An-Najah National University

Faculty of Graduate Studies

FREQUENCY OF RED CELL ALLOIMMUNIZATION IN HEMATOLOGICAL MALIGNANCIES IN WEST BANK, PALESTINE

By

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Dedication

This thesis is dedicated to:

My great teacher and messenger, Mohammed (May Allah bless and grant him), who taught us the purpose of life,

my mother's soul, who has been a constant source of support and encouragement during the challenges of life and always loved me unconditionally and taught me to work hard for the things that I aspire to achieve.

my father (May Allah protect you and prolong your live),

my beloved sister and best friend, Anwar,

everyone very dear to my heart, and

all cancer patients.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

FREQUENCY OF RED CELL ALLOIMMUNIZATION IN HEMATOLOGICAL MALIGNANCIES IN WEST BANK, PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: _____

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Date: _____

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Abstract

Background: Patients with malignant hematological diseases frequently have anemia. Blood transfusion therapy is fundamental in management in these patients. Repeated blood transfusions may cause the development of alloantibodies against one or more red cell antigens, which complicates subsequent transfusions.

Objectives: This study aimed to investigate the frequency and characteristics of RBC alloimmunization as well as the related risk factors among patients diagnosed with hematologic malignancies (mainly leukemia and multiple myeloma) in Palestine.

Materials and Methods: This cross-sectional study was performed between February and August, 2022 on oncohematological patients from clinics or admitted to the hospitals from three hospitals located north, middle and south of West Bank, Palestine: An-Najah National University Hospital in Nablus, the Istishari Arab Hospital in Ramallah and the Beit Jala Governmental Hospital in Bethlehem. A total of 94 of multi transfused patients were included. Demographic, medical data and history of transfusion were retrieved from patients` files. Alloantibody screening (indirect coomb's test) and identification (panel test) were performed from plasma samples of patients ,who have never done the test using commercial Capture-R Ready-screen and ID, IMMUCOR. In order to exclude autoantibodies, a patient's RBCs were analyzed in combination with each screen for autoantibodies.

Results: Twenty-nine participants had alloantibodies. The rate of alloimmunization observed in the present study was 30.85%. The most frequent alloantibodies were against Kell system antigens (Anti-K) (20.7%), followed by Anti-E (13.8%), and Anti-D (6.9%). One participant had multiple antibodies, and six participants (23.9%) had two

antibodies: Anti-E and K (6.9%), Anti-Jk^{a} and Kp^{a} (6.9%), Anti-E and Fy^{a} (3.4%), and Anti Fy^{a} and S (3.4%). Autoantibodies were found in this study (6.9%).

Conclusion: This study showed a high rate of alloimmunization among hematological malignancies mainly leukemia and multiple myeloma in different regions of West Bank, Palestine. The most frequently detected alloantibodies were against the Kell and Rh antigens. RBC compatibility in ABO, Rh, and Kell system as well as phenotyping of antigens may reduce the risk of the most of alloimmunizations.

Keywords: Red Blood Cell Transfusions; Antibody Screening; Hematological Malignancies; Palestine.

Chapter One

Introduction

1.1 Overview

Cancers that develop in blood-forming tissue, such as the bone marrow, or immune system cells are known as hematologic malignancies. Alloimmunization of erythrocytes is a significant issue in patients with hematological malignancies who frequently require blood transfusions for a critical intervention to resolve anemia (1). Patients with hematological malignancies, such as leukemia and multiple myeloma (MM), are commonly treated with intensive chemotherapy. Megakaryocyte proliferation and proplatelet formation are both impaired by this therapy and linked to a higher risk of clinically significant bleeding (2). Anemia is a common complication in cancer patients. Anemia is a reduction in the number of red blood cells (RBCs), the hematocrit, or the red cell hemoglobin content.

Insufficient erythropoietin (EPO) secretion in conjunction with inflammatory cytokines appears to play a crucial role in the development of anemia in MM disease. Low hemoglobin and hematocrit levels are linked with a poor quality of life and have an impact on the circulatory system (3). The correct management of blood transfusion has been associated with improvement in clinical outcomes.

Anemia has symptoms that are neither sensitive nor specific, making it difficult to distinguish between different anemias. The development of symptoms is typically accompanied by hemoglobin levels that are significantly below the patient's unique baseline and represent compensatory reactions to tissue hypoxia. When the anemia develops quickly or in people with low cardiopulmonary reserve, symptoms are typically more severe.

Despite the fact that most people regard blood transfusions to be safe, there is some risk of complications. Red blood cell (RBC) alloimmunization is frequently associated with transfusion and can result in severe adverse effects and significant consequences due to the presence of anti-RBC antibodies in the plasma of the recipient (4).

The percentages of cases of leukemia and multiple myeloma reported in the West Bank from all malignancies in 2021 are 5% and 2%, respectively, with a mortality rate of 5.7% and 2.7% for leukemia and multiple myeloma, respectively among the thirty-nine registered cancer sites. Thus, leukemia is considered the fourth leading cause of cancer deaths after lung, colon and breast cancers (5). Antibodies to cell surface antigens are produced during alloimmunization, which can occur after multiple RBC transfusions and make it difficult to cross-match and determine suitable blood transfusions in the future. RBC alloimmunization against the most clinically important antigens of Rh (D, C, c, E, and e), Kell (K, k, Kp^a, and Kp^b), Kidd (Jk^a and Jk^b), and Duffy (Fy^a and Fy^b) blood group systems interferes with the selection of compatible red blood cells for transfusion in the patients with hematologic malignancies although the ABO compatibility is performed (6).

The anti-CD38 daratumumab or darzalex, which was originally developed in 2012, is the standard therapy as monotherapeutic in MM (7, 8). Therapeutic CD38-targeting antibodies disrupt pretransfusion laboratory testing because the human erythrocytes have a weak expression of CD38 (9).

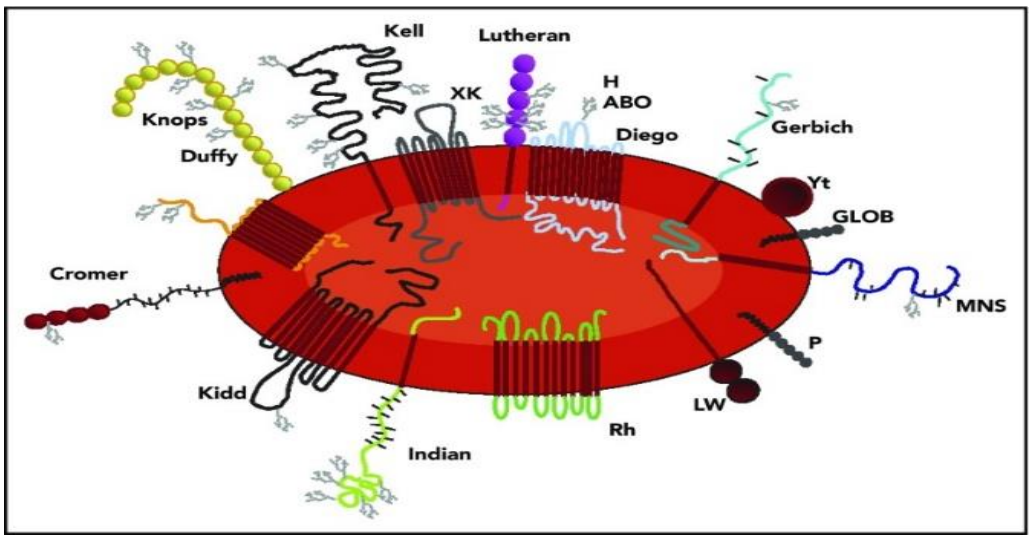
1.1.1 RBC antigen characteristics

The ABO blood types were discovered by Karl Landsteiner (1868–1943), who earned the Nobel Prize in 1930 for his research on the specificity of serological reactions (10). Blood transfusion-related mortality was reduced in the early stages of transfusion therapy because to the discovery of the ABO blood type system.

Red cell surface antigen terminologies are presently recognized by 43 blood group systems by the International Society of Blood Transfusion (ISBT) Working Party committee, which are genetically defined by 48 genes, and 349 red cell antigens as of September 2022. If given to a recipient who does not have the antigen, a blood group antigen can cause the formation of an antibody. Numerous blood group systems are carried on proteins with structural functions, which are used to maintain the normocyte (11).

Figure 1

RBCs blood group antigens. Elisabeth Sjoberg Webster



Note. adopted by Smart E, Armstrong B, Lee RfSEE. Blood group systems. ISBT Science Series. 2020; 15(S1):123-50.

Antigen-specific oligosaccharide building structures are used to identify a person's blood type; blood group antigens are seco=ndary gene products that play a supporting role in this process. Blood group antigens are present on red blood cells, platelets, leukocytes, plasma proteins, specific tissues in addition to being found in soluble form in bodily secretions, and different cell surface enzymes (12).

The most significant irregular RBC alloantibodies in blood transfusions are those against the blood group systems Rh (anti-D, -C, -E, -c and -e), Kell (anti-K), Fy (anti-Fy^a and -Fy^b), Jk (anti-Jk^a and -Jk^b), and MNS (anti-M, -S and -s). More than 80% of immunocompetent D negative people acquire alloimmunization after receiving a transfusion of D-positive erythrocytes, making the D-antigen the most immunogenic of them (13, 14).

Blood component production and use are not without risk. Despite advancements in blood component preparation, there are still issues that need to be resolved (15).

1.1.2 Guidelines for RBC transfusion

Insufficient quality evidence to justify practice recommendations has made it difficult to produce clinical practice guidelines for RBC transfusion. Hemoglobin (Hb) values below 7 to 8 g/dL should be taken into consideration for RBC transfusion, depending on the patient's features, and levels above 10 g/dL are typically not indicated (16).

The decision to transfuse RBCs should be made after a clinical evaluation of the patient that evaluates the benefits and risks of the transfusion. It is becoming more and more obvious that liberal transfusion procedures may expose patients to unnecessary risks and are not always associated with better outcomes as more data on RBC transfusion become available.

The Association for the Advancement of Blood & Biotherapies (known as the AABB) most recent published standards are based on a comprehensive analysis of randomized, controlled studies examining transfusion thresholds (17).

Patients who are actively bleeding may need an RBC transfusion, which should be determined by both a clinical evaluation of the patient and laboratory tests. The volume and oxygen-carrying features of an RBC transfusion must be examined separately when evaluating a transfusion's effectiveness. It is challenging to correlate any examination of the advantages gained from or attributed to the transfusion to a specific quality because these two properties are so linked and connected (18).

1.1.3 Formation and detection of RBC alloantibodies

Up to a certain point, transfusion burden causes an increase in the percentage of alloimmunized patients. Studies suggest that only 2% to 5% of recipients of general blood transfusions develop obvious RBC alloantibodies. (19). Plasma from a patient is commonly analyzed for antibodies using RBCs with known antigen specificities as reagents. Single antibodies, such as anti-D or anti-K, are simple to identify. The identification of numerous alloantibodies becomes more complicated and takes longer as a result of the complex structure and wide range of RBC antigens, despite the fact that methodologies for RBC alloantibody detection differ from those for human leukocyte antigen (HLA) antibody detection.

Anti-human globulin G (AHG) is the screening test's detecting antibody and for the identification of antibodies, specific subtypes or antibodies from distinct classes may require advanced reference laboratory testing. Alloantibody identification is followed by a crossmatch, which combines the patient's plasma with the antigen-negative donor RBCs to be transfused. This acts as a final compatibility check. Alloantibodies against the low incidence antigen may be found for the first time during the crossmatch if it was not present in the reagent screening cells.

Alloimmunization to RBC and other antigens caused by transfusion can be a clinically significant issue (20). For the best possible transfusion safety, patients' prior transfusion history must be obtained. Alloantibody detection that persists may be an indicator of the host's immune system's reaction to structural variations between antigens. This may also be due to the fact that certain antigens (such as RhD) have multiple potential antibody targets, whereas other antigens that are the result of single amino acid substitutions between the donor and recipient might possess only one target (21).

RBC alloimmunization has been linked to more frequent transfusion-related complications, such as:

1. Transfusion delays due to the discovery of new alloantibodies,
2. Difficulty finding compatible blood for highly alloimmunized people, and
3. Delayed hemolytic or serologic responses.

Alloimmunized individuals are also susceptible to acute hemolytic transfusion reactions, although they are uncommon. Depending on the conditions, transfusion of RBCs expressing an antigen against which the recipient has alloantibodies may result in varied clinical effects. Some RBCs that first appear to be incompatible may circulate in the transfusion recipient for the duration of their expected lifespan. Patients with chemotherapy-treated leukemia had lower RBC alloimmunization rates than would be expected based on transfusion burden (22, 23).

