrinogen Degradation Product
FEIBA	Factor Eight Inhibitor Bypassing Activity
FGF-4	Fibroblast Growth Factor 4
FGT	fibrin generation time
G-CSF	granulocyte-colony stimulating factor
GM-CSF	Granulocyte-Monocyte Colony Stimulating Factor
gMDSC	Granulocytic Myeloid-derived suppressor cell
GNB	gram-negative bacteria
GP	glycoprotein
GPB	gram-positive bacteria
GPIb	glycoprotein Ib
GSH	Groote Schuur Hospital
GVHD	graft-versus-host disease
HaploHCT	Haplo-identical haematopoietic cell transplantation
Hb	haemoglobin
HBoV	human bocavirus
HBV	hepatitis B Virus

HCC	hepatocellular carcinoma
HCT	haematopoietic stem cell transplantation
HCT-CI	HCT comorbidity index
HCV	hepatitis C virus
HDV	hepatitis delta virus
HFRS	hemorrhagic fever and renal syndrome
HHV-6	human herpesvirus 6
HIV	human immunodeficiency virus
HL	Hodgkin's lymphoma
HLA	Human Leukocyte Antigen
HLH	Hemophagocytic Lymphohistiocytosis
HMPV	human metapneumovirus
HPIV	human parainfluenza virus
HSC	haematopoietic stem cells
HCT	hematopoietic stem cell transplantation
HSP	Heparan Sulfate Proteoglycan
HUVEC	human vascular endothelial cells
IAV/IBV	influenza A/B virus
ICU	intensive care unit
Ig	immunoglobulin
IL-3, IL-6, IL-9	interleukins
INR	international normalized ratio
IQR	interquartile range
IRIS	immune reconstitution inflammatory syndrome
ISI	Immunodeficiency Scoring Index
ITP	immune thrombocytopenia purpura
IUT	intrauterine transfusion
IV	influenza virus
IVIG	intravenous immunoglobulin
JEV	Japanese encephalitis virus
KPC	*K. pneumoniae* carbapenemase
LCMV	lymphocytic choriomeningitis virus
LDH	lactate dehydrogenase
LFA	lateral flow assay
LL	lymphoblastic lymphoma
LMWH	low molecular weight heparin
LP	lumbar puncture

LRTD	lower respiratory tract disease
LRTI	lower respiratory tract infection
MAC	myeloablative chemotherapy
MC-FGT	modified fibrin generation time
MDR	multidrug-resistant
MDS	myelodysplastic syndrome
MF	mobilisation failure
MHC	major histocompatibility complex
MM	multiple myeloma
MP	microparticle
MPN	myeloproliferative neoplasm
MPV	mean platelet volume
MRD	matched related donors
MRD	minimal residual disease
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
MUD	matched unrelated donor
MV	measles virus
NAT	nucleic acid testing
NCD	non-communicable disease
NDM	New Delhi metallo-β-lactamase
NET	neutrophil extracellular trap
NHI	National Health Insurance
NHL	non-Hodgkin's lymphoma
NRM	non-relapse mortality
NS1	nonstructural protein 1
OR	odds ratio
OS	overall survival
OXA-48	oxacillinase-48
PAF	platelet activating factor
PAMP	pattern associated molecular pattern
PAR	protease activating receptor
PB	peripheral blood
PBCD34	peripheral blood CD34+ cell count
PBM	patient blood management
PBMC	peripheral blood mononuclear cells

