National profile of the characteristics of patients treated for TTP

An extension of the study: The characteristics of patients with TTP at the Universitas Haematology Department from 2010- 2017 (HSD 2018/0114)

Submitted in the fulfilment of the requirements in respect of the masters' degree M.Med. in the Department of Internal Medicine, Faculty of Health Sciences at the University of the Free State.

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I, Malekhetho Mohale, declare that this Master's degree mini-dissertation is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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- My Supervisor, Dr. C Barrett, for your endless support and guidance with regards to fundamentals of research.
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The protocol that has been approved by the University of the Free Sate Health Research Ethics Committee is, National profile of characteristics of patients treated for TTP which is an extension of the study: The characteristics of patients with TTP at Universitas Haematology department from 2010 – 2017.

This study relied solely on the provision of data from the WCBS, and not all the information could be supplied. This was initially planned as a national study, but data was only received from the Western Cape Province and the researchers were unable to obtain data from the South African National Blood Service (SANBS), limiting the study to the Western Cape.

The manuscript title has therefore been changed to; Thrombotic Thrombocytopenic Purpura in the Western Cape: Patient profile from the Western Cape Blood Service from 2010 to 2017.

Table of contents:

Summary

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening hematological disorder. It is caused by a deficiency in ADAMTS13. ADAMTS13 is a zinc-containing metalloprotease enzyme that cleaves Von Willebrand factor (VWF). The deficiency of ADAMTS13 results in VWF accumulation and causes platelet rich thrombi, resulting in haemolytic anaemia and thrombocytopenia. There is an increased risk of TTP among female patients, patients from black races and patients with blood group O, based on previous studies. It is also most commonly seen among patients with HIV. The mainstay of treatment is plasma exchange and fresh frozen plasma.

A retrospective study aimed at describing the national characteristics of TTP from 2010 to 2017 was done. Permission to conduct the study was obtained from the Health Sciences Research Ethics Committee of the University of the Free State (HSREC), Western Cape Blood Service (WCBS) ethics committee and South African National Blood Service (SANBS). The described characteristics are the age, gender, blood group and the blood products given to the patients.

Abbreviations:

ADAMTS13	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ARV	anti-retroviral treatment
FBC	full blood count
FFP	fresh frozen plasma
HAART	highly active anti-retroviral therapy
HIV	human immunodeficiency virus
HUS	haemolytic uremic syndrome
MAHA	microangiopathic hemolytic anemia
PEX	plasma exchange
RBC	red blood cell
TMA	thrombotic microangiopathy
TTP	thrombotic thrombocytopenic purpura
UAHC	Universitas Academic Hospital Complex
VWF	von Willebrand factor

Definitions

ADAMTS13: Processes ultra-large multimers of VWF secreted from Weibel Palade bodies of stimulated endothelial cells and platelets to VWF monomers (1).

MAHA: Fragmentation of red blood cells as they pass through platelet-rich microthrombi in microvascular system. Findings on peripheral smear: prominent schistocytes, helmet cells and triangular cells (1).

Von Willebrand Factor: Glycoprotein involved in blood clotting. It causes platelets to adhere to the endothelial layer of the cell (2).

Chapter 1

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease caused by reduced activity of ADAMTS13. It is characterized by small-vessel platelet-rich thrombi that cause thrombocytopenia and microangiopathic haemolytic anaemia (3). It is associated with advanced forms of HIV (1).

Thrombotic thrombocytopenic purpura (TTP) can be acquired, in which case it is an autoimmune disorder where there is an autoantibody inhibitor of ADAMTS13 activity, or hereditary, due to homozygous or compound heterozygous mutations in ADAMTS13 (4). It is important to note that there are a number of other causes of microangiopathic haemolytic anaemia (TMA), which may be difficult to differentiate from TTP. These include the following hereditary causes: complement-mediated TMA, metabolism-mediated TMA, and coagulation-mediated TMA. Acquired causes may be due to Shiga toxin-mediated TMA, drug-mediated TMA and complement-mediated TMAs (4).

Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome (HUS) were previously thought to be overlapping syndromes. They are similar disorders but HUS is more prevalent in children and frequently present with renal impairment (5) (6).

Epidemiology

Reported cases are four to eleven per one million adults per year in the USA (7). Acquired TTP is rare in children, and the median age is 41 years. There is an increased risk of TTP in females, patients belonging to black racial groups and patients with the blood group O (3) (8).

The risk of blood group O was postulated after a multi-centre study was performed in Oklahoma from 1995 to 2009, which showed that among patients with TTP and severe ADAMTS13 deficiency, the relative frequency of patients with blood group O was greater than expected, suggesting that blood group O could be a risk factor for severe ADAMTS13 deficiency (9).

In a study by Martino et al., a comparison was made between black and white people on survival and genetic risk factors in TTP. It was found that there was a low frequency of protective allele DRB1*04 in black people, which is a strong risk factor for developing TTP (10). Even though the frequency is much higher in the black population, it has been found that they have better survival rates than white patients. (10)

A study was conducted on 83 patients with autoimmune TTP at the University of Alabama at Birmingham Medical Center between April 2016 and June 2019. The study found that lower levels of ADAMTS13 and increased IgG of ADAMTS13 resulted in higher levels of relapse and exacerbation (11).

From the study, other important role players were found to be autoantibodies, genetic mutations, pregnancy, infection, obesity and HLA DRB1*11 (1).

Presentation

Thrombotic thrombocytopenic purpura (TTP) presents as a severe microangiopathic haemolytic anaemia and thrombocytopenia (3). Initial symptoms are fatigue, dyspnoea, petechiae or other bleeding. Patients are not always critically ill.

The common presenting features, with frequency expressed as a percentage in brackets, are the following (3):

- Microangiopathic hemolytic anemia (MAHA) and thrombocytopenia are the hallmark of TTP, and are present in 100% of cases.
- Neurological: severe (35%) - coma, seizures, stroke, focal signs
  minor (20.3%) - confusion, headache
  none (34%)
- Renal: acute kidney injury (6%), renal insufficiency (41%), normal renal function

(53%)

- Fever: (23%)
- Gastrointestinal: abdominal pains, nausea, vomiting or diarrhea
- Cardiac: chest pains, arrhythmias, sudden cardiac death, myocardial infarction, cardiogenic shock and heart failure

The complete pentad of clinical features namely MAHA, thrombocytopenia, renal failure, neurological symptoms and fever occur in 5% of patients (3).

Diagnostic testing

The condition should be suspected in those presenting with microangiopathy and thrombocytopenia, with or without organ involvement.

Supporting laboratory investigations include a full blood count (FBC), peripheral smear, creatinine, lactate dehydrogenase (LDH), unconjugated bilirubin, haptoglobin, coagulation testing, coombs and ADAMTS13 activity.

The diagnosis of TTP made when there is MAHA, thrombocytopenia, markedly increased LDH, increased bilirubin, negative coombs, decreased haptoglobin and decreased ADAMTS13 levels.

Systemic conditions associated with MAHA (3) (4)

- Systemic infection
- Systemic malignancy
- Severe pre-eclampsia, eclampsia, haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome
- Severe hypertension
- Systemic rheumatic disorders: systemic lupus erythematosus (SLE), scleroderma renal crisis,

and catastrophic antiphospholipid syndrome

- Hematopoietic stem-cell or organ transplantation due to bone marrow ablation, high doses of chemotherapy, immunosuppressive drugs, or rejection of transplanted kidneys
- Chemotherapy drugs associated with MAHA, e.g. mitomycin, cisplatin, daunorubicin and cytosine arabinoside, to mention a few other drugs associated with MAHA are cyclosporine, clopridrogrel, ticlopidine and quinine (12)
- Disseminated intravascular coagulation,
- Severe vitamin B 12 deficiency
- Pancreatitis

Treatment

This disease, if left untreated, is associated with a high mortality rate of >90%. Once suspected, treatment should be commenced speedily, because ADAMTS13 level results usually take long to come out. (1) The mainstay of treatment is plasma exchange, and the addition of steroids and rituximab has improved outcomes (1) (13).

Oral prednisone at a dose of 1mg/kg per day is prescribed if the patient can tolerate it, otherwise methyl prednisone may be used intravenously. The steroids are then tapered when the platelet count normalizes (13).

