SAPIENZA
UNIVERSITÀ DI ROMA

The ABO and Rh

system Transfusion

Medicine

Dr U. La Rocca 22 April 2022

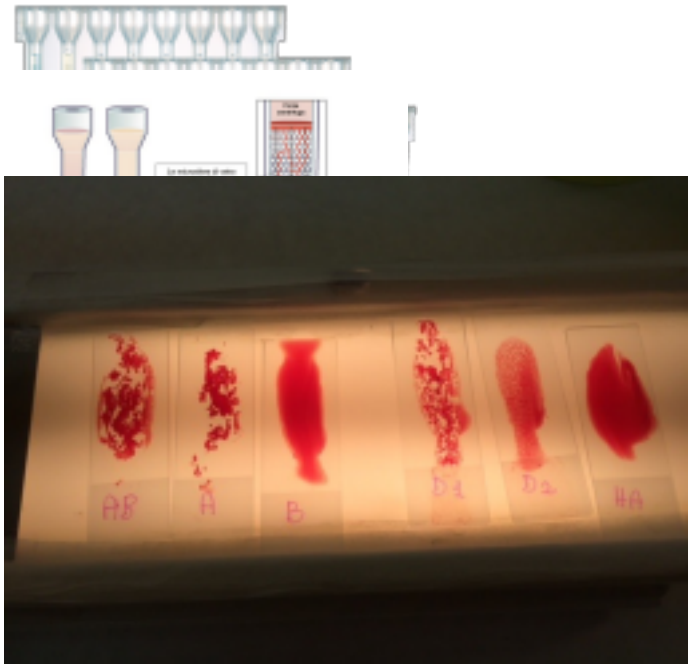
Main learning endpoints!

- ✔ **Chemical structure**
- ✔ **Inheritance pathways**
- ✔ **AB0 and Rh antibodies and their importance in transfusion**

✔ Principles of AB0 and Rh typing

IMMUNOHEMATOLOGY AND TRANSFUSION MEDICINE- LAB

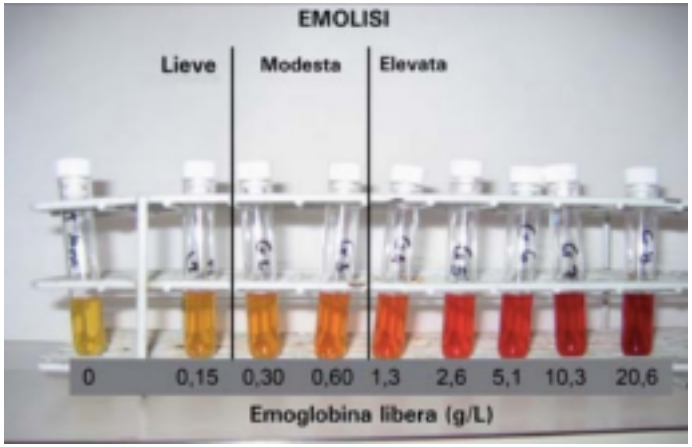
BLOOD GROUP METHODOLOGY TO DETERMINE THE ABO BLOOD TYPE IS BASED ON AGGLUTINATION REACTION and HEMOLYSIS.



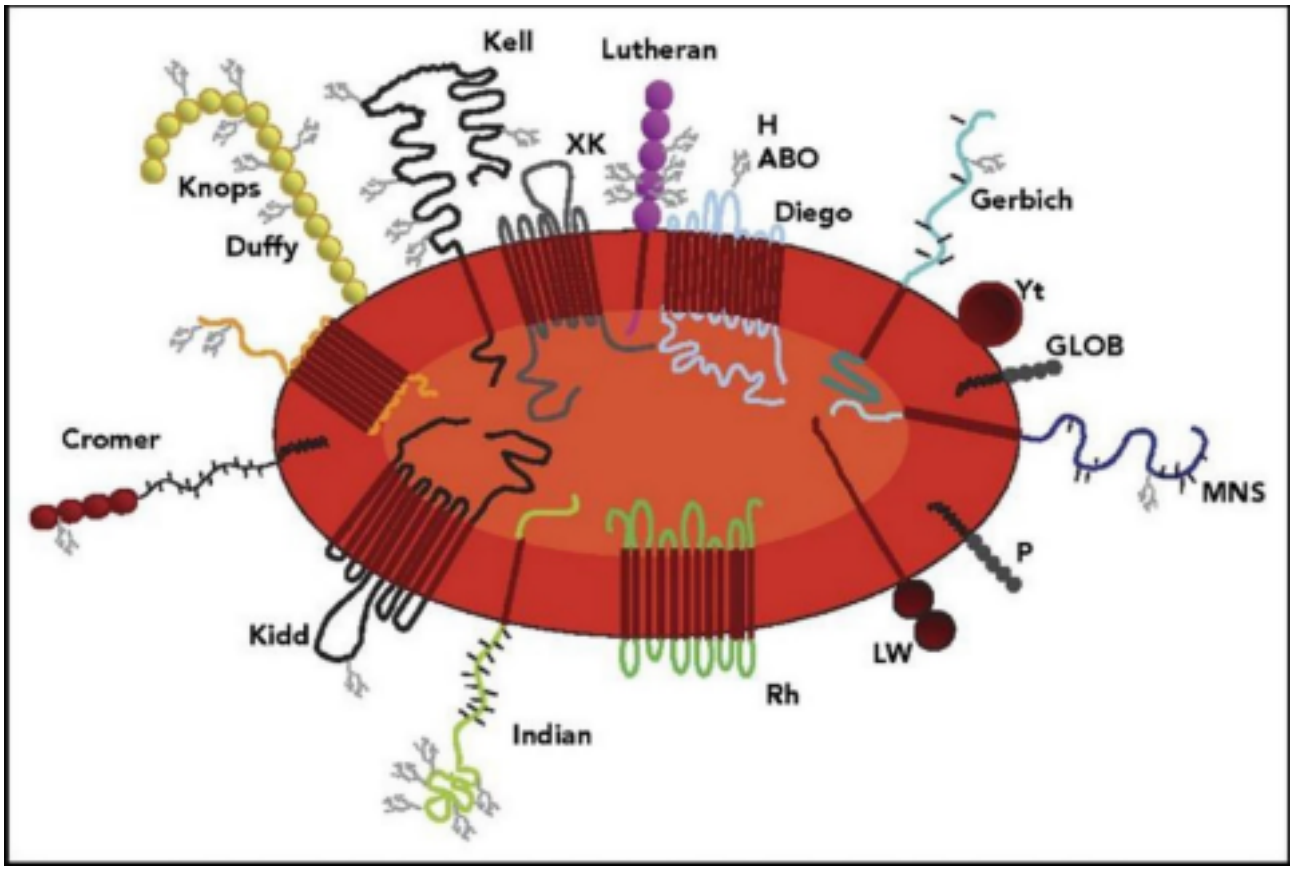
AGGLUTINATION IS THE PROCESS BY WHICH RED BLOOD CELLS AGGLUTINATE, MEANING CLUMP OR CLOG.