PBSC	peripheral blood haematopoietic stem cell
PCR	polymerase chain reaction
PDW	platelet distribution width
PE	pulmonary emboli
PF4	platelet factor 4
PLR	platelet lymphocyte ratio
PM	poor mobilisation
PMA	platelet-monocyte aggregate
PMP	platelet microbicidal peptide
POCT	point-of-care testing
PRR	pattern recognition receptor
PS	phospatidylserine
PSGL-1	P-Selectin Glycoprotein Ligand-1
PT	prothrombin time
PTCY	post-transplant cyclophosphamide
PUUV	puumala virus
PVB-19	parvovirus B19
RBC	red blood cell
RD	related donor
RI	relapse incidence
RIC	reduced intensity conditioning
RNA	ribonucleic acid
RotV	rotavirus
RR	relative risk
RSV	respiratory syncytial virus
SAA	severe aplastic anemia
SABMR	South African Bone Marrow Registry
SANBS	South African National Blood Service
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SASCETS	South African Stem Cell Transplant Society
sCD40L	soluble CD40 ligand
SD	standard deviation
SDD	selective decontamination of the digestive tract
SDF-1	Stromal Derived Factor 1
SFTS	severe fever with Thrombocytopenia Syndrome
SG	Spaarne Gasthuis Hospital
SM	super mobilisation

SOT	solid organ transplant
SSPE	subacute sclerosing panencephalitis
TA-GVHD	transfusion-associated graft-versus-host disease
Tat	transactivating factor
TB	tuberculosis
TBEV	tickborne encephalitis virus
TBI	total body irradiation
TF	tissue factor
TFPI	tissue factor pathway inhibitor
T-LL	T-lymphoblastic lymphoma
TLR	toll-like receptor
TPO	thrombopoietin
UCT	University of Cape Town
UD	unrelated donor
VHF	viral hemorrhagic fever
VIM	Verona Integron-Encoded Metallo-beta-lactamase
VL	viral load
VRE	vancomycin-resistant enterococci
VTE	venous thromboembolisms
VZV	varicella Zoster virus
WBC	white blood cell
WCC	white cell count
WDG	Wits Donald Gordon Medical Centre
WNV	West Nile virus
YFV	yellow fever virus
γHV68	murine gammaherpesvirus 68
ZIKV	Zika Virus

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Future directions

Several promising avenues for future research emerge from this PhD thesis:

1. **Monoclonal antibody prophylaxis in haematopoietic stem cell transplant (HCT) recipients:**
   Investigate the role of monoclonal antibodies for RSV prevention in post-HCT patients, either as pre-exposure prophylaxis during seasonal outbreaks or as post-exposure prophylaxis during conditioning therapy.
2. **RSV vaccination prior to HCT:**
   Assess the utility of RSV vaccination in HCT and haematology patients and determine whether prior immunisation improves outcomes in those who later acquire RSV infection.
3. **Ribavirin plus IVIG in early RSV disease:**
   Explore whether early intervention with ribavirin and IVIG in post-HCT patients with mild RSV infection reduces progression to severe lower respiratory tract disease and improves overall outcomes.
4. **Epidemiology of bloodstream infections in private HCT centres:**
   Evaluate the incidence and outcomes of bloodstream infections in HCT recipients at high-volume private transplant units in South Africa, as this may reveal higher MDR prevalence and mortality compared with public-sector hospitals.
5. **Standardising CARV testing policies:**
   Develop and implement a unified testing panel for community-acquired respiratory viruses (CARVs) across all bone marrow transplant centres in SA, followed by a prospective study to assess its impact on detection rates, clinical decision-making and patient outcomes.
6. **Optimising Etoposide dosing for stem cell mobilisation:**
   Conduct a prospective study comparing high-dose versus low-dose etoposide for peripheral blood stem cell mobilisation to determine whether lower doses provide adequate haematopoietic stem cell yields with reduced toxicity and lower costs related to cryopreservation.
7. **To conduct a comparative study of two graft-versus-host disease (GVHD) prophylaxis strategies using propensity score matching: post-transplant cyclophosphamide (PTCy) versus alemtuzumab "in-the-bag"**. This study will compare the PTCy strategy employed at Erasmus Medical Center for matched related and mismatched unrelated donor transplants with the alemtuzumab-based ex vivo T-cell depletion strategy used in South Africa. Primary outcomes will include overall survival, GVHD-free/relapse-free survival, non-relapse mortality, and the incidence of major post-transplant complications.

