UNIVERSITY of RWANDA

SCHOOL OF MEDICINE AND PHARMACY, COLLEGE OF MEDICINE AND HEALTH SCIENCES, DEPARTMENT OF INTERNAL MEDICINE

PROFILING CAUSES OF THROMBOCYTOPENIA AND HOSPITALIZATION OUTCOMES AMONG ADULT NON-HIV MEDICAL PATIENTS AT TERTIARY HOSPITALS IN RWANDA: CASE OF CHUK AND KING FAISAL HOSPITAL

A thesis submitted in partial fulfillment of the requirements for the award of the degree of master of medicine in internal medicine.

By NIYONZIMA Charles

Supervisor: **Dr.NKESHIMANA Menelas[1],MBBS(Hons),MMed (IM),MSc(Global Health Delivery)**

Cosupervisor: **Dr.HAVYARIMANA Sandra[2],MBChB,FCPath(Haem),MMed Haem (SA)**

1. Consultant Physician(CHUK) &Clinical Faculty(UR/SMP/CMHS)

2. Consultant Hematopathologist, King Faisal Hospital, Rwanda

September 2022

DECLARATION

I, NIYONZIMA Charles, to the best of my knowledge do hereby declare that the work presented in this dissertation entitled " ***Profiling causes of thrombocytopenia and hospitalization outcomes among adult non-HIV medical patients at tertiary hospitals in Rwanda: the case of CHUK and King Faisal Hospital*** " is original and has never been presented or submitted before. I have not copied from any other person's work or any other source, except where due references are highlighted in the text.

NIYONZIMA Charles Signature .....Date 10/09/2022

Approved for submission by:

1. Dr. NKESHIMANA Menelas, MBBS(Hons), MMed (IM), MSc(Global Health Delivery)
   Internal Medicine Consultant (CHUK) &Clinical Faculty/UR/SMP/CMHS
   Signature .....NKESHIMANA.....Date...../...../.....
   Digitally signed by Menelas NKESHIMANA
   Date: 2022.09.14 10:58:08 +02'00'
2. Dr. HAVYARIMANA Sandra, MBChB, FCPath(Haem), MMed-Haem (SA)
   Consultant Hematopathologist, King Faisal Hospital/Rwanda
   Signature .....Date 13/09/2022

DECLARATION

DEDICATION

To Jesus Christ: the abundant source of life and healing,

To the love of my life Jane, our children Steven and Hope: for your encouragement as a family

To the patients who have taught me so much over the years.

ACKNOWLEDGEMENT

My special gratitude goes to Dr. NKESHIMANA Menelas and Dr. HAVYARIMANA Sandra for their willingness to provide guidance and mentorship and keep me focused throughout this work.

I would like to thank all clinicians in all hospitals where we carried out the residency rotations, and the faculty from the University of Rwanda /School of Medicine and Pharmacy, College of Medicine and Health Sciences: for their devotion to teaching and sharing unconditionally knowledge and skills have shaped me as a medical doctor for today and tomorrow.

I thank my parents, my relatives, and all my friends mostly NIZEYIMANA Jean-Paul and Dr.TURATSINZE Marcel, your support in one way or another have been invaluable in my academic endeavor.

Last but not least, I am forever grateful to the Government of Rwanda, through the Ministry of Health for sponsoring my studies. I can't wait to give back to Rwanda serving to the best of my capabilities.

Once again, thank you all!

ABSTRACT

Background: A wide spectrum of factors affect platelet count in a patient**.** The severity of thrombocytopenia with associated medical conditions poses a relative risk of bleeding with adverse hospitalization outcomes. In this study, we aimed at determining the incidence, clinical characteristics, common factors, and immediate hospitalization outcomes of patients with thrombocytopenia at CHUK and KFH.

Methods: This was a prospective and cross-sectional study among a cohort of HIV-negative hospitalized medical patients with thrombocytopenia. It was conducted over 8 months from October 2021 through May 2022. Data from enrolled patients were analyzed and frequencies and proportions were expressed as percentages. Bivariate and multivariate analyses were conducted to determine significant factors associated with thrombocytopenia at a p-value of <0.05.

Results: Our cohort comprised 194 patients with thrombocytopenia. The incidence was 13.4 %. Cases of mild thrombocytopenia were the most common at 51.03%, followed by moderate thrombocytopenia at 31.9%, and cases with severe thrombocytopenia represented 17.01%.The most affected group were elderly patients (age > 61) representing 34.5% , mean age 50 (IQR: 38-56) ,males 1.6 times than females Among identified factors associated with thrombocytopenia , sepsis ranked first at 17.01% (OR 2 ,95%CI [1.152-4.611] , p=0.036) ,chronic liver disease came second in 13.4% (OR 2.47 ;95% CI[2.43-4.13]; p=0.041) , thirdly came malignancies at 11.85% (OR 3.5 ,95% CI [1.052-4.576] , p=0.045) , malaria at 8.24% (OR 12.6 ; 95%CI [2.44-15.46] , p=0.021), megaloblastic anemia in 5.15% (OR 14.63; 95% CI [1.300-16.36] , p=0.031). Chemotherapy, enoxaparin, and aspirin use proved an association with thrombocytopenia. Minor bleeding was the most common complication (WHO grade I-II) in 19 patients representing 9.79% (OR 4.23, 95%CI [1.23-15.2],p=0.022). At discharge, 57.73% of patients had normalized platelet counts while 42.27% had persistent thrombocytopenia.

Conclusion: Thrombocytopenia is common in medical wards, especially in the elderly. Timely investigations to make a diagnosis and treatment make a difference in patients with thrombocytopenia and prevention of its adverse outcome.

Keywords*: Thrombocytopenia, incidence, factors, complications, outcome*

LIST OF ABBREVIATIONS

ADAMTS-13: A Disintegrin and Metalloproteinase with Thrombospondin type 1 motif, member -13

AIHA: Autoimmune Hemolytic Anemia

ALL: Acute Lymphoblastic Leukemia

AML-M4: Acute Myelomonocytic Leukemia

ANA: Anti-Nuclear Antibody

ANCA: Anti-Neutrophil Cytoplasmic Antibodies

APLA: Antiphospholipid Antibodies

APML: Acute Pro-Myelocytic Leukemia

aPTT: activated-Partial Thromboplastin Time

BUN: Blood Urea Nitrogen

c-ANCA: Antineutrophil Cytoplasmic Antibody, Cytoplasmic

CHUK : Centre Hospitalier Universitaire de Kigali

CI: Confidence Interval

CMHS: College of Medicine and Health Sciences

CMV: Cytomegalovirus

DIC: Disseminated Intravascular Coagulation

DITP: Drug-Induced Thrombocytopenia

DVT: Deep Venous Thrombosis

EBV: Epstein-Barr Virus

EDTA: Ethylene-Diamine –Tetra-Acetic-Acid

ELISA: Enzyme-Linked Immunosorbent Assay

FBC: Full Blood Count

FFP: Flesh Frozen Plasma

GCS: Glasgow Coma Scale

GI: Gastro-Intestinal

GpIIb/IIIa: GlycoproteinIIb/IIIa

HCV: Hepatitis C virus

HepBSAg: Hepatitis B Surface Antigen

HIT: Heparin Induced Thrombocytopenia

HIV: Human Immunodeficiency Virus

HPA-1a: Human Platelet Antigen-1a

HUS: Hemolytic Uremic Syndrome

ICH: Intracerebral Hemorrhage

ICU: Intensive Care Unit

IgG: Immunoglobulin G

INR: International Normalized Ration

IQR : Interquartile Range

IRB: Institutional Review Board

ISTH: International Society on Thrombosis and Hemostasis

ITP: Immune Thrombocytopenia

KFH: King Faisal Hospital

LDH: Lactate dehydrogenase

LLN: Low Level of Normal

LMWH: Low Molecular Weight Heparin

MAHA: Micro-Angiopathic-Hemolytic Anemia

MDS: Myelodysplastic Syndrome

MPN-U: Myelodysplastic/myeloproliferative neoplasm, unclassifiable

OR: Odds Ratio

PBF: Peripheral Blood Count

PE: Pulmonary Embolism

PLEX: Plasma Exchange

PNH: Paroxysmal Nocturnal Hemoglobinuria

PRBCs: Packed Red Blood Cells

PT: Prothrombin Time

PTP: Post-Transfusion Purpura

PUD: Peptic Ulcer Disease

RDP: Random Donor Platelet

RF: Rheumatoid Factor

SDP: Single Donor Platelet

SMP: School of Medicine and Pharmacy

SPEP: Serum Protein Electrophoresis

SPSS: Statistical Product and Service Solutions

TMA: Thrombotic Microangiopathy

TTP: Thrombotic Thrombocytopenic Purpura

UFH: Unfractionated Heparin

UR: University of Rwanda

UTI: Urinary Tract Infection

VTE: Venous Thromboembolism

WHO: World Health Organization

LIST OF TABLES

Table 1. Socio-demographic and clinical characteristics of patients with thrombocytopenia..... 36

Table 2: Bivariate analysis of factors associated with thrombocytopenia 46

Table 3: Multivariate analysis of factors associated with thrombocytopenia 49

LIST OF FIGURES

Figure 1: Peripheral blood film..... 43

Figure 2:Bone marrow aspiration and trephine biopsy..... 44

Contents

DECLARATION 2
DEDICATION 3
ACKNOWLEDGEMENT 4
ABSTRACT 5
LIST OF ABBREVIATIONS 6
LIST OF TABLES 10
LIST OF FIGURES 11
CHAP I: BACKGROUND AND PROBLEM STATEMENT 14
1.1. Study hypothesis 16
1.2. Research question 16
1.3. Study Objectives 16
1.3.1. General objectives 16
1.3.2. Specific Objectives 16
CHAP II. LITERATURE REVIEW 17
2.1. Platelet production and definition of thrombocytopenia 17
2.2. Bleeding as a complication in patients with thrombocytopenia 17
2.3. Platelet transfusion thresholds and prevention of risk of bleeding 20
2.4. Risk factors for thrombocytopenia 21
2.4.1. Inherited thrombocytopenias 21
2.4.2 . Acquired thrombocytopenias 23
2.5. Diagnostic approach to thrombocytopenia 31
CHAP III. METHODOLOGY 33
3.1. Study design 33
3.2. Study site 33
3.3. Study period 33
3.4. Study population 33
3.5. Inclusion and exclusion criteria 33
3.5.1. Inclusion criteria 33
3.5.2. Exclusion criteria 33
3. 6 . Sample size 33
3. 7. Patients and data collection process 34

3.8. Data analysis 34
3.9. Study outcome measures 35
3.9.1. Primary outcome measure 35
3.9.2. Secondary outcome measures 35
3.10. Ethical consideration 35
3 .11 .Results dissemination 35
CHAP IV. RESULTS AND DISCUSSION 36
4.1. RESULTS 36
4.2. DISCUSSION 51
Study limitation 54
CHAP V. CONCLUSION AND RECOMMENDATIONS 55
5.1. Conclusion 55
5.2 .Recommendations 55
REFERENCES 56
ANNEXES 61
1. Data collection tool 61
2. Consent: English version 66
3.Consent : Kinyarwanda Version 69
4. IRB approval notice 72
5. CHUK approval notice 74
5.King Faisal Hospital Approval notice 75

CHAP I: BACKGROUND AND PROBLEM STATEMENT

Thrombocytopenia is a common hematological disorder defined when the platelet count falls below $150X10^3$ /μL. Clinically, the spectrum of factors that end up causing thrombocytopenia via different mechanisms ranges from laboratory artifacts to true and potentially life-threatening thrombocytopenia with a relative risk of bleeding. When bleeding occurs in vital organs such as the brain, the worst feared events like death or disability can happen. For a clinician, the full blood count (FBC) and peripheral blood film (PBF) on top of thorough bleeding history and physical examination make up an initial trail of diagnosis of thrombocytopenia (1),(2).

Stratifying findings from clinical examination and investigations which may be requested focusing primarily on enquiring on the fitting mechanism which has led to thrombocytopenia can identify one among the hypothesized triad of mechanisms to thrombocytopenia including decreased thrombopoiesis in the bone marrow, increased peripheral platelet destruction/sequestration in hypersplenism and increased consumption of platelets (1).

Balduini CL et al(3) reported a low prevalence of disorders with inherited thrombocytopenia to be 2.7 per 100,000 inhabitants. These disorders can be seen within the first weeks of life but their progression is associated with the intermittent and chronic bleeding pattern and their suspicion can be backed by specific phenotypical characteristics and genetic testing, both of which are considered to be invaluable tools for making a diagnosis (4).On the other side, the observation from many other studies position thrombocytopenia to be more common in acquired disorders and the prevalence has been increasingly changing, though, multifactorial according to the setting.

Nkambule et al in Kwazulu-Natal, South Africa reported a thrombocytopenia prevalence of 14.9%, oncology cases more represented at 55.2% among causes of thrombocytopenia, whereas 15.5 % of causes were found in non-hematology wards (1), while Vaughan JL et al in Soweto reported a thrombocytopenia prevalence of 18.7% in medical wards at a tertiary hospital and oncology cases accounting more of thrombocytopenia at a rate of 34.4% (5). Infectious causes in the medical emergency setting can decrease the platelet count acutely, and, as Teo CP et al remarked in one of Singapore hospital's medical emergency departments; 46.6% of patients with thrombocytopenia had an underlying infection as a culprit (6), and as Satoshi et al have found, DIC complicated sepsis and septic shock cases at a rate of 50.9% with concerning mortality of 24.7%, compared to the relatively low mortality of non-DIC patients, at a rate of 17.5% (7).

On the side of thrombocytopenia severity and transfusion requirements, Niyongabo et al(8) at Butaro cancer center found that 87.8% of transfused platelets were in cancer patients on chemotherapy whose side effects caused myelosuppression. Most of them had an increased risk of spontaneous bleeding, the worst feared being intracerebral(2). Leukemia, mostly acute lymphoblastic, was by far the most common cancer in transfused patients (p=0.01). Other than blood components like PRBCs, patients with that type of cancer required more therapeutic platelet transfusion than in any other pathologies (8).

In tropical countries where malaria is still endemic, cases of thrombocytopenia with variable severity have been reported. A study by Mboya et al in Kenya(9) found a higher incidence of thrombocytopenia at 87%, and he noted that there was a positive correlation between thrombocytopenia and malaria ($r=0.26$, $p<0.001$), and this was higher than the incidence of thrombocytopenia in malaria as found by Shiraz Jamal et al of 68% (10).

Cases of ITP are sporadic and not widely reported. Its reported incidence varies between 2-5/100,000 in the general population(11). Other rare conditions like thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) cause organ damage, mainly the renal vascular endothelium with a resulting fulminant renal failure, reported rates of associated renal failure occurrence to be as high as 58% in cases of TTP and 100% in cases of HUS (12)(13).

Atypical presentation of vitamin B12 deficiency can masquerade as TTP(14). This demands caution for a clinician not to miss a diagnosis of Vitamin B12 deficiency because it is a relatively common micronutrient deficiency in resource-limited settings (15).

Hepatitis B was reported to be associated with a thrombocytopenia prevalence of 6%, but this rate dramatically increases in cases of chronicity such as in cirrhosis, where thrombocytopenia prevalence was as overwhelmingly high as 68% (16). Auto-immune conditions like systemic lupus erythematosus (SLE) may be complicated by cytopenias, and thrombocytopenia alone has been reported at 20-40% in patients with lupus erythematosus (17).

Cases of drug-induced thrombocytopenia have been studied, and as Kuter JD et al's study heparin-induced thrombocytopenia (HIT) found(18), H IT was high in patients on unfractionated heparin at an incidence of 16%, and these patients had a high risk of death due to major bleeding which occurred in 48% among all cases.

Based on the above studies, we observe that it is common for clinicians to consult patients with thrombocytopenia daily. Causative factors, complications, and patient outcomes vary according to the setting and patients' characteristics. However, in Rwandan tertiary hospitals, the current situation of clinical characteristics of patients, common causative factors of thrombocytopenia, and the associated hospitalization outcomes are unknown. It is in this regard that we conducted this study so that we can inform clinicians on the current characteristics of patients with thrombocytopenia and, immediate outcomes and suggest recommendations for a better approach to patients with thrombocytopenia in our setting.

1.1. Study hypothesis

Thrombocytopenia is common in patients with febrile conditions and patients having chronic conditions at tertiary hospitals in Rwanda.

1.2. Research question

1. What is the incidence of thrombocytopenia in tertiary hospitals in Rwanda?

2. What are the common causes of thrombocytopenia and immediate hospitalization outcomes of patients with thrombocytopenia?

3. What are the complications of thrombocytopenia in hospitalized patients?

1.3. Study Objectives

1.3.1. General objectives

Determine the incidence, etiology, complications, and immediate hospitalization outcomes of patients with thrombocytopenia.

