RECENT DIAGNOSTIC TOOLS IN RED BLOOD CELLS IDENTIFICATION FOR REGULARY TRANSFUSED PATIENTS

ESSAY
Submitted for Partial Fulfillment of
The Master Degree in
Clinical and Chemical Pathology

By Heba Abu Bakr Tawfik M.B, B.Ch

Supervised by

Professor / Mona Ahmed Wahba Professor of Clinical and Chemical Pathology Faculty of Medicine, Ain Shams University

Professor / Amany Ahmed Osman

Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Professor/Soha Raouf Youssef

Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Faculty of Medicine Ain Shams University 2011

Introduction

Red cell transfusion should be based on a sound understanding of the indication of therapy, the principle of red cell selection, compatibility testing and possible adverse effects (*Daniels*, 2002).

One of the major indication of blood transfusion is the restoration of an adequate blood volume after its loss e.g. surgery. However, some patients require frequent blood transfusion for life e.g. congenital hemolytic anemia.

Multiple blood transfusion can be associated with various complications including iron overload, circulatory overload, blood born infection and hemolytic reaction (*Reid et al.*, 2004).

Although acute hemolytic reactions are currently rare, they are one of the most dangerous transfusion complications. In addition the risk of delayed hemolytic reaction are estimated to be 1-2% with each of RBCs transfused (*Yazer et al.*, 2006).

Therefore a major portion of pre-transfusion testing is directed toward proper RBCs identification. The processes of selecting RBCs for red cell antibodies and in vitro crossmatching (*Dariels et al.*, 2004).

Recently, new methods have been developed to enhance our capability for proper selection of RBCs for transfusion e.g. antibody identification and phenotyping. Extended red cell typing is required for the management of transfusion dependent patients to confirm the identity of suspected alloantibodies or to determine the specificity of potential additional antibodies that may be formed in the future. (*Brecher*, et al., 2002)

Aim of the essay

The aim of this essay is to review the currently used protocol for RBCs selection in poly-transfused patients in addition to the recently developed methodologies for RBCs identification.

Acknowledgment

My deepest gratitude and thanks to the most merciful for giving me the strength to complete this work.

I wish to express my sincere thanks and gratitude to prof. Dr. Mona Wahba for her great care, continuous guidance and support and for giving me the chance to work in the field that I like the most.

I would also like to express my gratitude to Prof. Dr. Amany Osman for her valuable advice, guidance and meticulous supervision during every step of this work.

I would like also like to thank Prof. Dr. Soha Raouf for her help and her valuable advice and work.

My deepest thanks to My Father and My Mother and all My Family for their great support and help.

List of contents

List of Contents

Title	page
List of abbreviations	Ш
List of tables	V
List of figures	VII
Introduction and aim of the work	1
Review of literature	
■ Chapter 1 : Blood Group Antigens and Antibodies	1
A-ISBT Terminology	3
B- Red cell antibodies	٨
C-Red cell blood group systems	1٣
 Chapter 2: Indications and complications of blood 	
Transfusion	51
A- Indications for regular packed RBCs transfusion	53
B- Complications of blood transfusion	58
1-Non immune complications	٥٩
2-Immune complications	63
i-Acute hemolytic reaction	64
ii-Delayed hemolytic reaction	65
C- Alloimmunization	
-Mechanism	69
-Effect of Alloimmunization	71
-Factors affecting Alloimmunization	77
D-Prevention of complications	86
Chapter 2 Dra transfersion testing	



List of contents

	A-A	ABO Grouping of the recipients	90
	1	- ABO Grouping	90
	2	- Rh Grouping	94
	3	- Extended Antigens phenotyping	97
	В	-Antibody screening and Identification:	98
		1-Antibodies screening	98
		2- Antibodies identification	101
	C-	-Cross matching	10 ^V
-		apter IV: Future of Pre-transfusion testing and Red Cell	110
		Genotyping	
	-	Red cells genotyping.	113
	-	DNA Based Methods for Red Cells Genotyping.	114
	-	The Molecular methods available for red cells genotyping.	
	-	Assays based on classical PCR	115
	-	Real time PCR.	117
	-	DNA microarrays.	117
	-	DNA based typing for ABO Antigens.	118
	-	DNA based typing for Rh Antigens.	121
	-	Red cell genotyping in HDOFN	124
	-	Applications of red cells genotyping in transfusion practice	130
	-	Applications in massive and repeated transfusion	135
	-	Prophylactic red cells genotyping .	137
	-	Assimilation of red $$ cells genotyping into routine practice .	140
	-	DNA arrays or gene chips.	141
•	Sur	nmary and conclusion	142
•	Ref	erences .	146

List of Tables

Table	Title	Page
Table (1)	Human Blood Group Systems	٥
Table (2)	Red Cell Antigen Collections	٧
Table (3)	Reactions of lectins with cryptantigens and with Sd(a++) and Hyde Park polyagglutinable cells	1۳
Table (4)	ABO grouping	17
Table (5)	Summary of ABO genes and antigens	14
Table (6)	Rh blood group antigens	2٦
Table (7)	Rh- blood group phenotytpes & frequencies	24
Table (8)	Some phenotypes and frequencies in the Kell system	٣٣
Table (9)	Phenotypes and frequencies in the Duffy system	٣٦
Table (10)	Phenotypes and frequencies in the Kidd system	٣٧