1.2 Objectives

1.2.1 Main objective

To investigate the frequency and characteristics of RBC alloimmunization as well as the related risk factors among patients diagnosed with hematologic malignancies (mainly leukemia and multiple myeloma) in Palestine.

1.2.2 Secondary Objectives

- Identification of the specific antibody that is present in multi-transfused patients with leukemia or multiple myeloma who have a positive antibody screen test (ICT).
- Identification of risk factors associated with the formation of antibodies in patients who have received multiple blood transfusions.

1.3 Significance

Alloimmunization is a serious clinical problem. In multi-transfused patients, the selection of blood for transfusion according to the ABO and Rh(D) typing to get compatible blood are not enough in hematological malignancies. The evaluation of antibody panel identification of these multi-transfused patients:

- Reduces the span of time required for selecting and matching appropriate blood needed for patients.
- Reduces the hospital costs and patient's stay period in the hospitals to achieve increased survival of patients in addition to improvement quality of life.
- Minimizing the adverse responses associated with blood transfusion.

1.4 Study Hypothesis

There is an alloimmunization of RBCs among hematological cancer's patients in Palestine.

Chapter Two

Literature Review

2.1 Pretransfusion Testing

2.1.1 ABO and Rh Typing

Blood that is intended for transfusion needs to undergo Food and Drug Administration (FDA)-approved testing. Before labelling units, valid test results must be provided. ABO, Rh, and testing for infectious diseases are among the tests. Blood may be discharged in an emergency before testing is finished, but this should only be done in severe cases and only with a physician's agreement and a medical necessity note.

Individuals generally develop antibodies against the A and/or B antigen(s) that are absent from their RBCs (naturally occurring antibodies). ABO antibodies are predominantly IgM antibodies that activate complement and react at room temperature or below (24, 25). Antigens on ABO red blood cells can be glycolipids, glycoproteins, or glycosphingolipids (26). Rh blood group system is second only to the ABO blood group system. It currently has 56 antigens, the most important of which are the D, C, c, E, and e antigens, particularly the D antigen. Rhesus blood type (Rh) determines whether or not RBCs contain the D antigen (27). A limited number of units undergo phenotyping for other red cell antigens, such as Duffy, Kidd, and MNS, to provide antigen-negative blood for alloimmunized patients (28).

Rh proteins serve a role in preserving the structural integrity of red blood cells and may be transporters. Rh glycoproteins are involved in the transport of NH3/NH4+ (29). The majority of Rh antibodies are IgG immunoglobulins that react optimally at 37°C or following antiglobulin testing with any antibody detection procedure. While the D antigen is extremely immunogenic, the c antigen is the most probable Rh antigen, followed by E, C, and e (30).

Clinically significant antibodies (CSAs) are those that induce a reduction in the survival of RBCs that contain the target antigen. Most of the published literature reported that these CSAs are generally IgG antibodies that are reactive at 37 °C or during the antihuman globulin (AHG) phase of an indirect antiglobulin test (IAT). Autoantibodies

target antigens expressed on an individual's RBCs. Utilizing an antibody identification panel to figure out the specificity of the antibody(s) present (31).

2.1.2 Compatibility Testing

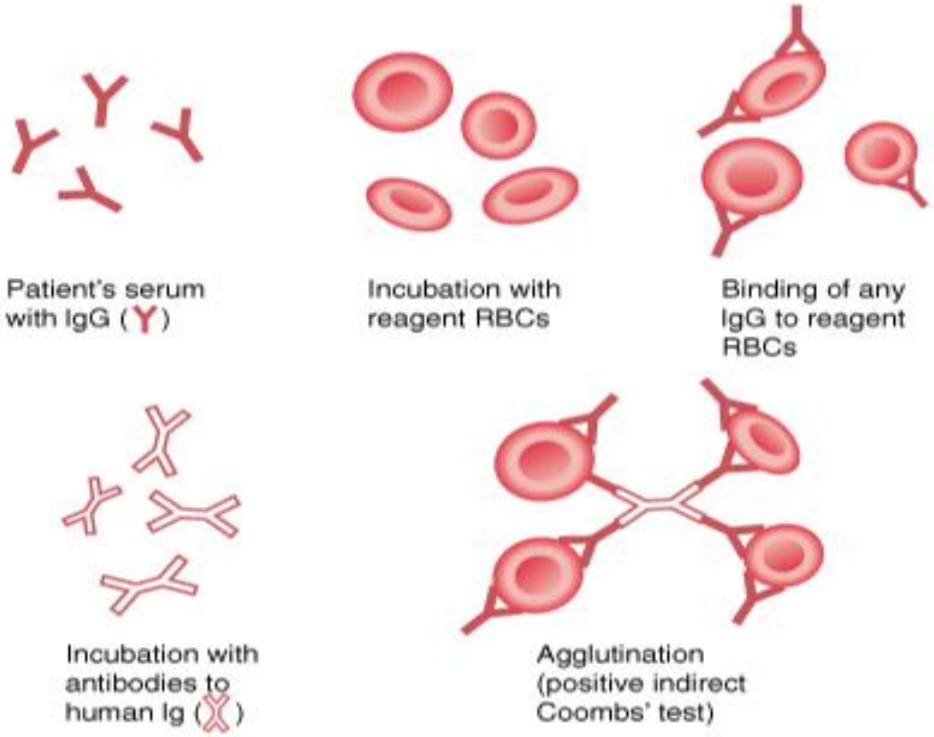
Testing for compatibility includes establishing the ABO/Rh classification of patients in addition to antibody screening and crossmatching. All of them seek to prevent a hemolytic transfusion reaction by identifying in vitro a potentially hazardous reaction between antigens and antibodies may result in complications when blood is transfused in vivo. All potential recipients are tested for pre-existing unexpected red cell antibodies e.g., antibodies to non-ABO antigens: Rh (C, c, D, E, e), Duffy, Kell, Kidd, etc…

The most important goals of the major crossmatch are to prevent the unexpected transfusion of ABO-incompatible blood and to identify previously unidentified clinically significant antibodies (CSAs) that react with red blood cell antigens (32). The antiglobulin test is a method for demonstrating the presence of antibodies or complements bound to red blood cell (RBC) membranes by using anti-human globulin to produce a visible agglutination reaction. Antiglobulin tests include both the direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT). This classification is determined by the sensitization environment, with the direct antihuman globulin test detecting sensitized RBCs in vivo and the indirect antihuman globulin test detecting sensitized RBCs in vitro (33).

The anti-globulin test was developed to detect immunoglobulin G (IgG) antibodies in human blood via agglutination. The negative surface charge of RBCs prevents them from approaching each other. Anti-IgG antibodies cross-link IgG molecules on one cell with those on nearby red cells (34). The purpose of the crossmatch is to assess whether the recipient has antibodies against the red blood cells of the donor.

Figure 2

Indirect antiglobulin (indirect Coombs) test used to detect red blood cell (RBC)-specific IgG antibodies in a patient's plasma



Note. MSD Manual. Available from: https://www.msdmanuals.com/ professional/multimedia/table/ indirect-antiglobulin-indirect-coombs-test.

2.1.3 Antibody Identification

Alloantibodies only react with allogeneic red cells and can be detected in any test that uses serum or plasma (e.g., ABO test, antibody detection test, crossmatch) or in an eluate made up of alloantibody-coated red cells. Red cell autoantibodies, on the other hand, react with the antibody producer's red cells. When an antibody is discovered, its specificity and clinical significance must be established. Clinically important red cell antibodies are those that decrease transfused red cells' ability to survive. Certain antibodies destroy incompatible red blood cells in minutes or hours, while others simply reduce survival by a few days, and yet others produce no obvious red cell destruction (35). The serum or plasma must be tested against a panel of chosen red cell samples with known antigen compositions for the major blood types in order to discover an antibody to a red cell antigen(s). They are frequently obtained from

commercial sources. Panel cells are group O, allowing serum/plasma from any ABO group to be tested (36).

Figure 3

A Reagent Red Cell Panel for Alloantibody Identification

Sample #	Rh Phenotype	Rh						Kell	Duffy		Kidd		P	Lewis		MNS			
		C	C^w	c	D	E	e	K	Fy^a	Fy^b	Jk^a	Jk^b	P$_1$	Le^a	Le^b	M	N	S	s
1	r′r	+	0	+	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+
2	R$_1^w$	+	+	0	+	0	+	+	+	+	0	+	+	+	0	+	+	+	+
3	R$_1$	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0
4	R$_2$	0	0	+	+	+	0	0	0	+	0	+	+	+	0	0	+	0	+
5	r″r	0	0	+	0	+	+	0	+	+	0	+	0	0	+	+	+	+	0
6	r	0	0	+	0	0	+	0	0	+	+	0	+	0	0	+	+	0	+
7	r	0	0	+	0	0	+	+	0	+	+	0	+	0	+	+	0	+	0
8	r	0	0	+	0	0	+	0	+	0	0	+	+	+	0	0	+	0	+
9	r	0	0	+	0	0	+	0	0	+	+	0	0	0	+	0	+	+	0
10	R$_0$	0	0	+	+	0	+	0	0	0	+	+	+	0	0	+	+	+	+

+ Denotes presence of antigen; 0 denotes absence of antigen.

Note. Adopted by Brecher M, Leger R. AABB, Technical Manual. 15 [sup] th ed. Bethesda: American Association of Blood Banks. 2005.

Knowing what happens when a serum interacts with autologous red cells may be useful. This aids in determining whether an alloantibody, autoantibody, or both are present. Risks associated with transfusion. Autoantibodies or autoantibodies along with alloantibodies are present when both reagent and autologous red cells react with them.

The purpose of the panel test is to figure out an unknown antibody. The serum of the patient is tested against a series of reagent cells containing various antigen combinations (37). You can begin to eliminate antigens once you have received your data for how the patient's serum tested with each reagent cell line. The first step is to examine all of the negative patient reactions, which indicate that there was no agglutination between the patient serum and the reagent cell line. Because there was no reaction with the patient serum, you can rule it out (also referred to as exclusion or cross-out).

When running an antibody panel in tubes, there will be three phases: immediate spin (IS), 37°C, and AHG (can also be called IAT phase). If there is no reaction, it is probable that the sample being tested lacks antibodies to the antigens on that panel cell. An antibody panel usually includes at least 10 panel cells (typically 8–14 reagent

RBCs). Panel RBCs are typically group O, allowing plasma from any ABO group to be tested. There are commercial RBC antibody identification panels available that allow the identification of common antibodies (e.g., anti-D, C, c, E, e, Fy^a, Jk^a, K, etc.) (38).

Figure 4

RBCs Identification Panel Test

			Rh - Hr						Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		Xg	CELL	PATIENT'S TEST RESULTS					
CELL	Special Type	Donor	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a							
1		RzR1 A3543	+	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	+	+	0	+	+	1						
2		R1wR1 B10586	+	+	0	0	+	+	0	+	+	+	0	+	+	+	+	0	0	+	0	+	+	0	+	0	+	+	2						
3	Di(a+)	R2R2 C5552	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	+	W	+	+	0	+	0	+	0	+	0	3						
4	Go(a+)	Ror D450	+	0	+	0	+	0	0	+	0	+	+	+	0	0	+	+	0	+	+	0	+	0	+	0	+	0	4						
5	Bg(a+)	r'r E739	0	+	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	+	5						
6		r"r F1037	0	0	+	+	+	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	+	0	+	+	6						
7	Co(b+)	rr N4291	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	0	7						
8		rr G1673	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	8						
9		rr H1411	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	0	+	+	9						
10		rr N4058	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	+	10						
11		rr N4250	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	0	+	+	11						
12		rr G1497	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	12						
13		rr H781	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+	13						
14	Mi(a+), GP.Mur	R1R1 B9666	+	+	0	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	14						
15		POSITIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	PC						
16		NEGATIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NC						

Note. Adopted from *IMMUCOR, INC. Norcross, GA 30071 USA.*

2.1.4 Some blood group alloantibodies

Anti-A:

High titer anti-A may be IgM or IgG. Group A RBCs are strongly directly agglutinated by both antibodies. The amount of powerful IgG complement-binding anti-A in the recipient's or the mother's serum is connected with the severity of transfusion responses or hemolytic disease of the newborn (HDN) (39).

Anti-B:

Strongly inducing direct agglutination of group B RBCs is anti-B, which can be IgM or IgG. Strong IgG complement binding anti-B is linked to the most severe clinical outcomes (39).