AGGLUTINIINS INVOLVED ARE CALLED HEMAGGLUTININ.

HEMOLYSIS IS THE RUPTURING (LYSIS) OF RED BLOOD CELLS AND THE RELEASE OF THEIR CONTENTS (HB) INTO THE PLASMA.



BLOOD GROUP ANTIGENS



The red cell is a complex structure, and the red cell membrane contains many surface proteins that are anchored to the membrane, cross the lipid bilayer one or more times or are adsorbed onto the surface of the red cells.

TABLE OF BLOOD GROUP ANTIGENS

System		Antigen number												Total
		001	002	003	004	005	006	007	008	009	010	011	012	
001	ABO	A	B	A,B	A1	...								4
002	MNS	M	N	S	s	U	He	Mi^a	M^c	Vw	Mur	M^g	Vr	49
003	P1PK	P1	...	P^k	NOR									3
004	RH	D	C	E	c	e	f	Ce	C^w	C^x	V	E^w	G	55
005	LU	Lu^a	Lu^b	Lu3	Lu4	Lu5	Lu6	Lu7	Lu8	Lu9	...	Lu11	Lu12	27
006	KEL	K	k	Kp^a	Kp^b	Ku	Js^a	Js^b	...	...	Ul^a	K11	K12	36
007	LE	Le^a	Le^b	Le^{ab}	Le^{bH}	ALe^b	BLe^b							6
008	FY	Fy^a	Fy^b	Fy3	...	Fy5	Fy6							5
009	JK	Jk^a	Jk^b	Jk3										3
010	DI	Di^a	Di^b	Wr^a	Wr^b	Wd^a	Rb^a	WARR	ELO	Wu	Bp^a	Mo^a	Hg^a	22
011	YT	Yt^a	Yt^b	YTEG	YTLI	YTOT								5
012	XG	Xg^a	CD99											2
013	SC	Sc1	Sc2	Sc3	Rd	STAR	SCER	SCAN						7
014	DO	Do^a	Do^b	Gy^a	Hy	Jo^a	DOYA	DOMR	DOLG	DOLC	DODE			10
015	CO	Co^a	Co^b	Co3	Co4									4
016	LW	...	...	...	...	LW^a	LW^{ab}	LW^b						3
017	CH/RG	Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	WH				Rg1	Rg2	9
018	H	H												1
019	XK	Kx												1

System		Antigen number												Total in system
		001	002	003	004	005	006	007	008	009	010	011	012	
020	GE	...	Ge2	Ge3	Ge4	Wb	Ls^a	An^a	Dh^a	GEIS	GEPL	GEAT	GETI	11
021	CROM	Cr^a	Tc^a	Tc^b	Tc^c	Dr^a	Es^a	IFC	WES^a	WES^b	UMC	GUTI	SERF	20
022	KN	Kn^a	Kn^b	McC^a	Sl1	Yk^a	McC^b	Sl2	Sl3	KCAM	KDAS			10
023	IN	In^a	In^b	INFI	INJA	INRA	INSL							6
024	OK	Ok^a	OKGV	OKVM										3
025	RAPH	MER2												1
026	JMH	JMH	JMHK	JMHL	JMHG	JMHM	JMHQ	JMHN						7
027	I	I												1
028	GLOB	P			PX2									2
029	GIL	GIL												1
030	RHAG	Duclos	Ol^a	DSLK†	... §									3
031	FORS	FORS1												1
032	JR	Jr^a												1
033	LAN	Lan												1
034	VEL	Vel												1
035	CD59	CD59.1												1
036	AUG	AUG1	At^a	ATML	ATAM									4
037	KANNO	KANNO1												1
038	SID	Sd^a												1

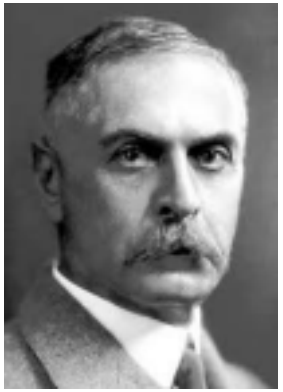
History

• **1900** Discovery of the AB0 system (K. Landsteiner) • **1907** the first successful blood transfusion was performed by Reuben Ottenberg at Mount Sinai Hospital in New York • **1915** The use of

sodium citrate as blood anticoagulant (R. Lewisohn)

- **1917** First Blood Bank
- **1921** «First blood donor Service» (O. Percy ,London) • **1943** ACD became the standard anticoagulant (J.F. Loutit, P.L. Mollison)
- **1950** The use of the plastic blood container (C.Walter,W.P. Murphy)
- **1951** First use of a cell separator (E. Cohn)

Karl Landsteiner



14th June birthday

The discovery of Blood group

In 1901, Karl Landsteiner discovered the AB0 blood group antigens.
By systematically mixing the RBC from a number of individuals (his colleagues) with the sera from others,
he found that the RBCs from some individuals were agglutinated by the sera from others.

He called the antigens A and B.
A third blood group without the agglutination properties of A and B, was later called O.
One year later, the fourth blood group, AB, was added
to the ABO blood group system (discovered by De Castello and Sturli)

A pattern of four major groups emerged: A, B, AB, or 0. Individuals have either A or B antigen on their cells, a combination of A&B, or neither (group 0).


SCIENCE

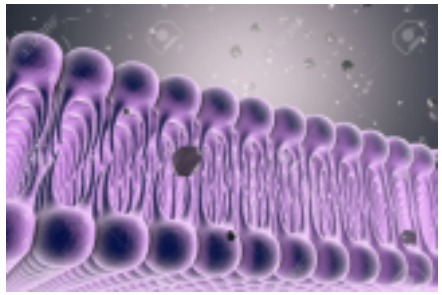
DR. KARL LANDSTEINER

This discovery earned Landsteiner a Nobel Prize

ABO System

The ABO blood group system is the first described of the human blood groups.

ABO antigens are carbohydrate antigens present on red cell membranes, as: ✔ glycoproteins
✔ glucose transporters
✔ glycosphingolipids



Anti-A , anti-B and anti-A,B antibodies are present only in the blood sera of individuals not possessing that specificity. This is the basis for typing humans into phenotypes dfined A, B, AB, and O.

BLOOD GROUP METHODOLOGY TO DETERMINE THE ABO BLOOD TYPE IS BASED ON AGGLUTINATION REACTION.