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Portfolio

Erasmus University Rotterdam

Faculteit der Geneeskunde en Gezondheidswetenschappen

13-11-2025

PHD PORTFOLIO

Justin Rudolph Du Toit
13-12-1980

Description	Organiser	FC
Supervising Master student Rory Shein (2020)		1.00
EBMT statistical course (2020)		0.50
Scientific Writing and Manuscript Preparation course (2020)	South Africa Haematology and Transfusion Medicine Fogarty Program	8.00
Supervising Master student Erkan Yildiz (2020)		2.00
EBMT transplant course (2021)		0.80
Poster presentation EBMT meeting 2022 (2022)		1.50
Oral presentation SASCeTS 2022 (2022)		1.00
Oral presentation EBMT deep dive (2023)		0.50
Scientific Integrity (2023)	Erasmus MC Graduate School	0.30
8th International Transplant and Cellular Therapy Course (2023)		1.00
PhD student network meeting Cirion foundation (2023)		0.20
EBMT Madrid Spain (2024)		1.00
Oral IDRC WMDA meeting (2024)		1.50
Supervising Master Student Adam Setzer (2024)		2.00
9th Congress on Controversies in Stem Cell Transplantation and Cellular Therapies (COSTEM) (2024)		1.00
MD Anderson invited lecture (2024)		0.50
Supervising Master student Tobias Neijzen (2025)		4.00
Invited lecture Spaarne Research meeting (2025)		0.20
Presentation ESWI Valencia (2025)		3.00
Optional		
Hematology Deep Dive lecture (2024)		0.50
BMT classroom lecture (2025)		0.50
BMT class room (2025)		0.50
		------ +
Total EC		**31.50**

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Dankwoord

A PhD is supposedly an individual academic achievement. Having now completed one, I can confidently say that this is completely untrue. There is absolutely nothing individual about it.

This thesis may have my name printed on the front, but there are fingerprints, coffee stains, reviewer comments, WhatsApp messages, statistics, late-night panic, friendship, love and probably a small amount of psychological damage from a very large number of people throughout it.

So, before anyone changes their mind and takes the degree back, I would like to thank the people who made this particular Mission Impossible possible.

First, to my promotor, Professor Eric van Gorp. Eric, thank you for always making time for our research meetings, for critically examining our ideas and, when necessary, gently bringing us back down to planet Earth. Importantly, however, you never brought me so far back to reality that I stopped believing this could actually be done. Thank you for giving me enough freedom to remain curious, enough criticism to make the science better, and enough encouragement to keep going. Most importantly, thank you for believing that I would eventually finish this. You were right. Eventually.

To my co-promotor and dear friend, Marco Goeijenbier. I think we should finally acknowledge publicly that this entire PhD was partly your fault. The conspiracy began in Cape Town during an internship at our haematology department, when Marco somehow convinced me that I had the potential to do a PhD. At the time, I believed him. Years later, somewhere around reviewer comment number 487, I occasionally reconsidered that decision. Marco, you are one of the hardest working and most enthusiastic people I know, but what I admire most is the way you combine your career with being such a devoted father and family man to your beautiful daughters, Josie and Liz. =Since those early Cape Town days, I have grown enormously – not only as a scientist and clinician, but as a person and as a friend. You inspire me.

And to Odette: without your Dutch discipline and strictness, there is a reasonable possibility that this thesis would still be sitting somewhere on my laptop entitled FINAL_version_17_really_final_this_time.docx. Thank you for taking such wonderful care of my dear friend Marco and your girls. I wish your family nothing but happiness and success.

To Professor Estelle Verburgh, my South African haematology guru. You are one of the brightest haematology minds I know. Your enthusiasm, your seemingly

inexhaustible energy and, above all, your love for haematology, for people and for our public-sector patients are extraordinary. You have always had my back. You believed in me long before I was entirely sure that I believed in myself, and I will always be grateful for that. South African haematology has accelerated with you at the steering wheel – and occasionally, I suspect without observing the speed limit. Watching what you have achieved leaves me inspired and in awe. May we continue working, plotting and conspiring together for many years to improve the lives of our patients.