Rituximab has been added to the first line treatment of TTP (with steroids and plasma exchange), unless it is contraindicated. It has been shown to cause less relapse, and reduces the risk of exacerbation. It is a Cluster or Differentiation 20 (CD20) monoclonal antibody inhibitor (13).

Plasma exchange (PEX) is part of first line treatment, and fresh frozen plasma infusions can be given while awaiting PEX. Oliguric renal failure may limit the amount of plasma that can be infused while awaiting PEX. Plasma exchange involves the removal of the patient's plasma and replacement with donor plasma. The rationale for this is that the plasma will be removed with other substances that

are causing damage to the patient. In TTP the plasma will be removed with VWF and autoantibodies against ADAMTS13. The plasma exchange is performed daily and is continued until the platelet count is more than $150x10^9$/L (14).

Complications of plasma exchange include the following (14):

- Citrate-induce hypocalcaemia
- Anaphylactic reaction
- Transfusion-related lung injury
- Risk of transfusion-transmitted disease
- Vascular catheter complications (Infection, pain, nerve damage, thrombosis, hematoma and air embolism)

When plasma infusion is considered, either FFP or cryo-poor plasma (cryo-supernatant) or freeze-dried plasma may be used.

- Fresh frozen plasma is prepared from a single unit of whole blood which contains all the coagulation factors (14).
- Cryo-poor plasma may be used instead of FFP as it is deficient of cryoprecipitant. Cryoprecipitate is the cold insoluble fraction of FFP and it contains factor VIII, VWF, fibrinogen, fibronectin and factor XIII (15).
- Bio plasma (National Bioproducts Institute) is a freeze-dried plasma made from pooled fresh human plasma, which is inactivated for lipoprotein-coated viruses found in HIV, Hepatitis B and Hepatitis C (15).

Previous studies have been conducted on outcomes of TTP in specific blood groups. In one study done at Cairo University Hospital from 2008 to 2016 on 33 patients with severe ADAMTS 13 deficiency, it was found that the patients with blood group O needed more plasma exchange sessions to achieve remission, and that cryo-supernatant improves outcomes in patients with refractory TTP (16).

TTP in HIV infected persons

The human immune deficiency virus (HIV) has been found to be a precipitant in the development of TTP. This is related to the direct effects of HIV causing endothelial damage. The presence of the antigen P24 in the endothelium of cells, suggests either a direct toxic effect or endothelium impairment. TTP has a greater incidence in HIV patients. It usually develops in advanced HIV, where patients have high viral loads, and in those who are not yet on ARVs (anti-retroviral therapy). Certain studies have indicated a reduction in the incidence of HIV since the introduction of ARVs. When it comes to treatment of TTP, the prompt initiation of ARVs has been shown to cause faster resolution of TTP than patients on plasma exchange only. The risk of relapse of TTP in HIV is reduced when the patient maintains a low viral load by taking ARVs (10) (17).

In a study by Louw et al. with 21 patients in South Africa it was found that HIV-related TTP was a cause for mortality, and the presentation is usually diverse (18).

Novitsky et al. did a descriptive study on 44 patients between 1996 and 2003 at Groote Schuur Hospital. The aim was to look at the response of TTP to therapy with or unrelated to HIV. It was found that HIV positive patients were highly responsive to plasma infusions when compared to HIV negative patients (1).

TTP and HIV viral load

In numerous published journal articles on patient case reports, it has been shown that TTP in HIV positive patients is associated with a high viral load, while in other instances the CD4 count can be normal. TTP associated with HIV will usually present in patients who are non-compliant with their treatment, indicating the causal effect of HIV on TTP (19) (20). Treatment of TTP in the setting of HIV is therefore based on immediate commencement of antiretroviral therapy, as this has been

shown to give a rapid response (21). It has also been demonstrated that the incidence of TTP has been reduced in the post-ARV era, compared to the pre-ARV era, in a study done by Becker et al (22).

Role of Anti-retroviral Therapy

South Africa has the largest ARV roll-out in the world. It was estimated in 2015 that about 3.1 million people in the country were on ARVs (23). The use of ARVs over the years in South Africa has changed according to CD4 count (Table 1). This might have had an impact on the incidence of TTP, possibly causing a decrease in HIV-associated TTP.

Table 1 Qualification for antiretroviral therapy per year in South Africa (23)

Year	**Trigger for initiation of ARVs**
Before 2012	CD4 count < 200
2012 to 2014	CD4 count < 350
2015 to August 2016	CD4 count < 500
From September 2016	All HIV+ patients qualify regardless of CD4 count

Rationale of research project

The rationale for this research is that TTP is an interesting and rare haematological disorder which can mimic many other causes of a microangiopathic haemolytic anaemia. It usually does not present with the usual pentad of MAHA, thrombocytopenia, renal failure, neurological symptoms and fever, as this only occurs in about 5% of patients. It would be interesting to see the epidemiology and the demographics in South Africa to better understand the disease and how it behaves. With regards to the demographics this study will investigate age, gender, blood group, the number of cases per year and blood products used. It will also look at the treatment regimens and blood products given to patients. Understanding the disease will assist in its timely detection and effective treatment.

Limitations and need for further research

This research project is a retrospective descriptive study, and therefore not all clinical data was available, for example laboratory results and the presenting features of the patients.

This study relied solely on the provision of data from the WCBS, and not all the information could be supplied. This was initially planned as a national study, but data was only received from the Western Cape Province and the researchers were unable to obtain data from the South African National Blood Service (SANBS), limiting the study to the Western Cape. One measurement error was that patients who had a diagnosis of TTP and did not receive FFP, would be missed.

A prospective study can be done where certain data can be obtained, like clinical features, laboratory results, concomitant medication, patient outcome and the specific treatment modality.

Aims and objectives

The aim of this research project was to describe the characteristics of patients with TTP from 2010 to 2017 in South Africa (which is an extension of the initial project of describing the characteristics of patients diagnosed with TTP at the Universitas Haematology Department from 2010 to 2017).

Approval was obtained from the WCBS and SANBS ethics committees for the provision of data for the time period 01 January to 31 December 2017, for patients above the age of 18 that received fresh frozen plasma for indications of TTP, microangiopathic haemolytic anaemia and haemolytic uremic syndrome. The data that was collected from the archives of WPBTS and information received was from the Western Cape, which included the demographics of the patients such as

their age, the gender, the blood group, the name of hospital, the province, the date of first plasma product ordered and the type and number of blood product ordered. We unfortunately did not receive the required data from SANBS despite getting approval.

The demographics and characteristics that have been described in the manuscript are the age, gender, blood group, number of TTP cases per year and the products given.

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Chapter 2

Thrombotic Thrombocytopenic Purpura in the Western Cape: Patient Profile from the Western Cape Blood Service from 2010 to 2017.

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Abstract

Introduction: Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening haematological disorder caused by a deficiency in the Von Willebrand factor (VWF) cleaving enzyme, ADAMTS13. Deficiency of ADAMTS13 results in the accumulation of ultra-large VWF multimers and causes formation of platelet-rich thrombi, with subsequent haemolytic anaemia and thrombocytopenia. An increased risk of TTP has been described in female patients, people with black ethnicity, and patients with the O blood group. It is also commonly seen among patients with HIV. The mainstay of treatment is plasma exchange and infusion of fresh frozen plasma. Conflicting results regarding best treatment options, necessitates further research into TTP management.

Aim: This retrospective study aims to describe the profile of people treated for TTP by the blood transfusion services in the Western Cape Province from 2010 to 2017. Permission to conduct this study was obtained from the Health Sciences Research Ethics Committee of the University of the

Free State (HSREC) and the Western Cape Blood Service (WCBS).

Results: The median age of TTP patients was 35.5 years, and the majority of the patients was female (72.43%). The B blood group was overrepresented in our study population, when compared to the reported prevalence in South African blood donors. The Rh-negative blood groups were found to be underrepresented, and proportionally less common than reported in the healthy South African blood donor population. The most commonly used blood product was fresh frozen plasma.