The AB0 System and the Landsteiner's Law

- ✔ **Two antigens (A and B) found on RBCs, tissue cells, fluid and secretions**
- ✔ **Four different groups: A, B, AB, 0**
- ✔ **… whichever ABO antigens are lacking on a given person's RBCs, that person will always have the corrisponding antibody or isohemagglutinin**

The ABO System and the Landsteiner's Law

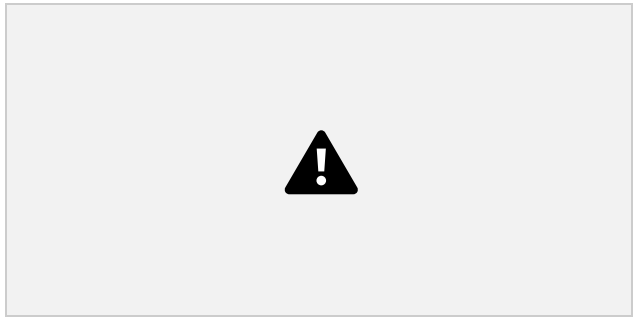
- ✔ Group A individuals always have anti-B in their plasma
- ✔ Group B individuals always have anti-A in their plasma
- ✔ Group O individuals

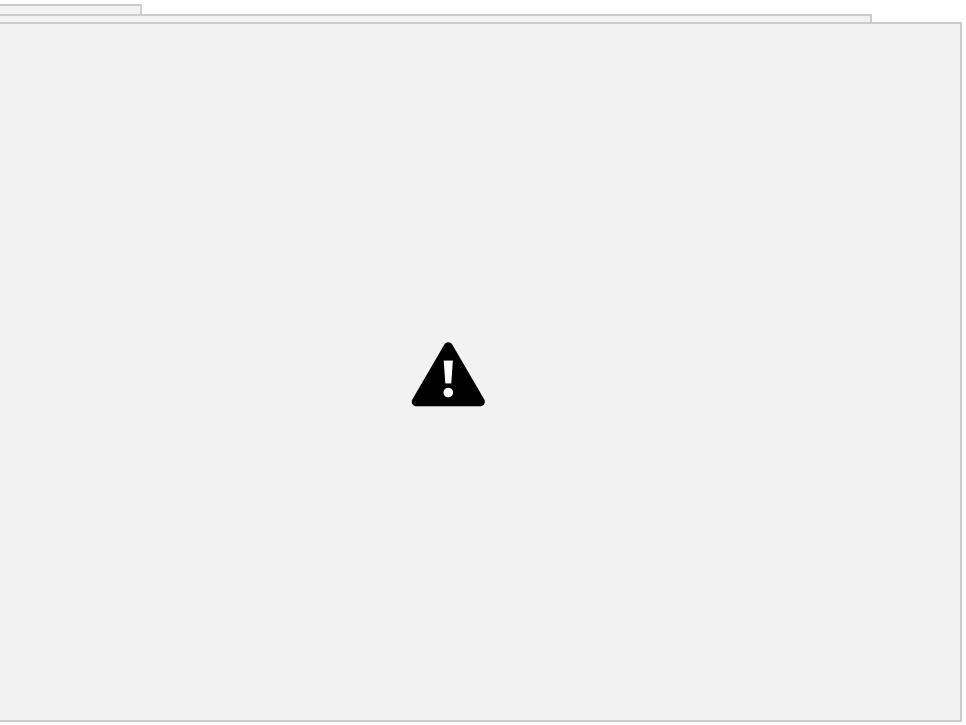
always have anti-A and anti-B and anti-AB

✔ Group AB individuals don't have any isohemoagglutinins

ABO ANTIBODIES

… following the Landsteiner law





IgM

NATURAL

“COLD” (REACT AT 20-24°C)

IgG

IMMUNE

“WARM” (REACT AT 37°C)

Whether they are IgG or IgM, ABO antibodies can activate complement readily !!!
INCOMPATIBILITIES CAN CAUSE LIFE THREATENING TRANSFUSION REACTIONS!!!

Why do we have natural antibodies?

Antibodies are usually not present at birth but are present in most individuals by about 6 months of age.

In 1960s Springer and colleagues showed ABO isohemoagglutinins were produced as a response to bacterial antigens.

Infants are exposed to a variety of microorganisms and foodstuffs which have antigenic determinants that are cross reactive with the blood

group substances and which can thus provide the stimulation for isoantibody formation (ie E. coli has type B like Ag).

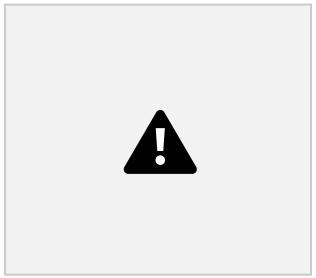
These cross reacting Ags induce formation of Abs in individuals lacking these antigens because epitopes are too similar to self and a state of self tolerance to the epitopes should exist.

The AB0 Genes

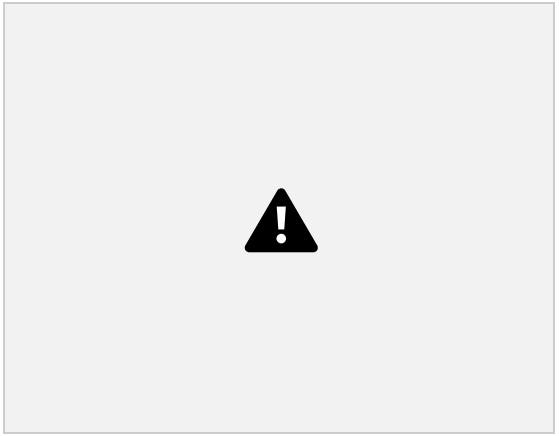
✔**DO NOT ENCODE A AND B ANTIGENS DIRECTLY**

✔**They encode TRANSFERASE ENZYMES which catalyse the addition of specific monosaccharides to oligosaccharide precursor chain having a terminal galactose**

✔**H antigen form a precursor oligosaccharide necessary to form A antigen and B antigen**