Anti-M:

The majority of anti-M examples are IgG, however they only agglutinate M-positive RBCs when they react below body temperature. In most cases, technical issues with this antibody in the compatibility test can be avoided by rigidly conducting the reaction at 37°C (40).

Anti-N:

Around 70% of unrelated blood samples will react with anti-N, which is typically a direct agglutinin. Usually, by strictly performing the compatibility test at 37°C, technical issues with this antibody can be avoided (41).

Anti-S:

The antihuman globulin test reactions can indicate anti-S presence. S-negative random blood samples account for approximately 48% of the population (41).

Anti-s:

Direct agglutinating cold agglutinins are some examples of anti-s that can be reactive by the antiglobulin test. The s-negative blood percentage is about 12% (42).

Anti-P1:

Anti-P1 is almost always a low-temperature-active, direct-agglutinating antibody. Approximately 21% of blood specimens are P1-negative. Keeping the temperature of the compatibility test at 37°C will typically prevent technical troubles with this antibody. Anti-P1 could be suppressed by soluble P1 material (43).

Anti-D:

Anti-D is typically composed of IgG, and both the antiglobulin test and enzyme techniques react favorably to it. IgG anti-D does not activate the complement cascade, unlike other antibodies in this blood group system (44). Certain antisera contain an IgM

component that agglutinates D-positive RBCs directly. Approximately 85 percent of random blood samples contain D.

Anti-C:

Anti-C is typically an immunological IgG antibody, however some IgM cases seem to be naturally occurring. Anti-C is present in approximately 30% of sera. 73% of blood donated by African Americans and about 33% of blood donated at random are C-negative, and most blood that is D-negative is also C-negative. Nevertheless, sera containing IgM antibody typically result in direct agglutination of C-positive RBCs. Anti-C reacts through antiglobulin and enzyme methods (45).

Anti-E:

Anti-E antibodies are typically IgG that can be activated by enzymes or antiglobulins. Some examples contain an IgM component that agglutinates E-positive RBCs directly. Almost all D-negative RBC samples are also E-negative, whereas only approximately 62% of randomly selected D-positive blood samples are also E-negative. Anti-E appears to be prevalent in the natural world (46).

Anti-c:

Anti-c typically reacts with antiglobulin or enzyme methods and is typically IgG. Nearly all D-negative people are c-positive, although only about 17% of random donors are c-negative (44).

Anti-e:

Anti-e is generally immune and reacts using enzyme or antiglobulin methods. Blood that is D-negative will almost always be e-positive.

Anti-K:

Anti-K is typically IgG reactive when tested with an antiglobulin. IgM saline agglutinating components may be present in a small percentage of antisera. As 91% of donors are K-negative, it is simple to find compatible blood through random screening. It is advised that compatibility testing be performed utilizing an indirect antiglobulin approach with saline suspended RBCs because the antibody may not react well to low ionic strength solution (LISS) protocols (47).

Anti-k:

Anti-k is a rare IgG antibody that reacts to an antigen that is opposite to K in the antiglobulin test. K-negative blood donors will make up about 1 in 500 random blood donors (48).

Anti-Kp^{a}:

Antiglobulin test results often indicate that the antibody is IgG and reactive. Around 2% of randomly chosen people are Kp(a+) .

Anti-Kp^{b}:

Anti-Kpb is often IgG reactive when tested with an antiglobulin. The likelihood of finding suitable blood by screening random donor units is low, as only around one in 5,000 donors will have Kp(b-).

2.2 Leukemia Types

For diagnosis of cancer, in general, one or more of tests may be indicated in patients with a suggestive history or physical or laboratory findings such as imaging tests, biomarkers, biopsy, grading and molecular tests.

Leukemia is a malignant disease characterized by an overproduction of immature or abnormal leukocytes, which ultimately inhibits the production of normal blood cells and causes cytopenia-like symptoms (49). The American Cancer Society estimates that in the United States in 2023 there will be approximately 59,610 new cases of leukemia (of all forms) in adults and children, with approximately 23,710 fatalities (50).

A variety of factors probably are etiologically involved in leukemia (51). There are various forms of leukemia. Some forms of leukemia are more prevalent in young people. Adults are typically affected by other forms of leukemia. Acute and chronic leukemia are classifications based on the rate of progression of the disease. Other classification is based on the type of affected white blood cell and includes lymphoid and myeloid leukemia.

Acute leukemias:

Most of the cells in acute leukemias are immature and poorly differentiated (usually blast forms). There are various types of acute leukemia:

- Acute lymphoblastic leukemia (ALL).
- Acute myeloid leukemia (AML).

Compared to acute leukemias, Cells in chronic leukemias are more mature. They usually manifest in otherwise asymptomatic people as leukocytosis with or without cytopenias. Results and management vary greatly between:

- Chronic lymphocytic leukemia (CLL),
- Chronic myeloid leukemia (CML).

The likelihood of having leukemia is higher in those with:

- Exposure to ionizing radiation or chemicals, such as benzene, certain pesticides, or tobacco smoke contains polyaromatic hydrocarbons; can cause acute leukemias.
- Some types of ALL can sporadically be brought on by viral infection (e.g., human T lymphotropic virus types 1 and 2, Epstein-Barr virus); this is most frequently seen in areas where viral infections are prevalent, including Asia and Africa.
- History of prior hematologic conditions, such as myelodysplastic syndromes and myeloproliferative neoplasms, which can cause AML.
- Others.

Table (1) summarizes the major types of leukemia.

Table 1

Different types of Leukemia and their attributes

Types	Cell involved	Cytology	Symptoms	Statistics	Prevalence
AML	Immature myeloid WBCs	Oncogene mutations, single myeloblast mutation, cytogenetic abnormalities	Anemia, spontaneous bleeding	Both adults and children	80%
CML	Myeloid stem cells	Chromosomal translocation, granulocytes	Anemia, low platelet count, enlarged spleen	Rare in children	90%
ALL	Immature B or T cell and macrophages	Chromosomal aberration	Disturb marrow function	Common in children	33%
CLL	Lymphoid B or T cell	Chromosomal abnormalities	Swelling of lymph nodes	Commonly effect over 55 age	30%

Table (2) contains a list of myeloproliferative neoplasms (MPN). Tyrosine kinase inhibitors (TKI) therapy decreased the likelihood of illness progression to advanced stages, and 80–90% of CML patients approximately survive 10 years (52).

Table 2

Myeloproliferative neoplasm

Chronic myeloid leukemia
Polycythemia vera
Essential throbmocythemia
Primary myelofibrosis
Chronic neutrophilic leukemia
Chronic eosinophilic leukemia
Juvenile myelomonocytic leukemia
Myeloproliferative neoplasm

Note: Adopted from Pizzi M, Croci GA. The Classification of Myeloproliferative Neoplasms: Rationale, Historical Background and Future Perspectives with Focus on Unclassifiable Cases. 2021;13(22).

In leukemia, abnormal and functionless WBCs are formed as a result of immature cells or a deficiency in bone marrow cells, which make them unable to fight infection and protect the body against foreign substances.

The following tests are used to diagnose leukemia:

1. Complete blood count test (CBC): for cells counts and other features in the bloodstream to diagnose and monitor numerous diseases.
2. Blood film: for examination of blood cells noting any abnormal differences in size, shape and count.
3. Tissue biopsy: from the lymph nodes or bone marrow to determine the presence, type, and growth rate of leukemia.
4. Genetic abnormality test: to determine whether leukemic cells contain the Philadelphia chromosome (a specific genetic aberration in chromosome 22 associated with CML) or a genetic defect, Cytogenetics is for diagnosis and prognosis.
5. Cytochemistry: to identify the tissue structure of bone marrow cells.
6. Flow cytometry provides a complete description of the phenotypic of leukemic cells to aid in their identification, particularly in the identifying and monitoring of minimum residual disease (MRD) during treatment.
7. The lumbar puncture is a spinal fluid test used to determine whether leukemia has progressed to the central nervous system.

Acute leukemias spread quickly because they involve stem cells, known as blasts, which divide rapidly—both normal and malignant blood cells. World Health Organization (WHO) has proposed a classification of acute leukemia that includes genetic, immunologic, morphological and epidemiologic characteristics.

Table 3

WHO myeloid neoplasm and acute leukemia classification

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1);*DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome
B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Note. Adopted by: Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.

Bleeding is a frequent feature in hematological malignancies with bone marrow failure (53), its risk with declining platelet count is increasing in patients with acute leukemia (54).

2.3 Leukemia Classification Systems

Leukemia experts from France, the United States, and Britain collaborated to develop the French-American-British (FAB) classification system of acute myeloid leukemia (AML) in the 1970s. AMLs were divided into subtypes ranging from M0 to M7. Based on the cell type and cell maturity that gave rise to the leukemia, this was determined. The FAB categorization was based on how leukemia cells appeared under a microscope following routine staining. Now, the WHO classification is used instead of the FAB. The WHO system only requires 20% of marrow blasts to diagnose AML.

Table 4

Classification of AML

Category	Diseases
MPN	CML, PV, PMF, ET, mastocytosis
MDS	Refractory cytopenias (RA, RAEB, RARS, etc.)
MDS/MPN	CMML, aCML, JMML
AML	With/ without recurrent genetic abnormalities, MDS-related, therapy- related, DS- related

Note. Adopted by Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117(19):5019-32.

The patterns of occurrence for the various types of leukemia vary by age, sex, and racial and ethnic group. For instance, ALL is most common in children aged 2-4, whereas chronic lymphocytic leukemia (CLL) is uncommon before the age of 30, and is most common in the elderly. African-Americans are more likely than Caucasians to develop CML, but Caucasians are more likely than Asians to get CLL. Leukemia in children and adults has unknown origins, although there are some risk factors including; environmental, host and genetic predisposition that have been associated with increased or decreased leukemia risk.

2.3.1 Pathophysiology of ALL

Acute lymphoblastic leukemia is primarily caused by a spectrum of inherited genetic abnormalities. The majority of malignant transformation occurs at the level of pluripotent stem cells, although it can occasionally involve committed stem cells with a restricted capacity for self-renewal. Malignant cells supplant normal blood components due to aberrant proliferation, clonal expansion, aberrant differentiation, and decreased apoptosis (programmed cell death).

2.3.2 Pathophysiology of AML

AML is the most type of acute leukemia with genetic defects. Acute myeloid leukemia resulting from a variety of inherited genetic mutations. Occasionally, committed stem cells may play a role in the development of cancer since they have a reduced capacity for self-renewal, but pluripotent stem cells account for the vast majority of malignant transformation.

Malignant cells replace normal blood components due to abnormal proliferation, clonal expansion, aberrant differentiation, and decreased apoptosis (programmed cell death) (55).

2.3.3 Pathophysiology of CLL

The CD5+ B cells in chronic lymphocytic leukemia change from benign to malignant. Via the accumulation of mutations that cause monoclonal B-cell lymphocytosis, the B cells continuously become activated. B cells are continually activated due to the accumulation of mutations that result in monoclonal B-cell lymphocytosis. CLL is developed by the progressive accumulation of genetic mutations and the ensuing oncogenic transformation of monoclonal B cells.

Lymphocytes progressively spread from the bone marrow to the lymph nodes and other lymphoid tissues, causing splenomegaly, hepatomegaly, and systemic symptoms including fatigue, fever, night sweats, early satiety, and unexplained weight loss.

Anemia, neutropenia, thrombocytopenia, and a reduction in immunoglobulin synthesis are symptoms of advanced CLL caused by abnormal hematopoiesis. Up to two-thirds of people may have hypogammaglobulinemia, which increases the likelihood of

developing infectious problems. With a positive direct antiglobulin test, the risk of developing autoimmune thrombocytopenia and hemolytic anemia increases (56).

2.3.4 Pathophysiology of CML

The Philadelphia (Ph) chromosome is present in 90–95% of chronic myeloid leukemia cases. The Ph chromosome (9;22) is the result of a translocation between chromosomes 9 and 22. During this translocation, an area of chromosome 9 containing the oncogene Abelson leukemia virus homology (ABL) is transferred to chromosome 22 and joined to the breakpoint cluster region (BCR) gene. The chimeric fusion gene BCR-ABL produces the oncoprotein tyrosine kinase bcr-abl.

Untreated CML develops in three stages (57):

1. Phase I of the chronic condition is a languid initial stage that can last between five and six years.
2. Accelerated phase: Therapy failure, severe anemia, thrombocytopenia or thrombocytosis that is progressing, persistent or worsening splenomegaly, clonal proliferation, rising blood basophil counts, and rising marrow or blood blast counts.
3. The blast phase, which is characterized by an increase in the quantity of blasts in extramedullary locations such the bone, brain, lymph nodes, and skin as well as a 20% increase in the quantity of blasts in the blood or marrow.