Conclusion: TTP is more prevalent in females than males in the Western Cape population. The median age of TTP patients in our study is lower than what is reported in other countries. The B blood group could be a risk factor for TTP, however, the lower incidence in Rh-negative blood groups could indicate it as a protective factor against TTP. We recommend that further investigation into the aetiological factors of TTP in South Africa is performed.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening haematological disorder caused by a deficiency in the Von Willebrand factor (VWF) cleaving enzyme, ADAMTS13. When ADAMTS13 is deficient, ultra-large VWF multimers accumulate in the circulation system, causing the formation of platelet-rich thrombi, subsequently resulting in microangiopathic haemolytic anaemia (MAHA) (1). An increased risk of developing TTP has been reported in females, people with black ethnicity, and people belonging to blood group O (2). Infection with the human immune deficiency virus (HIV) is an additional risk factor for the development of TTP (3). Other important contributors are the presence of anti-ADAMTS13 autoantibodies, certain genetic mutations, pregnancy, infection, obesity, and HLA variants (HLA DRB1*11, DRB1*3 andDRB1*04) (4)(5).

It has been previously argued that blood group O could be a partially protective element against TTP because of the presence of a lower level of VWF as compared to other blood groups (6). However, a study done in Oklahoma from 1995 to 2009, which included 301 patients, found that among patients with TTP and severe ADAMTS13 deficiency, the relative frequency of patients with blood group O was greater than expected, suggesting that blood group O could be a risk factor for severe ADAMTS13 deficiency (7).

HIV infection has been found to be a precipitating factor in the development of TTP. It has been stated that this is related to the direct effects of HIV-related endothelial damage, as the presence of the antigen P24 in the endothelium suggests either a direct toxic effect or endothelium impairment (8). TTP is reported to occur in patients with advanced HIV infection who have high viral loads, and those who have not yet started on anti-retroviral treatment (ART) (9). Prompt initiation of ART has been shown to cause faster resolution of TTP compared to plasma exchange alone. The risk of relapse of TTP in HIV-infected patients is reduced when a patient maintains a low viral load by taking ART(8). Although some studies show a decline in the incidence of TTP since the introduction of ART (10) (11), HIV infection remains an important risk factor for thrombotic microangiopathies.

The mainstay of treatment of TTP is plasma exchange, while the addition of steroids and rituximab

has improved outcomes. In HIV positive patients, the prompt initiation of ART assists with faster resolution of TTP (10). In a South African study by Louw et al. (2018), it was found that TTP was more common in HIV-infected patients (77%), and had a female predominance (76%). Even though the patients showed a good overall survival on plasma exchange treatment (96.5% survival rate), HIV-infection was a risk for mortality. The presentation of TTP in HIV-infected patients is usually diverse, further complicating diagnosis and management (12). Another South African study found that HIV-associated TTP patients were more responsive to plasma infusions than HIV-negative TTP patients. None of the HIV-infected patients required apheresis compared to the roughly 43% of the HIV-negative patients who required apheresis (17% of these patients died regardless of receiving aphaeresis) (3). Therefore, with the two studies recommending different treatment approaches to HIV-associated TTP in South Africa, further exploratory research on the aetiological factors associated with HIV-linked TTP-like syndrome in our setting is vital.

The lack of current TTP data in South Africa and controversy around the role of blood group and HIV in TTP prompted this study. The researchers aimed to determine the demographics of patients treated for TTP in the Western Cape Province of South Africa. The Western Cape Blood Service (WCBS) was approached, and the data of all patients that received blood products for the reported diagnosis of thrombotic thrombocytopenic purpura, TTP, and MAHA was requested. The described characteristics included age, gender, blood group and the blood products given.

Methodology

Study design

A retrospective study was performed using data from the Western Cape Blood Service, South Africa.

Participant selection

All archived electronic records of patients who were issued blood products for the recorded indication of ‘thrombotic thrombocytopenic purpura’, ‘TTP’, ‘microangiopathic haemolytic

anaemia', 'MAHA', 'haemolytic uraemic syndrome', and 'HUS', from 01 January 2010 to 31 December 2017 were included in the study.

Measurement

The electronic data was retrieved from Blood Bank electronic records by the Information Technology data analysts, and sorted by WCBS senior staff. Demographic data including age, gender, and treating hospital was collected, as well as Blood Bank data (blood group, type of blood product ordered, quantity of blood products, and dates of first and last orders).

Bias

It is acknowledged that due to the retrospective nature of this study, some selection bias may be present. Only cases that were reported as TTP, MAHA and HUS were included in the study. The diagnosis of TTP was not confirmed by laboratory testing. There may be cases that were missed due to other recorded diagnoses. Patients who received other plasma products not issued by the WCBS (e.g. freeze-dried plasma or Bioplasma®(NBI)) were not represented in this data.

Ethics

Approval to conduct this study was obtained from the Health Sciences Research Ethics Committee (**HSD 2018/0114**) of the University of the Free State (HSREC) and permission to use this data was obtained from the management of the WCBS.

Statistical methods

The Department of Biostatistics at the University of the Free State performed the data analysis. Continuous variables were summarised by means, standard deviations or medians and percentiles. Categorical variables were summarised by frequencies and percentages. Differences between groups (e.g. blood groups) were evaluated using appropriate statistical tests and confidence intervals for unpaired data.

Results

A total of 342 cases with at least one of the specified recorded diagnoses were included in the study. This number includes patients treated at public (91.8%, n=314) as well as private (8.2% n=28) hospitals. The majority of the cases were from two public academic hospitals, namely Tygerberg (39.8% n=136) and Groote Schuur (37.1%, n=127).

Of the 342 cases included in the study, 293 had confirmed TTP, one had HUS and 48 had a diagnosis of 'possible TTP'. The HIV status was recorded in only 13 patients and they were all HIV-infected. Systemic lupus erythematosus (SLE) was present in five cases, four of the SLE cases had TTP and one had 'possible TTP'. Disseminated intravascular coagulation was noted in seven cases.

The median age of the cases was 35.5 years (IQR, 30-43 years). The youngest case was 19 and the oldest was 81 years of age. Females represented 72.43% (n=247) of the study population.

Over the study period, the mean number of cases per annum was almost 43 new cases per year. The highest number of cases of TTP was reported in 2017 (59 cases) and the lowest number of cases was 36 (2011 and 2012). The number of cases per year is indicated in Figure 1.

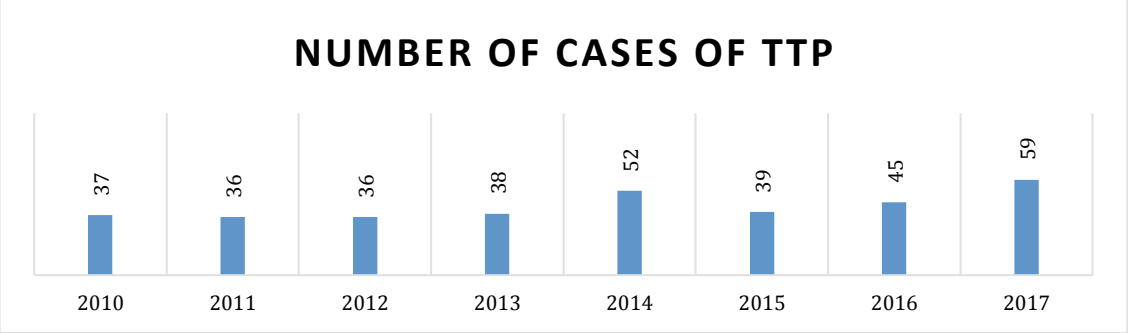

Figure 1. Number of cases of TTP per year

One hundred and thirty-one (131; 38.53%) participants were blood group O positive (OP), followed by 109 (31.76%) who were A positive (AP). Only one participant (0.29%) was blood group AB negative (ABN), and three (0.88%) were B negative (BN). The blood groups of the participants are presented in Table 1.

Table 1. Prevalence of blood groups in the Western Cape TTP and South African blood donor populations (13)

Blood Group	n	TTP Study %	95% confidence interval	SA Blood donors %
ABN	1	0.29	0.1% - 1.6%	1
ABP	20	5.88	3.8% - 8.9%	3
AN	5	1.47	0.6% - 3.4%	5
AP	109	31.76	27.0% - 36.9%	32
BN	3	0.88	0.3% - 2.6%	2
BP	64	18.82	15.0% - 23.3%	12
ON	8	2.35	1.2% - 4.6%	6
OP	131	38.53	33.5% - 43.8%	39
Unknown	1	0.29		

Key: ABP AB positive, AN A negative, AP A positive, BN B negative, BP B positive, ON O negative, OP O positive.