1.3.2. Specific Objectives

-Determine the incidence, demographic and clinical profile of patients with thrombocytopenia,

-Determine the relationship of severity of thrombocytopenia with hospitalization outcomes,

-Compare the treatment of severe thrombocytopenia with hospitalization outcome.

CHAP II. LITERATURE REVIEW

2.1. Platelet production and definition of thrombocytopenia

Platelets are anucleated cells produced in millions each day through the shedding of megakaryocytes, the latter being specialized cells with self-renewal capacity in the myeloid lineage of hematopoiesis in the bone marrow. Megakaryocytes are regulated by thrombopoietin, a hematopoietic growth factor produced in the liver. Platelet count in the peripheral circulation and the spleen reflect the stimulatory response of the bone marrow by thrombopoietin(19).

Platelets make a small portion of blood volume which is estimated at 1%. About 40%-45 % of blood volume is composed of red blood cells, whereas the big volume is made of plasma at 55%-60% and less than 1% of blood volume is composed of white blood cells as known as leukocytes. Almost 2/3 (66.6%) of platelets are in the peripheral circulation while 1/3 (33.3%) of platelets are stored in the spleen. Platelets have a short life span ranging between 5 to 12 days, but this life span dramatically becomes as shorter as 24 hours in clinical stressful situations such as in severe acute infectious processes. In normal subjects, platelets are replenished continually to maintain a constant supply and balance to keep up with a watchful task of restoring vascular integrity through primary hemostasis by initiating clotting mechanisms alongside other clotting factors to form a stable clot when there is a vascular injury(20),(21).

The normal platelet count ranges from 150 $x10^3$ /µL to 450 x 10^3 / µL. An absolute decrease below the lower limit of normal (LLN:150 $x10^3$ /µL) is called thrombocytopenia and this low limit of normal threshold is used in this study (1).

2.2. Bleeding as a complication in patients with thrombocytopenia

The clinical significance of thrombocytopenia relies on patients being at an increased risk of bleeding. This depends on how severe the depletion has been and on the underlying condition of the patient. However, it is still unknown why some patients with severe thrombocytopenia bleed and others do not. As Kuter DJ et al(18) remarked, there might be bleeding in some patients despite having seemingly safe platelet counts; because other factors may compound the bleeding risk.

Those factors may be but are not limited to anemia, coagulopathy, vascular integrity impaired by infection, malignant disease, or chemotherapy(18).

For platelet counts above 100$x10^3$ /µL-150$x10^3$µL, no data were found reporting a bleeding event. However, WHO grade I bleeding (**table-a**) can occur with platelet counts less than 150$x10^3$ /µL

to 75x10 ^{3}/μ L, WHO grade II bleeding episodes with platelet count less than 75x10^{3}/μL to 50x10^{3} /μL, WHO grade III bleeding with platelet count less than 50x10^{3}/μL to 25x10^{3}/μL while WHO grade 4 bleeding becomes mostly expected in patients with very low counts below 25x10^{3}/μL. For platelet count below 20x10^{3} /μL, there is an increased risk of spontaneous, life-threatening bleeding and this risk dramatically increases when counts fall below less than 10x10^{3}/μL, and some studies have classified this as very severe thrombocytopenia requiring emergency therapeutic platelet transfusion (21).

Intracerebral hemorrhage (ICH) is one of the worst events and life-threatening complications of severe thrombocytopenia. In a study by Estcourt et al (2), they reported an incidence of ICH of 0.5% at 95% CI [0.39%-0.64%], and in a study by Rost NS. et al, the size or volume of the hematoma and the low GCS (below 9/15) at the time of hemorrhage were the important predictors of mortality and functional outcome after ICH(22).On the other hand, Friedmann et al(23) evaluated factors that may increase the risk of bleeding in patients with thrombocytopenia and found that severe hypoalbuminemia (serum albumin <2g/dL) and uremia (BUN >50mg/dL or serum urea >17.9mmoL/L, as might be the case in HUS) were associated with an increased risk of bleeding (for uremia: bleeding episode had significant odds: OR 1.64;95%CI [1.40 -1.92] and for severe hypoalbuminemia, the bleeding episode has an odd ratio of 1.45, 95%CI [1.33 - 1.79] (23).

Webert K et al (24) analyzed the bleeding pattern in patients with acute myeloid leukemia having thrombocytopenia and found that the presence of fevers (temperature above 38^{o} C) increased the risk of mild bleeding in WHO grades I and II, (**table-a**) by 52% ; (RR 1.52; 95%CI [1.25-1.85], p <0.005), and the presence of elevated body temperature increased the risk of significant bleeding in WHO grades III-IV by 87% (RR 1.87;95% CI 1.40-2.49). As Kuter DJ et al have found(18) , all patients with very low counts (<5x10^{3}/μL) were at increased risk of spontaneous bleeding (OR 3.1;95% CI[2.0-4.8]) compounded with other factors including having low hematocrits < 25% (OR 1.29;95% CI [1.11—1.49] ,INR > 1.2-1.5 (OR,1.46; 95%CI [1.17—1.83] and INR>1.5 (OR,2.05; 95%CI [1.43-2.95] and prolonged aPTT between 30-<50 seconds(OR : 1.40 ; 95%CI [1.08-1.81] ,p <0.01) ,aPTT > 50 seconds (OR 2.34, 95%CI [1.54- 3.56] (18).

Table-a: Adapted WHO bleeding grades (21)

WHO Grade	Bleeding event
Grade 0	Absence of bleeding
Grade I	Petechiae, purpura, ecchymosis, occult blood in body secretions, epistaxis <30min, gum bleeding, mild vaginal spotting
Grade II	Evidence of gross hemorrhage not requiring RBC transfusion within 24 hours (epistaxis, hematuria, hematemesis)
Grade III	Hemodynamically stable patients with hemorrhage requiring 1 or more units of RBC transfusion within 24 hours of onset
Grade IV	Debilitating bleeding/life-threatening bleeding causing hemodynamic instability or bleeding into a vital organ (intracranial, pericardial, or pulmonary hemorrhage) or fatal bleeding.

2.3. Platelet transfusion thresholds and prevention of risk of bleeding

Even though the rate of platelet transfusion in a study by Valsami S. et al (25) was 30.26% in medical wards, hematological disorders, mostly cancers, claimed more platelets than others, needing 26.2% of all platelet transfusions. Valsami S. et al remarked that the indications of platelet transfusion were relative and varied profoundly depending on the clinical situation and patient's characteristics. Some of the indications were therapeutic and others were prophylactic, considered when the risk of bleeding was potentially high. On the other side, platelets as human tissue, are part of allogenic blood and the risk of zero to transfusion-related effects doesn't exist. However, in clinical practice, transfusion restriction to needy patients with a high risk of bleeding is strongly recommended.

Mostly, the random donor platelets(RDP) from more donors are used in wards, and usually, units to transfuse fall into the judgment of the clinician, (1 unit of platelet almost contains 45-65 mL, with expected platelet count raise of about 10 x10 ^{3}/μL in a 70 kg patient); as opposed to single donor platelets(SDP) or apheresis platelet, which undergo apheresis, a process where one unit volume contains approximately 20mL-300mL with a benefit of leukocyte antigen-reduction and increase the platelet count up to $50x10^{3}$/μL (21).

Even though controversies still exist on defining the actual threshold of thrombocytopenia requiring platelet transfusion, the morning platelet count is still being used as a trigger to decide on therapeutic platelet transfusion for counts below $10x10^{3}$/μL, unless other factors can increase the risk of bleeding such as in sepsis, which is a situation with an active infectious process where there is thinning and fenestration of vessels which increases platelet reactivity to close the formed gap, thus, in sepsis, counts are recommended to be kept above $100x10^{3\mu}$/μL to prevent bleeding. Therefore, the morning platelet threshold is a poor predictor of bleeding in situations like sepsis, when a patient has coagulation/hemostasis disorders, chronic liver disease, or in patients with a history of bleeding or who have active disease(23).

Relative precautions are taken in different clinical situations. Examples may be in case of elective surgical or invasive procedures (like as insertion of a central line, or lumbar puncture), prophylactic platelets transfusion is indicated to maintain counts > $50x10^{3}$/μL, and for more aggressive procedures, the platelet counts are to be maintained higher. A typical example is in the case of ophthalmic or neurosurgery where platelets are to be maintained above $100x10^{3}$/μL.

Withholding anticoagulation therapy for platelet counts less than $50x10^3$ /µL is strongly recommended. Premedication before platelet transfusion is required in patients with a history of urticaria, and the evaluation of response to platelet transfusion is obtained post platelet transfusion count 30 to 60 minutes after infusion. Other measures include the administration of plasma to replenish clotting factors when a patient is actively bleeding or when facing refractoriness to platelet transfusion. Progestational agents are prescribed to decrease menstrual bleeding and drug-induced or disease-related bleeding is treated with steroids, and cancer chemotherapy is withheld for counts less than $50\text{-}100x10^3$ /µL (21),(26).

2.4. Risk factors for thrombocytopenia

Factors can be patient-related, disease-related, or treatment-related. They can be subdivided into inherited or acquired.

2.4.1. Inherited thrombocytopenias

Inherited thrombocytopenias(IT) represent a group of rare disorders with variable severity of thrombocytopenia and clinical phenotypes range from mild to life-threatening bleeding disorders recognizable right from the first few weeks of life up to adulthood. Balduini et al estimated IT incidence in Italy at 2.7per 100.000 inhabitants(3). Mutations in genes that regulate megakaryocyte growth in the bone marrow and shedding to produce platelets result in defective, dysfunctional, and decreased platelet production with a shorter lifespan and patients experience chronic bleeding disorders (4).

The clinical suspicion of inherited thrombocytopenia anchors on stratification of different factors including age at onset of thrombocytopenia, chronicity of bleeding episodes on top of a thorough review of systems to look for specific associated non-hematological disorders which help to fit thrombocytopenia in a likely clinical syndrome (examples being hearing loss, immunodeficiency disorders, disorders of the skeletal system as highlighted in **Table-b**). The emphasis is put on morphological changes in platelets as might be seen on a peripheral blood film (PBF) to differentiate small platelet size, and normal platelet size, versus the presence of large platelets, or presence of abnormal platelet granules, neutrophil inclusions known as Döhle bodies, or erythroid "tear-drop" cells or hairy cell leukemia which may give a clue to a specific inherited thrombocytopenic disorder (4).

Table- b: Inherited thrombocytopenias classified by genetic mutations and associated clinical findings (4).

Syndrome	Gene mutation	Chromosomal location	Associated findings
MYH9-related thrombocytopenias			
May-Hegglin's anomaly	MYH9	22q11	Neutrophil inclusions, sensorineural hearing loss, nephritis, cataracts
Fechtner syndrome	MYH9	22q11	
Epstein syndrome	MYH9	22q11	
Mediterranean thrombocytopenia/ Bernard Soulier's carrier	GP1BB	17pter-p12	None
Bernard-Soulier syndrome	GP1BA,GP1BB	17pter-p12	None
Velocardiofacial/ DiGeorge syndrome (CATCH22)	?GP1BB	22q11	Cardiac, facial, parathyroid, and thymus anomalies, cognitive/learning impairment
Familial platelet disorder/Acute myeloid leukemia	AML1	21q222	Myelodysplasia, acute myeloid leukemia
Chromosome10/THC2	?FLJ14813	10p12-11.2	None
Paris-Trousseau thrombocytopenia/ Jacobson syndrome	?FLJ1	11q23	Psychomotor retardation , facial anomalies (Jacobson syndrome)

Gray platelet syndrome	Unknown	Unknown	None
Congenital amegakaryocytic thrombocytopenia	*MPL*	*1p34*	Marrow failure during the second decade
Thrombocytopenia and absent radii	*Unknown*	*Unknown*	Shortened/absent radii bilaterally
Thrombocytopenia and radial synostosus	*HOXA11*	*7p15-p14.2*	Fused radius, incomplete range of motion
Wiskott-Aldrich syndrome	*WAS*	*Xp11.23-p11.22*	Immunodeficiency, eczema, lymphoma
X-linked thrombocytopenia	*WAS*	*Xp11.23-p11.22*	None
GATA-1 mutation	*GATA1*	*Xp11.23*	Anemia, dyserythropoietic, thalassemia

2.4.2 . Acquired thrombocytopenias

2.4.2.1.Immune thrombocytopenia (ITP)

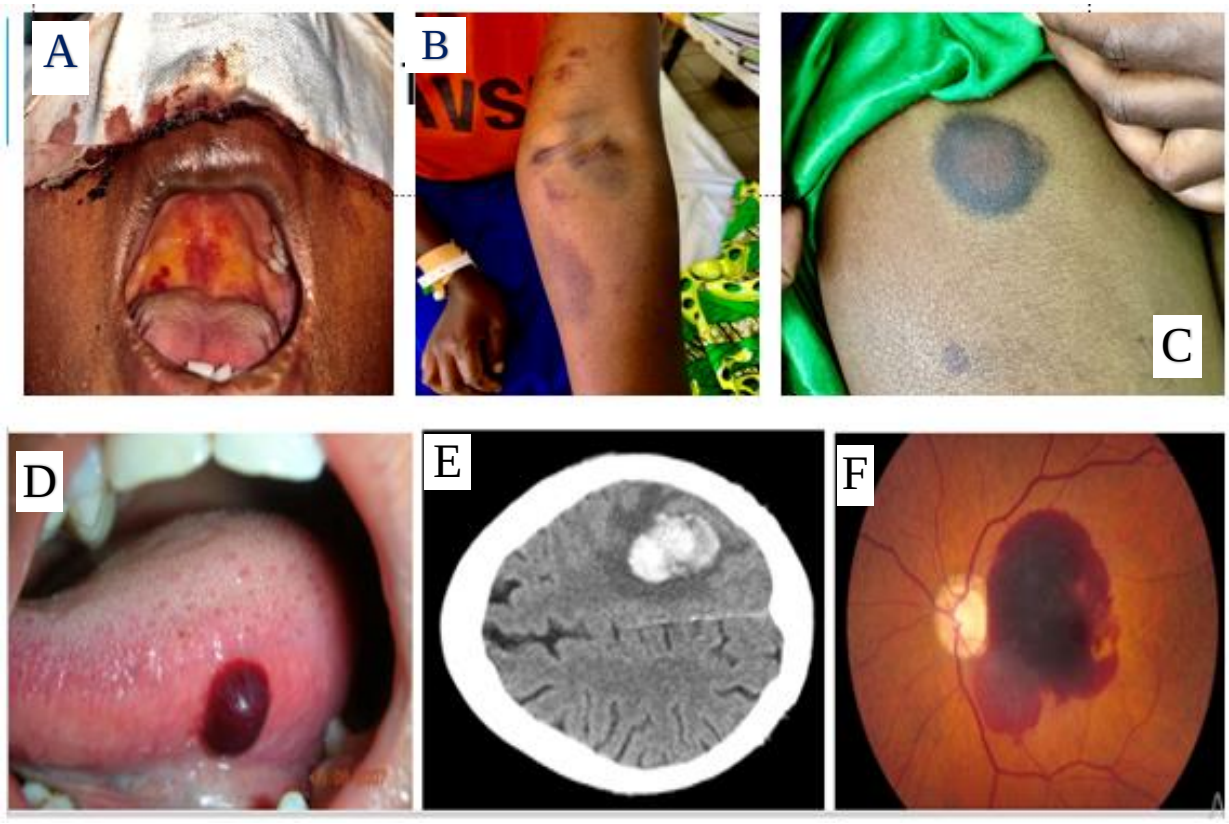
ITP is mostly diagnosed in children and young adults, with females mostly affected than males. Its incidence is estimated to be 2-5/100.000 in the general population (11). For unknown reasons, autoantibodies (immunoglobulins G) attack platelets receptor GpIIb/IIIa, and the complex is attacked and phagocytized by macrophages in the spleen and they are destroyed, resulting in thrombocytopenia. Around 20-30% of patients with ITP are asymptomatic at the time of diagnosis. For patients who do have symptoms **(Photo-1)**, the spectrum of bleeding varies from mild episodes like epistaxis, petechiae, purpura, and gum bleeding, to life-threatening overt gastrointestinal bleeding, heavy menses, hematuria (representing 9.4%), or patients can develop an intracerebral hemorrhage, reported at 1.4% (27).

Paradoxically in ITP, there is a weak correlation between low platelet count and the bleeding risk, and the incidence of cases of venous thromboembolism (VTE) and arterial thrombosis complicating ITP is reported to be 2.75% (28). Isolated thrombocytopenia on FBC and PBF revealing severe thrombocytopenia are typical findings in the case of ITP(**Photo-2).** Sometimes PBF can show platelet anisocytosis, characterized by small and large platelets.