AB0 genetics



- Chromosome 9q34.1 – q34.2

- There are three main allelic forms: A, B and

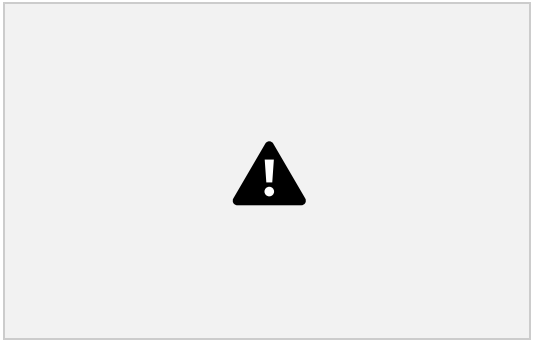
O • A and B co-dominant

- 0 is the recessive form encoding a non functional enzyme

- Each individual has a pair of chromosomes so has two genes for the AB0 group

Phenotype vs genotype

Two chromosomes Two genes



A B AB A 0 A

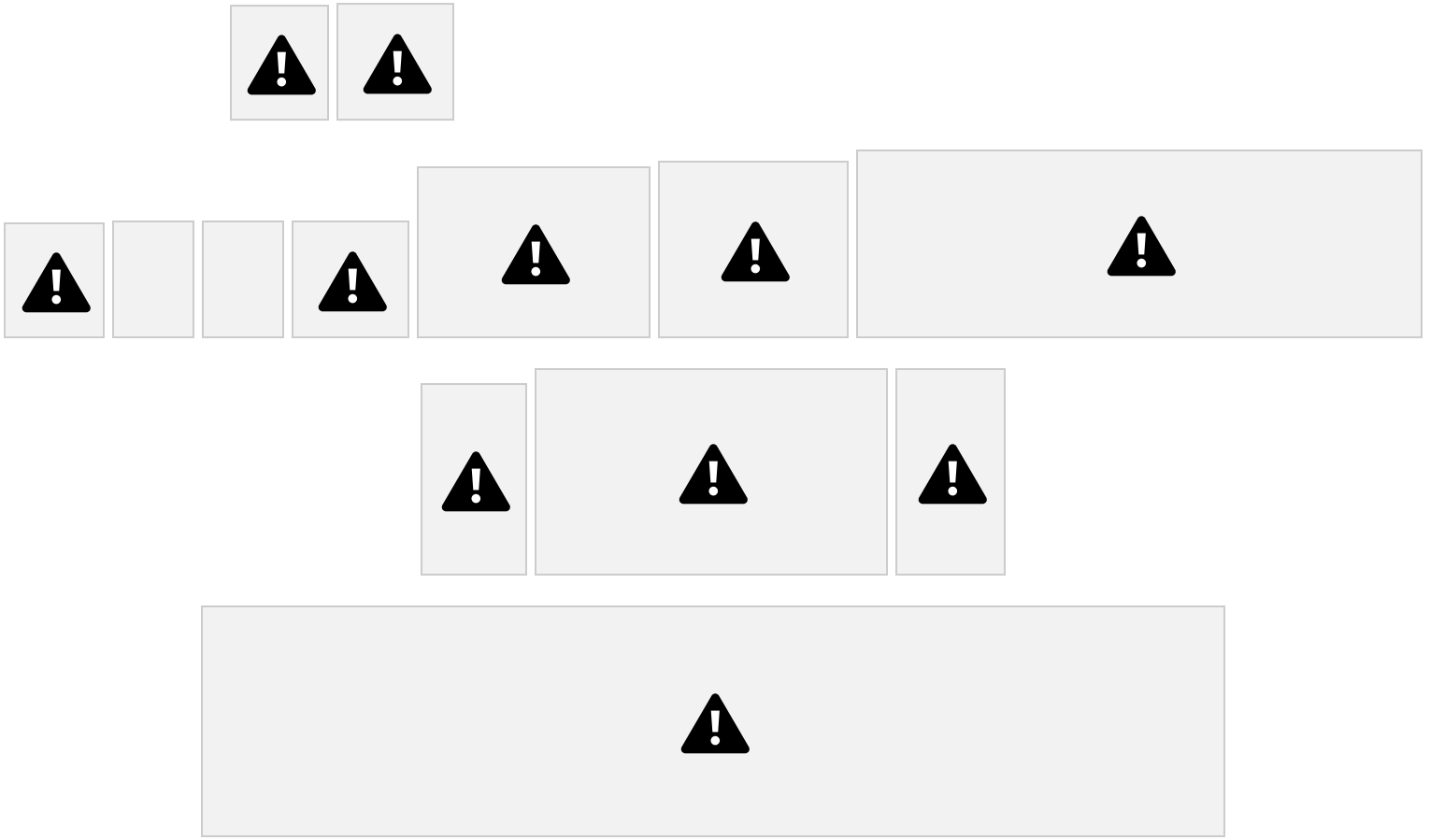
A A A

B 0 B

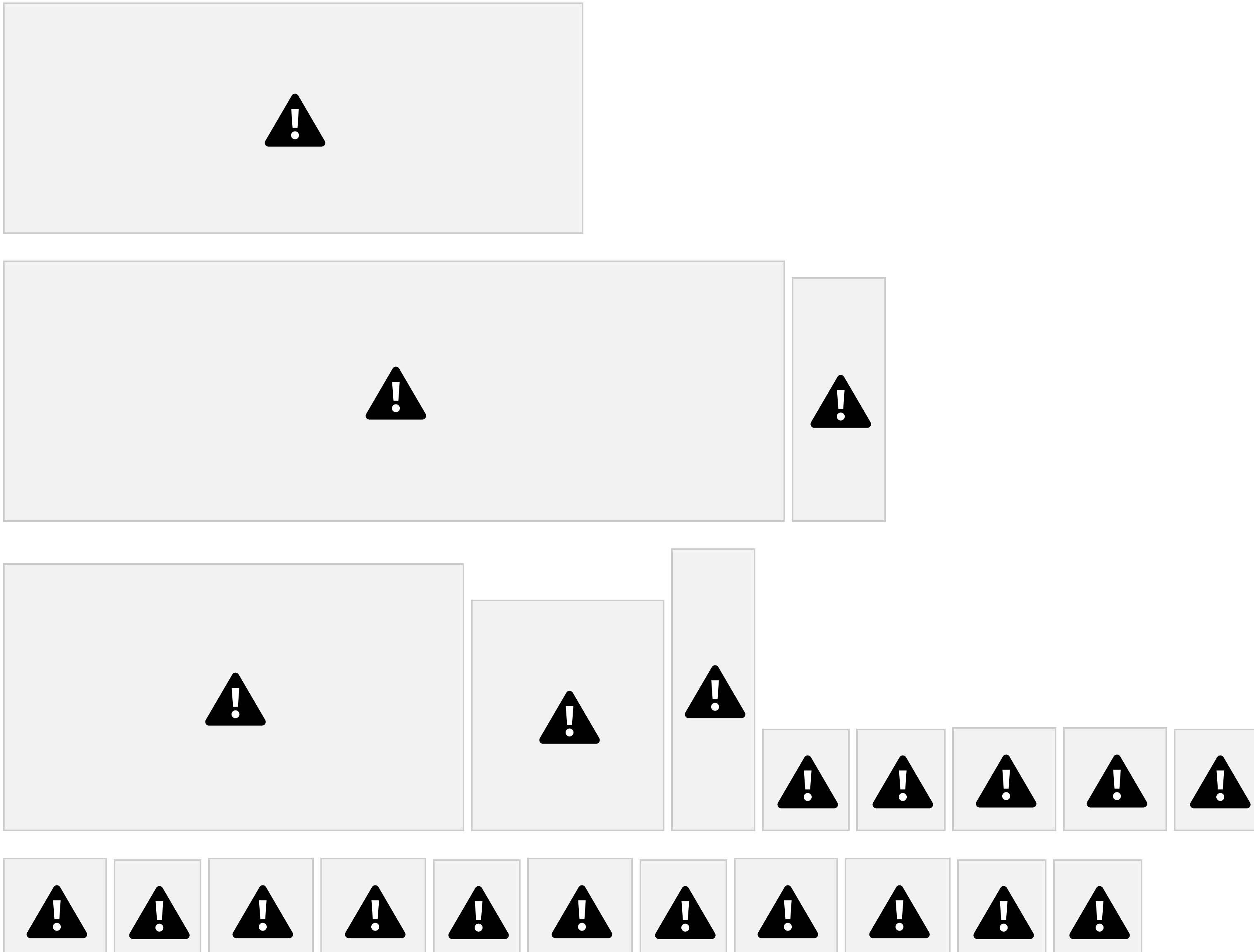
B B B

0 0 0

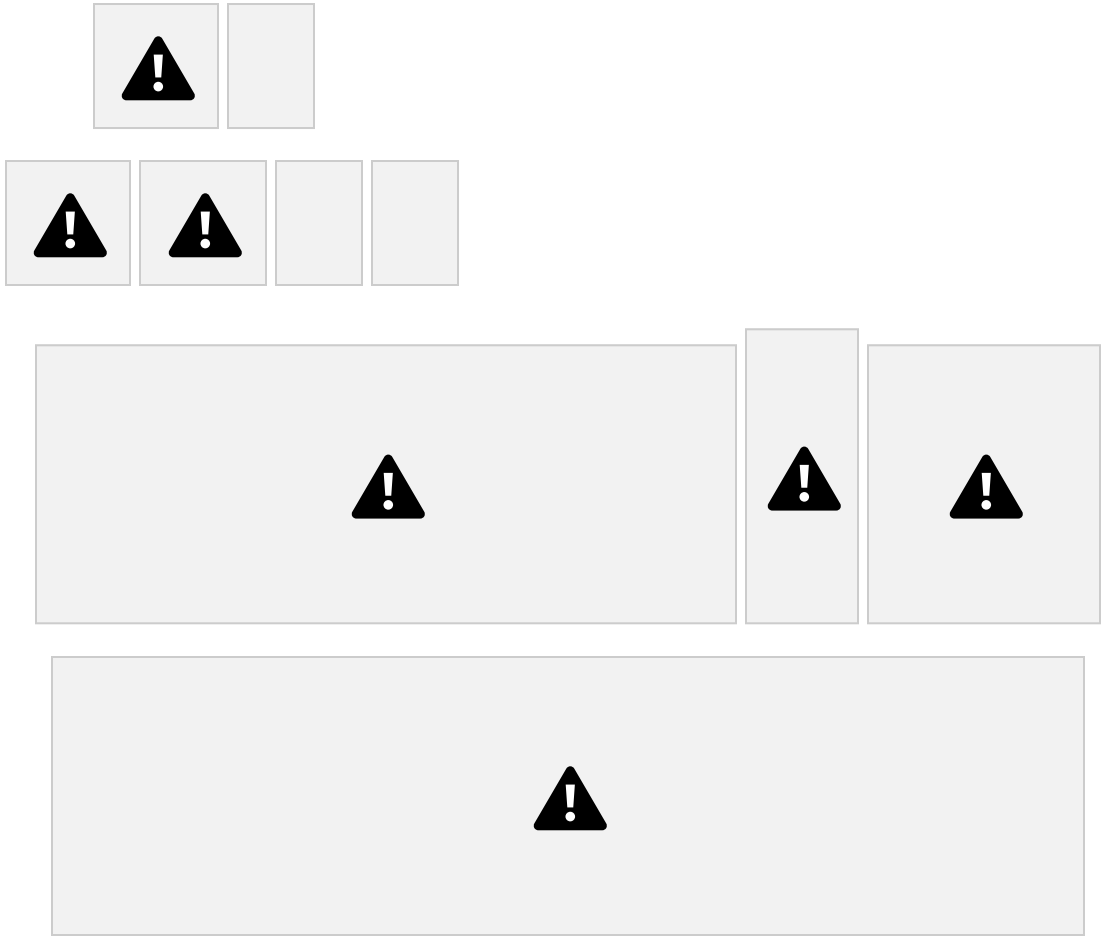
Phenotype vs genotype

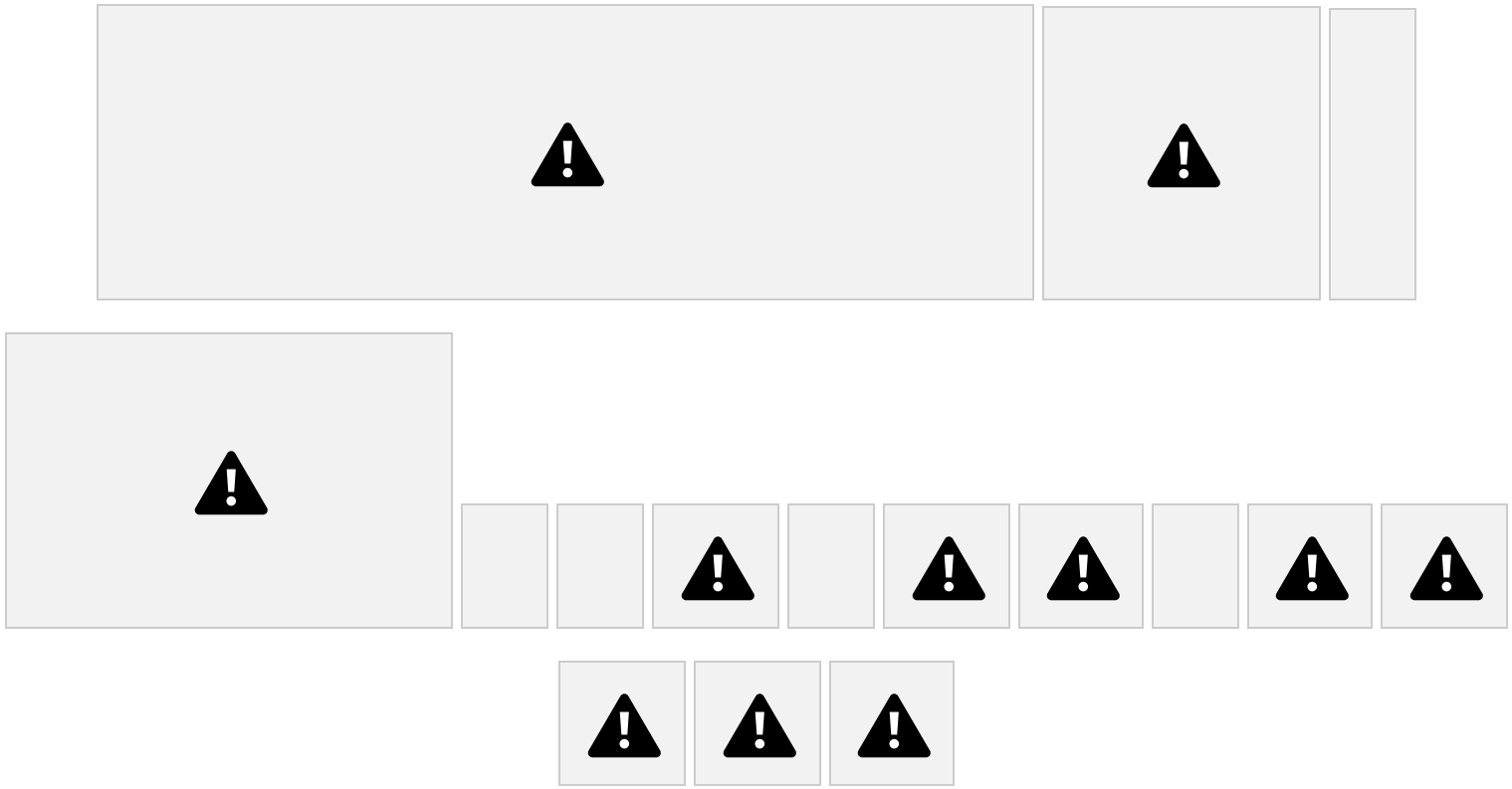


Phenotype vs genotype



Phenotype vs genotype





Phenotype vs genotype



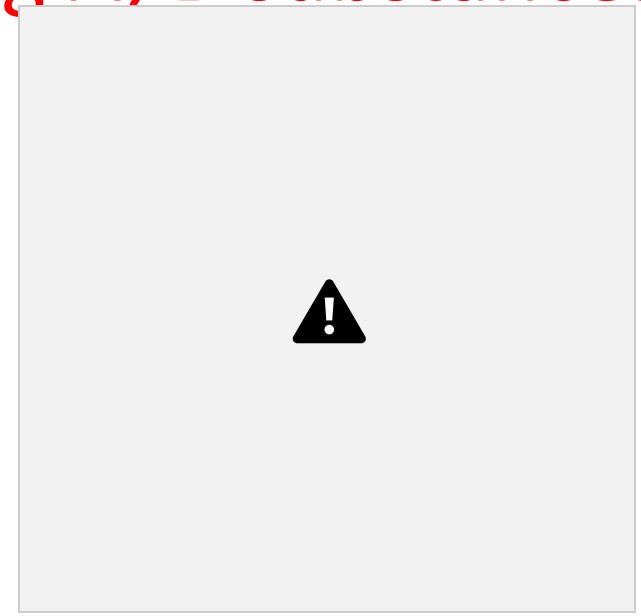