Blood products that were issued to the cases are summarised in

Table 2 .

Table 2: Blood products issued to cases

Blood product	Cases receiving blood product (%, n)	Median number of units issued (IQR)	Maximum number of units issued to a single patient
FFP[#]	84.5%, 289	9 (5-27)	169
Cryo-poor plasma[*]	55.8%, 191	19, (8-41)	260
Paediatric pooled plasma	1.16%, 4	5 (3.5-9)	12
Cryoprecipitate[^]	1.75%, 6	20 (10-40)	40
RCC (including leukodepleted RCC)	68.4%, 234	3 (2-4)	26
Platelets	14.3%, 49		4

Key: IQR interquartile range, FFP fresh frozen plasma, RCC red cell concentrate.
[#] FFP is the total plasma content of a whole blood donation. [^] Cryoprecipitate is collected from thawing a frozen FFP unit and collecting the precipitate. [*] Cryo-poor plasma (or cryosupernatant) is the remaining product after the cryoprecipitate has been extracted from thawed FFP

Seventy-two (72) units of platelets were issued to 49 (14.3%) patients. TTP was diagnosed in 67.3% (n=33/49) of the patients receiving platelets, while the remainder (n=16/49) had a diagnosis of

'possible TTP'. Random donor pooled platelet products (POOLP) were issued to 73.5% (n=36/49) of these participants. The maximum number of POOLP units issued to a single patient was four units. Thirty-two (n=32/36) patients from this group who received POOLP were diagnosed with TTP and four (n=4/36) were recorded as "possible TTP" cases. Single donor apheresis platelet products (PLAPH) were issued to 30.6% (n=15/49) of the total number of patients who received platelets. The maximum number of PLAPH units received by a single patient was four units. Three (n=3/15) patients from this group had confirmed TTP and ten (n=10/15) had "possible TTP". Two patients received both POOLP and PLAPH units.

Discussion:

The characteristics of TTP that have been described are age, gender, incidence, blood groups and blood products.

Demographics

In our study the majority of the TTP population was females (72.43%). Considering that during roughly the same time period (2011 to 2016) females only comprised between 50.9% (2011) and 50.7% (2016) of the Western Cape Province population, and nationally remained constant at 51% of the total population (2011 and 2016), females are over-represented in the TTP group by more than 20% (14). The results of this study therefore support the findings of previous studies that found that females are at a higher risk of developing TTP (1) (7) (15) (16) (17).

The median age of our study population was 35.5 years. This finding is similar to the mean age of 34.3 years found in another South African study (15). The mean age of onset for TTP in South Africa seems to be slightly younger when compared to other areas of the world where the median age was determined to be 41 years (1).

Incidence

TTP is a life-threatening haematological disorder that is associated with advanced forms of HIV infection. Another South African study (15) showed that 78.0% of patients with TTP were also HIV

infected. With millions of people in South Africa infected with HIV, a high incidence of HIV-related TTP can be expected. From September 2017 every HIV-infected patient in South Africa has been eligible to be started on ART regardless of their CD4 count (18). This was a very positive development in the South African healthcare system, and it was hoped that the widespread use of ART would also result in a decline in HIV-associated TTP. Unfortunately, the HIV status was only recorded for 13 patients in our study cohort. Therefore, we could not evaluate the effect of ART treatment on HIV-related TTP. However, we could evaluate the incidence of all TTP cases before and directly after the rollout of ARV treatment in South Africa. Alarmingly, the incidence of TTP in the Western Cape was found to be increasing from an average of 40 cases per year (2010 to 2016) to 59 cases in 2017 alone. However, it may be too soon to assume that the rollout of ART did not have an effect on the incidence of TTP. Therefore, a follow-up study would be needed covering a longer timespan following implementation of the revised ART programme, in order to establish if there was a significant change in the incidence of TTP or not.

Blood groups

For the purposes of this study, blood group patient data is compared to blood group data from the donor population of the South African National Blood Service (SANBS). SANBS provides a service to all the provinces of South Africa with the exception of Western Cape. Furthermore, the blood donor population may not be representative of the South African population as a whole. Despite changes in donation policies in post-apartheid South Africa, the majority of blood donors in South Africa are White. Thus the general blood donor population in South Africa may not be a true representation of the region's demographics, resulting in a systematic bias of our results.

It was previously postulated that blood group O would have a lower incidence of TTP, due to a lower level of VWF in that blood group (2). A 2009 study in Detroit, USA, found that the O blood group had a lower incidence in patients with TTP (36%) when compared to the rest of the local population (44%). However, the differences they described were not statistically significant (19). Another retrospective study in the USA in 2009 also found no statistical difference between group O and non-O TTP patients (2). In 2011, a large multicentre study on the Oklahoma TTP registry

surprisingly found that blood group O was a risk factor for TTP associated with ADAMTS13 deficiency (7). In the TTP study population for our current study, it was found that the O blood group is less prevalent when compared to the SANBS donor population (40.9% vs 45%). However, there was a similar prevalence of O-positive people the groups (38.5% vs 39%), but O-negative was nearly three times less prevalent in the TTP group than in the SANBS donor population (2.4% vs 6%). The AB blood group was over-represented in the TTP group (6.2% vs 4%). However, the AB-negative group was much less prevalent in the TTP group (0.23% vs 1%), with the AB-positive group representing nearly double the prevalence than that of the general population (5.9% vs 3%). The A-positive blood group showed a similar prevalence between the two groups (31.8% vs 32%), however, the A-negative group was more than three times less prevalent in the TTP group (1.5% vs 5%). The B-positive blood group was more prevalent in the TTP group (18.8% vs 12%), but the B-negative group was again under-represented in the TTP group (0.9% vs 2%). The clear pattern that emerged from our data is that Rh-negative was under-represented in our study population for all blood groups (5.0% vs 14%). Two blood types were over-represented in the TTP group, namely AB-positive and B-positive (5.9% vs 3%, and 18.8% vs 12%, respectively). Therefore, it may be hypothesised that Rh-negative grouping is a possible protective factor in the development of TTP, whereas the B blood group and Rh D expression serve as a possible risk factors. We believe that our data warrants further investigation into the correlation between TTP and blood groups in our setting.

Treatment options

The mainstay of treatment for TTP is plasma exchange, fresh frozen plasma and steroids. Rituximab, which is a CD20 monoclonal antibody, has been added to the first line treatment. (1) It has been shown that patients with blood group O need more plasma exchange sessions to achieve remission (6). The same study showed that treatment with cryo-supernatant improved the clinical outcome in blood group O patients with refractory disease, which may be due to the fact that cryo-supernatant is devoid of VWF. This study was not designed to evaluate the response to treatment or differences in response to treatment options, but we noted that fresh frozen plasma was administered to 84.5% of the cases (average of nine units were given per patient) and 55.8% of the

patients were treated with cryo-poor plasma. Fresh frozen plasma as well as cryo-poor plasma was issued to 139 patients (40.6%). Red cell concentrates were issued to 68.4% of the cases, as patients with TTP develop haemolysis as part of the disease process and require red cell transfusions.

Platelet products were issued to 49 patients (14.3%). Platelet products are usually considered inappropriate therapy in patients with TTP without significant bleeding, as they may worsen patient outcomes. Platelet transfusion has been shown to increase the platelet-rich thrombi, which worsens thrombotic complications, although a systematic review on this subject showed uncertain causation of platelet transfusion and harm (20). This is supported by a more recent publication (21) that reported no additional harmful thrombotic complications in TTP patients who received platelet therapy. This should be read in caution, as these observations were made at sites where plasma exchange was routinely available for the management of TTP, which is not the situation in many sites in South Africa. Additionally, platelet products should not be withheld from patients with clinically important bleeding or where indicated for invasive procedures. As this was a retrospective study using Blood Bank data, we could not establish the indication for the platelet transfusion, thus, we cannot comment on appropriateness of transfusion or effect on outcome.

Limitations

This study relied solely on the provision of data from the WCBS, and not all the information could be supplied. This was initially planned as a national study, but data was only received from the WCBS and the researchers were unable to obtain data from the South African National Blood Service (SANBS), limiting the study to the Western Cape. One measurement error was that patients who had a diagnosis of TTP and did not receive FFP, would be missed. Further were the retrospective nature of the study, and unavailability of certain data points and treatment modalities. Furthermore, presenting features, concomitant medication, clinical course and patient outcomes could not be measured.