The American Society of Hematology (ASH) does not recommend doing bone marrow for ITP cases, except in elderly patients above 60 years old, where malignancies could be underlying. Above age 40, myelodysplasia can be a possibility to exclude associated hematological disorders to ITP.

ITP treatment becomes challenging with the dual risk of bleeding and thrombosis, which is 2 times higher in ITP than in the general population. However, treatment aims at restoring platelets to safe levels to prevent future bleeding. Although 20-30% of patients do not respond to the 1st line steroid therapy, rituximab (an immunoglobulin), produces an immediate response rate in increment of platelet counts in 60% of cases which is sustained in 50% for 2 years period. Thrombopoietin receptor agonists like eltrombopag can be used and splenectomy is considered the most effective therapy of ITP with an estimated 86.7% of response rate and 60-70% of long-lasting remission, but this radical option is delayed at least for 1 year hoping for ITP spontaneous remission(27).

Photo 1:Bleeding spectrum in patients with severe thrombocytopenia in different locations (29)



1

[1] ***-On panel A*** *,you see nasal packs due to epistaxis and scattered mucosal bleeding lesions located in the mouth on the soft palate representing petechial hemorrhage which is due to severe thrombocytopenia ,typical of ITP.*

*-**Panels B and C** show subcutaneous round and not well-demarcated lesions representing ecchymosis in ITP.*

*-**Panel D** shows a raised pocket of sublingual mucosa representing a blood blister lesion in a patient with severe thrombocytopenia.*

*-**Panel E** shows a computed tomography scan of the brain revealing an intra-cortical enhancement typical of an acute hemorrhagic stroke in a patient with severe thrombocytopenia.*

*-**Panel F** shows a fundoscopic image of a retina revealing a round, dark lesion near the optic disc representing retinal hemorrhage in a patient with severe thrombocytopenia.*

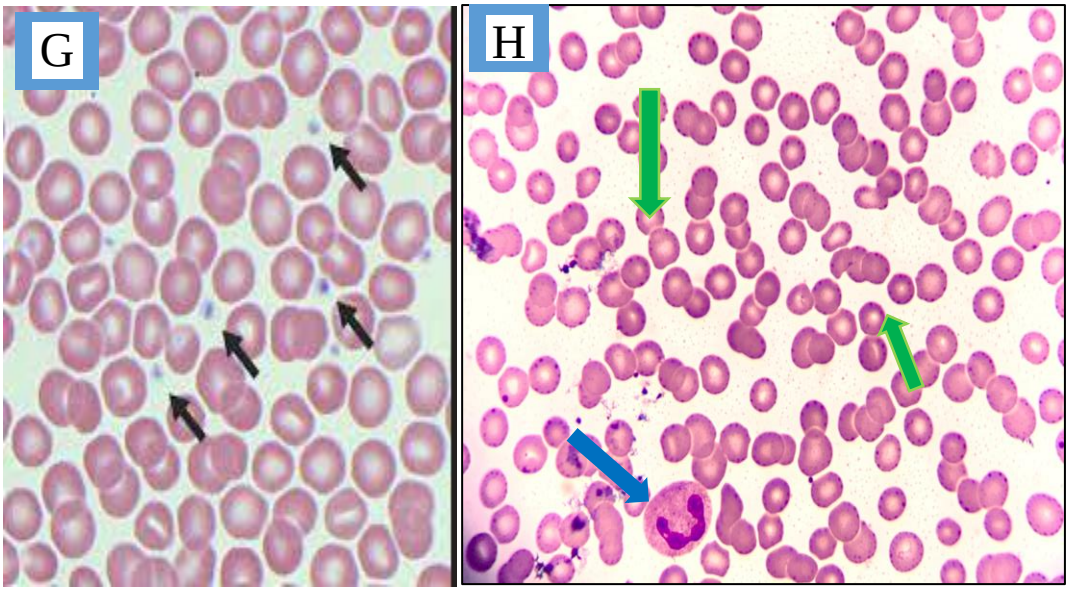
***Terminology**: **1.Petechiae**: is a very small, pinpoint bleeding lesion on the skin less than 4 millimeters(mm) in size.*

***2.Purpura** : is a larger bleeding lesion under the skin typically between 4mm and 10 mm in size*

***3.Ecchymosis**(also known as **bruising**): is an area of bleeding lesion under the skin larger than 10 mm*

***4.Epistaxis-** a medical term for nosebleed*

Photo 2: Courtesy of a PBF showing severe thrombocytopenia in an ITP patient at CHUK (29)



[2]

[2] ***-Panel G*** *shows platelet morphology on a peripheral blood smear/film (highlighted in* ***black arrows****)*

(Source:Platelet morphology/Blood film-Online/MedSchool,August2022)

- ***On panel H****, you see a peripheral blood smear from a patient with ITP at CHUK. The smear shows a decreased number of platelets, a normal appearing neutrophil (****blue arrow****) and normal appearing erythrocytes(* ***green arrows****).ITP is diagnosed by excluding other diseases; therefore, the absence of other findings from the peripheral smear is at least as important as the observed findings. This smear demonstrates the absence of immature leukocytes(blasts, as in leukemia) and fragmented erythrocytes,schistocytes(as in thrombotic thrombocytopenic purpura) and no clumps of platelets seen (as in pseudothrombocytopenia).*

2.4.2.2. Post-Transfusion Purpura, Heparin Induced Thrombocytopenia, and Paroxysmal Nocturnal Hemoglobinuria

Post-transfusion purpura (PTP) is a rarely reported auto-immune disorder that may happen after a blood transfusion. According to Van Kogelenberg et al (30), this autoimmune condition can occur 3-10 days after transfusion of blood products containing platelets at an estimated incidence of 1/50,000-1/100,000 blood transfusions and has a female to man ratio of 5:1.There is a febrile reaction as a result of the formation of alloantibodies (HPA-1a) against the transfused platelet (Human Platelet Antigen, HPA) and this reactivity ends up in severe thrombocytopenia which can be accompanied by both intracranial and gastrointestinal bleeding (31). PTP is a diagnosis of exclusion after the possibility of HIT/drug-induced thrombocytopenia or TTP/HUS/ DIC has been ruled out. Transfusion of HPA-1a negative platelet concentrate, a high dose of IV immunoglobulin (1g/kg) and prednisolone (1-2mg/kg) help to reduce the destruction of platelets and stabilizes the immune system from attacking platelets (31).

The cross-reactivity of administered heparin and platelet factor-4 causes heparin-induced thrombocytopenia (HIT), highly suspected in the presence of thrombocytopenia, the timing of thrombocytopenia with respect to heparin exposure, thrombosis or other sequelae of HIT and possibility of other causes of thrombocytopenia other than heparin. Its incidence is 0.5%-1% in hospitalized patients receiving a therapeutic dose of heparin or recently discharged patients (in 1-2 weeks) who were exposed to unfractionated heparin (UFH).On the contrary, HIT incidence is lower at 0.065 % of in-patients receiving LMWH. When using LMWH, antibody formation is at 8%, an estimate which is low than when using UFH, where antibody formation is reported at 17%, especially during thromboprophylaxis (32). Thrombosis, being the most serious complication of HIT has been reported in 20%-64%, and can develop after 4-10 days if no previous heparin exposure or on the same day if previous exposure to heparin (18).

ELISA helps to identify Platelet factor-4 antibodies (IgG) and is a sensitive test (tests alpha granules of the platelets) but has low specificity to diagnose HIT. Serotonin release assay (which tests dense granules of platelets) confirms that platelet has been activated and aggregating to cause thrombosis and is to be requested in case of suspected HIT. The most dangerous form of HIT with thrombosis has been associated with a high mortality rate of up to 20 % (1 in 5 patients dies). If

HIT is diagnosed, heparin is stopped and the antidote, argatroban, a direct thrombin inhibitor is given or lepirudin, as an alternative. Prophylactic platelet transfusion is not recommended unless there is life-threatening bleeding and warfarin is contraindicated until platelet normalization (33).

Paroxysmal nocturnal hemoglobinuria (PNH), is characterized by chronic intravascular hemolysis which is marked by a triad of thrombophilia, hemolytic anemia, and bone marrow failure. Iron deficiency anemia, hemoglobinuria, thrombocytopenia, and painful abdominal crises can be present and this rare disorder has been reported to have a prevalence of 1-5 cases/million worldwide it occurs due to activation of the complement system and the situation becomes worse as there are no complement regulators on red blood cells in susceptible patients (34).

2.4.2.3. Thrombotic microangiopathies

Various thrombotic micro-angiopathies including disseminated intravascular coagulation(DIC), thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS) induce thrombocytopenia due to shortened platelet circulation and increased clearance/ consumption: a thrombus formation decreases platelet count and induces RBCs shearing and organ dysfunction alongside with severe bleeding episodes which may occur(13). Satoshi Gando et al(7) reported cases of severe sepsis complicated with DIC at a prevalence of 50.9% in ICU patients and these patients had a poor prognosis with an associated mortality of 24.7 % compared to non-DIC patients' mortality at 17.5%. DIC can be overt **(Table-c)** or non-overt. Byuk Sung Ko et al in South Korea using the DIC ISTH score found cases of overt DIC prevalence at 17.6% (35).

Table-c: Scoring system for overt DIC by ISTH (35)

SCORE	POINTS
Platelet count (× 10^3/µL)	
< 50	2
≥ 50 and < 100	1
≥ 100	0
Fibrin-related markers	
Strong increase	3
Moderate increase	2
No increase	0
Prothrombin time (in seconds)	
≥ 6	2
3-6	1
< 3	0
Fibrinogen level (g/mL)	
< 100	1
≥ 100	0
Calculate score	
If ≥ 5, compatible with overt DIC; repeat scoring daily	
If < 5, suggestive (not affirmative) for non-overt DIC; repeat next 1 to 2 days	

In the case of TTP, platelet aggregation and the associated reno-vascular endothelial damage results in hemolytic uremic syndrome (HUS), a clinical situation mostly accompanied by bloody diarrhea, especially in children. In adults, diarrhea is rare and HUS follows a Shiga-like toxin produced by E.coli which can be ingested from unpasteurized milk, undercooked meat or drinking contaminated water, or consuming contaminated raw vegetables(13).

Atypical HUS can be associated with a genetic mutation in the genes encoding the complement system, identifiable in 60% of cases. Both TTP and HUS are more concerning: they cause kidney injury, and the incidence of kidney injury is reported to be as high as 58 % in cases related to TTP and 100 % in cases related to HUS (12)(13). Fever and neurological symptoms are typical in TTP and finding ADAMTS-13 enzyme activity depleted (< 10%) can be diagnostic.

These 2 conditions (TTP and HUS) will not require platelet transfusion unless in case of life-threatening bleeding. No antibiotics are indicated. Plasmapheresis or plasma exchange (PLEX) in TTP can be done. As Friedmann et al reported (23), uremia (defined as blood urea Nitrogen > 50 mg/dL which equals urea > 17.9mmol/L) was associated with an increased risk of bleeding in case of HUS (OR 1.64;95% CI [1.40 to 1.92] and dialysis is urgently needed.

2.4.2.4. Pseudo-thrombocytopenia

Being a laboratory artifact, pseudo-thrombocytopenia is not clinically significant as it is not associated with bleeding and does not require treatment. It is a relatively common phenomenon caused by platelet clumping due to the presence of EDTA anticoagulant in a sampling tube. Its incidence accounts for 15-20 % of all patients with isolated thrombocytopenia (36).To rule out thrombocytopenia, another sample is sent in a citrate tube which does not induce platelet clumping and this prevents reporting factitious thrombocytopenia.

2. 4.2.5. Thrombocytopenia in patients with malaria

A study in Kenya by Mboya et al (9) found a thrombocytopenia incidence to be 87% and there was a positive correlation between thrombocytopenia and anemia with the diagnosis of malaria (P.falciparum) ($r=0.26$, $p<0.001$) as compared to findings by Shiraz Jamal et al (10) of thrombocytopenia incidence of 68 % in P.faciparum malaria and both Mboya et al and Shiraz et al correlated the presence of thrombocytopenia and fever with malaria. In malaria cases, bleeding is rare and thrombocytopenia happens via increased peripheral destruction, platelet consumption by disseminated intravascular coagulation as well as excessive platelet removal by splenic pooling. Finding an adequate or increased number of megakaryocytes on bone marrow samples analysis

makes decreased thrombopoiesis unlikely in thrombocytopenia from malaria and of this fact Mboya et al recommend against unnecessary platelet transfusion in malaria patients (9).

2.4.2.6. Thrombocytopenia in chronic diseases and nutritional deficiencies

Various infectious agents such as hepatitis C, cytomegalovirus (CMV), Epstein-Barr Virus (EBV), human immunodeficiency virus (HIV), and disseminated tuberculosis, among others, can infiltrate the bone marrow and cause secondary myelofibrosis which results in pancytopenia. Chronic alcoholism or viral infections like hepatitis C and B can lead to chronic liver failure causing decreased production of thrombopoietin and decreased thrombopoiesis in the bone marrow. The resulting deficiency of Vitamin K-dependent factors due to liver failure compound the coagulation abnormalities with an associated bleeding risk (21).

Immuno-deficiencies due to nutrient deficiencies such as vitamin B12 were reported to be associated with cytopenias and ill health (15). Disseminated TB was reported to be a factor in 5-10% of the causes of thrombocytopenia whereas hypo-coagulation states from paraneoplastic syndromes, DIC, and thromboses in cancer patients increase the use of platelets which results in thrombocytopenia (21).

2.4.2.7. Drug-induced thrombocytopenia

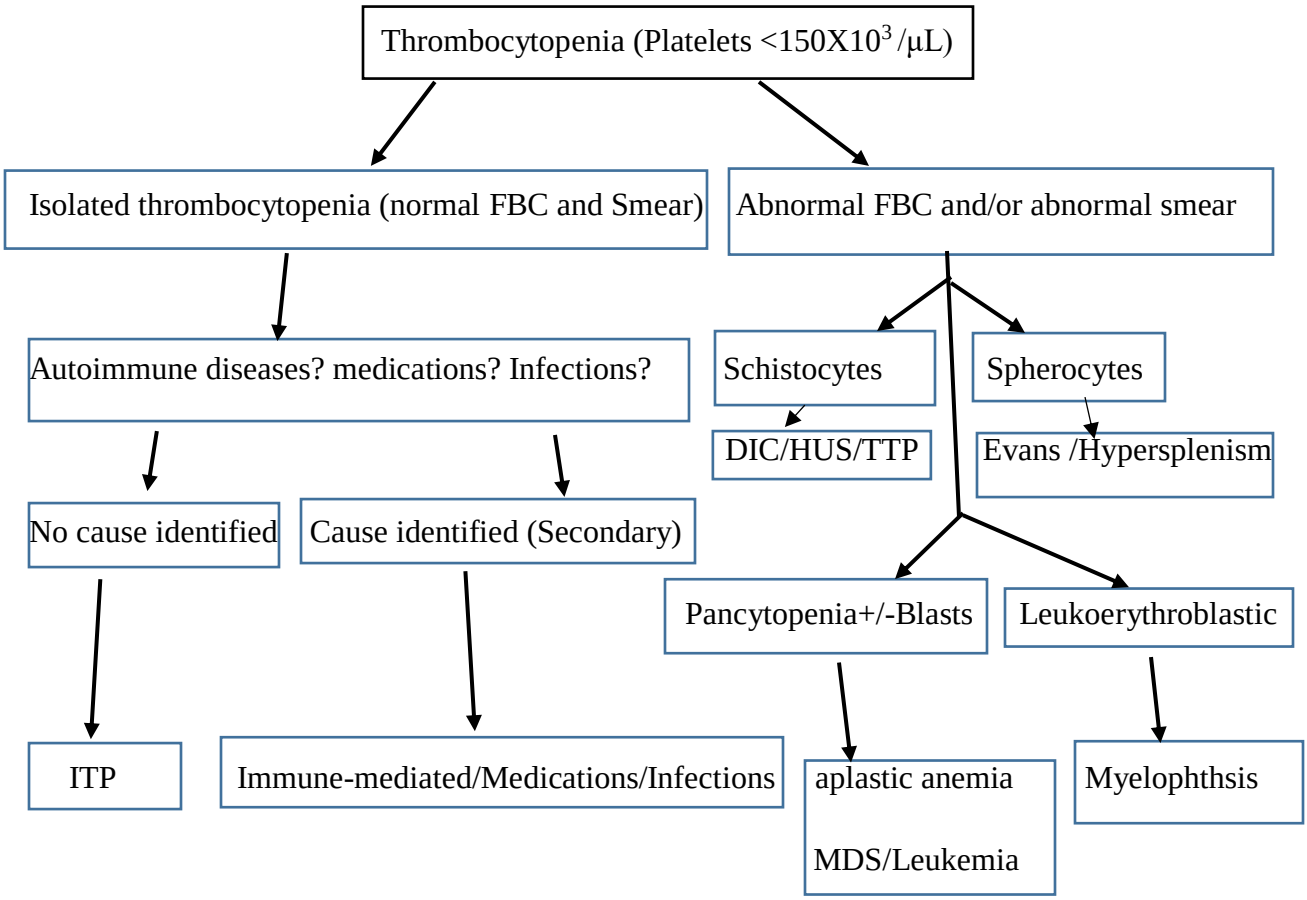
Severe thrombocytopenia can occur after initiating an antithrombotic agent that blocks fibrinogen binding to platelet GpIIb-IIIa, such as abciximab, tirofiban, and eptifibatide. Rivaroxaban was reported to cause 0.3% of cases of thrombocytopenia. In many patients, the drug etiology is usually not recognized. Recovery from DITP usually begins in 1-2 days after stopping the drug and is completed within a week. Chemotherapy in cancer patients causes myelosuppression which becomes the underlying cause of low platelet counts (21).