2.3.5 Pathophysiology of MDS

A set of clonal hematopoietic stem cell diseases known as myelodysplastic syndromes is defined by particular mutations in hematopoietic stem cells, most frequently in RNA splicing-related genes. Ineffective and dysplastic hematopoiesis is a hallmark of myelodysplastic disorders.

Myelodysplastic syndrome's cause is unknown. Age-related risk factors include clonal proliferation and dominance of a particular hematopoietic stem cell, exposure to radiation, benzene, and chemotherapy (especially long or intense regimens and those that include alkylating agents, hydroxyurea, and/or topoisomerase inhibitors), and possibly other factors may be facilitated by somatic mutations. Anomalies of the chromosome, including deletions, duplications, and structural anomalies, are regularly discovered.

Both hypocellular and hypercellular bone marrow are possible. The ineffective hematopoiesis causes:

- Anemia: which is the most prevalent symptom of inefficient hematopoiesis,
- Neutropenia,
- Thrombocytopenia, or
- Marrow aplasia.

Iron overload eventually occurs in patients with severe, resistant, or chronic anemia. The most prevalent symptom is anemia, which is typically accompanied by macrocytosis and anisocytosis (58). Instead, a compromised potential for self-renewal and differentiation is the physiologic hallmark of these stem cells in myelodysplastic disorders (59).

2.3.6 Patterns of metastasis

Various leukemia subtypes have different metastatic patterns to different organs, such as the BM, lymph nodes, liver, spleen, central nervous system, skin, and testicles. The extrahepatic biliary tract, ureters, prostate, cervix uteri, central nervous system, thymus, ovaries, and pituitary were all areas where acute lymphoblastic leukemia had metastases in excess (60). Although leukemias are generally thought of as disorders of the hematopoietic and circulatory systems, as well as their metastatic spread of extramedullary (non-bone marrow (BM)) locations plays a key role in the disease's progression, severity, and patient prognosis. The spleen is a unique organ for metastasis of solid tumors, although it is a common location for the metastasis of leukemia (61).

Using flow cytometry as a diagnostic method to assess hematological neoplasms is without any doubt effective. In reality, together with molecular research and cytogenetics, it is now a crucial component in the diagnosis and categorization of numerous disorders such leukemias and lymphomas. The classification and diagnosis of acute leukemia highly depend on flow cytometry. This is demonstrated by the volume of literature articles on the identification of minimum residual disease by flow cytometry. While the morphology of the cells may suggest to a particular diagnosis, immunophenotyping and genetic examination of the cells are used (62).

2.4 Multiple Myeloma (MM)

Myeloma multiple is a plasma cell cancer that invades and destroys adjacent bone tissue while generating monoclonal immunoglobulin. Increased osteoclast activity surrounding MM cells leads to MM-induced bone destruction. Multiple myeloma is regarded as treatable but ultimately incurable in the end. (63).

Diagnosis and differentiation from other malignancies generally require a number of characteristics:

- Clonal bone marrow plasma cells or plasmacytoma.
- M-protein in plasma and/or urine.
- Organ dysfunction (hypercalcemia, renal failure, anemia, or skeletal abnormalities).

Clinical presentation:

- Bone pain and pathological fractures,
- Anemia (bone marrow failure),
- Recurrent infections (related to immunoparesis),Hypercalcemia,
- Renal failure,
- Abnormal bleeding (due to platelet dysfunction).

Fatigue and bone pain are the two presenting symptoms of MM most frequently. Around 75% of patients have anemia, which increases tiredness. In 80% of patients, osteolytic bone lesions are detectable (64). As a result, if MM is suspected, it is advised to conduct serum protein electrophoresis, serum immunofixation, and either a serum free light chain (SFLC) assay or a 24-hour urine protein electrophoresis with immunofixation. The International Staging System (ISS) classifies albumin and the protein β_2-microglobulin as following (65):

Table 5

Stages of MM according to ISS

Stage 1	Stage 2	Stage 3
B_2 microglobulin Albumin > 35 g/L	Neither 1 nor 3	B_2 microglobulin > 5.5 mg/dL

Note. Adopted by Firth J. Haematology: multiple myeloma. Clinical medicine (London, England). 2019;19(1):58-60.

Almost all MM patients will suffer from anemia at some point in their illness. This is often related to either the myelosuppressive effects of antimyeloma treatment or the impairment of erythropoiesis caused by the expansion of plasma cells in the bone marrow (66).

Individuals with MM are more likely to get an infection, with the risk of infection-related mortality being highest in the first three months following diagnosis (67, 68). Patients with MM now have better survival rates because to new treatment choices offered in recent years. As MM patients live longer, it is more crucial than ever to manage the disease's and treatment's side effects, including infections, thrombosis, and neuropathy.

In multiple myeloma, the imbalance of bone remodeling, which is caused by the activation of osteoclastogenesis and the concurrent inhibition of osteoblastogenesis, as well as the rise in osteocyte death, is the etiology of lytic lesions and progressive bone mass loss (69).

Table 6

When should a myeloma be considered during diagnosis?

Finding	When to consider myeloma
Anemia (normocytic or macrocytic)	Vitamin B12, folate and iron studies normal No history of blood loss No haemolysis No clear alternative explanation such as renal impairmen
Hypercalcemia	Parathyroid hormone appropriately suppressed Vitamin D normal No history of malignancy, sarcoidosis or use of medications such as thiazides
Renal impairment	No clear explanation including prerenal causes, primary renal disorders or obstructive conditions
Bony pain or fractures	Evidence of bony lesions on imaging Crush fractures in a young patient Pathological fractures in unusual sites
Monoclonal paraprotein	Usually required to confirm a diagnosis of multiple myeloma A small proportion of cases may be non-secretory with undetectable paraprotein

Note. Adopted by Eslick R, Talaulikar D. Multiple myeloma: from diagnosis to treatment. Australian family physician. 2013;42(10):684-8.

Steroids and chemotherapy can result in remissions. Bisphosphonates and radiation treatment are both used to treat pain caused by bone lesions (70). Daratumumab, a human CD38 monoclonal antibody, has been approved as a treatment of multiple myeloma (71-73), this substance binds red blood cells (RBCs), causing panagglutination during compatibility tests. During these investigations, routine blood screening revealed routinely positive findings from the indirect antiglobulin test (IAT) in daratumumab-treated patients. Daratumumab and SAR650984, two monoclonal antibodies that target CD38, have demonstrated potential in treating relapsed, refractory MM. Daratumumab's ability to bind to endogenous CD38 on the surface of RBCs may interfere with ICT results. CD38 is a prominent cell-surface receptor on multiple myeloma (MM) cells with a broad spectrum of functions, including signaling, receptor-mediated adhesion, and enzymatic activity (74).

Daratumumab interference with ICT may result in needless extra testing and may delay the release of blood products in MM patients, who are usually anemic and regularly require RBC transfusions. First-line treatment differs between patients initially classified as transplant eligible and those who are considered as nontransplant eligible. Patients who are transplant-eligible get a primary proteasome inhibitor (PI)-based induction, along with an immunostimulating medication, a monoclonal antibody directed against CD38, high-dose melphalan therapy, an autologous stem cell transplant, and a lenalidomide-based maintenance therapy. Patients who are considered to be ineligible for transplantation are treated upfront with a continuous combination regimen that includes either a CD38-directed monoclonal antibody in conjunction with the immunomodulator lenalidomide or a lenalidomide-PI combination followed by lenalidomide maintenance. Current methods for enhancing maintenance treatment techniques include investigating combination therapies to improve the rate of MRD and sustained MRD-negative remissions (75).

Smoldering MM (SMM) is a premalignant plasma cell disease with a higher tumor burden (M-protein ≥30 g/L and/or BM plasma cells 10–60%).

The accuracy of antibody screening and matching issues must be resolved as soon as anti-CD38 monoclonal antibodies are used. Before receiving a blood transfusion, patients should consent to RBC antigen phenotypic screening. When scheduling an appointment to receive blood, patients should let the blood bank know that they have received anti-CD38 monoclonal antibodies (76).

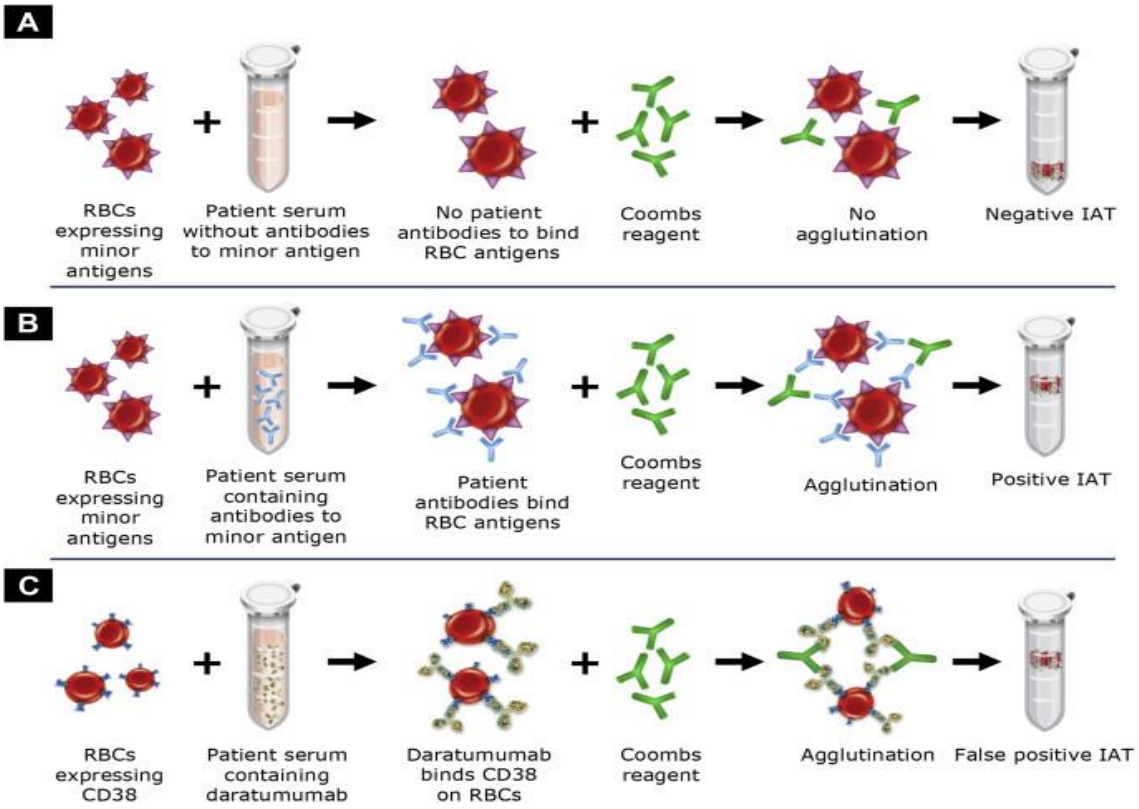
When patients who have received CD38 monoclonal antibodies require a blood transfusion and get positive IAT results or incompatible crossmatches, a clinical choice should be made based on the patient's situation. If necessary, an emergency blood transfusion shouldn't be delayed.

In an emergency situation, red blood cells that are ABO/RhD compatible can be used without crossmatching. Instead, the importance of the clinical situation should be compared to the risks of allogeneic immunity. If possible, dithiothreitol or DTT, proteolytic enzymes, and CD38-deficient RBCs from neonatal cord blood could be used to perform reagent RBC in IAT. Anti-CD38 monoclonal antibodies in serum might be interfered with using papain, an excessive amount of soluble CD38 protein, and anti-

CD38 monoclonal antibodies; RBC antigen genotyping could be done before transfusion. At present, DTT is the most prevalent method. It should be mentioned that individuals who have undergone anti-CD38 monoclonal antibody transfusions may not necessarily have positive IATs that are falsely positive; in particular, patients with a history of blood transfusions may generate RBC alloantibodies (77).

Figure 5

Mechanism of Daratumumab Interference in indirect Coomb's test (indirect antiglobulin test)



Note. Adopted by Chari A, Arinsburg S, Jagannath S, Satta T, Treadwell I, Catamero D, et al. Blood Transfusion Management and Transfusion-Related Outcomes in Daratumumab-Treated Patients With Relapsed or Refractory Multiple Myeloma. Clinical lymphoma, myeloma & leukemia. 2018;18(1):44-51.

The neutralization method, which involves adding anti-idiotype antibody or soluble CD38 to the patient's sample to bind daratumumab, inhibit interaction between daratumumab and RBCs, and neutralize daratumumab interference, however, it is expensive and the reagents are scarce. Genotyping has also been utilized successfully to provide blood products to MM patients treated with daratumumab (78).