A and B antigens

✔Not fully developed at birth
(few copies of antigens on the cells)

✔Antigens detectable as early as 5 weeks after conception

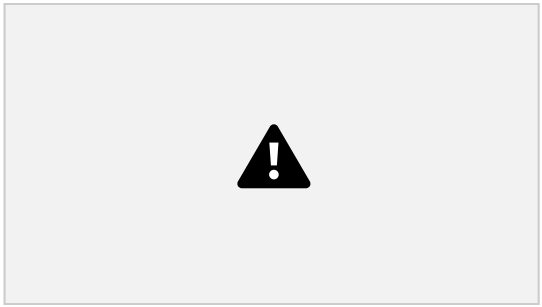
✔Complete expression at 6 months

Other cells holding A, B substances



- ✔Plasma
- ✔Leucocytes
- ✔Platelets
- ✔Epithelial cells
- ✔Amniotic liquid cells
- ✔Sperms
- ✔Cells of the endothelium of: capillaries, veins, arteries

The H gene

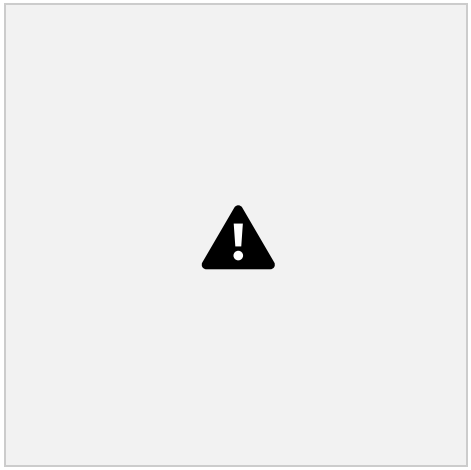


• The H locus is found on chromosome 19 • Why is therefore included in the AB0 blood group system?

Although H is encoded by a gene on a different chromosome from ABO, the H blood group system is considered in this chapter because H is a