2.5. Diagnostic approach to thrombocytopenia

The start point is the patient's history enquiring about any bleeding, infectious history, underlying comorbidities, and performing a physical examination that looks for features of any inherited disorders (3), bleeding signs, presence of hepato-splenomegaly, or lymphadenopathies. The FBC with differential can identify isolated thrombocytopenia versus multi-lineage involvement. PBF will give clues on signs of decreased production, and increased destruction, like large platelets, schistocytes, or platelet clumping. Additional and customized tests are requested when the clinical situation dictates like doing flow cytometry after bone marrow aspiration and biopsy, SPEP,

ADAMTS-13 in case of TTP, or autoimmune tests. If anemia, request reticulocyte count, LDH, haptoglobin, and bilirubin to detect hemolysis are recommended. If suspected hemolytic anemia can enquire on PT, PTT, fibrinogen, D-dimers, and coombs. The bone marrow aspiration and trephine biopsy, immunohistochemistry can be done for unexplained thrombocytopenia in patients with splenomegaly (37)

Summarized approach to thrombocytopenia (37)



CHAP III. METHODOLOGY

3.1. Study design

This is a prospective and cross-sectional study

3.2. Study site

Medical departments of CHUK and KFH.

3.3. Study period

The study period was from October 2021 through May 2022.

3.4. Study population

Patients hospitalized in internal medicine wards having thrombocytopenia (Platelets<$150x10^3$/µL)

3.5. Inclusion and exclusion criteria

3.5.1. Inclusion criteria

All patients having thrombocytopenia (<$150x10^3$/µL) with or without bleeding in medical wards who have given an informed consent

3.5.2. Exclusion criteria

- HIV-positive patients having thrombocytopenia
- Patients with pseudo-thrombocytopenia
- Patients who did not consent

3. 6 . Sample size

We estimated the sample size using the formula:

$$\mathbf{N = (Z1-\alpha/2)^2 \times p(1-p) / d^2} \quad (38)$$

where **Z1-α/2** is a standard normal variate,

d is absolute error or precision and **p** is the expected proportion in a population based on the previous studies.

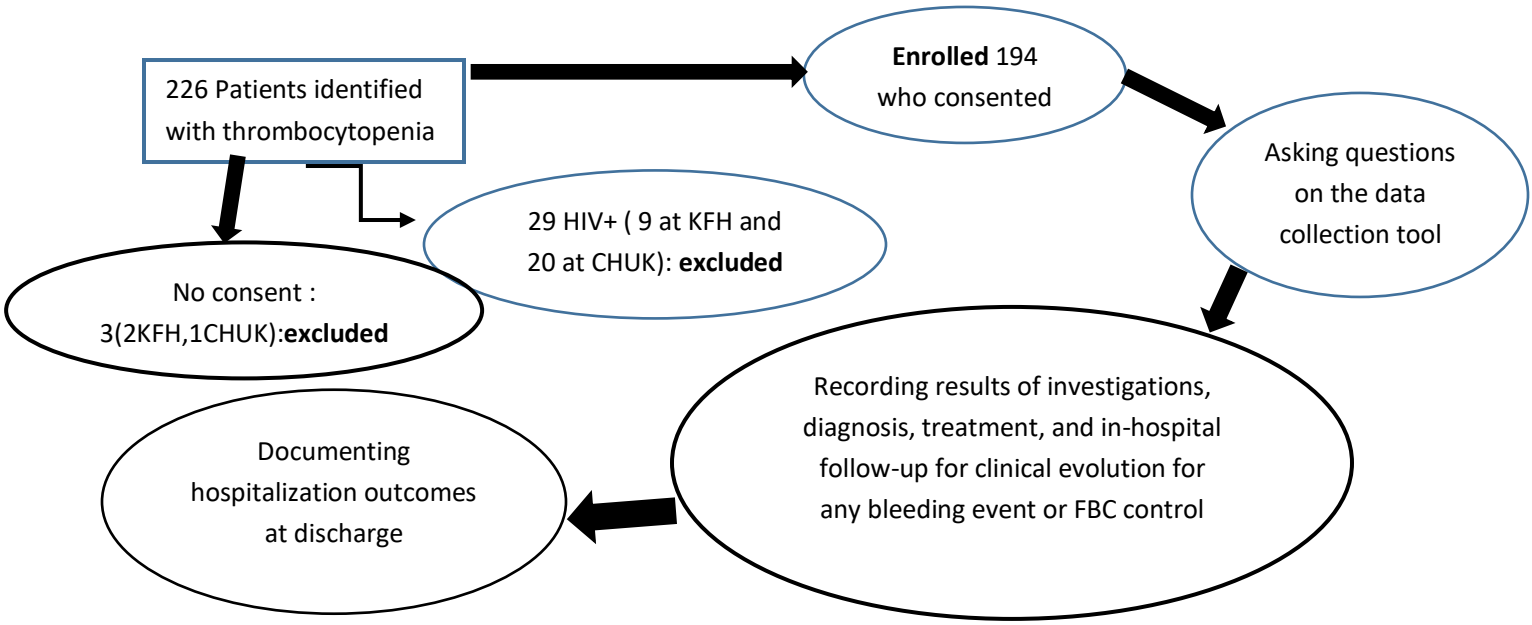
Therefore, choosing the absolute error **d** at 5% which is considered a type 1 error where **p <0.05**

Z1-α/2 becomes **1.96**. Referring to the study on thrombocytopenia in patients in medical wards from a South African hospital, the reported proportion of its prevalence was 14.9 % (1) therefore, the sample size becomes $\mathbf{N = (1.96)^2 x\ 0.149\ (1-0.149) / (0.05)^2 = 194\ patients}$

3. 7. Patients and data collection process

Patients with thrombocytopenia were approached for informed consent to participate. There was consent in the Kinyarwanda version and English to facilitate the communication. The purpose of the study was explained. Asked questions were customized to the study objectives on the data collection tool (**annex-1**). Questions included the patient's age, sex, smoking, alcohol intake, reason of admission, any bleeding sign(s), pre-existing comorbidities, medications history, physical examination findings, laboratory findings, diagnosis, treatment, cause of thrombocytopenia, and outcomes of hospitalization. Patients were followed-up during their hospitalization for any updates in the labs, treatments, and clinical evolution up to discharge. A code on the data collection tool for confidentiality was assigned for each patient.

Summary of the data collection process



3.8. Data analysis

Data collected were entered and analyzed using a statistical software SPSS version 26.0. The incidence of thrombocytopenia in the study population was expressed as a percentage. Descriptive characteristics of patients having thrombocytopenia were presented using tabulations expressing frequencies and proportions. The correlation between thrombocytopenia and different associated

factors was determined using bivariate analysis. Multivariate analysis of factors associated with thrombocytopenia was done for a p-value < 0.05.

3.9. Study outcome measures

3.9.1. Primary outcome measure

Our primary study outcome was patients with thrombocytopenia (<150x10 ^{3} /μL).

3.9.2. Secondary outcome measures

We evaluated :

1. Frequency of minor/major bleeding episodes

2. Need for ICU admission

3. Need for blood (platelet) transfusion

4. Sepsis-induced thrombocytopenia

5. ITP/TTP/HUS/DIC-related thrombocytopenia

6. Non-resolving thrombocytopenia at discharge

7. Resorption of thrombocytopenia at discharge

8. Frequency of chronic diseases

9. Median length of hospitalization

10. Death

3.10. Ethical consideration

The study proposal was presented and approvals were obtained after review from the UR Institutional Review Board (IRB) and both hospital research and ethics committees at CHUK and KFH. Participants signed the informed consent after explanations of the study objectives. There were no risks associated with this study and the confidentiality of patients was ensured by using the code number instead of participants' names on the data collection tool.

3 .11 .Results dissemination

Final report submitted to the research departments of the hospitals and the University of Rwanda College of Medicine and Health Sciences, School of Medicine and Pharmacy research department.

CHAP IV. RESULTS AND DISCUSSION

4.1. RESULTS

Our study enrolled 194 patients with thrombocytopenia after excluding HIV patients who were 29 (9 HIV + patients from KFH and 20 HIV+ patients from CHUK). All 194 patients were from 1448 admissions at CHUK and KFH (940 admissions at CHUK and 508 at KFH, respectively). The incidence of thrombocytopenia was 13.4 %. Patients from CHUK were 138 (71.1%), and patients from KFH were 56 (28.9%). The mean age of participants was 50(IQR 38-56), with the youngest patient having 19 years and the oldest at 94 years. A big number of patients were elderly 67(34.5%), followed by middle-aged patients (41-60) who were 64(33%). Males were 120 (61.85%), representing 1.6 times more than females who were 74 (38.14%).

TABLE 1. SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH THROMBOCYTOPENIA

	CHUK n (%)	KFH n (%)	Total n(%)
Age (range in years)	138(71.1%)	56(28,9%)	(100%)
≤ 20	9(6.52)	1(1.78)	10(5.15)
21-40	40(28.9)	13(23.21)	53(27.31)
41-60	44(31.8)	20(35.71)	64(33)
≥61	45(32.6)	22(39.28)	67(34.5)
Sex			
Male	80(57.97)	40(71.42)	120(61.85)
Female	58(42.03)	16(28.58)	74(38.14)
Health insurance			
Community-based	113(81.8)	12 (21.42)	125(64.43)
Private	15(10.86)	38(67.85)	53(27.3)
No insurance	10(7.24)	6910.71)	16(8.24)
Tobacco smoking			
Yes	33(23.9)	14(25)	47(24.2)
Alcohol consumption			
Yes	30(21.73)	18(32.14)	48(24.74)

Reason of admission	CHUK(n%)	KFH(n%)	Total (n%)
Difficulty in breathing	21(15.21)	9(16.07)	30(15.46)
Fevers	19(13.76)	13(23.21)	32(16.49)
Altered level of consciousness	11(7.97)	4(7.14)	15(7.73)
Abdominal distension	18(13.04)	6(10.71)	24(12.37)
Dizziness	8(5.79)	4(7.14)	12(6.18)
Hematemesis/melena	12(8.69)	3(5.35)	15(7.73)
Epistaxis	5(3.62)	2(3.57)	7(3.60)
Epigastric pain	13(9.42)	5(8.92)	18(9.27)
Chest pain	8(5.79)	4(7.14)	12(6.18)
Chemotherapy	0(0.00)	11(19.64)	11(5.67)
Seizures	4(2.89)	1(1.78)	5(2.57)
Joint pain	8(5.79)	3(5.35)	11(5.67)
Other reasons*	11(7.97)	9(16.07)	20 (10.30)
Pre-existing conditions			
Arterial hypertension	16(11.59)	7(12.50)	22(11.34)
Diabetes mellitus	11(5.67)	4(7.14)	15(7.73)
Chronic liver disease	12(6.18)	5(8.92)	17(8.76)
Old stroke	9(6.52)	1(1.78)	10(5.15)
Chronic kidney disease	11(7.97)	4(7.14)	15(7.73)
Malignancies	6(4.34)	8(14.28)	14(7.21)
COPD/Asthma	2(1.44)	3(5.35)	5(2.57)
Kidney transplant recipient	0(0.00)	3(5.35)	3(1.54)
No-comorbidity	71(51.44)	21(37.5)	92(46.90)

Discharge diagnosis	CHUK(n%)	KFH(n%)	Total(n%)
Sepsis	28(20.28)	5(8.92)	33(17.01)
Chronic liver disease	19 (13.76)	7(12.5)	26(13.40)
Malignancy	12 (8.69)	11(19.64)	23(11.85)
Malaria	6 (4.34)	10(17.85)	16(8.24)
Gastric ulcers	10(7.24)	3(5.35)	13(6.70)
Stroke	7(5.07)	3(5.35)	10(5.15)
Uncontrolled Diabetes	10(7.24)	2(3.57)	12(6.18)
Congestive heart Failure	8(5.79)	2(3.57)	10(5.15)
Megaloblastic anemia	8(5.79)	2(3.57)	10(5.15)
Tuberculosis	6(4.34)	2(3.57)	8(4.12)
Thromboses	7(5.07)	3(5.35)	10(5.15)
ITP	2(1.44)	1(1.78)	3(1.54)
Thyroid disorders	1(0.72)	1(1.78)	2(1.03)
AIHA	6(4.34)	2(3.57)	8(4.12)
Others	8(5.79)	2(3.57)	10(5.15)
Medications			
Enoxaparin	34(24.63)	12(21.42)	46(23.7)
Aspirin	12(8.69)	8(14.28)	20(10.30)
Clopidogrel	0(0.00)	2(3.57)	2(1.030)
Warfarin	3(2.17)	0(0.00)	3(1.54)
Unfractionated heparin	0(0.00)	1(1.78)	1(0.51)
Rivaroxaban	0(0.00)	3(5.35)	3(1.54)
Alteplase	0(0.00)	1(1.78)	2(1.03)
Chemotherapy	0(0.00)	11(19.64)	11(5.67)
Filgrastim	0(0.00)	8(14.28)	8(4.123)
Steroids	11(7.97)	6(10.71)	17(8.76)
MMF +Taclorimus	0(0.00)	3(5.35)	3(1.54)

Severity of thrombocytopenia	CHUK(n%)	KFH(n%)	Total(n%)
Mild (100- 149x10^3/µL)	75(54.34)	24(42.85)	99(51.03)
Moderate (50-100x10^3 /µL)	43(31.16)	19(33.92)	62(31.95)
Severe (< 50x10^3 /µL)	20(14.50)	13(23.21)	33(17.01)
Associated cytopenias			
Anemia			
Male(Hb<13 g/dl)	59(39.07)	18(28.12)	77(35.81)
Female(Hb<12.5g/dl)	38(25.16)	14(21.87)	52(24.18)
Leukopenia(WBCs<4x10^3 /µL)	32(16.49)	19(29.68)	51(23.72)
Pancytopenia	22(14.56)	13(20.31)	35(16.27)
Hepatitis C Antibodies			
Positive	17(73.91)	6(26.09)	23(100.0)
Hepatitis BsAg			
Positive	12(70.58)	5(29.42)	17(100.0)
Elevated INR (above 1.12)	3(23.07)	10(76.92)	13(100.0)
Low serum albumin (< 3.5mg/dL)	36(72.0)	14(28.0)	50(100.0)
Elevated creatinine (>106µmol/L)	41(77.35)	12(22.64)	53(100.0)
Low serum VitB12 (<25.1pmol/L)	8(80.0)	2(20.0)	10(100.0)
Auto-immune work-up			
ANA(positive, n=2)	0(0.00)	2(25.0)	2(14.28)
ANCA(positive ,n=2)	0(0.00)	2(25.0)	2(14.28)
C-ANCA (positive,n=2)	0(0.00)	2(25.0)	2(14.28)
Intrinsic factor antibodies (positive, n=1)	0(0.00)	1(12.5)	1(7.14)
RF (Positive, n=2)	2(33.4)	0(0.00)	2(14.28)
Coombs test(Positive, n=5)	4(66.6)	1(12.5)	5(35.71)
Thrombophilic work-up			
Factor VII (n=1) 81%(50-185)	0(0.00)	1(20.0)	1(20.0)
AntithrombinII(n=1)I 69%(83-118)	0(0.00)	1(20.0)	1(20.0)
ProteinCfunction(n=1)76%(70-140)	0(0.00)	1(20.0)	1(20.0)
Free Protein S (n=1) 73% (70-140)	0(0.00)	1(20.0)	1(20.0)

Thrombin gene mutation (not detected)	0(0.00)	1(20.0)	1(20.0)
Positive thick smear for malaria	6(37.5)	10(62.5)	16(100)
Abdominal ultrasound			
Splenomegaly/hepatomegaly	16(34.04)	9(47.36)	25(37.87)
Ascites	31(65.95)	10(52.63)	41(62.13)
Upper GI Endoscopy			
Bleeding esophageal varices	11(28.20)	3(16.66)	14(24.56)
Ulcers	23(58.97)	7(38.88)	30(52.64)
Gastric tumor	5(12.82)	8(44.46)	13(22.80)
Brain CT-Scan			
Hemorrhagic stroke	2(15.40)	1(25)	3(17.64)
Ischemic stroke	11(84.60)	3(75)	14(82.36)

As highlighted in the above table-1,non-remitting fevers ranked first as the reason for hospitalization in 32 (16.49%) patients, followed by difficulty in breathing in 30 patients (15.46%). On the other side, bleeding as a reason for admission was not common, apart from hematemesis/melena as a reason for admission in 15 patients (7.73%) and epistaxis in 7 patients (3.60%). Of those who came for chemotherapy at KFH were 11 patients (5.67%).In the other reasons* category, we had 6 patients who had diarrhea, 3 patients with dysuria and pelvic discomforts, and 2 patients with a psychiatric disorder at CHUK. At KFH: 4 patients with itchy skin lesions, 5 with difficulty in urination.