Conclusion

Utilising data from 342 patients, we can conclude that TTP is indeed more common in females, with

our study population being 70% female. It is also noteworthy that the median age in our study population was 35.5 years, which correlates with other South African data, but is younger than reported elsewhere in the world. Therefore, our results indicate that TTP occurs earlier in life in the South African population than described globally. Unsurprisingly, the most commonly transfused blood product was FFP, which is the mainstay of TTP treatment in South Africa, and corresponds to the most commonly used TTP treatment regimen in the country. Our results further suggest that Rh-negative blood groups could possibly be a protective factor against TTP, as the Rh-negative groups were underrepresented in all TTP patients, when evaluated against the known South African blood donor population. However, the B and AB blood groups were over-represented in our TTP population, which could indicate that the B antigen is a risk factor for the development of TTP in our population. We believe that these findings provide enough scientific evidence to warrant further in-depth investigation into the role of HIV and blood groups in the development of TTP.

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Appendices

A. Letter of approval from the University of the Free State Health Sciences Research Ethics committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

28-Nov-2018

Dear **Dr Malekhetho Mohale**

Ethics Number: UFS-HSD2018/0114/3107

Ethics Clearance: **The characteristics of patients with Thrombotic Thrombocytopenic Purpura (TTP) at Universitas Haematology Department from 2010 to 2017**

Principal Investigator: **Dr Malekhetho Mohale**

Department: **Internal Medicine Department (Bloemfontein Campus)**

SUBSEQUENT SUBMISSION APPROVED

With reference to your recent submission for ethical clearance from the Health Sciences Research Ethics Committee. I am pleased to inform you on behalf of the HSREC that you have been granted ethical clearance for your request as stipulated below:

- Amendments made to the protocol
- Added that Co-supervisor is working in the human molecular biology unit and addition of the listed collaborators:
  - Dr Walter Janse van Rensburg
  - Biomedical Scientist, Lecturer, Human Molecular Biology Unit, University of the Free State
  - Collaborators:
  - Nadia Mundey
  - Blood Bank Supervisor: Western Province Blood Transfusion Service
  - Dr C Hilton
  - Transfusion Medical Specialist: Western Province Blood Transfusion Service
  - Dr PL Wessels
  - Lead consultant: Patient Blood Management, South African National Blood Service (SANBS)
  - Dr C Poole
  - Lead consultant: Specialised Transfusion and Therapeutic Services, SANBS

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this request for ethical clearance and we wish you continued success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00006240; REC 230408-011; IORG0005187; FWA00012784
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

B. Letter of approval from Western Cape Blood Service

WP Blood Transfusion Service
Do something remarkable

www.wpblood.org.za / info@wpbts.org.za
Head Office
Old Mill Road, Pinelands, 7405 • PO Box 79, Howard Place, 7450
T: 021 507 6300 / F: 021 531 0322

25 October 2018

University of the Free State Health Sciences Research Ethics Committee

To Whom It May Concern,

Participation of the Western Province Blood Transfusion Service (WPBTS) in the M.Med thesis: **National profile of the characteristics of patients treated for TTP** (an extension of the study: The characteristics of patients with TTP at Universitas Haematology Department from 2010 - 2017 (HSD 2018/0114))

This letter serves to confirm that WPBTS is willing to participate in the above-mentioned study by supplying data and blood product usage of patients treated for thrombotocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS) between 2010 - 2017 provided that ethics approval is granted from your institution.

All supplied information will be treated in a strictly confidential manner as per standard research etiquette.

Further queries can be directed to Dr Caroline Hilton (WPBTS Transfusion Medical Specialist) - caroline@wpbts.org.za.

Kind regards,

Dr Gregory Bellairs

Chief Executive Officer / Medical Director WPBTS
greg@wpbts.org.za

Paarl
263 Main Road, Paarl, 7646
PO Box 422, Paarl, 7620
T: 021 871 1030 / F: 021 872 5945

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26 Napier Street, Worcester, 6850
PO Box 194, Worcester, 6849
T: 023 342 2450 / F: 023 342 7556

George
Medical Centre, Courtenay Street
PO Box 65, George, 6530
T: 044 874 2074 / F: 044 874 6097

WP Blood Transfusion Service NPC
Pr. No: 7800045 / Reg. No: 1943/016692/08

Directors: GRM Bellairs, AR Bird, MR Burton, NB du Toit, BdL Figaji, A Huggett, C Ingram, V Louw, DM Ndebele, R Ramsbottom, PK Slack (Chairman), PJ Veldhuizen
Company Secretary: I van Schalkwyk

C. Letter of approval from the South African National Blood Service

Toll Free: 0800 11 9031

Head Office or Zone
Head Office
1 Constantia Boulevard
Constantia Kloof Ext 22
1709

Postal Address: Private Bag X14, Weltevreden Park, 1715

Tel: 011 761 9290

Email: Jackie.thomson@sanbs.org.za www.sanbs.org.za

SANBS
South African National Blood Service

Registration No. 2000/026390/08

Per email:

HREC

30 October 2018

To whom it may concern,

Re: Study Protocol: Re "NATIONAL PROFILE OF THE CHARACTERISTICS OF THE PATIENTS TREATED FOR TTP"
The SANBS is able and willing to transfer data for the study purposes.

Yours Sincerely

Dr Jackie Thomson

Medical Director

D: Letter of permission from Free State Department of Health

health
Department of Health
FREE STATE PROVINCE

18 June 2018

Miss M Mohale
Dept. of Internal Medicine
UFS

Dear Miss M Mohale

Subject: The characteristics of patients with Thrombotic Thrombocytopenic Purpura (TTP) at Universitas Haematology Department from 2010 to 2017

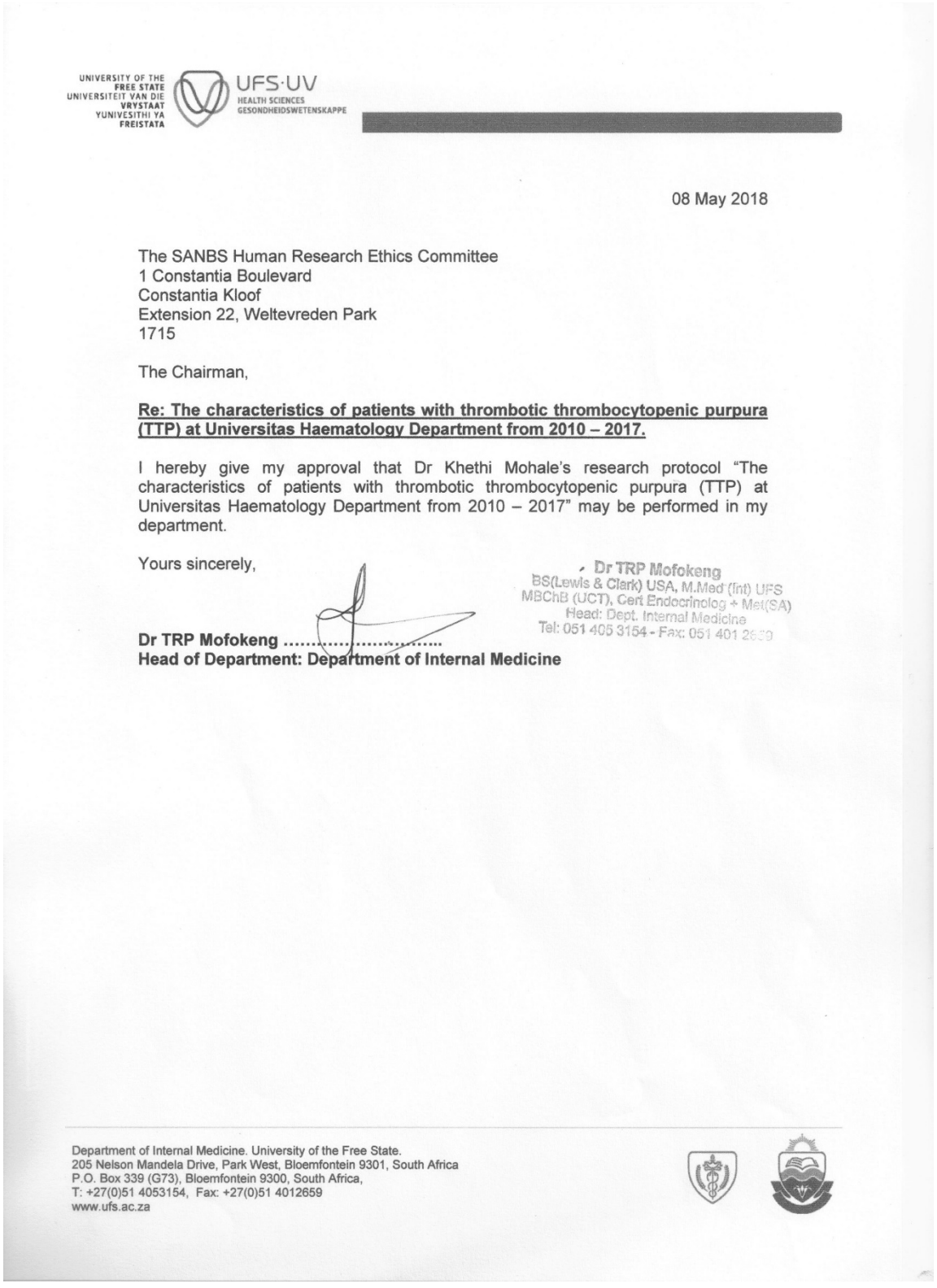
- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of Universitas Hospital nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** lithekom@fshealth.gov.za or sebeelats@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution manager/CEOs on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see http://nhrd.hst.org.za