To Jurjen Versluis. Jurjen, may I one day be able to think as clearly as you do. And may I one day understand statistics the way you do. I accept that the second request may require another lifetime. Thank you for answering haematology questions at completely unreasonable hours and for so generously sharing your knowledge with our transplant unit in South Africa. PTCy is now well underway in our unit, and through your willingness to teach and collaborate, your influence is already reaching patients thousands of kilometres away. I have no doubt that as you grow, Erasmus MC Haematology will continue to grow with you.

To Tobias Neijzen. Tobias, as your self-appointed South African parents, Vincent and I want to thank you for coming all the way to South Africa to help with the data capturing and, apparently having not learnt your lesson, for now joining us on a systematic review. Your energy, enthusiasm, kindness and zest for life are wonderfully contagious. You arrived to help with research and very quickly became part of the family. We hope South Africa keeps pulling you back – not only for the science, the sunshine and the people, but also for the entirely legitimate academic pursuit of eating some of the best steaks on Earth. Come back often. There will always be another dataset, a table that needs fixing and, most importantly, a very good steak waiting for you.

To my assessment committee, Professor de Vlas, Professor Sander Herfst and Professor Tutu van Furth: thank you for the enormous honour of assessing this work. Sander, you are quite possibly the hardest-working virology nerd I know – and in the world in which I now move, that is genuinely a compliment. Professor Tutu van Furth, it is particularly meaningful to me that your life intersected with our beloved Archbishop Desmond Tutu, a man who played such an extraordinary role in South Africa's truth and reconciliation process and in our country's continuing journey towards healing. It is a great privilege to have all of you as part of this milestone.

And then, to my dear husband, Vincent Jones. You have walked – sometimes quite literally – this very long and winding road with me. There were many

days when I would see 20 or 30 patients, come home late, finish treatment plans and then discover, waiting patiently for me, another beautiful collection of reviewers' comments. Nothing says romance quite like: "Reviewer 2 has several additional concerns." There was moaning. There was complaining. There was occasionally language unsuitable for an academic thesis. There were nights when I worked until far too late and mornings when I woke far too early to continue. And throughout all of it, you were there. When I felt that I had had enough, you encouraged me to continue. When continuing was clearly becoming counterproductive, you had the equally important wisdom to prescribe a distraction – usually involving travel, food, a walk, wine or some other highly evidence-based intervention.

Thank you for your patience, your encouragement and your endless love. Thank you for understanding the sacrifices this journey demanded without ever allowing the PhD to become more important than life itself. Without you, I genuinely could not have done this. I love you.

To my dear parents, Rudolph and Marie. Thank you for encouraging me to study, for allowing me to pursue my dreams and for teaching me that curiosity is something to be treasured rather than controlled. You have always told me that I am a free spirit. I suspect that at several points you may have wondered whether encouraging that particular quality was wise. But you never stopped believing in me. You gave me the freedom to explore, to make mistakes, to travel, to learn and eventually to find my own path. Everything I have achieved rests, in some way, on the foundation you gave me. I love you to the ends of the Earth.

To my late grandfather Van der Merwe and his wife, Nella van der Walt. Without the smell of freshly baked bread, the discipline you instilled in me and Sunday afternoons spent watching David Attenborough, I doubt my curiosity would have developed quite the way it did. Somewhere between a loaf cooling in the kitchen and another impossibly strange animal appearing on television, you taught me that the world was worth paying attention to. You played a fundamental role in shaping who I am and where I am today. I will forever be grateful.

To my late Granny du Toit (née Jooste), quite simply the toughest woman I have ever known. You were tough as nails – and, frankly, you had to be. You single-handedly raised six children, including my apparently rather naughty father, which I think should qualify as its own doctorate. You taught me one of life's least glamorous but most useful lessons: self-pity gets you absolutely nowhere. Get up, carry on and preferably feed everyone first. I still miss your spaghetti bolognese, our conversations about your travels through Europe, and our visits

to my Aunty Noddy/Maudie. Those stories made the wider world feel close long before I began travelling through it myself. I miss you deeply, and I carry your toughness, humour and refusal to feel sorry for yourself with me.