Myeloma anemia is usually normocytic and normochromic. It is caused by tumor cells invading the bone marrow and cytokines inhibiting normal red blood cell synthesis (hematopoiesis). Kidney dysfunction may occur due to proteins secreted by the malignant cells. Typically, a bone marrow biopsy is carried out to determine the proportion of bone marrow occupied by plasma cells. This percentage is utilized in the diagnosis of myeloma. Immunohistochemistry (staining specific cell types with antibodies against surface proteins) is able to identify plasma cells that express immunoglobulin in the cytoplasm but rarely on the cell surface. Plasma cell clonality is frequently determined via flow cytometry. Cytogenetics can also be used to predict myeloma prognosis (79).

2.5 Pathophysiology of Alloimmunization

It is necessary to have a better understanding of the presentation of RBC antigen as well as the behavior of alloantibodies in order to create novel therapeutics. A diversity of factors affect the immune system's response. Frequently, the immune response to carbohydrate antigens occurs independent of the thymus (80).

IgM antibodies are produced when multivalent antigens directly activate B cells to generate antibodies without the assistance of helper T cells. People who lack a specific blood group antigen for carbohydrates on their red blood cells may have "naturally occurring" IgM antibodies (81). Reduced CD4/CD8 ratio, elevated B lymphocyte levels, and a lack of Treg cells were the most important immunological changes seen in alloimmunized patients (82). It is believed that the clinical circumstances of the RBC transfusion affect the possibility of alloimmunization in the recipient. It is believed that patients who get blood transfusions in their baseline stages of health have a lower risk of developing alloimmunization than those who receive blood transfusions during an inflammatory condition (83). A substantial risk factor for developing alloimmunization is suffering from acute pulmonary syndrome, as well as a viral illness or another inflammatory disorder at the period of the transfusion (84).

Several previous studies showed that RBC alloimmunization rates in sickle cell disease patients are among the highest of any population, followed by myelodysplastic syndrome (MDS), thalassemia, and autoimmune conditions (85, 86).

2.6 Mechanism of Alloimmunization

Antigenic epitopes are delivered to the T cell receptor of CD4 positive T cells when they bind to the groove of HLA class II molecules during the processing process. B cells that are able to recognize the antigenic epitopes are then activated by CD4 positive T cells, which causes the B cells to generate antibodies against the relevant epitopes. CD4+ T/T follicular helper (Tfh) cells are needed for the production of antibodies and cytotoxic T effector cells. Recognition between a foreign peptide presented by class II antigens of the major histocompatibility complex and the T cell receptor (TCR) of naïve CD4+ T cells is crucial to this process major histocompatibility complex (MHC II) (82).

2.7 RBCs alloimmunization in pregnancy

An uncommon disease of pregnancy is maternal red blood cell alloimmunization which results from the maternal immune system producing antibodies to the erythrocyte surface antigen that she does not have which induce the synthesis of IgG (immunoglobulin G) antibodies. Rh-negative women who are carrying Rh-positive infants occasionally develop Rh antibodies. If a fetus is Rh-positive for the particular erythrocyte surface antigens, these IgG antibodies might cross the placenta and cause hemolysis, a disease known as haemolytic disease of fetus and newborn (HDFN). It is thought that administering anti-D to the woman after the first birth may reduce this issue (87).

Red blood cell (RBC) alloimmunization in mothers is the most frequent reason for anemia in the fetus. The mechanism by which anti-Kell alloimmunization causes adverse fetal outcomes is more intricate. As anti-Kell antibodies inhibit erythropoiesis directly, fetal hemolysis is accelerated (87).

2.8 Adverse effects of blood transfusion

Adverse reactions that happen during blood donation or blood transfusion are examples of nonconformances that need to be reported and investigated. Transfusion services shall notify the blood collecting agency where the product was collected of any negative donor blood-related occurrences. This includes assessing instantaneous and delayed responses, as well as potential disease transmission.

Absolute iron deficiency in cancer patients is caused mostly by bleeding. Most importantly, iron homeostasis is frequently impaired in cancer patients due to the production of proinflammatory cytokines and the elevation of hepcidin, the major regulator of iron absorption and release (88). Intravenous (i.v.) or oral administration of iron are given to correct iron deficiency anemia, reduced RBC transfusions as well as to increase response to erythropoiesis-stimulating agents. However, the European Medicines Agency (EMA) suggests that only qualified professionals should provide intravenous iron to assess and manage allergic and anaphylactoid reactions, and only when resuscitation facilities are nearby (89).

Transfusion iron overload correlates positively with the frequency of blood transfusions. The iron content of a blood transfusion is usually between 200 and 250 milligrams. Patients who receive 10–20 units of blood are at increased risk of developing iron overload (90).

2.8.1 Acute non-infectious blood transfusion adverse reactions

1. Acute haemolytic transfusion reactions
2. Febrile nonhaemolytic transfusion reactions
3. Allergic reactions
4. Urticaria
5. Anaphylaxis
6. Transfusion related acute lung injury
7. Acute non-immune mediated adverse reactions (Transfusion related sepsis)
8. Non immune haemolytic reactions
9. Transfusion associated circulatory overload
10. Transfusion associated dyspnea
11. Acute hypotensive transfusion reaction
12. Metabolic and haemostatic derangement
13. Citrate toxicity
14. Hyperkalemia
15. Hypocalcaemia
16. Coagulopathy
17. Hypothermia
18. Air embolism

2.8.2 Delayed serologic reactions (occurring after 24 hours or up to month/years after transfusion)

It is impossible to completely avoid delayed hemolytic transfusion reactions (DHTRs). Nevertheless, their risk can be decreased by avoiding unnecessary transfusions and by giving RBCs that are prophylactically antigen matched for at least C/c, E/e, and K (and perhaps for Fy^a/Fy^b, Jk^a/Jk^b, and S/s as well) (91).

2.8.2.1 Delayed immune mediated reactions

1. Delayed haemolytic transfusion reactions (DHTRs)
2. Alloimmunization
3. Transfusion associated immunomodulation
4. Transfusion-associated graft versus host disease
5. Posttransfusion purpura

2.8.2.2. Delayed non-immune mediated reactions

Table 7

Acute and delayed complications of blood transfusion

COMPLICATION	SYNONYM or RELATED
Acute Hemolytic Reaction	Immune mediated hemolysis, acute febrile hemolytic, ABO incompatibility
Acute Lung Injury	Transfusion related acute lung injury (TRALI)
Air Embolism	
Anaphylaxis	Severe Allergic reaction Anaphylactic reaction
Bacterial Contamination	Sepsis
Febrile Non-Hemolytic Reaction	Simple febrile reaction
Hypothermia	
Metabolic Abnormalities:	
Citrate Toxicity	Hypocalcaemia
Hyperkalemia	
Impaired Oxygen Delivery	Blood storage defect
Mild Allergic Reaction	Urticarial, hypersensitivity
Volume Overload	Transfusion-associated circulatory overload (TACO)
Infection:	
Parasites	
Prion	
Virus	
Iron Overload	Transfusion-related hemochromatosis
Sensitization to non-RBC Antigens:	
HLA Alloimmunization	
Platelet Refractoriness	
Post-Transfusion Purpura	PTP

Note. Adopted by Roback JD GB, Harris T, Hillyer CD, eds. Technical Manual. Washington, DC: AABB. 2011.

2.8.3 Risks or limitations of RBC transfusions

Blood transfusion risks and side effects have increased along with the extensive use of blood, and they should be minimized to the greatest extent possible.

There are some risk of complications:

• Reactions to transfusion and circulatory overload

• Pathogens that are known or unknown

• Surgical treatment for certain cancers is associated with a lower survival rate

• Due to immune suppression, a higher likelihood of infection (92).

Alloimmunization may be more common in these locations due to a lack of awareness of alternative blood group system screening methods. Alloimmunization can cause fatal or severe hemolytic transfusion reactions, and kidney failure could result from this hemolysis. By giving Rh(D) negative and Kell negative blood to Rh(D) negative and Kell negative patients, alloimmunization to the D and K (Kell) antigens is reduced (93).

Universal leukocyte reduction (ULR), which eliminates the donor's WBCs to avoid WBC apoptosis, necroses, and cytokine release, plays a significant role in transfusion safety to minimize the frequency and severity of febrile transfusion reactions, cytomegalovirus transfusion-transmission risk as well as reduce recipient mortality and organ dysfunction (94).

Patients with hematologic malignancies, bone marrow transplantation, hereditary immune system abnormalities, and other illnesses that predispose patients susceptible to transfusion-associated graft-versus-host disease (TA-GvHD) should receive irradiated cellular blood products (95).

2.9 HLA antibodies

Individual predisposition to producing alloantibodies affects alloimmunization. It is critical to understand the clinical conditions that raise the likelihood of alloimmunization since it can have an impact on how a patient is managed and help researchers had better understand the causes of alloimmunization-related transfusion responses.

One of the key components of recipient-impact alloimmunization is the genetic component that causes the diversity of Human Leukocyte Antigen (HLA). Three mechanisms are contributed in HLA antibody generation: transfusions, pregnancy and transplantation (96).

The primary immunological factor in the degradation of transfused platelets (PLTs) is HLA alloimmunization (97). HLA alloimmunization is not a common occurrence in PLT multitransfused patients. In a study including 114 patients with non-lymphocytic acute leukemia 58%, of them did not develop alloimmunization during induction or within six to eight weeks after the onset of chemotherapy (98).

The transfusion product or the immunological state of the recipient may need to be adjusted in order to reduce alloimmunization and alloimmune refractoriness. The most properly studied approaches focus on particular products, such as single-donor platelets, prophylactic HLA-matching, and leukoreduction or irradiation of cellular blood products (99). Additionally, there are factors of alloimmunization that are independent of the recipients. Some components of transfused RBC units that are not RBCs include leukocytes and their remnants, platelets and their remnants, and soluble components like cytokines. One immune response to foreign antigens is called erythrocyte alloimmunization, and the alloantigens are foreign erythrocyte antigens. The proteins containing the alloantigenic epitopes are processed after erythrocytes are phagocytosed by macrophages, monocytes, or dendritic cells.

2.10 Irradiated blood components

Irradiation of whole blood and cellular components is used to prevent transfusion-associated graft: versus-host disease (TA-GvHD). The suggested method to avoid TA-GvHD is gamma or X-irradiation of blood components using validated systems.

It is desirable to irradiate any components that could contain viable T cells. Red cells, platelets, and granulocytes, which are made either from whole blood or by the apheresis procedure, are among them. (100). Skin rashes, difficulties with liver function, and digestive system issues are all signs of an immunological attack by the lymphocytes in donor blood that is frequently lethal. Graft-versus-host response is frequently unresponsive to treatment, thus, the preferred course of action is prevention. It has never been documented that receiving irradiation blood can cause a graft-versus-host response. Irradiation, however, can also damage RBC units, leading to leakage of RBC contents, instability of the membrane, and a greater postinfusion clearance rate.

The elevated potassium content of the red blood cell products is the primary issue with irradiation. Irradiated blood's potassium content tends to rise during storage more quickly than that of regular, non-irradiated products, and the level of potassium is inversely proportional to the radiation dosage. All human leukocyte antigen (HLA)-selected components must be radioactively eliminated, despite the patient's immunocompetence (101, 102).

2.11 Washed Red Blood Cells

Red blood cells that have undergone washing are those in which the majority of the plasma, platelets, and white blood cells have been removed and replaced with saline or another kind of preservation solution.

If all patients who require RBC transfusions received saline-washed RBC instead of packaged RBC, the incidence of adverse reactions to blood transfusions could be reduced further.

Due to potassium leakage from the RBCs and RBC lysis, serum potassium content in RBC units significantly rises with time (103). When erythrocytes are washed with 0.9% sodium chloride utilizing automated cell washers, plasma and consequently potassium are removed to produce washed RBCs. Transfusions of washed RBC can reduce the potassium dose. When hemodialysis patients require transfusion, washed RBCs are advised during dialysis-free intervals to prevent transfusion-related hyperkalemia.

Chapter Three

Methodology

3.1 Study context

Antibody identification Panel test was performed. Reagent cells licensed by the FDA for this purpose must express the following antigens: D, C, E, c, e, M, N, S, s, P1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b.

The data collection sheet was prepared to collect demographic, medical and transfusion history for all study participants. Data about the patient's age, gender, ABO and Rh blood groups, and the total number of blood components transfused, frequency, indication for transfusion and specificity of alloantibodies, disease staging and medications obtained through direct patient interviews or medical records.