The most associated pre-existing co-morbidity reported was arterial hypertension in 22 (11.34%) patients, followed by 17(8.76%) patients with chronic liver disease and 15 patients with diabetes mellitus. We had 3 (1.54%) patients with a background history of kidney transplantation who were followed at King Faisal hospital. Among the most common diagnoses at CHUK, sepsis was on top in 28 patients representing 20.28%: it included 14 cases of community-acquired pneumonia, 8 cases of UTI, 3 cases of meningitis, and 3 cases of spontaneous bacterial peritonitis (SBP). Cases of chronic liver disease came second in 19(13.76%), followed by malignancies in 12(8.69%)

patients: 3 cases of hematological malignancies,5 cases of gastric adenocarcinomas, 2 cases of prostatic cancers, and 2 cases of colon adenocarcinomas.

At King Faisal Hospital, cases were mostly represented by malignancies in 11 patients representing 19.64%, including 2 cases of gastric adenocarcinomas, 3 cases of colon adenocarcinomas, 4 cases of hematological malignancies,1 case of prostatic cancer and 1 case of lung adenocarcinoma. Cases of malaria were 10 (17.85%) then thirdly were cases of chronic liver disease in 7 patients (12.5%).ITP cases were 2 at CHUK and 1 case at KFH, the same for auto-immune hemolytic anemia cases in 2 patients at King Faisal Hospital and 6(4.34%) cases at CHUK. In the “others" category, there were 8 cases at CHUK (2 cases of rheumatoid arthritis,3 cases of atrial fibrillation on ischemic stroke, 1 case of ischemic heart disease, and 2 cases of asthmatic exacerbations) whereas, at KFH, we had 3 cases in "other" category represented by one case of SLE, 1 case of dermatomyositis and 1 case of asthmatic exacerbations.

Thyroid disorders were in one patient with hyperthyroidism at CHUK and a patient with hypothyroidism at KFH. Thromboses comprised one patient with PE having hemodynamic instability which indicated thrombolysis at KFH using 100mg alteplase. Also, unfractionated heparin was used in this patient. The other 2 cases were of DVT. At CHUK, there were 7 cases of thrombosis (cases of DVT were 5 and PE cases were 2).

Enoxaparin was prescribed in 46 patients (23.7%), mostly on a prophylactic basis in hospitalized patients and patients with atrial fibrillation, sometimes given bridged with warfarin on a therapeutic basis, and also in patients with PE and DVT. Aspirin was given in 20 patients (10.3%) and clopidogrel was used in 2 patients at KFH (ischemic stroke, ischemic heart disease) and chemotherapy was in 11 patients (5.67%) at the KFH oncology unit. Chemotherapy drugs cause myelosuppression resulting in severe pancytopenia and the patient becomes increasingly vulnerable to opportunistic infections due to severe neutropenia. In these patients on chemotherapy, when they developed severe pancytopenia, filgrastim could be administered subcutaneously once a day for three days to boost hematopoiesis and formation of blood cells, and this support many transfusions with PRBCs and platelets to increase circulating blood cells.

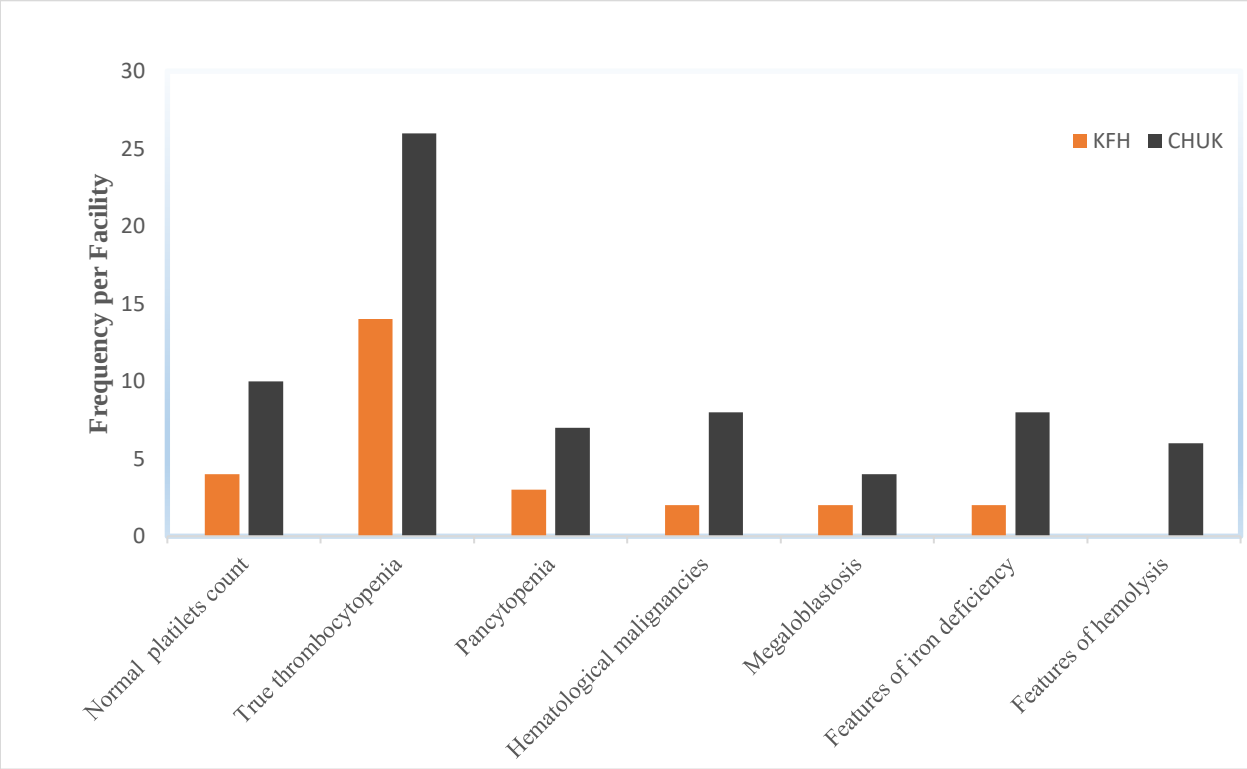
By severity, 99 patients(51.03%)patients had mild thrombocytopenia, 61 (31.44%) had moderate thrombocytopenia and 33 (17.01 %) had severe thrombocytopenia .Anemia was the most common association with thrombocytopenia in 129 (60%), followed by leukopenia in 51 (23.72%) and pancytopenia was less common in 35 patients representing 16.27%. Other labs reported hepatitis(hepatitis B in 17 patients, and Hepatitis C in 23 patients).

Megaloblastic anemia could be diagnosed by low serum levels of Vitamin B12 in 5 patients, and in some cases, vitamin B12 deficiency could also be seen on PBF in 6 cases (**Figure:1**). Auto-immune work-up was done in a limited number of patients, reason being non-availability of tests in the hospital and limited accessibility as patients were not able to pay to do the investigations in outside private laboratories. In a patient who had PE with hemodynamic instability, thrombophilic work-up was non-revealing as there were normal activity levels of the tested factors.

Imaging studies supported the diagnosis in the quest of identifying the cause of thrombocytopenia. Abdominal ultrasound could reveal splenomegaly, hepatomegaly or intrahepatic lesions, and ascites in patients with chronic liver disease. Upper GI endoscopy could reveal esophageal varices, and gastric tumors, among others.

PERIPHERAL BLOOD FILM AND BONE MARROW ASPIRATION AND BIOPSY

FIGURE 1: PERIPHERAL BLOOD FILM

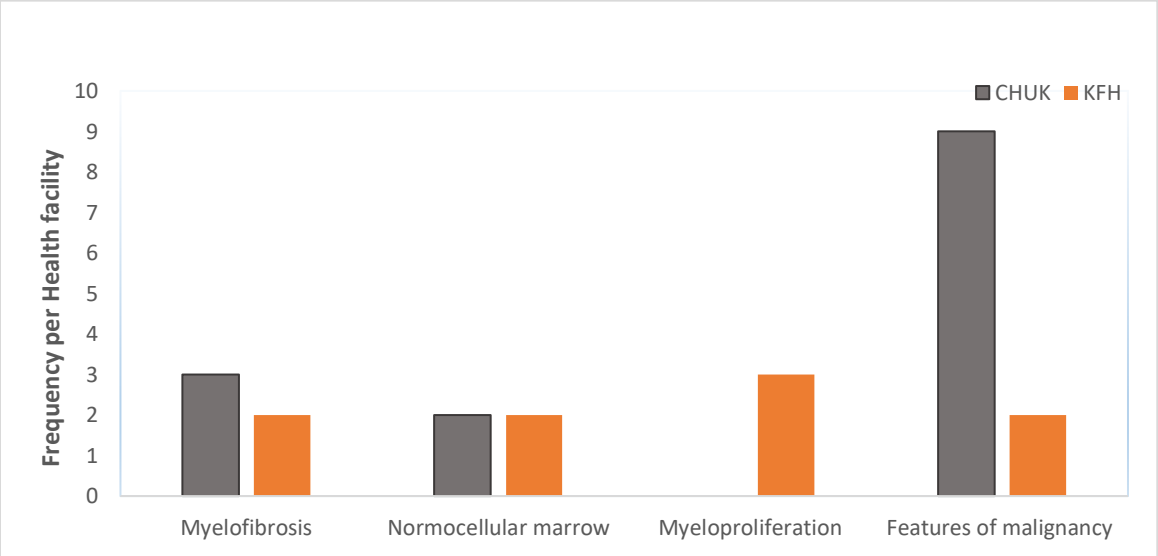


At KFH (**figure-1: red bars**) we had a total of 17 PBFs representing 30.35% of all 56 cases. During a slide reading, 1 case of PBF could be a source of different findings. Therefore, we had 14 findings (among the total of 27) of true thrombocytopenia representing 51.85%, which was marked by visualized decreased platelet count in a field and occasional large platelets with no clumps. Normal platelet counts were in 4 cases on PBF(14.81%). Other findings were pancytopenia in 3 cases (11.11%), and 2 cases (7.40%) of hematological malignancies (APML and MDS/MPN-U) with numerous blasts, and no Auer rods were seen. Features of megaloblastic were seen in 2 patients (7.40%), with macro-ovalocytes, anisocytosis, and teardrop cells. We noted also 2 cases (7.40%) with features of iron deficiency marked with hypochromic cells, pencil cells, occasional teardrop cells (dacrocytes), and some target cells.

At CHUK (**figure-1: black bars**), we had 34 PBFs done in 138 patients, representing 24.63 %. True thrombocytopenia cases were found in 26 (37.68%), the normal platelet count in 10 cases (14.49%), pancytopenia in 7 cases(10.14), hematological malignancies in 8 cases (11.59%) (5ALLs,2 cases of acute myeloid leukemia(with Auer rods), 1 plasma cell neoplasm or multiple myeloma), megaloblastic in 4 cases (5.79%), features of iron deficiency in 8 cases (11.59%) and features of hemolysis in 6 cases (8.69%), marked with spherocytes, rouleaux formation, and red cell clumps.

Combining all PBFs at both hospitals, they were 51 representing 26.28% of the cohort. Findings of true thrombocytopenia were in 40 slides, representing 41.66% (41/96 of all PBF findings), normal platelet count was in 14 (14.58%), pancytopenia in 10 (10.41%), hematological malignancies 10 (10.41%), megaloblastic cells in 6 cases (6.25%), iron deficiency in 10 (10.41%) and hemolysis features in 6 cases (6.25%) with a possibility of underlying cold agglutination.

FIGURE 2:BONE MARROW ASPIRATION AND TREPHINE BIOPSY



At KFH (**red bars, Figure 2**), we had 4 cases of bone marrow aspiration and trephine biopsies, representing 7.14% (n=4/56). Same as for PBF, one bone marrow could be the source of different findings; therefore, we had 2 cases of myelofibrosis representing 22.22%, then 2 cases of normocellular marrow (22.22%), 3 cases of hyper-cellular marrow/myeloproliferative (33.33%), and 2 cases with features of hematological malignancies (MPN-U and APML) at 22.22%.

At CHUK (**black bars, figure 2**), bone marrow aspiration and biopsy were performed in 11 patients (n=11/138) and they found 3 cases of myelofibrosis representing 21.42%, 2 cases of normocellular marrow (14.28%), and 9 cases of hematological malignancies (64.28%) (4cases of acute lymphoblastic leukemias, 2 cases of plasma cell neoplasms (or multiple myeloma), Acute myelogenous leukemia -AML(M4), Anaplastic Large Cell Lymphoma). Combined at both hospitals, total bone marrow aspiration and biopsies were 16, representing 8.24% with findings of myelofibrosis in 5 slides (21.7%), normocellular marrow in 4 cases(17.39%),myeloproliferation in 3 cases (13.04), and 11 cases of hematological malignancies (47.82%).

TABLE 2: BIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH THROMBOCYTOPENIA

Factor	Thrombocytopenia Mild	[Moderate+ Severe,n(%)]	95% CI	P-value
Hospital stay, median (IQR)	7(4,11)	8(3,13)	[0.104,0.48]	0.021*
Length of Hospital stay			[1.019,1.420]	0.029*
<5 days	32(32.32)	27(28.43)		
5-15 days	63(63.64)	59(62.10)		
≥16 days	4(4.04)	9(9.47)		
Anticoagulants/chemotherapy			[0.0112-3.22]	0.0399*
Enoxaparin	34(62.96)	12(36.36)		
Aspirin	12(22.22)	8(24.24)		
Clopidogrel	0(0.00)	2(6.06)		
Warfarin	3(5.55)	0(0.00)		
Rivaroxaban	3(5.55)	0(0.00)		
Unfractionated Heparin	1(1.85)	0(0.00)		
Alteplase	1(1.85)	0(0.00)		
Chemotherapy	(0.00)	11(33.34)		
Discharge diagnosis			[0.0763,0.101]	0.033*
Sepsis	14(14.14)	19(20.0)		
Chronic liver disease	9(9.09)	17(17.89)		
Malignancies	5(5.05)	18(18.94)		
Malaria	7(7.07)	9(9.47)		
PUD/Gastritis	10(10.10)	3(3.15)		
Uncontrolled diabetes	10(10.10)	2(2.10)		
Congestive heart failure	4(4.04)	6(6.31)		
Megaloblastic anemia	4(4.04)	6(6.31)		
Thromboses	7(7.07)	3(3.15)		
AIHA	2(2.02)	6(6.31)		
Stroke	7(7.07)	3(3.15)		
Tuberculosis	8(8.08)	0(0.00)		

ITP	0(0.00)	3(3.15)
Thyroid disorders	2 (2.02)	0(0.00)
Others	10(10.10)	0(0.00)

Factors in above **table 2** with a significant p-value<0.05 (as marked with an *), were analyzed in **table 3** to determine the causative association to thrombocytopenia.