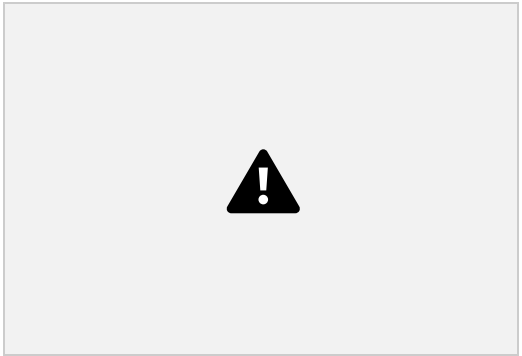
precursor of A

ABH System Hh genes



- Two alleles: **H** and **h**
- Located on **Chromosome 19**
- **H gene**, dominant, has a higher frequency (> 99,9%)
- **h gene** is called “amorphous gene”; homozygosity (hh) is extremely rare

The product of H gene is a α-L-fucosiltransferase, that adds a **L-fucose molecule** on the common substance, **with the consequent formation of H substance**

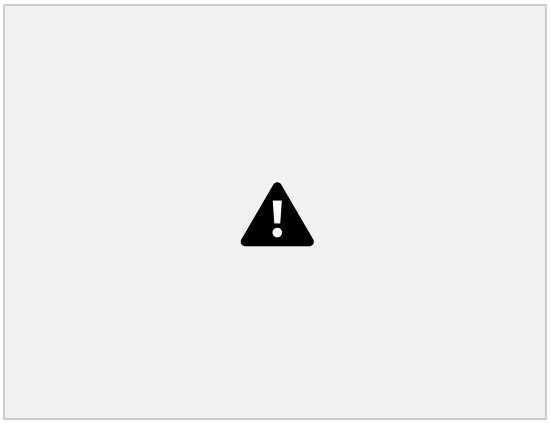


This is a crucial step for developing A and B specifities

Chemical Structure

All normal individuals synthesize a common core glycan called the **H Ag** that is attached to a polypeptide backbone (precursor).