3.2 Study design

This study was conducted using cross-sectional design. Oncohematology patients from the An-Najah National University Hospital in Nablus, the Istishari Arab Hospital in Ramallah and the Beit Jala Governmental Hospital in Bethlehem were considered.

3.2.1 Population

Patients with blood cancers, such as leukemia, multiple myeloma, myelodysplastic syndromes (MDS) and other hematological malignancies who sought treatment in hematology and oncology clinics or who were admitted to the hospitals. We used the e Raosoft® sample size calculator (http://www.raosoft.com/samplesize.html).

3.2.2 Inclusion and exclusion criteria

Patients, at any age or gender, were eligible to participate in the research provided they met the following criteria: Patients with positive indirect coomb's test diagnosed with hematological malignancies: leukemia or multiple myeloma; anemic and received blood transfusion. The last transfusion occurs at least before two weeks.

Patients with negative indirect coomb's test admitted to the hospital and diagnosed with hematological malignancies and never taken blood. These groups were excluded.

3.2.3 Ethical Considerations

The study's ethical approval was given by Institutional Review Board (IRB) at An-Najah National University. Ref: Mas. Feb. 2022/19 (Appendix A).

A brief justification was provided to the individual patients or their guardians. Following that, the patients were requested to sign a form (Appendix C) authorizing their participation as well as blood sample gathering.

3.3 Laboratory Investigations

About three milliliter of whole blood was drawn from each participant in an ethylene di-amine tetra-acetic acid (EDTA) tube. The samples were stored in a refrigerator at 4º C until the tests were performed within the day. The serum samples were stored at -20ºC until the tests were performed. The collected samples were sent to the blood bank at An-Najah National University Hospital for testing.

3.4 Methodology

The data generated from the data collection sheet, which included the patient's medical information and sample analysis, were analyzed. This study spanned February to August of 2022. Blood samples were taken from each patient. Utilizing a commercial three-cell panel, plasma was screened for alloantibodies (Capture-R I-II-II) if the patient had not had recent result of ICT. A commercial eleven-cell panel also served to establish the antibody specificity of all positive samples.

The screening test results were done automatically using IMMUCOR instrument (Appendix D). Reagent cells authorized by the Food and Drug Administration (FDA) for panel test expressed the following antigens: D, C, E, c, e, M, N, S, s, P1, Le^a, Le^b, K, k, Fy^a, Fy^b, Jk^a, and Jk^b. In order to exclude out autoantibodies, an autocontrol was conducted on patients' RBCs against their own plasma in combination with each screen. Crossing out the types of antibodies, which did not interact for each result got out from the machine, was done manually for the identification of specific antibodies.

Solid phase technology was used in the capture antibody/antigen test method. A microtiter well was filled with LISS and plasma or serum. The RBCs to be tested were previously fixed to the well bottom of the wells. The antibody/antigen reaction is then allowed to take place during an incubation period of 37 degrees in the wells. For the

panel's identification, antigens that did not react were manually matched and crossed out.

3.5 Statistical analysis

To compare the two groups, positive and negative ICT, descriptive statistical analysis was done. The population frequency distribution for each covariate was computed. Group comparisons for quantitative and qualitative variables employed the t-test, Mann–Whitney test, or Chi-squared test, depending on the statistical distribution of variables. Univariate and multivariate regression analysis were performed for factors associated with bleeding. A two-sided *P*-value < 0.05 was considered to indicate statistical significance. Chi-square (categorical variables) or Fisher's exact test (linear variables) were used to compare between the two groups. R version 4.1.1 has been utilized for analyzing the data gathered (https://www.R-project.org).

Chapter Four

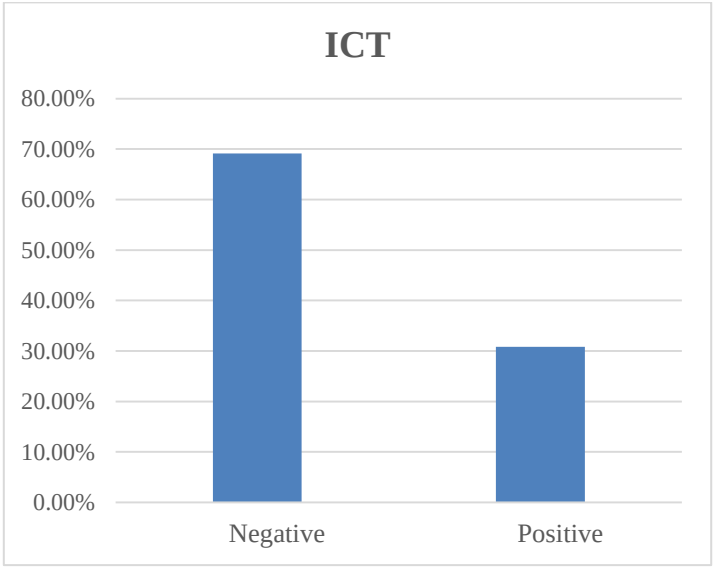
Results

4.1 Patient's characteristics

This study included approximately 94 participants, of whom 65 (69.15%) had negative ICT and 29 (30.85%) had positive ICT. The population had an equal distribution of males and females. The mean age was 28.37± 16.99 years. Roughly (87.23%) were positive for Rh (D). The majority of participants carried blood type A (42; 44.68%), followed by O (29; 30.85%), B (20; 21.28%), and AB (3; 3.19%). About (21.28%) of participants receiving treatment in West Bank hospitals were from the Gaza Strip.

Figure 6

Frequency of ICT results and red cell alloimmunization in hematological malignancies in Palestine



Additionally, 10.5% of males and 19.5% of females had positive ICT results. The average age of participants with positive ICT was 30.41± 13.3, and the majority of them were ≥ 18. Furthermore, 82.8% of participants who had positive ICT were adults. Blood type A positive was present in 11 (37.9%) of the positive ICT participant. 36.17% of participants had an acute leukemia, and 22.34% had chronic leukemia. The percentage of multiple myeloma patients in this study was 14.89%. Three participants (3.19%) had MDS. Additionally, the majority of participants with positive ICT (51.7%) had CML diagnoses.

Table 8

Population demographic characteristics and ICT descriptive statistics

	ICT			
Variable	Total (%)	Negative	Positive	p
Gender				
Female	47 (50%)	28 (43.1%)	19 (65.5%)	0.044 a
Male	47 (50%)	37 (56.9%)	10 (34.5%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Age				
n (Missing)	94 (0)	65 (0)	29 (0)	0.45 c
Mean ± Std-Dev	28.37 ± 16.99	27.46 ± 18.4	30.41 ± 13.3	
Age				
< 18	31 (32.98%)	26 (40.0%)	5 (17.2%)	0.03 a
≥ 18	63 (67.02%)	39 (60.0%)	24 (82.8%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Region				
Gaza	20 (21.28%)	17 (26.2%)	3 (10.3%)	0.084 a
West Bank	74 (78.72%)	48 (73.8%)	26 (89.7%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	

Table 9

Medical data of population with ICT results and effects on alloimmunization

	Total (%)	Negative ICT	Positive ICT	p
Blood Group (ABO)				
A	42 (44.68%)	31 (47.7%)	11 (37.9%)	0.771 a
AB	3 (3.19%)	2 (3.1%)	1 (3.4%)	
B	20 (21.28%)	14 (21.5%)	6 (20.7%)	
O	29 (30.85%)	18 (27.7%)	11 (37.9%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Blood Group (Rh)				
Negative	12 (12.77%)	11 (16.9%)	1 (3.4%)	0.071 a
Positive	82 (87.23%)	54 (83.1%)	28 (96.6%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Diagnosis				
ALL	27 (28.72%)	26 (40.0%)	1 (3.4%)	<0.001 a
AML	7 (7.45%)	7 (10.8%)	0 (0.0%)	
CLL	4 (4.26%)	2 (3.1%)	2 (6.9%)	
CML	17 (18.09%)	2 (3.1%)	15 (51.7%)	
CMML	1 (1.06%)	0 (0.0%)	1 (3.4%)	
MDS	3 (3.19%)	2 (3.1%)	1 (3.4%)	
MM	14 (14.89%)	9 (13.8%)	5 (17.2%)	
Unspecified	21 (22.34%)	17 (26.2%)	4 (13.8%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Diagnosis				
Acute	34 (36.17%)	33 (50.8%)	1 (3.4%)	<0.001 a
Chronic	21 (22.34%)	4 (6.2%)	17 (58.6%)	
other	25 (26.6%)	19 (29.2%)	6 (20.7%)	
MM	14 (14.89%)	9 (13.8%)	5 (17.2%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Number of received RBCs units				
< 16	40 (42.55%)	24 (36.9%)	16 (55.2%)	0.098 a
≥ 16	54 (57.45%)	41 (63.1%)	13 (44.8%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	
Stage of disease				
other	27 (28.72%)	19 (29.2%)	8 (27.6%)	0.006 a
In relapse	26 (27.66%)	12 (18.5%)	14 (48.3%)	
In remission	41 (43.62%)	34 (52.3%)	7 (24.1%)	
Total	94(100%)	65 (100.0%)	29 (100.0%)	

Table 10

Alloimmuniztion in hematologic malignancies patients, Palestine

Total (100%)		Neg ICT	Pos ICT
Antibody Identification (Panel Test)			
Anti-D	2 (2.13%)	0 (0.0%)	2 (6.9%)
Anti-E	4 (4.26%)	0 (0.0%)	4 (13.8%)
Anti-E, Fya	1 (1.06%)	0 (0.0%)	1 (3.4%)
Anti-E,K	1 (1.06%)	0 (0.0%)	1 (3.4%)
Anti-Fya,S	1 (1.06%)	0 (0.0%)	1 (3.4%)
Anti-Jka,Kpa	2 (2.13%)	0 (0.0%)	2 (6.9%)
Anti-K	6 (6.38%)	0 (0.0%)	6 (20.7%)
Anti-K, E	1 (1.06%)	0 (0.0%)	1 (3.4%)
Autoab-unspecified	2 (2.13%)	0 (0.0%)	2 (6.9%)
Multiple Abs	1 (1.06%)	0 (0.0%)	1 (3.4%)
Unspecified	8 (8.52%)	0 (0.0%)	8 (27.6%)
Total	94(100%)	65 (100.0%)	29 (100.0%)

Figure 7

Red Cell Antibody Panels

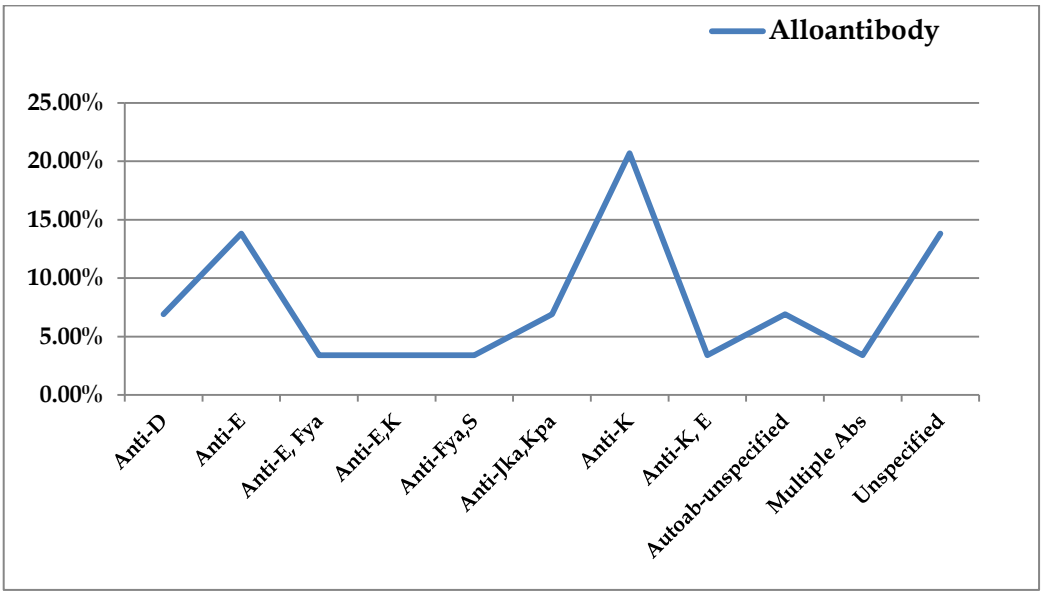
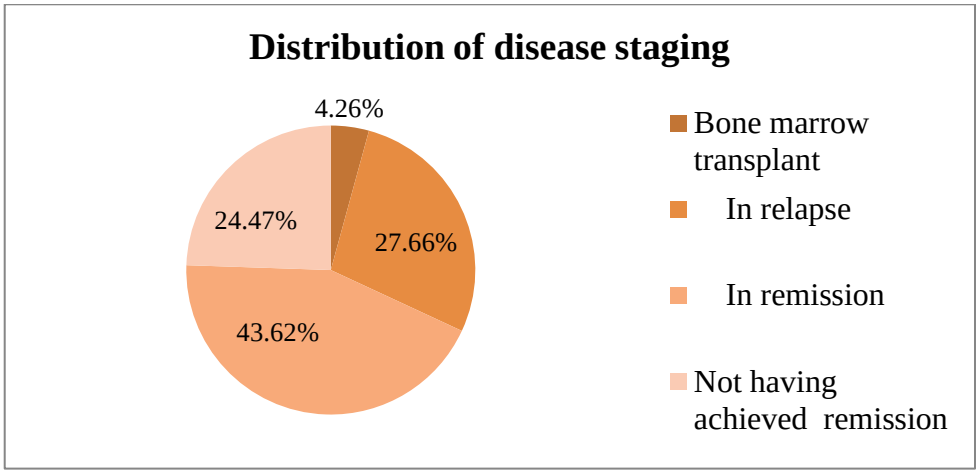


Figure 8

Distribution of disease staging



4.2 Alloantibody Screening and Specificity

The majority of the participants had a single alloantibody for Anti-K (20.7%), Anti-E (13.8%), and Anti-D (6.9%) with an equal percent (20.7%) of Kell and Rh (both E and D) antibodies. One participant (3.4%) had multiple antibodies and six participants (23.9%) had two antibodies and were distributed as follows: Anti-E/K (6.9%), Anti-Jk^a/Kp^a (6.9%), Anti-E/Fy^a (3.4%), and Anti Fy^a/S (3.4%).