Table (11)	Phenotypes and frequencies in the MNS system	٣٨
Table (12)	Phenotypes and frequencies in the Lewis system	3٨
Table (13)	Phenotypes and frequencies in the Lutheran system in the whites	٤١
Table (14)	Difference between whole blood and packed red blood cells	٥٧
Table (15)	Interpretation of ABO testing results	9 Y
Table (16)	Reagent red cell panel for alloantibodies identification	1.7
Table (17)	Useful application for red cell genotyping	150

List of Figures

Figure	Title	Page
Figure 1	Red blood cell antigens	2
Figure 2	Red blood cell groups	14
Figure 3	Coomb's test	101
Figure 4	A panel of blood cell identification	102

List of Abbreviations

Ab Antibody

ADCC Antibody-dependent cellular cytotoxicity

Ag Antigen

AHG Anti human globulin

AIHA Auto-immune hemolytic anemia

CAT Column agglutination technology

CD Clusters of differentiation

CLL Chronic lymphocytic leukaemia

CLT Chemiluminescence Test

CMV Cytomegalovirus

DAF Decay-accelerating factor

DAT Direct antiglobulin test

DHTRs Delayed hemolytic transfusion reactions

DIC Disseminated intravascular coagulation

DL Donath-Landsteiner

DNA Deoxyribose nucleic acid

ELISA Enzyme linked immunosorbent assay

gp glycoprotein

GVHD Graft versus host disease

HDFN Hemolytic disease of fetus and newborn

HLA Human Leukocyte Antigen

IAT Indirect antiglobulin test

Ig Immunoglobulin

IHTRs Immediate hemolytic transfusion reactions

ISBT International society of blood transfusion

IVIG Intravenous immunoglobulin

LISS Low ionic strength saline

MAIEA Monoclonal antibody- specific immobilization of erythrocyte

antigens assay

MMA Monocyte monolayer assay

NAT Nucleic acid testing

PCH Paroxysmal cold haemoglobinuria

PCR Polymerase chain reaction

PEG Polyethylene glycol

RBCs Red blood cells

RFLP Restricted fragment length polymorphism

Rh Rhesus

SLE Systemic lupus erythematosus

SNP Single- nucleotide polymorphism

SPH Solid phase

ZZAP ZZ-activated papain

I-BLOOD GROUP ANTIGENS AND ANTIBODIES

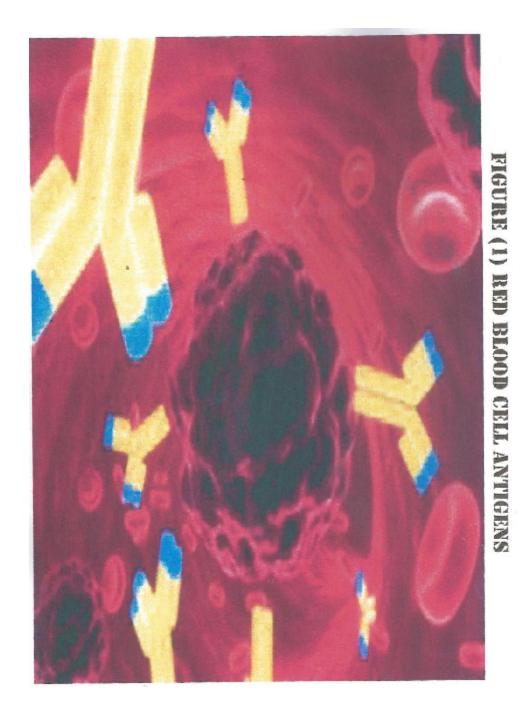
Introduction

The observation by Landsteiner in 1900 that red cells of some individuals could be agglutinated by the serum of others led to the discovery of the ABO blood group system. Following the identification of the A and B blood group antigens, blood group serology blossomed throughout the 20th century, such that in humans approximately 302 blood group antigens were identified (Figure 1), most of which belong to 1 of 29 genetically discrete blood group systems (Contreras and Daniels, 2005).

The genes representing the 29 systems have been located on specific chromosomes. All are autosomal except XG and XK, which are X- borne and MIC2, which is on both the X and Y chromosomes. All the genes have been cloned, with the exception of P1 (Webert et al., 2004).

Blood group antigens may be proteins, glycoproteins or glycolipids. Most red cell antigens are synthesized by the red cells, however, some antigens such as those of Lewis, are







adsorbed onto the red cell membrane from the plasma. Some red cell antigens are specific to the red cells, however others are found on other cells throughout the body (Lewis et al., 1990).

Antibodies to many of these antigens have the potential to be clinically significant; that is, they can facilitate accelerated destruction of red cells carrying the corresponding antigen. It has been recognized that knowledge and understanding of blood groups are essential for transfusion therapy. This is because individuals who lack antigens on their red blood cells can be alloimmunized, if they are exposed to blood expressing the antigen. This might occur with transfusion of blood products or during pregnancy. Antibodies that react with red blood cell antigens can cause problems such as delayed and immediate hemolytic transfusion reactions (HTRs) and hemolytic disease of the newborn (Menitove, 1997).

A-International Society of Blood Transfusion (ISBT) Terminology:

The ISBT working party on terminology for red cell surface antigens was established in 1980 with the goal of creating a uniform nomenclature. Blood group antigens are now