COMPLICATION OF THROMBOCYTOPENIA, TREATMENT, AND HOSPITALIZATION OUTCOMES

Severity of thrombocytopenia					
	Mild n (%)	**[Moderate +Severe n(%)]**			
Complication(Bleeding)			**OR**	**95%CI**	**P-Value**
WHO grade 0:No bleeding	**93(93.93)**	**67(70.52)**	**1**	**NA**	**NA**
WHO grade I-II bleeding	**6 (6.06)**	**13 (13.68)**	**4.3**	**[1.23-15.2]**	**0.02**
Gum bleeding	2 (2.02)	4 (4.21)			
Epistaxis	0(0.00)	7 (7.36)			
Ecchymosis	3(3.03)	2 (2.10)			
Hematuria	1(1.01)	0(0.00)			
WHO grade III-IV bleeding	**0(0.00)**	**15(15.78)**	**7**	**[0.861-56.89]**	**0.06**
Hematemesis & Melena	0(0.00)	15 (15.78)			
Treatment					**Total n(%)**
Platelet transfusion	0(0.00)	21 (22.10)			21(10.82)
PRBCs	4 (4.04)	15 (15.78)			19(9.79)
FFP	0(0.00)	6 (6.31)			6 (3.09)
Steroids	8(8.08)	12 (12.63)			20 (10.30)
Filgrastim	0(0.00)	8(72.72)			8(72.72)
Hospitalization outcomes					**Total n(%)**
Resolution of thrombocytopenia	77(39.69)	35(18.04)			112 (57.73)
Persistent thrombocytopenia	22(11.34)	60(30.92)			82 (42.27)
ICU admission	1(0.51)	7(3.60)			8(4.12)

Died	12(6.18)	20(10.30)	32 (16.49)

From the table above, the majority of patients with thrombocytopenia 160 (82.4%) did not develop any sign of bleeding. We had 15 (7.73%) cases of major bleeding (WHO grade III-IV), OR 7; 95% CI [0.861-56.89] which was marked with hematemesis and melena causing hemodynamic instability requiring blood transfusion, as compared to minor bleeding (WHO grade I-II) which occurred in 19 patients 9.79% (OR 4.3; 95%CI [1.23-15.2], p=0.022).

Thrombocytopenia is expected to resolve when the underlying cause is treated. The decision to transfuse after assessing the relative risk of bleeding was individualized and packed red blood cell transfusion was given in 22 patients (11.34%), mainly in those who had major bleeding (WHO grade III-IV) and 15(78.94%) among those who needed a blood transfusion. Platelets transfusion was given in 21 patients with severe thrombocytopenia (33), representing 63.63% while FFP was given in 6 patients who had major bleeding and underlying end-stage liver disease.

Steroids were initiated in ITP cases and cases of autoimmune diseases, and steroids were indicated as adjunctive to antibiotics in case of meningitis. Filgrastim, a booster of the blood cell production in the bone marrow and growth was used at King Faisal hospital in patients with severe pancytopenia related to chemotherapy. At discharge, thrombocytopenia resolved in 112 patients, representing 57.73% and thrombocytopenia persisted in 82 patients, representing 42.27%. Mortality was 16.49% and was not directly related to thrombocytopenia as a standalone factor, but due to the underlying medical condition and comorbidity.

TABLE 3: MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH THROMBOCYTOPENIA

	Thrombocytopenia				
	Mild [Mod+ Severe n(%)]		**Odds Ratio**	**95%CI**	**P-value**
Duration of hospitalization					
< 5 days	32(32.32)	27(28.43)	1	NA	NA
5-15 days	63(63.64)	59(62.10)	0.93	0.58-0.995	0.019*
>16	4(4.04)	9(9.47)	2.3	0.601-2.56	0.903
Discharge diagnosis					
Sepsis	14(14.14)	19(20.0)	2	1.152-4.611	0.036*
Decompensated liver cirrhosis	9(9.09)	17(17.89)	2.47	2.43-4.13	0.041*
Malignancies	5(5.05)	18(18.94)	3.5	1.052-4.576	0.045*
Malaria	7(7.07)	9(9.47)	12.6	2.44-15.46	0.021*
PUD/Gastritis	10(10.10)	393.15)	1.057	0.022-1.514	0.445
Uncontrolled Diabetes	10(10.10)	2(2.10)	0.72	0.67-3.485	0.528
Congestive heart failure	4(4.04)	6(6.31)	2.67	0.75-3.76	0.191
Megaloblastic anemia	4(4.04)	6(6.31)	14.63	1.300-16.36	0.031*
Thromboses	7(7.07)	3(3.15)	0.514	0.483-1.832	0.172
Stroke	7(7.07)	3(3.15)	0.85	0.21-1.89	0.207
AIHA	2(2.02)	6(6.31)	0.7	0.08-1.77	0.097
Tuberculosis	8(8.08)	0(0.00)	8.1	0.094-9.11	0.094
ITP	0(0.00)	3(3.15)	0.63	0.514-3.44	0.445
Thyroid diseases/others	12(12.12)	0(0.00)	1	NA	NA
Medications					
Enoxaparin	34(62.96)	12(36.36)	1.78	0.582-2.86	0.048*
Aspirin	12(22.22)	8(24.24)	1.32	1.23-7.44	<0.001*
Clopidogrel	0(0.00)	2 (6.06)	0.78	0.02—10.34	0.178
Warfarin	3(5.55)	0(0.00)	0.98	1.07-9.77	0.245
Rivaroxaban	3(5.55)	0(0.00)	0.65	0.099-13.5	0.0692
Unfractionated Heparin	1(1.85)	0(0.00)	1.02	1.1-8.04	0.998

Alteplase	1(1.85)	0(0.00)	1	NA	NA
Chemotherapy	0(0.00)	11(33.34)	2.25	0.692-7.30	0.0417*

(*) : significant p-value <0.05 , NA : Non-applicable

4.2. DISCUSSION

In our study cohort, the incidence of thrombocytopenia was 13.4%. Elderly patients (age>61) were more affected (34.5%) than other age ranges. In Rwanda, there was no similar study done before evaluating thrombocytopenia in hospitalized medical patients, but studies done in South African tertiary hospitals by Nkambule et al (1) and Vaughan et al(5) found a higher prevalence of thrombocytopenia in medical patients at 14.9% and of 18.7%, respectively. The relative difference in the incidence of thrombocytopenia in these studies could be due to different reasons, among others, the setting, inclusion, exclusion criteria, and study period.

Non-remitting fevers were among the most common reason for hospitalization in 16.4% of patients, correlating with a high occurrence of thrombocytopenia in patients with sepsis, accounting for 15.9% of all medical hospitalizations (OR:2,95%CI [1.15-4.61], p=0.036). This was low compared to that found in sepsis-induced thrombocytopenia in other settings like medical emergencies or ICUs where patients are much more critically ill in severe sepsis or septic shock. Apart from findings by Nkambule et al who reported thrombocytopenia due to sepsis in medical patients to be 27.6% (1), Teo CP et al's study in Singapore found a prevalence of thrombocytopenia in the medical emergency department to be as high as 46.6% (6), and this was in line with the observation by Satoshi et al in South Korea, where he studied cases of DIC in severe sepsis and septic shock, and they noted that DIC complicated cases of severe sepsis or septic shock in 50.9% of patients (7) and Teo CP et al reported increased mortality at 24.7% of DIC in case of DIC on top of severe sepsis or septic shock in ICU patients.

In Brasil, Boechat et al reported thrombocytopenia in sepsis to be ranging between 33.8% to 60%, and thrombocytopenia is regarded as an important prognostic factor in septic patients (39). We did not find similar studies in Easter-African countries. Correlating thrombocytopenia with sepsis relies on the pathophysiological fact of having an active infectious process that triggers an inflammatory response and associated thinning and fenestration of vessels, and this causes an increased platelet reactivity and consumption to repair the gap to maintain vascular integrity (23).

Another situation has been the presence of thrombocytopenia in malaria patients, showing an alarming positive correlation (OR 12.6, 95%CI[2.44-15.46], p=0.021). In East Africa and other countries across the world where malaria is still endemic, this observation is not strange.

In a study by Mboya et al in Kenya (9), they observed that in malaria patients, thrombocytopenia was much more frequent at 87% (against 10% in the non-infected group) while severe and moderate anemia was present in 78% (against 47% in the non-infected group) and both thrombocytopenia and anemia proved to have a positive correlation in patients with malaria ($r=0.26$; $p<0.001$). This observation support findings from a study cohort in Pakistan conducted by Shiraz Jamal et al evaluating the presence of malaria among patients with fevers and thrombocytopenia, where they found that among 53% of patients who proved to have malaria;68% had falciparum malaria while 38% had vivax infection. Thus, all these studies report thrombocytopenia to be an important predictor of malaria in the adult population until proven otherwise(10).

On the other side, malignancies had significant odds to cause thrombocytopenia (OR: 3.5,95%CI[1.052-4.57], p=0.048). Moreover, there is a dual compounding effect of malignancies causing thrombocytopenia on top of the toxicity from chemotherapy drugs inducing thrombocytopenia due to myelosuppression (OR 2.25; 95% CI [0.692-7.30], p=0.043). This effect of malignant cells infiltrating the bone marrow with resulting suppression of normal megakaryopoiesis, thus the reduced platelet production with resulting thrombocytopenia of variable severity(8),(21),(24).

Severe thrombocytopenia (as part of pancytopenia) is associated with the worst feared adverse events of bleeding in a vital organ, like intracerebral bleeding (2) and an increased risk of vulnerability to opportunistic infections due to severe neutropenia (ANC <500 cells/ µL or <1000cells/µL with predicted nadir <500 cells/µL)(37). Even though we could not experience an event of bleeding in our patients who were on chemotherapy, therapeutic platelet transfusion was given in this oncologic emergency as patients on chemotherapy had very low counts of $<10x10^3$ /µL. As Niyongabo et al(8), Friedmann et al(23), Valsami et al(25) and Webert et al(24) remarked, many units of platelets are more transfused in cancer patients during chemotherapy than in any other medical situation.

Alongside transfusion, hematopoietic growth factors like filgrastim (Neupogen) were given as primary prophylaxis to boost counts in peripheral circulation, and empiric regimens of antibiotics are also indicated in the case of severe neutropenia(37).

Blood transfusion was not only for cases of cancer patients on chemotherapy, but also was given in cases of upper GI bleeding due to ruptured esophageal varices from portal hypertension on

decompensated liver cirrhosis causing thrombocytopenia, and this was having significant odds (OR 2.47 95%CI [2.43-4.13], p=0.041. Clinically we see patients presenting with hematemesis/melena, and in our study cohort, thrombocytopenia compounding to the effect of chronic liver disease,(WHO grade III-IV, OR 7, 95% CI [0.861-56.89],p=0.069), the bleeding with hemodynamic instability requiring blood transfusion of PRBCs, platelets, and FFPs due to decreased clotting factors, raised INR and prolonged PTT (23).

Deficiencies like vitamin B12 cause megaloblastic anemia and in our cohort, it was found to be associated with thrombocytopenia in 10 patients (5.15%), with odds remarkably high: *OR 14.6; 95% CI [1.300-16.36] p=0.031*.In our setting, factors for vitamin B12 deficiency could be due to inadequate nutritional intake of foods of animal origin, alcoholism, and a big representation of elderly patients (34.5%). Another factor from our study was a decreased absorption due to autoimmune-related vitamin-B12 deficiency where antibodies to intrinsic factors were positive. Astonishingly; unusual circumstances of vitamin B12 deficiency can masquerade as TTP and the diagnosis becomes tricky as Almadani Houssam et al in Saudi Arabia remarked in patients having anemia, thrombocytopenia, and schistocytes (14).

Locally in Rwanda, Nkeshimana et al evaluated vitamin B12 deficiencies in different studies and, among other findings, disturbed hematopoiesis causing cytopenias, like thrombocytopenia leading to the ill-health of those affected (15),(40). By this, we draw the fact that we should not overlook doing a thorough investigation on the road to making the diagnosis of the deficiency, and this slow-progressing disorder effects can be warded-off from a patient from developing complications related to vitamin B12 deficiency, like neuro-psychiatric manifestations reported to be reversible if treatment initiated before 6 months of disease onset(37)

As Adewoyin et al observed, as much as 70% of clinical decisions and diagnoses are supported by laboratory medicine(41), and in this regard, having a patient with thrombocytopenia must prompt requesting a PBF or doing a bone marrow aspiration and biopsy both of which provide invaluable and informative findings to the possible cause of thrombocytopenia, not only in megaloblastic anemia but also in other conditions. The PBFs performed in our cohort were in 51 patients among 194 representing 26.2 %. Features of megaloblastic anemia found were in 6 patients with macroovalocytes, anisocytosis, and teardrop cells, among others. And the bone marrow aspiration and trephine biopsy could be revealing in support of the diagnosis (7.73%, n=15/194). Among findings from a study by Magesa et al in Tanzania analyzing samples of bone marrow aspiration

and biopsy, features of nutritional anemia were common at a rate of 71.4% (n=5/7) of iron deficiency and 28.6% (n=2/7) (42) of megaloblastic anemia. A study of the cause of iron deficiency anemia was beyond the scope of this study.

Finally, conditions like ITP were rare and sporadic in our study (3 patients 1.54%) and have been associated with a minor bleeding episode (WHO grade I-II: OR 4.3,95%CI [1.23-15.20];p=0.022) (11), and we could not find a case of microangiopathy like TTP, DIC or HUS(13) nor did we found a case of suspected inherited thrombocytopenia(3) (4), the reason being the rarity of these cases and the need for an extended period of study to include a bigger sample size as some cases can be found in some setting like DIC is common in ICU patients with sepsis) (35).

Investigating the risk factors for likely cases of thrombophilia in patients with thromboses was not affordable for some patients as they were to pay for investigations in private laboratories during hospitalization.For presumed drug-induced thrombocytopenia ,aspirin use (OR 1.32 95% CI[1.23-7.44] p<0.001) and enoxaparin(OR 1.78 ; 95%CI[0.582-2.86] ,p=0.048 ,were having an association to causing thrombocytopenia.

Resolution of thrombocytopenia at discharge occurred in 57.73% against persistent thrombocytopenia in 42.27%. Thrombocytopenia was multifactorial and the underlying comorbidities alongside a big representation of elderly patients, and a limited follow-up time to trend thrombocytopenia have been the possible reasons for this big number of unresolved thrombocytopenia.

Study limitation

The main limitation of this study is that the results might not be generalizable in all settings due to the small sample size and a short study period as it could lead to underestimation of the reported incidence and profile of patients with thrombocytopenia. Some patients had financial limitations to pay for non-available special investigations in private laboratories which could help in making a diagnosis, therefore the causes of thrombocytopenia might not be complete at discharge. Another limitation was a short follow-up period and this has led to having partial information on the cause and resolution of thrombocytopenia.

CHAP V. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Thrombocytopenia was common in medical wards. On top of clinical examination findings, specific tests to confirm the diagnosis are strongly needed in the tertiary hospitals in Rwanda. We have noted a thrombocytopenia incidence of 13.4%. Commonly associated causes included sepsis, chronic liver disease, malignancies, malaria, and megaloblastic anemia. The results of our study cohort showed thrombocytopenia predilection in patients with chronic diseases mainly malignancies and chronic liver disease, and this was in line with other studies' findings.

5.2 .Recommendations

This study provides us with quantitative and qualitative data on the current characteristics of patients with thrombocytopenia. Therefore, it would be of interest to continue to track the clinical presentation and do investigations for better treatment approaches for patients. To be able to do this, different actors must work closely to identify and solve gaps in the diagnosis and management of patients with thrombocytopenia.

1. For the MoH/RBC: For patient access to non-available tests in the hospitals, the RBC/MOH would do advocacy with private laboratories and insurance companies to pay the same price as they do in the public hospitals
2. For the hospital hematology department: to continue raising awareness in clinicians to ask for a PBF on a systematic basis in all patients with thrombocytopenia on FBC, perform a bone marrow when indicated, and advise clinicians on special tests when needed.
3. Clinicians should follow up on patients with thrombocytopenia beyond the hospitalization period.
4. Patients must be advised to seek care as early as possible for timely prevention of any complications of the underlying disease.

REFERENCES

1. Jali AGP, Nkambule BB. Prevalence and etiology of moderate and severe thrombocytopenia in a tertiary and quaternary center in KwaZulu-Natal. Afr J Lab Med. 2020 Aug 24;9(1):799. doi: 10.4102/ajlm.v9i1.799. PMID: 32934907; PMCID: PMC7479432.

2. Estcourt LJ, et al . Intracranial hemorrhage in thrombocytopenic hematology patients: a nested case-control study: the InCiTe study protocol.BMJ Open.2014 Feb7;4(2):e004199.doi:10.1136/BMJ open-2013-004199

3. Balduini CL, Pecci A, Noris P. inherited thrombocytopenias: the evolving spectrum.Hemostaseologie,2012;32(4):259-270,Doi:10.5482/ha12050001.