Trust you find the above in order.
Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 27/06/18

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za

E. Permission from Head of Department



UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

08 May 2018

The SANBS Human Research Ethics Committee
1 Constantia Boulevard
Constantia Kloof
Extension 22, Weltevreden Park
1715

The Chairman,

Re: The characteristics of patients with thrombotic thrombocytopenic purpura (TTP) at Universitas Haematology Department from 2010 – 2017.

I hereby give my approval that Dr Khethi Mohale's research protocol "The characteristics of patients with thrombotic thrombocytopenic purpura (TTP) at Universitas Haematology Department from 2010 – 2017" may be performed in my department.

Yours sincerely,

Dr TRP Mofokeng
BS(Lewis & Clark) USA, M.Med (Int) UFS
MBChB (UCT), Cert Endocrinolog + Met(SA)
Head: Dept. Internal Medicine
Tel: 051 405 3154 - Fax: 051 401 2659

Dr TRP Mofokeng .....
Head of Department: Department of Internal Medicine

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www.ufs.ac.za

F. Protocol approved by HSREC

National profile of the characteristics of patients treated for TTP

An extension of the study: The characteristics of patients with TTP at Universitas Haematology Department from 2010- 2017. (HSD 2018/0114)

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Table of contents:

Summary of protocol in layman's terms:

The study is about looking at the presenting features of thrombotic thrombocytopenic purpura (TTP) in Universitas Haematology Department from the time period 2010 to 2017.

Von Willebrand factor (VWF) is a protein vital in platelet adhesion to wound sites within blood vessel walls.

Thrombotic thrombocytopenic purpura (TTP) is a haematological disorder were VWF cannot be broken down because of a deficiency of an enzyme known as ADAMTS13. This results in an accumulation of ultra large VWF molecules, that then spontaneously binds to platelets in the blood stream, which results in the blocking of small blood vessels. Red blood cells (RBC) are cut into fragments as they pass through these platelet rich thrombi. Hence these patients will present with blood breaking down (fragmentation) and low platelets (thrombocytopenia). The presence of an active infection, such as HIV, may trigger the onset of TTP.

Therefore, the study will describe the common features that these patients present with. Their clinical presenting features, laboratory findings and outcomes of treatment.

The extended study aims to review the national profile of patients treated for TTP with plasma products from both the Western Province Blood Transfusion Service (WPBTS) and SANBS, the South African National Blood Service over the same period as the main study. The initial study was only getting this data from patients treated at Universitas Hospital. Limitations are that there is some data from the National Study that will not be available, yet other important demographic and epidemiological data will be collected through this protocol ammendment, which will strengthen the study.

Abreviations:

ADAMTS13	A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13
ARV	anti-retroviral treatment
FBC	full blood count
FFP	fresh frozen plasma
HAART	highly active anti-retroviral therapy
HIV	human immundefeciency virus
HUS	haemolytic ureamic syndrome
MAHA	microangiopathic haemolytic anaemia
PEX	plasma exchange
RBC	red blood cell
TMA	thrombotic microangiopathy
TTP	thrombotic thrombocytopenic purpura
UAHC	Universitas academic hospital complex
VWF	Von Willebrand factor

Definitions/ function

ADAMTS13: Processes ultra-large mutlimers of VWF secreted from Weibel palade bodies of stimulated endothelial cells and platelets to VWF monomers. [2]

MAHA: Fragmentation of red blood cells as they pass through platlet-rich microthrombi in microvascular. Findings on peripheral smear: prominent schistocytes, helmet cells and triangular cells. [2]

Von Willebrand Factor: it is a glycoprotein involved in blood clotting. It causes platelets to adhere to the endothelial of the cell. [10]

Introduction:

Background:

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease caused by reduced activity of ADAMTS13. It is characterized by small-vessel platelet-rich thrombi that cause thrombocytopenia and microangiopathic haemolytic anaemia.[1] It is associated with advanced forms of HIV.[2]

Thrombotic thrombocytopenic purpura (TTP) can be acquired, which is an autoimmune disorder where there is an autoantibody inhibitor of ADAMTS13 activity, or hereditary due to homozygous or compound heterozygous mutations in ADAMTS13.[3] It is important to note that there are a number of other causes of microangiopathic haemolytic anaemia (TMA), which may be difficult to differentiate from TTP. These include the following hereditary causes: complement-mediated TMA, metabolism-mediated TMA, coagulation-mediated TMA, and acquired causes may be due to Shiga toxin-mediated TMA, drug-mediated TMA and complement-mediated TMA's.[3]

Epidemiology

Reported cases are four to eleven per one million adults per year in the US.[4] Acquired TTP is rare in children. The median age is 41 years. There is increased risk in females, black race and blood group O.[1,5]

The risk of blood group O was postulated after a multi-centre study was performed in Oklahoma from 1995 to 2009 which showed that among patients with TTP and severe ADAMTS 13 deficiency the relative frequency of patients with blood group O was greater than expected.[6]

In a study by Martino et al., a comparison between black and white people on survival and genetic risk factors in TTP was made. It was found that there was a low frequency of protective allele DRB1*04 in black people and it is a strong risk factor for developing TTP.[7]

Presentation

Thrombotic Thrombocytopenic purpura (TTP) presents as a severe microangiopathic haemolytic anaemia and thrombocytopenia.[1] Initial symptoms are fatigue, dyspnoea, petechiae or other bleeding. Patients are not always critically ill.

Presenting features with frequency expressed as a percentage in brackets:[1]

- Microangiopathic haemolytic anaemia and thrombocytopenia are the hallmark of TTP, are present 100% of the time.
- Neurological: severe (35%) - coma, seizures, stroke, focal signs
  Minor (20.3%) - confusion, headache
  None (34%)
- Renal: Acute kidney injury (6%), renal insufficiency (41%), normal renal function (53%)
- Fever (23%)
- Gastrointestinal: abdominal pains, nausea, vomiting or diarrhoea
- Cardiac: chest pains, arrhythmias, sudden cardiac death, myocardial infarction, cardiogenic shock and heart failure

The complete pentad of clinical features namely MAHA, thrombocytopenia, renal failure, neurological symptoms and fever occur in 5% of patients.[1]

Diagnostic testing

Suspected in those presenting with microangiopathy and thrombocytopenia, with or without organ involvement.

Supporting laboratory investigations: Full blood count (FBC), peripheral smear, creatinine, lactate dehydrogenase (LDH), unconjugated bilirubin, haptoglobin, coagulation testing, coombs, ADAMTS13 activity.

Diagnosis of TTP made when there is MAHA, thrombocytopenia, markedly increased LDH, increased bilirubin, negative coombs, decreased haptoglobin and decreased ADAMTS13 levels.