- ✔ **A INDIVIDUALS POSSESS AN A GENE , GTA, A GLYCOSYLTRANSFERASE THAT ADDS A TERMINAL N-ACETYLGALACTOSAMINE TO THEIR H AGS**
- ✔ **THE B ALLELE ENZYME GTB, A DIFFERENT GLYCOSYLTRANSFERASE ADDS A TERMINAL GALACTOSE TO THE H AGS.**
- ✔ **TYPE 0 BLOOD HAVE ONLY H SUBSTANCE BECAUSE iT HAVE A NON-FUNCTIONAL GENE.**



Precursor

L- Fucose

N-Acetylglucosamine

H gene

D-Galactose

H Substance

N-Acetylgalactosamine

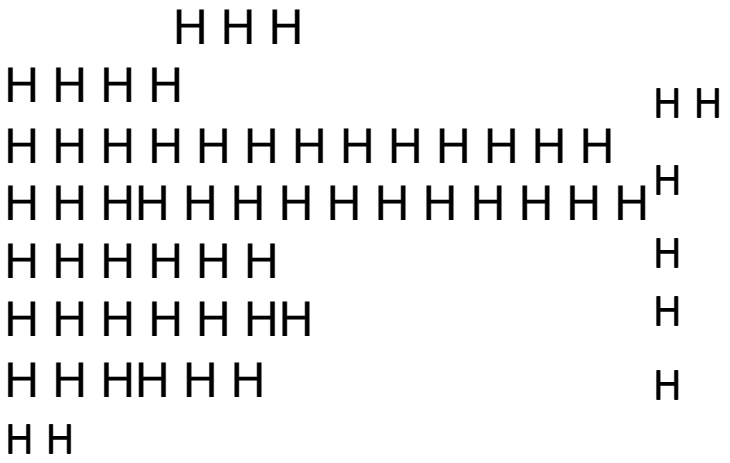
A Gene B Gene

A Substance B Substance

✔ GTA and GTB are almost identical, with only four aminoacid changes.

H ANTIGEN CONCENTRATION
IN RED BLOOD CELLS

0 > B > A > AB



Bombay Phenotype:
a dangerous recipient

Homozygosity for genes h (Oh)

Detected in Bombay by Bhende et al., 1952

- Absence of H substance -> absence of A and/or B substance
- Presence in serum of anti-A,-B,-H
- Definition of “apparent 0” o “0_h”
- Not recognized until serum tested against group 0 cells and causes strong agglutination.
  - Have anti-A, -B, -A,B and –H
  - Can only be transfused with Bombay blood <0.01%

ABO frequency

- Frequencies differ in selected populations and ethnic groups

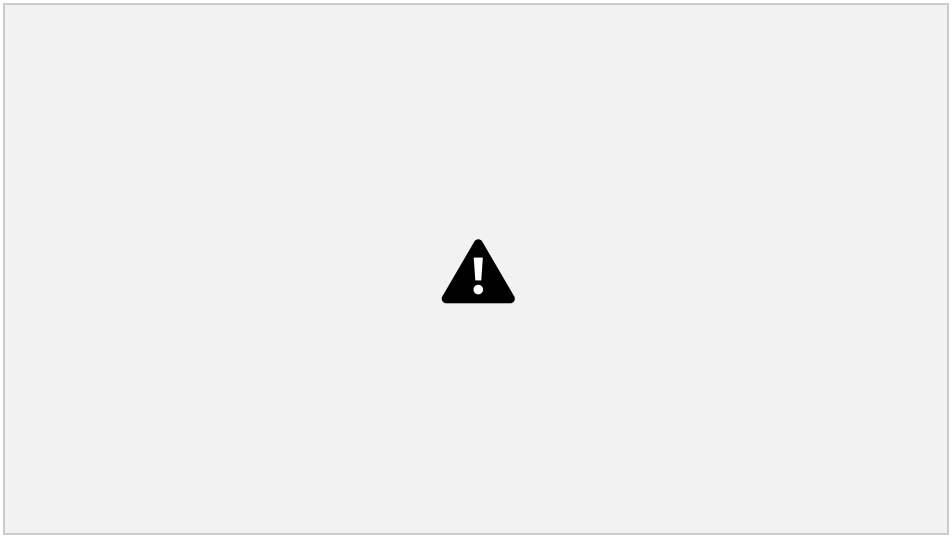
• Group B is higher in African and Asian populations • Frequency in caucasian population:

✔ group 0 45%

✔ group A 40%

✔ group B 11%

✔ group AB 4%.