4. Jonathan G.Drachman: Inherited thrombocytopenia: when a low platelet count does not mean ITP/Reviews in translational hematology.January 15, 2004.Blood(2004)103(2):390-398,https://doi.org/10.1182/blood-2003-05-1742

5. Vaughan JL, Fourie J, Naidoo S, Subramony N, Wiggill T, Alli N. Prevalence and causes of thrombocytopenia in an academic state sector laboratory in Soweto, Johannesburg, South Africa. *S Afr Med J*. 2015;105(3):215–219. 10.7196/samj.8791

6. Teo CP, Kueh YK. Incidence of thrombocytopenia in an acute care hospital. *Ann Acad Med Singapore*. 1989;18(4):379–381

7.Satoshi Gando et al , Disseminated intravascular coagulation in Severe sepsis/Chest Journal/Volume156,Issue4,supplementA1122/Oct.2019.Doi:https://doi.org/10.1016/j.chest.2019.08.1025

8. Niyongabo FN et al: Platelet Transfusion Practice at Butaro Cancer Centre of Excellence in Rwanda /East-African Health Research Commission/Research for Health and Prosperity/East-African Science 2022/Volume 4/Number1.www.eahealth.org

9. Mboya P.K et al; the correlation between thrombocytopenia and anemia in Plasmodium falciparum malaria among patients in Kisumu County-Western Kenya [Afri J.Health Sci.2016;29(2):76-88]

10. Shiraz Jamal Khan, Yasir Abbass, et al; thrombocytopenia as an indicator of Malaria in the adult population. Malaria research and treatment, Vol.2012. doi.org/10.1155/2012/405981

11. Abrahamson PE, Hall SA et al; the incidence of idiopathic thrombocytopenic purpura among adults, a population-based study and literature review.Eur J Haematol 2009,83(2):83-89

12.Nguyen TC,Cruz MA,Carcillo JA. Thrombocytopenia is associated with multiple organ failure and acute Kidney injury. Crit Care Clin.2015;31;661-74

13. George JN.Nester CM.Syndromes of thrombotic microangiopathy. N Engl J;2014;371:654-66.

14. Housam Almadani et al: case report: Pseudothrombotic Thrombocytopenic Purpura and Cobalamine deficiency.International Journal of Advanced Research.5(12),1523 1528.Doi:10.21474/IJAR01/6115

15. Nkeshimana et al: The prevalence of vitamin B12, folic acid and iron deficiency in healthy relatives of patients admitted at regional referral hospitals in Rwanda: a cross-sectional study.Rwanda Medical Journal.Vol 75(1), March 2018.

16. Peck-Radosavljevic M. Thrombocytopenia in chronic liver disease. Liver Int. 2017 Jun;37(6):778-793. doi: 10.1111/liv.13317. Epub 2016 Dec 27. PMID: 27860293.

17. Galanopoulos N, Christoforidou A, Bezirgiannidou Z, Lupus thrombocytopenia: pathogenesis and therapeutic implications.Mediterr J Rheumatol.2017 Mar 28;28(1):20-26.doi:10.31138/mjr.28.1.20.

18. Kuter DJ, Konkle BA, Hamza TH, Uhl L, Assmann SF, Kiss JE, Kaufman RM, Key NS, Sachais BS, Hess JR, et al . Clinical outcomes in a cohort of patients with Heparin-induced-thrombocytopenia.Am J Hematol.2017;92:730.doi:10.1002/ajh.24759

19. Kaushansky K. Thrombopoietin and the physiology of platelet production. Leuk Res. 1996 Sep;20(9):705-7. doi: 10.1016/0145-2126(96)00037-9. PMID: 8947578.

20. Dean L: Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 1, Blood and the cells it contains. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2263/

21. Itano KJ et al.Core Curriculum for Oncology Nursing 5th edition (2016).Chapter 27:Alterations in Hematologic and Immune Function. The section on Thrombocytopenia (pages 331-338).

22. Rost NS, Smith EE, Chang Y, Snider RW, Chanderraj R, Schwab K, FitzMaurice E, Wendell L, Goldstein JN, Greenberg SM, Rosand J. Prediction of functional outcome in patients with primary intracerebral hemorrhage: the FUNC score. Stroke. 2008 Aug;39(8):2304-9. doi: 10.1161/STROKEAHA.107.512202

23. Friedmann AM, Sengul H, Lehmann H, Schwartz C, Goodman S. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A re-evaluation of prophylactic platelet transfusions. Transfus Med Rev. 2002 Jan;16(1):34-45. doi: 10.1053/tmrv.2002.29403

24. Webert K, Cook RJ, Sigouin CS, Rebulla P, Heddle NM. The risk of bleeding in thrombocytopenic patients with acute myeloid leukemia. Haematologica. 2006 Nov;91(11):1530-7. Epub 2006 Oct 17.

25. Valsami S, Pouliakis A, Gavalaki M, et al. Platelets transfusion in Greece: Where, when, why? A national survey. Asian J Transfus Sci. 2020 Jul-Dec;14(2):158-166. doi: 10.4103/ajts.AJTS_72_18. Epub 2020 Dec 19.

26 . Neunert C et al. Severe bleeding events in adults and children with primary immune thrombocytopenia: a systematic review. J Thromb Haemost. 2015 Mar;13(3):457-64. doi: 10.1111/jth.12813.

2 7.American Society of Hematology 2019 guidelines for immune thrombocytopenia. *Blood Adv* (2019) 3 (23): 3829–3866.https://doi.org/10.1182/bloodadvances.2019000966

28. Tan Y, Yan M, Cheng Z, Pan X. Pulmonary Thromboembolism in Immune Thrombocytopenia: A Report of Five Cases and a Review of the Literature. Int J Gen Med. 2021 Aug 13;14:4479-4483. doi: 10.2147/IJGM.S323146.

29. Evidence-based management of immune thrombocytopenia. Courtesy Pictures obtained from the Grand Round Presentation at CHUK in 2020 by Dr. Pedro Gueva Vianna, A Johnson-Johnson Globar Health Scholar at Stanford University/Internal Medicine.

30.Kogelenberg VN et al : The complex diagnosis of post-transfusion purpura; a case report/BJH4-2019-Art-Slomp.

31. Hayashi T, Hirayama F, et al . Advances in alloimmune thrombocytopenia: perspectives on current concepts of human platelet antigens-Antibody detection strategies and genotyping.Blood transfus.2015;13(3):380-90.

32. Dhakal B, Kreuziger LB et al: Disease burden, complication rates, and health care costs of heparin-induced thrombocytopenia in the USA: A population-based study.Lancet Haematol.2018;5:e220-e231.doi:10.1016/s2352-3026(18)30046-2

33. Warkentin TE. The clinical picture of heparin-induced thrombocytopenia (HIT) and its differentiation from non-HIT thrombocytopenia. Thromb Haemost. 2016 Oct 28;116(5):813-822. doi: 10.1160/TH16-06-0435. Epub 2016 Sep 22. PMID: 27656712.

34. Risitano AM, Perna F, Selleri C. Achievements and limitations of complement inhibition by eculizumab in paroxysmal nocturnal hemoglobinuria: the role of complement component 3. Mini Rev Med Chem. 2011 Jun;11(6):528-35. doi: 10.2174/138955711795843301. PMID: 21561403.

35. Byuk Sung Ko et al: The Prevalence and Significance of Overt Disseminated Intravascular Coagulation in Patients with Septic Shock in the Emergency Department According to the Third International Consensus Definition Korean J Crit Care Med > Volume 31(4); 2016 DOI: https://doi.org/10.4266/kjccm.2016.00339

36. Payne BA, Pierre RV. Pseudothrombocytopenia: a laboratory artifact with potentially serious consequences. Mayo Clin Proc. 1984 Feb;59(2):123-5. doi: 10.1016/s0025-6196(12)60247-x. PMID: 6422167.

37. Mark S. Sabatine 's Pocket Medicine 7th edition. section 5-7 on platelet disorders /The Massachusetts General Hospital Handbook of Internal Medicine

38. Jaykaran Charan et al : How to Calculate Sample Size for Different Study Designs in Medical Research, 2013, 35(2): 121–126

39.Boechat Tde O, Silveira MF, Faviere W, Macedo GL. Thrombocytopenia in sepsis: An important prognosis factor. Rev Bras Ter intensiva. 2012;24(1):35–42.

40.Nkeshimana, Menelas & Ndayambaje, B. & Masaisa, Florence. (2016). Reversible megaloblastic hematopoiesis due to severe vitamin B12 deficiency in a well-lived man with 2 years history of transfusion-dependant anemia: - A silent tragedy. 73. 16-18

41. Adewoyin AS, Nwogoh B. Peripheral blood film - a review. Ann Ib Postgrad Med. 2014 Dec;12(2):71-9. PMID: 25960697; PMCID: PMC4415389

42. Magesa et al: Causes of pancytopenia based on bone marrow examination in adult patients presenting at Muhimbili National Hospital: A case report Published by OJS /TMJ V30.September 2019. Doi: doi.org/10.4314/tmj.v30i1.275

ANNEXES

1. Data collection tool

UNIVERSITY OF RWANDA — COLLEGE OF MEDICINE AND HEALTH SCIENCES

INTERNAL MEDICINE DEPARTMENT

Study title:

Profiling causes of thrombocytopenia and hospitalization outcomes among adult non-HIV medical patients at tertiary hospitals: the case of CHUK and KFH

PART I: PATIENT IDENTIFICATION&SOCIAL DETAILS

Patient' ID: …… Phone: …….

Sex : M ☐ F ☐ Age:……..

Admitted From……..

Occupation: …….. ……Insurance:…….

Marital status: Single/Married/Widow/Divorced

Alcohol consumption: Yes ☐ No ☐ If yes, For how long:……..

Smoking: Yes No If yes: Pack-years:……..

PART II: CLINICAL DETAILS

Reason for admission: …….

Vital signs at admission: BP: HR : T: RR: O2Sat:

-Altered mental status: Yes No If Yes, GCS: …….

-Epistaxis: Yes No -Gum bleeding: Yes No

-Petechiae: Yes No -Purpura: Yes No

-Ecchymosis: Yes No -Hematuria: Yes No

-Menorrhagia: Yes No -Melena: Yes No

-Splenomegaly: Yes No -Hematemesis: Yes No

-Hepatomegaly: Yes No -Hemarthrosis: Yes No

-Sub-conjunctival hemorrhage: Yes No

-Intracranial hemorrhage: Yes No

-Diarrhoea: Yes No

-Other: ……

…….

…….

Co-Morbidities : HIV + : Yes No

Diabetes: Yes No

Arterial hypertension: Yes No

Chronic liver disease: Yes No

Malignancy: Yes No: If Yes, type:……..

History of Stroke/MI: Yes No, if yes, type:……

Other: ……..

……..

List of Pre-admission medications, duration, and doses

1……Dose……Duration……

2……..Dose…….Duration…….

3……..Dose…….Duration…….

Laboratory investigations

FBC at admission

WBCs ☐ RBCs ☐ Platelets ☐ Hb ☐ Hct ☐ MCV ☐

Neutrophils ☐ Lymphocytes ☐

FBC Control before discharge

WBCs ☐ RBCs ☐ Platelets ☐ Hb ☐ Hct ☐ MCV ☐

Neutrophils ☐ Lymphocytes ☐

Others

Hepatitis B: …….. Hepatitis C: …… H pylori test:……..

Bleeding Time: ___ seconds Clotting Time:___seconds

PT/INR _____ aPTT _____ ADAMTS-13 _____

ALT___ AST ___ urea _____ Creatinine _____

Coombs test: Direct ___ Indirect _____ D-dimers _____

Serum fibrinogen levels _____ Bilirubins: Direct _____

Total bilirubin _____

Albumin _____Haptoglobin _____Homocystein levels……

Serum vitamin B12 levels _____ Blood smear for malaria _____

Peripheral blood Film report (PBF) _____

_____

Bone marrow aspiration and biopsy report:

_____

_____

Blood Culture/Urinalysis report:……..

Factor VIII…….Protein C ……Protein S ……

Anti-thrombin III……Antiphospholipid antibodies……..Factor V Leiden……..

Prothrombin gene mutation……

Work-up for auto-immune diseases: ANA…….. anti-ds-DNA,……

Brain CT scan for any hemorrhage/ ischemic infarct……..

Abdominal ultrasound……

Diagnosis: _____

Medications are taken during admission, doses, and duration :

1. ……2……..3……

Time of development of thrombocytopenia :

-At admission: Yes No

-During admission: Yes No

The severity of thrombocytopenia :

Mild: Yes No

Moderate: Yes No

Severe: Yes No

Very severe: Yes No

Blood transfusion: PRBCs: ……Units

Platelets: …… Units or apheresis platelets ……

FFP:……

Cryoprecipitate: ……..

Cause of thrombocytopenia identified Yes No.

If yes, specify…….. ……..

ITP: Yes No TTP: Yes No HUS: Yes No

DIC: Yes No HIT: Yes No MAHA: Yes No

Evans syndrome Yes No

Drug-related: Yes No Chemotherapy-induced: Yes No

Sepsis: Yes No

Sources of Infection: Urinary Tract

Pneumonia

Intraabdominal…….

Skin /soft tissue

Septicemia

Meningitis

Malaria

Disseminated TB

Liver disease: Yes No

Megaloblastic anemia : Yes No

Aplastic anemia: Yes No

Malignant marrow infiltration: Yes No

Hypersplenism: Yes No

Spurious thrombocytopenia: Yes No

Other cause:……

Cause of thrombocytopenia unknown: Yes No

PART III: HOSPITALIZATION OUTCOME

Resolution of thrombocytopenia by hospital discharge: Yes No

Patient transferred to ICU: Yes No

Transferred to another center: Yes No

Patient clinically improved /cured /dead

Duration of hospital stay: _____ days

2. Consent: English version

UNIVERSITY OF RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

INTERNAL MEDICINE DEPARTMENT

PARTICIPANT INFORMED CONSENT

Participant's names: ……ID……Age…..

1. You are being asked by Dr. NIYONZIMA Charles, to participate in the study entitled " ***Profiling Causes of thrombocytopenia and hospitalization outcomes among adult non-HIV medical patients at tertiary hospitals: case of CHUK and KFH*** "

2. **Rationale**: As you are invited to participate in the study, you have the right to be informed about

i). The study procedures so that you can freely consent to participate. If there is any word or information that you do not understand, please ask for clarification.

ii). The right to know what you will be asked to do so that you can accept or decline participation: your participation is voluntary. If you decline to participate, there will be no consequences to you and it will not affect your clinical management.

iii). The fact that those who are conducting the study will not be involved or will not influence the treating team on choices of treatments or investigations.

3. Specific clarifications:

a)Why is this Study being done?

-The purpose is to know the incidence and clinical profile of thrombocytopenia in adult patients in medical departments at CHUK and KFH.

b) How many patients will be in the study?

-Patients who meet the inclusion criteria will be enrolled as they present. The enrolment is non-random

c)What I am being asked to do as a participant?

-You are required to consent so that the investigator can have access to your medical records and you are requested to answer some questions on the data collection tool of your clinical history, your physical examination findings, and investigation reports

d) How long will I be in the study?

This study will extend to an 8-month period to complete.

e) What are the benefits for me being in the study?

-Your participation will contribute to knowing the current situation of thrombocytopenia in adults patients hospitalized at CHUK and KFH

f) What are the risks of being in the study?

-There are no risks expected as we will be asking you questions and checking findings on your physical examination and recording data from your medical charts. We shall not take part in deciding what labs or investigations to request nor shall we decide on any medical treatment options: prescribing is the responsibility of your treating physician.

g)What is the cost of being in the study?

There is no additional cost that you will pay apart from the bill due to your medical care expenses in the hospital.

h)What shall I be given to participate in the study?

-Your participation is voluntary. There are no incentives or payments to give you to have you participate.

i) Confidentiality

-We guarantee you that the information in this study will not reveal your name. We will use your hospital identification number for reference purposes. The information will be securely kept in a specific file and no third party will have access to it except the research team or if required by law.

j) Who do I contact if I have questions/concerns or complaints?

You may ask any question at any time. You may contact Charles NIYONZIMA, the investigator by calling 0783194713, e-mail: mngcalny16@gmail.com)

-You may also contact Dr.NKESHIMANA Menelas, physician and clinical faculty at CHUK at 0784732678 or contact Dr.HAVYARIMANA Sandra, hemato-pathologist at King Faisal Hospital at 0786529761.

-If you feel any pressure to enroll or to continue to participate in this study, you may contact the IRB at the College of Medicine and Health Sciences/UR at researchcenter@ur.ac.rw P.O Box 3286 Kigali, Rwanda, or contact the hospital ethics committee chairperson: at CHUK, Dr.RUSINGIZA KAMANZI Emmanuel at erkamanzi@gmail.com or Tel: +250785466254.At KFH at email: research.education@kfkigali.com for any complaints.

SIGNATURES

I have read this consent form and my questions have been answered by the investigator. My signature below confirms that I want freely to participate in this study. I know that I can withdraw from the study at any moment with no negative repercussions for my medical care.

Participant ……Signature…..……

Next of kin (In case unable to sign by him/herself):……...

Investigator receiving consent: Dr. NIYONZIMA Charles, Signature …….