Systemic conditions associated with MAHA[1,3]

- Systemic infection
- Systemic malignancy
- Severe pre-eclampsia, eclampsia, haemolysis elevated liver enzymes low platelet count (HELLP) syndrome
- Severe hypertension
- Systemic rheumatic disorders: systemic lupus erythematosus (SLE), scleroderma renal crisis, catastrophic antiphospholipid syndrome
- Haematopoietic stem-cell or organ transplantation due to bone marrow ablation, high doses chemotherapy, immunosuppressive drugs, or due to rejection of the transplanted kidney.
- Disseminated intravascular coagulation
- Severe vitamin B 12 deficiency
- Pancreatitis

Treatment

This disease is associated with a high mortality if not treated, with a mortality rate of >90%. Once suspected, treatment should be commenced speedily, because ADAMTS13 levels usually take long to recover.[2] The mainstay of treatment is plasma exchange, and the addition of steroids and rituximab has improved outcomes. [2,11]

Oral prednisone at a dose of 1mg/kg per day is prescribed if the patient can tolerate it, otherwise methylprednisone may be used intravenously. The steroids are then tapered when the platelet normalizes. [11]

Rituximab has been added to first line treatment of TTP (with steroids and plasma exchange) unless contraindicated. It has been shown to cause less relapse and reduces risk exacerbation. It is a Cluster or Differentiation 20 (CD20) monoclonal antibody inhibitor. [11]

Plasma exchange (PEX) is part of first line treatment and fresh frozen plasma infusion can be given

temporarily while awaiting PEX. Oliguric renal failure may limit the amount of plasma that can be infused whilst awaiting PEX. Plasma exchange involves the removal of the patient's plasma and replacement with donor plasma, the rationale being that the plasma will be removed with other substances that are causing damage to the individual. In TTP the plasma will be removed with Von Willebrand factor and autoantibodies against ADAMTS13. The plasma exchange is performed daily; it is continued until the platelet count is more than 150. [12]

Complications of plasma exchange [12]

- Citrate induce hypocalcaemia
- Anaphylactic reaction
- Transfusion-related lung injury
- Risk of transfusion-transmitted disease
- Vascular catheter complications (Infection, pain, nerve damage, thrombosis, haematoma and air embolism)

When plasma infusion is considered, either FFP or cryo-poor plasma (cryo-supernatant) or freeze dried plasma may be used.

- Fresh frozen plasma is prepared from a single unit of whole blood which contains all the coagulation factors.[12]
- Cryo-poor plasma may be used instead of FFP as it is deficient of cryoprecipitant. Cryoprecipitate is the cold insoluble fraction of FFP and it contains factor 8, vWF, fibrinogen, fibronectin and factor 13. [13]
- Bio plasma (National Bioproducts Institute). Is a freeze dried plasma made from pooled fresh human plasma, which is inactivated for lipoprotein- coated virus found in HIV, Hepatitis B virus and Hepatitis C virus. [13]

HIV and TTP

The human immune deficiency virus (HIV) has been found to be a precipitant in TTP by causing endothelial damage. HIV can occasionally present with TTP and thrombotic microangiopathy in patients with advanced HIV or those with poor adherence to antiretrovirals (ARVs). Treatment of patient with TTP associated HIV should be directed at prompt commencement of highly active antiretroviral therapy (HAART) to suppress viral load and plasma exchange.[7] Starting HAART has been shown to result in recovery and no relapse in patients compliant with their HAART.[8]

TTP and viral load

In numerous journals on patient case reports, it has been shown that TTP in HIV positive patients is associated with a high viral load, in other instances the CD4 count can be normal. TTP associated with HIV will usually present in patients who are non-compliant with their treatment, this showing causal effect of HIV on TTP. [14,15] Treatment of TTP in the setting of HIV is therefore based on immediate commencement of antiretroviral therapy, and this has been shown to give a more rapid response. [16] It has also been demonstrated that the incidence of TTP has been reduced post HAART era compared to pre HAART era, in a study done by Gervasoni C , Ridolf AL et al. [17]

Roll of ARVs

South Africa has the largest ARV roll out in the world. It was estimated in 2015 about 3.1 million people were on ARVs.[9] The roll out of ARVs over the years in South Africa, might have had an impact on the incidence of TTP, possibly causing a decrease in HIV associated TTP.

Qualification for ARV's in South Africa according to year [9]	
Year	Trigger for initiation of ARV's
Before 2012	CD4 count < 200
2012 to 2014	CD4 count < 350
2015 to August 2016	CD4 count < 500
From September 2016	All HIV+ patients qualify regardless of CD4 count

Aim:

1. To describe the characteristics of patients diagnosed with TTP at Universitas Haematology Department from 2010 to 2017.
2. To better understand the epidemiology of TTP and the treatment regimes used to treat patients with TTP in South Africa

Objective:

1. To describe the characteristics of patients diagnosed with TTP at Universitas Haematology Department in the period Jan 2010 to Dec 2017.
2. To describe the national profile of patients treated as TTP
3. To describe the plasma products issued for patients with the diagnosis TTP for whom FFP/ cryopoor/ aphaeresis plasma were prescribed for the period 2010 to 2017.

Methodology:

Study design:

This is a retrospective descriptive study. Collecting data from the database at SANBS, National Health Laboratory Services (NHLS), patient files from 01 January 2010 to December 2017.
Additional information will be obtained from the databases of the South African National Blood Service as well as the Western Province Blood Transfusion Service (WPBTS)

Participant selection:

Inclusion criteria:

- Diagnosis of:
    - TTP
    - Thrombotic thrombocytopenic purpura
    - Microangiopathic haemolytic anaemia

- HUS
- Haemolytic uraemic syndrome

- All patients diagnosed with TTP in the time period 01 January 2010 to Dec 2017
- Age > 18 years

Exclusion criteria:

- Patients who did not receive plasmapheresis or plasma infusion.

Measurement:

How the data will be collected:

- We will apply to WPBTS and the SANBS ethics committee for the following data for the period Jan 2010 to 31 Dec 2017:
  - Number of patients with the diagnosis "TTP"/ "thrombotic thrombocytopenic purpura"/ "microangiopathic haemolytic anaemia"/ "HUS"/ "Haemolytic uraemic syndrome" for whom fresh frozen plasma/ cryo-poor plasma/ plasma exchange were requested for. We will also request the blood group of these patients.
  - A list of patients treated at Universitas Hospital for whom FFP/ cryo-poor FFP or plasma exchange were ordered for the indications mentioned above.
- We will then draw the patient records to confirm the diagnosis of TTP.

The following will data will be collected: (Only data from point 1 and 2 will be included in National Study)

1. Demographics information collected (data will be obtained from the meditech system as well as and records at WPBTS and SANBS)

- Age
- Gender
- Date of first plasma product ordered
- Blood group
- Name of hospital

- Province
- Baseline aboratory parameters
    - Haemoglobin (as reported on initial request form)
    - Platelet count (as reported on initial request form)

2: Treatment:

- TTP specific treatment received:
    - Steroids
    - Rituximab
    - Initiation of HAART
    - Plasma products: FFP/PEX/bioplasma/cryopoor plasma
        - Start date
        - Stop date
        - Number of units/ volume used
        - Number of units of platelets used
        - Number of units of red cell concentrate used
    - Complications of treatment with plasma products as reported to Haemovigilance office.
    - Outcome: Died, intensive care unit (ICU) admission, discharged.

3: Condition at diagnosis of TTP (data will be obtained from the clinical notes)

- Co-morbidities
- Concomitant medication used, including herbal preparations
- Presenting symptoms
- Vital signs at presentation
- Organ involvement
    - Renal failure
    - Myocardial ischaemia
    - Central nervous system (Glasgow coma scale)
- HIV status: CD4 count, viral load and duration of treatment.

4: Antiretroviral therapy

- Regimen
- Start date

5: Laboratory parameters (data will be obtained from NHLS Labtrack system)

- Haemoglobin
- Platelets
- % fragmentation at diagnosis
- DIC screen
- LDH
- Creatinine
- ADAMTS13 Activity
- ADAMTS13 Antibodies
- Haptoglobin
- Coombs
- Troponin T and CK_{MB}
- Antinuclear antibodies, Antiphospholipid antibodies, Extractable nuclear antibodies

Data collection:

Data will be collected from the archives of meditech, patient files, NHLS Labtrack and the databases of WPBTS and SANBS.