To my cousin Jason and my uncle Wally, thank you for always taking such a genuine interest in my life, for your support, the adventures and the travels. It means more to me than you know.

To my dear friend Ronwyn. Your quirkiness, stubborn optimism and wonderfully crazy energy are forces of nature. Your willingness to travel to virtually the ends of the Earth with us is perhaps either proof of extraordinary friendship or questionable judgement. Probably both. Your friendship and inspiration carried me through many parts of this journey. Without you, this PhD would have been considerably less colourful and quite possibly would have fallen flat. I adore you, and I will always have your back.

To Mart, quite simply one of the most intelligent people I know. I will never forget our Master's years, when you supported me in ways both intellectually profound and extremely practical – including buying me coffee and, on at least one memorable occasion, pants, when I had no money. That is friendship at its most fundamental level. You introduced me to your family, and to your father Bart and mother Maria: thank you for welcoming me with such extraordinary kindness and making me feel part of your family.

Through you, I learnt how to taste a proper espresso, how to cook a mysterious Dutch vegetable called pastinaak with duck, how to approach durian – the world's most antisocial fruit – and somehow accumulated memories involving fish and swimming in Big Bay in Hong Kong that still make me smile. These wonderfully strange experiences have been some of the highlights of my life. Thank you for being part of it.

To Linda, another of the brightest people I know, better at football than I will ever be, fellow traveller, co-foodie and one of the very few people capable of challenging Mart – which I continue to find both fascinating and entertaining. You essentially define our social life.

And to Leo, the happiest little chap who simply loves people: we love you enormously and care for you as though you were our own.

To Tami, my friend with unquestionably superior knowledge of Asian teas. Thank you for continually educating me – and for never making me feel entirely

uncultured while doing so. Your curiosity about the world is something I deeply connect with. Yunnan is still waiting for us.

To Carmelo, the finest Italian-stallion haematologist I know. Admittedly, this is a highly specific category. Thank you for your collaborative spirit, your advice, your guidance and your friendship. I hope the next chapter includes a South African safari and your return to teach us even more haematology at SASMO and SASCETS.

To my dear colleague John Tilley and partner Calvin, thank you for the cooking clubs, the Michelin-quality food, the extraordinary internal-medicine knowledge and for simply always being available when advice was needed. John, I have never met anyone who can run an ICU quite like you. I remain simultaneously impressed and slightly terrified.

And finally, there are so many other people who have shaped my career and my life, sometimes through mentorship, sometimes through friendship, sometimes through collaboration, and sometimes simply by appearing at exactly the right moment: Mariza Tunmer, Mark Phipson, Jackie Thomson, Nandi Sigedle, Jennifer Geel, Matthew Seftel, Vernon Louw, Andrew McDonald, David Brittain, Nicolas Novitzky, Alan Davidson, Wendy Spearman, Mark Sonderup, Sharlene Parasnath, Cecile du Toit, Adam Setzer, Jenna Damelin, Tanya Glatt and Sue Tager. Thank you.

When you reach the end of something as long as a PhD, it is tempting to look at the thesis and see a collection of papers, tables, statistics and references. But when I look at it, I see people. I see mentors who challenged me. Colleagues who taught me. Friends who fed me, travelled with me, bought me coffee – and occasionally pants. Patients who reminded me why the science matters. Parents who gave me the freedom to dream. And a husband who stood beside me while that dream became considerably more complicated than originally advertised. Science is built on curiosity. Medicine is built on humanity. And a life worth living, I have learnt, is built on the people with whom we share both.

So although my name may appear on this PhD, this achievement belongs, in some small way, to every one of you. Thank you for believing in me. Thank you for walking this road with me. And thank you for helping me prove that this particular Mission Impossible was, after all, possible.