Date: ……

3.Consent : Kinyarwanda Version

KAMINUZA Y'u RWANDA

ISHAMI RYIGISHA UBUVUZI

INYANDIKOISABA UBURENGANZIRA UMURWAYI MU KWITABIRA UBUSHAKASHATSI

<u>Igenekereza ry'nyito y'ubushakashatsi</u> :*Gushaka impamvu zituma abarwayi bari mu bitaro bagira igabanuka ry'uturemabuzimangiro dutuma batava amaraso n'ingaruka bibagiraho*.

Wowe,(Amazina y'umurwayi cyangwa umuhagarariye)……..Imyaka ……,twifuza kugusobanurira ibijyanye n'ubushakashatsi turimo gukora kugirango umenye icyo bugamije bityo uduhe uburenganzira bwo kwitabira unatwemerera kukubaza ibibazo bimwe na bimwe no kutwemerera kureba mu ifishi ibyagukorewe uvurwa kugirango tubone amakuru twifuza ajyanye n'intego z'ubu bushakashatsi.

Njye ukora ubushakashatsi nitwa NIYONZIMA Charles (Tel: 0783194713), nkaba ndi umuganga wiga mu cyiciro cya gatatu muri kaminuza y'u Rwanda mu buvuzi bw'abantu, ishami ry'indwara zo mu mubiri.Turimo kubukorera mu bitaro bya CHUK no ku bitaro byitiriwe umwami Faisal.

IKIGAMIJWE :

1. Kumenya ijanisha n' impamvu zituma habaho igabanuka ry'uturemabuzimangiro dutuma hatabaho kuva amaraso bityo bikaba byafasha mu kumenya icyiciro cy'abarwayi bibasiwe kurusha abandi , uburyo bavuwe , igihe bamaze mu bitaro n'ingaruka byabateye.
2. Ibi biramutse bimenyekanye, bigira inyungu rusange ku barwayi mu kurushaho gusobanukirwa uburwayi ndetse n'abaganga bakamenya abibasiwe kurusha abandi bakarushaho kubakurikirana mu buryo bwihariye.

UBURYO UBU BUSHAKASHATSI BUZAKORWAMO :

1. Tubaza umurwayi/umurwaza uhagarariye umurwayi ibibazo byateguwe ku rupapuro rukusanya amakuru,
2. Twifashisha amafishi y'umurwayi n'ahandi hari ububiko bujyanye n'amakuru y'abarwayi (mudasobwa, ishinguranyandiko ry'ibitaro) tureba ibiba byakorewe umurwayi .
3. Duteganyako ubu bushakashatsi buzamara amezi 8.

ABITABIRA UBUSHAKASHATSI

Ni abarwayi basobanuriwe kandi bemeye kwitabira ku bushake bwabo nta gahato,barwariye aho bavurira indwara zo mu mubiri.

MU KWEMERA KWITABIRA :

1.Nta ngaruka ubu bushakashatsi buzagira ku buzima bwawe cyangwa ku buvuzi uzaba urimo guhabwa.

2.Tuzagira ibanga amakuru uzaba waduhaye: nta na rimwe izina ryawe rizagaragara ku rupapuro rukusanya amakuru , tuzifashisha nomero yawe y'ibitaro mu kumenya urupapuro rwanditseho amakuru y'uburwayi bwawe.

3.Nta bihembo cyangwa amafaranga uzahabwa kugirango ubone kwitabira ubu bushakashatsi

4. Ibizava muri ubu bushakashatsi bizamenyeshwa urwego rwa kaminuza n'amashami y'ibitaro ashinzwe ubushakashatsi

5.Ukeneye ubundi busobanuro ku kibazo cyose wagira, wakwifashisha nimero za telefoni zikurikira z'abaganga cyangwa ukabaza mu nzego zigenzura ko ubu bushakashatsi bukurikiza ibisabwa mu kubahiriza uburenganzira bw'abitabira:

-Mu bitaro bya CHUK :Dr.NKESHIMANA Menelas , Tel 0784732678 (Umuganga mu bitaro bikuru bya kaminuza bya CHUK akaba n'umwarimu muri kaminuza y'u Rwanda , ishami ry'ubuvuzi,

-Mu bitaro byitiriwe umwami Faisal,mwabaza Dr.HAVYARIMANA Sandra , Tel 0786529761 (Umuganga w'inzobere mu burwayi bw'amaraso)

6.Uramutse ushizwe ku nkeke ngo witabire ubushakashatsi mu buryo ubwo ari bwo bwose, wakwisunga inzego nkuru za kaminuza cyangwa z'ibitaro zikurikira :

-Muri kaminuza y'u Rwanda : Wamenyesha urwego rushinzwe ubushakashatsi kuri researchcenter@ur.ac.rw /P.O Box 3286 Kigali, Rwanda

-Mu bitaro bikuru bya kaminuza bya Kigali (CHUK) :wamenyesha ushinzwe ishami rigenzura ubushakashatsi bukorerwa mu bitaro ariwe Dr.RUSINGIZA KAMANZI Emmanuel kuri telephone +250785466254 cyangwa ukohereza ubutumwa kuri erkamanzi@gmail.com

-Mu bitaro byitiriwe umwami Fayisali , wamenyesha Dr.Edouard NGENDAHAYO, umuyobozi w'ishami rishinzwe ubushakashatsi no kwigisha kuri Telefoni +250788659635 cyangwa ukohereza ubutumwa kuri research.education@kfkigali.com

GUSHYIRA UMUKONO KURI IYI NYANDIKO

Nemeje ko nasobanuriwe bihagije ikigamijwe mu gukora ubu bushakashatsi. Nemeye nta gahato kugira uruhare muri bwo nemera gusubiza ibibazo mbazwa kandi ntanga n'uburenganzira bwo kureba ku ifishi navuriweho no muri mudasobwa cyangwa mu ishinguranyandiko ry'ibitaro amakuru ku burwayi n'ubuvuzi nahawe.Igihe icyo ari cyo cyose nshobora kwivana muri ubu bushakashatsi kandi hakaba ari nta ngaruka byagira mu buvuzi bwanjye.

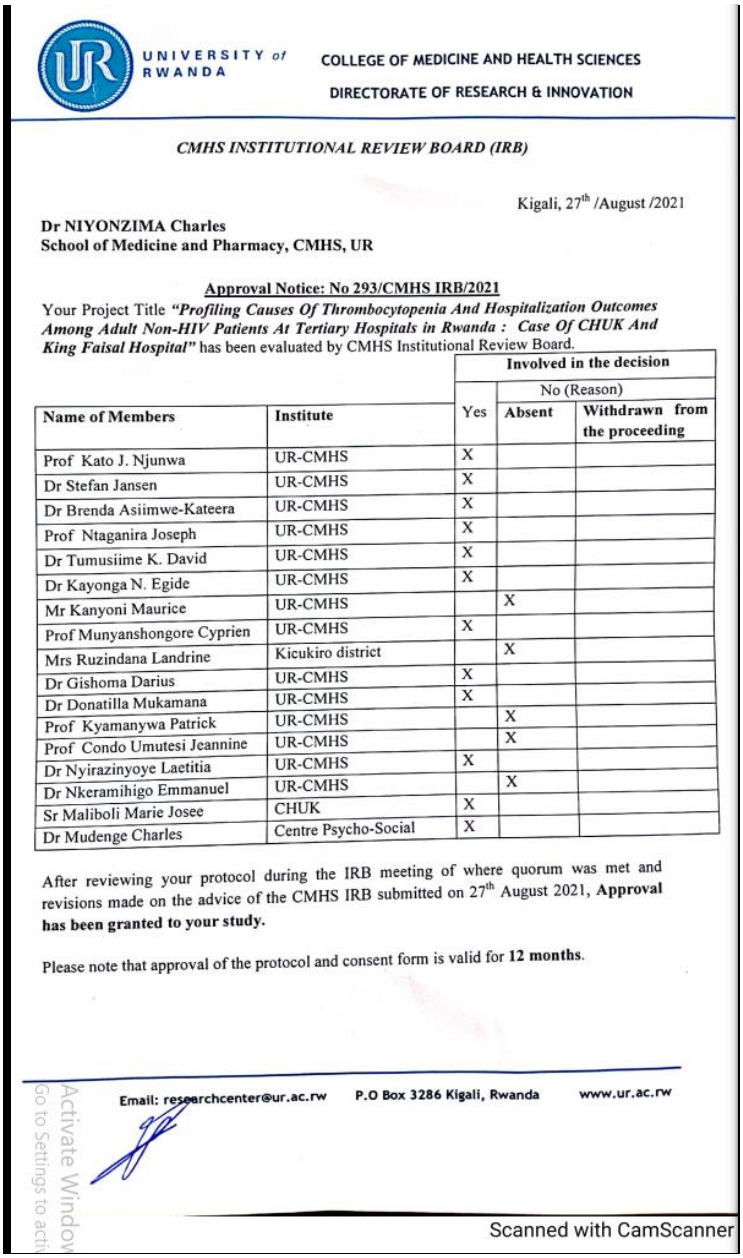
AMAZINA Y'UTANZE UBURENGANZIRA…….
UMUKONO……

UHAGARARIYE UMURWAYI (IGIHE ADASHOBOYE KWISINYIRA)……
UMUKONO……

UMUKONO W'UHABWA UBURENGANZIRA :Dr.NIYONZIMA Charles ……..

ITALIKI……

4. IRB approval notice



UNIVERSITY of RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DIRECTORATE OF RESEARCH & INNOVATION

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 27th /August /2021

Dr NIYONZIMA Charles
School of Medicine and Pharmacy, CMHS, UR

Approval Notice: No 293/CMHS IRB/2021

Your Project Title ***"Profiling Causes Of Thrombocytopenia And Hospitalization Outcomes Among Adult Non-HIV Patients At Tertiary Hospitals in Rwanda : Case Of CHUK And King Faisal Hospital"*** has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
		Yes	No (Reason)	
			Absent	Withdrawn from the proceeding
Prof Kato J. Njunwa	UR-CMHS	X		
Dr Stefan Jansen	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS		X	
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS	X		
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 27th August 2021, **Approval has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months**.

Email: researchcenter@ur.ac.rw P.O Box 3286 Kigali, Rwanda www.ur.ac.rw

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 27th August 2021

Expiration date: The 27th August 2022

Dr Stefan Jansen
Ag. Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Cc:
- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

Email: researchcenter@ur.ac.rw P.O Box 3286 Kigali, Rwanda www.ur.ac.rw

5. CHUK approval notice

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Quality Health Care
Training & Research

Ethics Committee / Comité d'éthique

28th Oct,2021 Ref.:EC/CHUK/118/2021

Review Approval Notice

Dear Charles NIYONZIMA,

Your research project: ***"Profiling causes of thrombocytopenia and hospitalization outcomes among adult non-HIV patients at tertiary hospitals in Rwanda : Case of CHUK and King Faisal Hospital "***

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 28th Oct,2021 to evaluate your request for ethical approval of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your research project.

You are required to present the results of your study to CHUK Ethics Committee before publication by using this link:www.chuk.rw/research/fullreport/?appid=452&&chuk.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

Dr Emmanuel Rusingiza Kamanzi
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

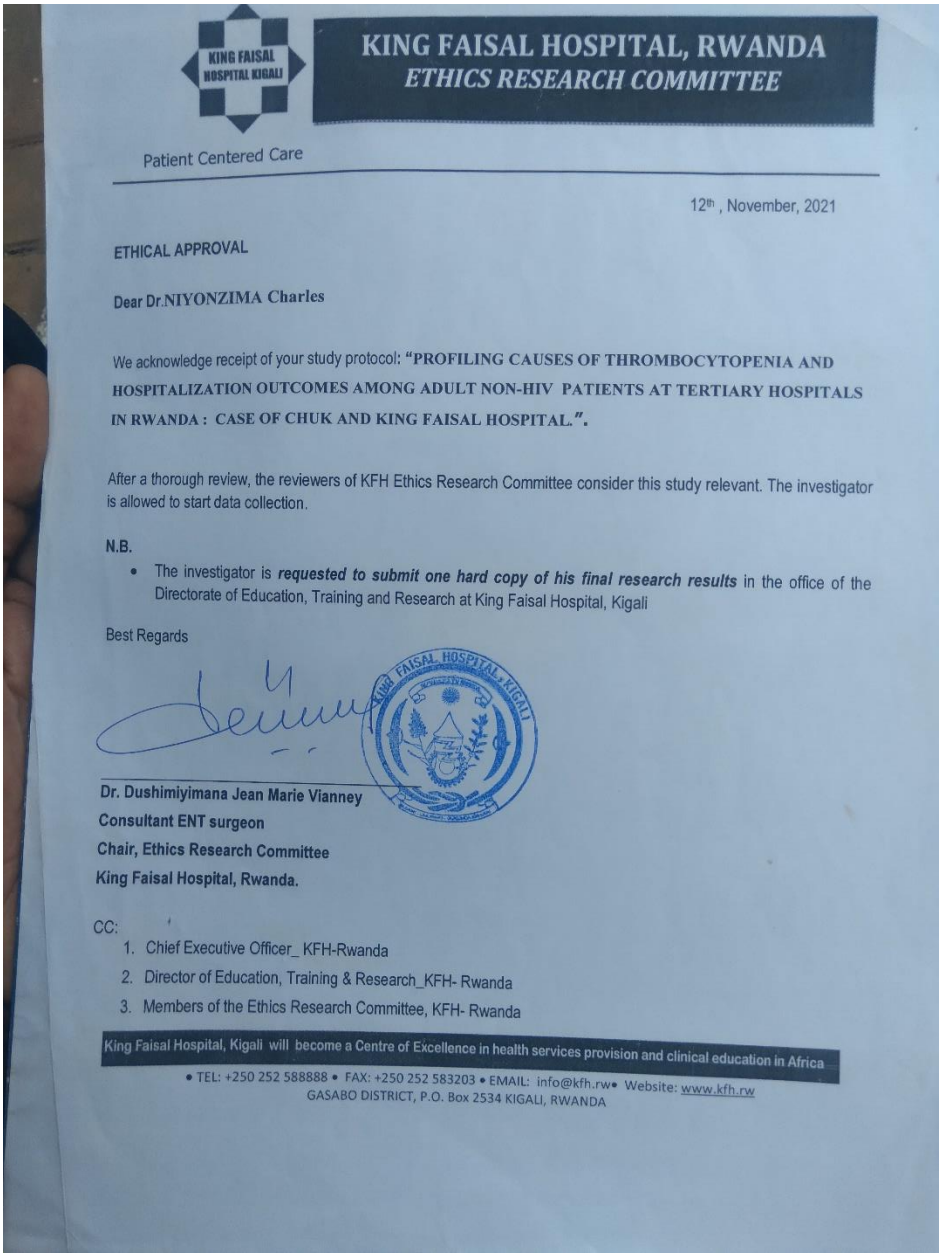
ETHICS COMMITTEE
CHUK

Scan code to verify.

" University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations "

Web Site : www.chuk.rw ; B.P. 655 Kigali- RWANDA Tél.: 00 (250) 252575462. E-Mail: chuk.hospital@chuk.rw

5.King Faisal Hospital Approval notice



KING FAISAL HOSPITAL KIGALI

KING FAISAL HOSPITAL, RWANDA
ETHICS RESEARCH COMMITTEE

Patient Centered Care

12th , November, 2021

ETHICAL APPROVAL

Dear Dr.NIYONZIMA Charles

We acknowledge receipt of your study protocol: **"PROFILING CAUSES OF THROMBOCYTOPENIA AND HOSPITALIZATION OUTCOMES AMONG ADULT NON-HIV PATIENTS AT TERTIARY HOSPITALS IN RWANDA : CASE OF CHUK AND KING FAISAL HOSPITAL."**.

After a thorough review, the reviewers of KFH Ethics Research Committee consider this study relevant. The investigator is allowed to start data collection.

N.B.

- The investigator is ***requested to submit one hard copy of his final research results*** in the office of the Directorate of Education, Training and Research at King Faisal Hospital, Kigali

Best Regards

KING FAISAL HOSPITAL KIGALI

Dr. Dushimiyimana Jean Marie Vianney
Consultant ENT surgeon
Chair, Ethics Research Committee
King Faisal Hospital, Rwanda.

CC:

1. Chief Executive Officer_ KFH-Rwanda
2. Director of Education, Training & Research_KFH- Rwanda
3. Members of the Ethics Research Committee, KFH- Rwanda

King Faisal Hospital, Kigali will become a Centre of Excellence in health services provision and clinical education in Africa

• TEL: +250 252 588888 • FAX: +250 252 583203 • EMAIL: info@kfh.rw• Website: www.kfh.rw
GASABO DISTRICT, P.O. Box 2534 KIGALI, RWANDA