Methodological and measurement errors:

- Incomplete patient notes or no notes
- Incomplete data given on request forms to WPBTS and SANBS
- Missing files
- Patients may be missed due to not receiving FFP/ therapeutic aphaeresis
- Diagnosis not entered into forms requesting FFP from blood bank
- Patients may be missed as diagnosis not made before patient demise/ discharge
- Due to the nature of this being a National study, some data points may be missed as not all information will be available. However, we believe that the critical data points will be available according to the feasibility discussions that we have had with the stakeholders.

Pilot study:

A pilot study of five patients will be performed, if there is no change to the data sheet/ protocol these patients will be included in the final data.

Data analysis:

The Department of Biostatistics (UFS) will assist the researcher with the analysis of data. Continuous variables will be summarised by means, standard deviations or medians and percentiles. Categorical variables will be summarised by frequencies and percentages. Differences between groups will be evaluated using appropiate statistical tests and confidence intervals for unpaired data.

Outcomes:

- To better understand the epidemilogy of TTP in South Africa
- To use this study towards the research component (NAMB) of the degree, Mmed (Internal Medicine)
- To present the data at the UFS Faculty Forum in 2019
- To publish the findings in a peer reviewed journal or at a local or international congress.

Time schedule (for short and medium term outcomes):

Outcome	Time frame	Responsibility
Protocol submission	February 2018	Researcher
Second submission	March 2018	Researcher
Ethics evaluation	April 2018	Ethics committee
Data collection	June to August 2018	Researcher
Data analysis	October 2018	Researcher and biostatistics
Protocol amendment	September 2018	Researchers

Time implications for implementation of protocol:

Task	Person responsible.	Anticipated time to complete
Data collection	Researcher. This can be done out of work hours. Should not affect work flow.	2 months
Data analysis	Researcher. This can be done out of work hours. Should not affect work flow.	1 month

Budget:

All costs will be covered by the researcher.

Item	Number	Cost	Total cost
Printing of data sheets	100	R 1.00	R 100.00
Stationery	5	R 35.00	R 175.00
Language editing	20 pages	R 100.00	R 2000.00
Total cost:			R 2775.00

Ethical considerations:

The protocol will be submitted to the Health Sciences Research Ethics Committee of the University of the Free State for their consideration and approval. A unique study number will be allocated to each participant to ensure confidentiality.

The protocol will also be submitted to the Western Province Blood Transfusion Service as well as the South African National Blood Bank Ethics Committees for their approval as the data will be obtained from their data bases for request of information on the patients given FFP over the time period 2010- 2017 at Universitas Hospital for the indication of TTP and other microangiopathic haemolytic anaemias.

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Data Sheet

Study site: Study number:

Characteristics			
Age			
Gender			
Date of first plasma product ordered			
Date of last plasma product ordered			
Blood Group			
Name of Hospital			
Province			
Diagnosis			
HIV status: • CD4 count • Viral load • Treatment commencement date			
Other co-morbidities			
Symptoms: • Dyspnoea • Fatigue • Petechiae • Bleeding • Confusion • Other			
Parameters at diagnosis			
Platelets			
LDH			
Haemoglobin			
Creatinine			
ADAMTS13 level			
ADAMTS13 antibodies			
Haptoglobin			
% Fragmentation			
Coombs			
Troponin T			
CK_{MB}			
Treatment			
Treatment option:	Plasma exchange	Plasma infusion without PEX	
Total number of plasma product used	Fresh Frozen Plasma _____units	Cryo-poor plasma _____ units	
Total number of other blood products	Red cell concentrate _____ units	Platelets _____ units	
Additional blood products used			
Additional therapies used:	Steroids: Dose	Rituximab: Dose	
Treatment complications (plasma products) • TRALI • TACO • other			

Outcome (may have 2 options) • Discharged • ICU • Died	

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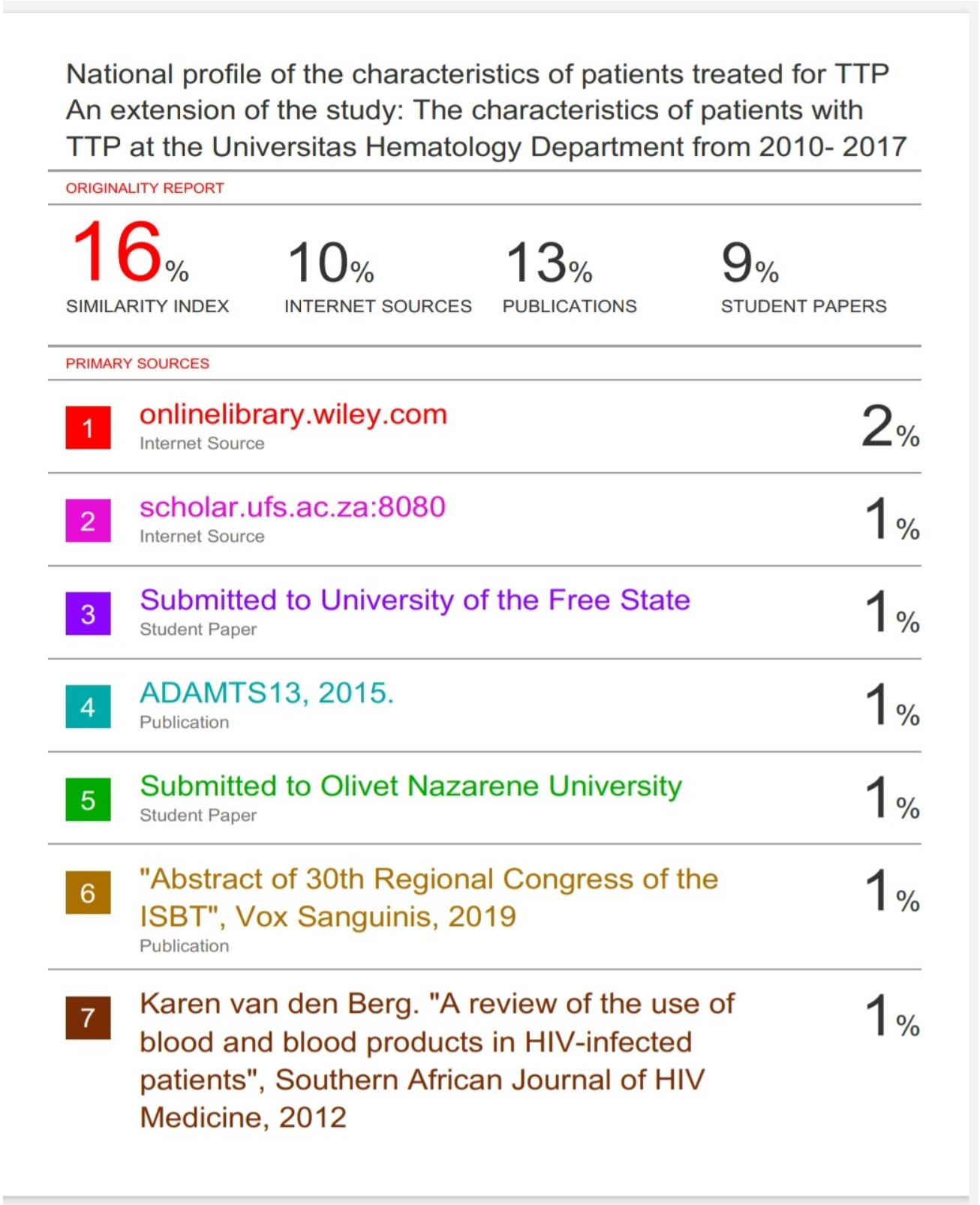

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TABLE OF CONTENTS

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INTRODUCTION

INTRODUCTION

Transfusion and Apheresis Science brings comprehensive and up-to-date information to physicians and health care professionals involved in the rapidly changing fields of transfusion medicine, hemostasis and apheresis. The journal presents original articles relating to scientific and clinical studies in the areas of **immunohematology**, **transfusion practice**, **bleeding and thrombotic disorders** and both **therapeutic** and **donor apheresis** including **hematopoietic stem cells**. Topics covered include the collection and processing of blood, compatibility testing and guidelines for the use of blood products, as well as screening for and transmission of **blood-borne diseases**. All areas of apheresis - therapeutic and collection - are also addressed. We would like to specifically encourage allied health professionals in this area to submit manuscripts that relate to improved patient and donor care, technical aspects and educational issues.

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