Our data revealed that females are more likely to be alloimmunized (65.5%) than males (34.5%). According to the disease staging, seven participants (24.1%) were in remission while eight (27.6%) were not in remission, and fourteen participants (48.3%) were in relapse. Fifteen patients were diagnosed with CML (51.7%).

4.3 Risk factor associated with ICT

To find the relationship between the ICT group and the explanatory factor, univariate and multivariate linear regression were used. The ICT were modified for gender, age, and disease stage in the multivariate model. Table E1 demonstrates that participants between the ages of 18 years or more (adults) experience an increase in the positive ICT risk (AOR, 0.25, 95%CI, 0.08-0.81). Additionally, the chronic stage disease has a positive ICT more risk increase (AOR, 0.09, 95%CI, 0.02-0.43). Furthermore, an acute stage has a risk (AOR, 9.58, 95%CI, 1.01-90.6) There was not increase in the positive ICT risk for number of RBC units received sixteen units or more (AOR, 2.02, 95%CI, 0.72-5.70).

Chapter Five

Discussion

5.1 Rate of alloimmunization

Patients with leukemias are among the most frequent transfusion recipients for about 24,000 annual deaths in the United States (104). In several studies (105-109), it has been debated whether patients who require blood transfusions for a prolonged period of time should be matched for antigens in addition to ABO and D for individuals with conditions like sickle cell disease or thalassemia in order to prevent the formation of RBC alloantibodies.

For chronic types of anemia like myelodysplasia, the purpose of RBC transfusion is to improve quality of life and function and to minimize the negative consequences of long-term RBC transfusion exposure (110). Up to 90% of MDS patients receive a blood transfusion (111).

This study shows a quantitative description of RBC immunization in patients who have received many transfusions diagnosed with leukemia and multiple myeloma in Palestine. The overall immunization rate was 30.8% in Palestine compared to 9.0 % in another study from Netherlands (81) which has been conducted on patients with hematologic and oncologic malignancies.

The rate of alloimmunization observed in the present study was 30.85%, which is higher than that reported in many studies of RBC alloimmunization. It can be due to variety of types of blood cancers and stages of multi transfused patients. The most frequent alloantibody was Anti-K (20.7%) close to the rate of the same alloantibody in China and Southeast Asia (22.0%)(112). Anti-K is the next most common immune red cell antibody after ABO and Rh system. In this study, 20.7% of alloantibodies were against antigens from the Rh-system and 20.7% were against antigens from the Kell-system.

Anti-Rh antigens and anti-Kell antigens were the most common in regards to antibody specificity in our study similar to different previous studies, with alloimmunization rate of 23% in Brazil, 3.4% in North India, 23% in China, 4.5% in Iraq Kurdistan, 12% in South Australia and 9.4% in Taiwan. Table (E1) in the appendix E shows the comparison with many studies with patients of hematological malignancies and hemoglobinopathies, who are multi-blood transfused in different countries.

In this study, alloimmunization is greater than in the thalassemic population; it affects 10–20% of thalassemia patients who depend on blood transfusions (106, 113). As mentioned in the results of our study, Anti-K was the highest frequency somewhat similar to a study about sickle cell disease in Palestine (105).

Antibody prevalence rates are known to vary throughout countries, most likely as a result of differences in transfusion practices, testing methods, and gene frequencies. There is no association found between RBC's storage time and anti-K formation in Netherlands (114). Kell phenotypes with homozygous expression of the K antigen (K+k-) are regarded uncommon, with K-k+ being the most prevalent. Transfusion of K+ units is a major cause of alloimmunization in Canadian prenatal patients (115). Several authors state that patients who may develop a long-term transfusion dependency should be phenotyped for the Rh/K antigens so they can be transfused according to that phenotype, preventing alloimmunization. Females seem to be risk factors for alloimmunization as seen in this study (81) (116).

The frequency of alloimmunization detected in transfused patients in China and Southeast Asia was 22%, it was found that the most prevalent alloantibodies are those directed against antigens from the Kell-system (112). Additionally, it was shown that the most prevalent alloantibodies in Punjab (42.8%) were those belonging to the Kell-system (117).

Thalassemia, sickle cell anemia, and myelodysplastic syndrome were linked to the high number of transfusions (118, 119). Some studies indicate a relationship between the number of prior pregnancies and the rate of alloimmunization as a result of higher allogeneic exposure, and thus puts females at higher risk for RBC alloimmunization (120).

A total of 6.9% of participants were had autoantibodies, these high autoantibody concentrations have a hiding effect that prevents the identification of alloantibodies (121). However, the identification procedure of autoantibodies was not performed.

The differences in antigenicity among blood donors and recipients, immune condition of blood recipients, and immune system alterations in the body impact of transfusions on the recipient's are at least three primary factors that account for the differences in alloimmunization rates (112).

Previous studies may have found a lower alloimmunization rate than what was observed in our Palestinian population study. This might be because all Palestinian thalassemia patients receive post-storage leukoreduced blood.

In addition, our study found a noteworthy correlation between alloimmunization and those aged 18 years or older ($p < 0.05$), as well as between alloimmunization and leukemic patients (acute and chronic) other than MM. In addition, there is no statistically significant link observed between the quantity of received packed red blood cells (RBCs) and the severity of disease ($p > 0.05$). In the current investigation, we found that no conclusive correlation between alloimmunization, ABO blood group, and Rh-D.

5.2 Limitations

There are limitations of this study; it is notable that the results presented from three medical centers mainly specialized not governmental hospitals (due to the lack of data documentation) that necessarily affect the interpretation of these results in addition to insufficient sample size for statistical measurements. The association between age (pediatric and adult) and alloimmunization rates was insignificant.

5.3 Conclusions and Recommendations

This study showed a high rate of alloimmunization among hematological malignancies mainly leukemia and multiple myeloma in different regions of West Bank, Palestine. The predominant alloantibodies were mostly directed against the Kell and Rh antigens. RBC compatibility in ABO, Rh, and Kell system may reduce the risk of the most of alloimmunizations and consequently transfusion reactions in these patients.

One method of preventing RBC alloimmunization is examined transfusion or transfusion avoidance. For hematological malignancies patients, matching for a few blood group antigens is advised to reduce the development of alloantibodies such as D, C/c, E/e and K.

List of Abbreviations

Abbreviations	Meaning
AHG	Anti-human globulin
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
BM	Bone marrow
CBC	Complete blood count
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CSAs	Clinically significant antibodies
DAT	Direct antiglobulin test
DHTRs	Delayed hemolytic transfusion reactions
DTT	Dithiothreitol
EDTA	ethylene diamine tetra-acetic acid
EMA	European Medicines Agency
EPO	Erythropoietin
FAB	French-American-British
FDA	Food and Drug Administration
Hb	Hemoglobin
Hct	Hematocrit
HDFN	Hemolytic disease of fetus and newborn
IAT	Indirect antiglobulin test
ICT	Indirect coomb's test
IgG	immunoglobulin G
IS	Immediate spin
ISBT	International Society of Blood Transfusion
ISS	International Staging System
IV	Intravenous

LISS	Low ionic strength solution
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MM	Multiple myeloma
MPN	Myeloproliferative neoplasms
MRD	Minimal residual disease
PLTs	Platelets
RBCs	Red Blood Cells
Rh	Rhesus
SFLC	Serum free light chain
SMM	Smoldering multiple myeloma
TAGvHD	Transfusion-associated graft-versus-host disease
TCR	T cell receptor
Tfh	T follicular helper
TKI	Tyrosine kinase inhibitors
TRALI	Transfusion-related acute lung injury
VTE	Venous thromboembolism
WHO	World Health Organization

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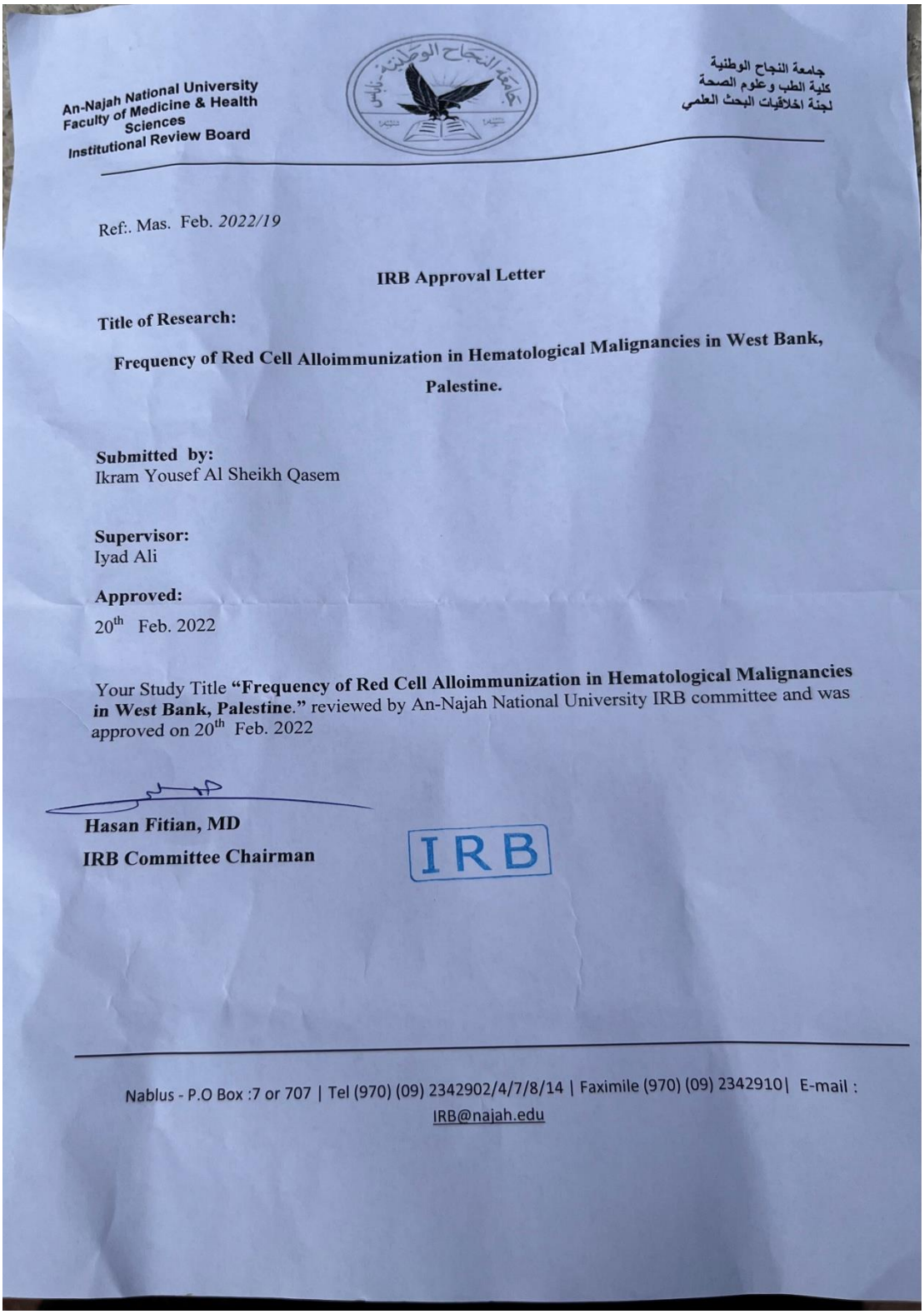
Appendices

Appendix A

IRB



An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref:. Mas. Feb. *2022/19*

IRB Approval Letter

Title of Research:

Frequency of Red Cell Alloimmunization in Hematological Malignancies in West Bank, Palestine.

Submitted by:
Ikram Yousef Al Sheikh Qasem

Supervisor:
Iyad Ali

Approved:
20th Feb. 2022

Your Study Title **"Frequency of Red Cell Alloimmunization in Hematological Malignancies in West Bank, Palestine."** reviewed by An-Najah National University IRB committee and was approved on 20th Feb. 2022

Hasan Fitian, MD
IRB Committee Chairman

IRB

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix B

Data Collection Sheet

Frequency of Red Cell Alloimmunization in Hematological Malignancies in West Bank, Palestine

Hospital/ Department/Clinic:	
Age: ☐ <18 ☐ 18-24 ☐ 25-30 ☐ 30-39 ☐ 40-50 ☐ >65	**Date of birth** ---/---/-----
Sex: ☐ Female ☐ Male	**Diagnosis:** **Date of Diagnosis** ☐ AML -----/----- ☐ ALL mon/yr ☐ CML ☐ CLL ☐ MM ☐ Lymphoma ☐ MDS
Did the patient receive transfusion: ☐ No ☐ Yes, (PRBCs, FFP, Plts) Number of units transfused…….. Filtered PRBCs/Non-Filtered	**What are the transfusion's interval between previous unit and next one(s)?** ☐ Once a week ☐ Multi-units a week …….. ☐ Once every 2 weeks ☐ Once a month ☐ Once after each months ☐ Rare

Patient Blood Group: **□ O (Rh(D)-Positive, Rh(D)-Negative)** **□ A (Rh(D)-Positive, Rh(D)-Negative)** **□ B (Rh(D)-Positive, Rh(D)-Negative)** **□ AB (Rh(D)-Positive, Rh(D)-Negative)**	
Is the patient frequently anemic? **□ No** **□ Yes, most recent Hb was in g/dL:** **pre-transfusion …….** **post-transfusion ……**	**Indirect antiglobulin test (IAT):** **□ Negative** **□ Positive (1+/2+/3+/4+)**
Most recent creatinine result ……mg/dL **Most recent bilirubin result ……..mg/dL**	**Direct Coomb's Test (DAT):** **□ Negative** **□ Positive (1+/2+/3+/4+)** **Autocontrol ……..**
For MM cases: **Was the patient receive (darzalex, bortezomib, dexamethasone)?** **□ No** **□ Yes**	**Was the Panel Test done?** **□ No (the current Ab(s) is/are ……)** **□ Yes, (the type was ……)**
Had the blood unit been evaluated the red cell antigens (phenotyping)? **□ No** **□ Yes ……..**	**Medications …….** **Adverse reaction occurred?** **□ No reaction** **□ Yes, cause:** **Allergy** **Febrile non-hemolytic** **Acute hemolysis** **Bacterial contamination** **Viral infection** **Delayed hemolysis** **TRALI** **TACO** **Anaphylaxis**

Appendix C

Consent Form

Appendix C

Consent Form

جامعة النجاح الوطنية

كلية الدراسات العليا

معدل حدوث أجسام مضادة وخصائصها لدى مرضى سرطانات الدم في إلضفة الغربية– فلسطين

Consent Form/نموذج الموافقة

بعد تكون الأجسام المضادة لأنتيجينات خلايا لدم الحمراء من أحد الأسباب المرتبطة بمضاعفات نقل الدم. لا سيما عند المرضى الذين يتم نقل الدم لهم بشكل دوري.

فإنني أنا الطالبة إكرام الشيخ قاسم طالبة في كلية الدراسات العليا في جامعة النجاح الوطنية بصدد إجراء دراسة بحثية للتعرف على الأجسام المضادة لأنتيجينات خلايا الدم الحمراء المتكونة عند مرضى سرطانات الدم ومن أهمها اللوكيميا بأنواعها بهدف العمل على البحث عن البات للحد من تكون مثل هذه الأجسام المضادة عند تكرار نقل الدم وتسهيل عملية البحث عن وحدات دم منطابقة للمريض والتقليل من مضاعفات نقل الدم المرتبطة بوجودها.

ونؤكد لك بأن نتائج هذا الفحص سوف تستخدم لغرض البحث العلمي فقط. ويمكنك وطبيبك المختص الاطلاع على نتائج الفحوصات الخاصة بك. هذا البحث يتضمن بعض الاسئلة عن طبيعة حالتك المرضية وتاريخك المرضي الشخصي ومن ثم سوف يتم سحب عينة دم منك وفحصها لتحديد وجود من عدم وجود أجسام مضادة ومعرفة نوعها. في حال موافقتك على المشاركة في الدراسة. نحيطك علما انه يمكنك الانسحاب من الدراسة في اي مرحلة دون الحاجة لشرح الاسباب ودون التأثير على علاجك. وان معلوماتك سرية ولن يتم استخدامها الا لغرض البحث العلمي.

شكرا لتعاونكم

في حال كان المريض قاصرا على ولي الامر ان يوافق على مشاركته ومن ثم التاكد من رأي المريض نفسه.

توقيع المشارك/ولي الامر :

التاريخ :

Appendix D

IMMUCOR Device

Appendix E

Table of Study

Table E1

Univariate and multivariate association on between ICT and other factors

		95%CI				95%CI		
Variable	OR	Low	Hight	p	AOR	Low	Hight	p
Gender								
Male	2.51	1.01	6.24	0.05	2.23	0.82	6.08	0.12
Female	Ref							
Age								
< 18	Ref							
≥ 18	0.31	0.11	0.92	0.04	0.25	0.08	0.81	0.02
City								
West Bank	0.330	0.087	1.220	0.100	0.48	0.12	1.97	0.31
Gaza	Ref				Ref			
Blood Group								
A	1.41	0.12	17.12	0.79	2.44	0.17	34.21	0.51
B	1.17	0.09	15.46	0.91	1.99	0.13	31.49	0.62
O	0.82	0.07	10.12	0.88	1.68	0.12	24.38	0.70
AB	Ref				Reg			
Stage of disease								
Acute	10.42	1.17	93.21	0.04	9.58	1.01	90.60	0.047
Chronic	0.07	0.02	0.31	<0.001	0.09	0.02	0.43	<0.001
MM	0.57	0.14	2.37	0.44	0.67	0.12	3.78	0.65
Other	Ref				Ref			
Number of received								
RBCs *units*								
≥ 16	2.10	0.86	5.11	0.10	2.02	0.72	5.70	0.18
< 16	Ref				Ref			
Stage of disease								
In relapse	0.36	0.12	1.12	0.08	0.37	0.11	1.28	0.12
In remission	2.04	0.64	6.52	0.23	2.04	0.61	6.79	0.25
	Ref				Ref			

Table E2

Comparison between studies on alloimmunization in multitransfused patients

Studies	Region	Year	Total number of patients studied	Number of patients alloimmunized	Alloimmunization rate (%)	Most common alloantibody detected	Percentage of Anti-K (%)
Our study	Palestine	2023	94	29	30.8	Anti-K	20.7
Singer *et al* [112]	China &Southeast Asia	2000	64	14	22	Anti-K	22
Pereira Bueno *et al* [122]	Brazil	2021	29,128	79	0.27	Anti-E	10.7
Luzo *et al* [123]	Brazil	2010	302	70	23	Anti-E	4
Valle Neto *et al* [124]	Brazil	2018	153	17	11.1	anti-E, anti-K	21.8
Thakral *et al* [125]	North India	2008	531	18	3.4	anti-c	0
Handa *et al* [117]	Punjab, India	2020	100	42.8	7	Anti-K	42.8
Chao *et al* [126]	Taiwan,	2013	64	6	9.4	anti-E	0
Cheng *et al* [127]	China	2012	382	88	23	Anti-E	0.9
Al-Mousawi *et al* [128]	Iraq Kurdistan	2015	401	18	4.5	Anti-E	15
Singhal, Deepak *et al* [129]	South Australia	2017	817	98	12	Anti-E	10
Samarah, Fekri *et al* [105]	Palestine	2018	116	9	7.76	Anti-K	33.3
Abu Taha, Adham *et al* [106]	Palestine	2019	215	27	12.6	Anti-D	25.9

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

معدل حدوث أجسام مضادة وخصائصها لدى مرضى سرطانات الدم في الضفة الغربية– فلسطين

إعداد

اكرام يوسف عبد الرحمن الشيخ قاسم

إشراف

د. إياد العلي

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2023

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الدكتور اياد العلي

الملخص

مقدمة: غالباً ما يعاني مرضى أورام الدم الخبيثة من فقر الدم. يعد العلاج بنقل الدم أمراً أساسياً لدى هؤلاء المرضى. قد تتسبب عمليات نقل الدم المتكررة في تكوين أجسام مضادة ضد واحد أو أكثر من مستضدات الخلايا الحمراء، ما يعقد عمليات نقل الدم اللاحقة.

الأهداف: هدفت هذه الدراسة إلى التحقق من نسبة وخصائص تكوين اجسام مضادة لخلايا الدم الحمراء، بالإضافة إلى عوامل الخطر ذات الصلة بين المرضى الذين تم تشخيص إصابتهم بأورام الدم الخبيثة (سرطان الدم والورم النخاعي المتعدد بشكل رئيسي) في فلسطين.

منهجية الدراسة: أجريت هذه الدراسة المقطعية في الفترة ما بين شباط وأغسطس 2022 على مرضى الأورام من عيادات أو تم إدخالهم إلى المستشفيات من ثلاثة مستشفيات تقع شمال ووسط وجنوب الضفة الغربية، فلسطين. تم تضمين ما مجموعه 94 من المرضى الذين تتكرر لهم عمليات نقل الدم. تم جمع البيانات الديموغرافية والطبية وتاريخ نقل الدم. تم إجراء فحص لوجود أجسام مضادة في الدم (اختبار coomb غير المباشر) وتحديده –ان وجد– من عينات البلازما للمرضى الذين لم يسبق لهم القيام بذلك من قبل بطريقة Capture-R باستخدام جهاز IMMUCOR. تم اختبار وجود من عدم وجود اي جسم

مضاد لخلايا الدم الحمراء للمرضى باستخدام البلازما الخاصة بهم بالتوازي مع كل فحص لاستبعاد الأجسام المضادة الذاتية.

النتائج: وجد أن 29 عينة مشاركة بالدراسة تحوي أجسام مضادة لمرضى أورام الدم الخبيثة بمعدل (30.85%) وهو أعلى من ذلك المبلغ عنه في العديد من الدراسات. كانت أكثر الأجسام المضادة شيوعاً Anti-Kell (20.7%)، تليها Anti-E (13.8%) و Anti-D (6.9%).

كان لدى أحد المشاركين عدة أجسام مضادة، وكان لدى ستة مشاركين (23.9٪) جسمان مضادان على النحو التالي:

S و $Anti\ Fy^a$ (3.4%), Fy^a و Anti-E (6.9%), Kp^a و $Anti-Jk^a$ (6.9%), Anti-E وK (3.4%).

أيضاً كانت نسبة المشاركين الذين لديهم اجسام مضادة ذاتية (6.9%).

الخلاصة: أظهرت هذه الدراسة ارتفاع معدل حدوث أجسام مضادة بين مرضى الأورام الدموية الخبيثة خاصة اللوكيميا والورم النخاعي المتعدد في مناطق مختلفة من الضفة الغربية، فلسطين. كانت الأجسام المضادة الأكثر شيوعًا التي تم اكتشافها ضد مستضدات Kell و Rh. قد يؤدي توافق كرات الدم الحمراء في نظام ABO و Rh و Kell إلى تقليل مخاطر حدوث معظم الأجسام المضادة وبالتالي تأثير نقل الدم الانحلالي في هؤلاء المرضى.

الكلمات المفتاحية: نقل خلايا الدم الحمراء، فحص الجسم/ الأجسام المضادة، أورام الدم الخبيثة، فلسطين.