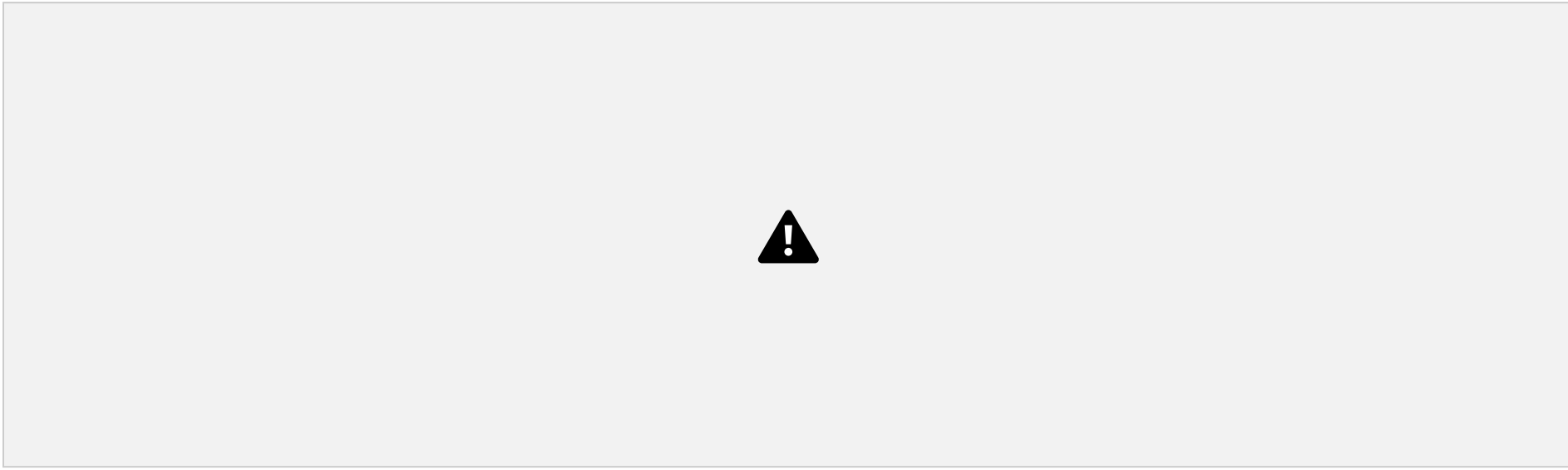


Distribution of A blood type

Distribution of B blood type

Distribution of 0 blood type

Functions of blood groups



The structures of the different blood group carrier molecules and their antigens have been studied extensively, and a wealth of information has become available, particularly since the development of molecular genetic techniques and the data from the human genome project.

However, only a little is known about the function of the blood groups.

The functions of some of the red cell membrane proteins have been identified

The ABO, H, I, P1PK blood groups are carbohydrate structures on the red cell

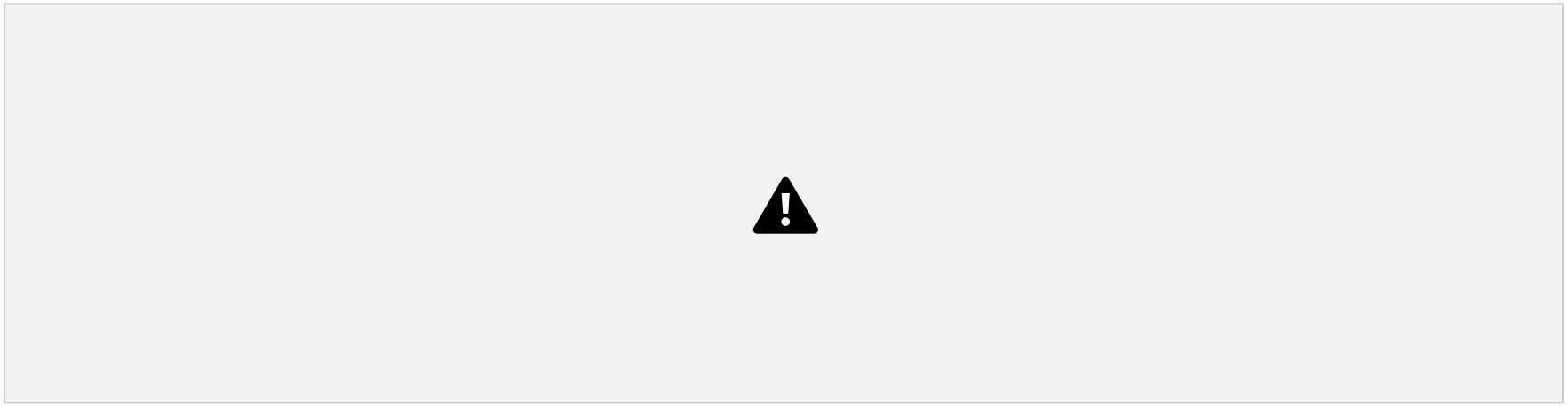
membrane glycolipids and glycoproteins and less is known about their function..

ISBT Science Series (2020) 15, 123–150

in the development of many pathologies. One of the most important examples is represented by malaria

AB0 frequency

Many authors tried to identify the role of blood antigens

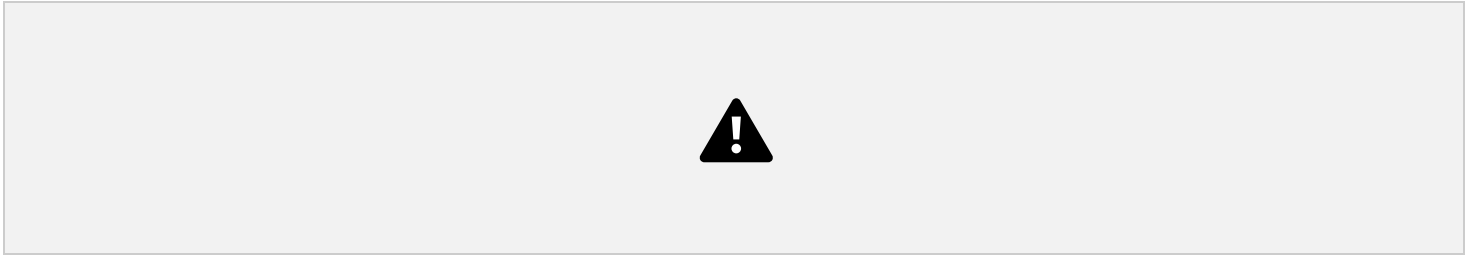


Frequencies differ in selected populations and ethnic groups as a consequence of the geographical spread and a continuos process of natural selection against environmental factors such as diseases, climate, humidity, altitude…

The ABO system is important because the original allele, encoding glycosylation with the A sugar, acts as an adhesion ligand with infected red blood cells thus promoting rosette formation with uninfected red blood cells and adhesion to vascular endothelium, which

cause vaso-occlusion and severe disease. The least rosette formation is observed in individual with blood group O, thereby explaining the prevalence of this blood group in areas in which **malaria is endemic**

Blood Type Biochemistry and Human Disease



Over the last years, our knowledge on hundreds of blood groups antigens, classified into 38 blood systems, in terms of structural homology, secondary structure and

biological functions (structural proteins, enzymes, transporters, channels, receptors) has increased, but there is still much research to be done …

ASSOCIATIONS BETWEEN BLOOD TYPE AND DISEASE HAVE BEEN STUDIED SINCE THE EARLY 1900S WHEN RESEARCHERS DETERMINED THAT ANTIBODIES AND ANTIGENS ARE INHERITED.

Ewald DR, Sumner SC.. *Wiley Interdiscip Rev Syst Biol Med*. 2016;8(6):517–535.

Blood Type Biochemistry and Human Disease

Blood antigens can serve as receptors and ligands for microbes, and may play a role…

Although the **exact mechanisms are not yet known** that will explain all of the reported associations **between blood group antigens and disease**, what is known about their structure and functions provides some intriguing clues.

An unexpected number of the antigenic structures found on RBCs act as **cell adhesion molecules**; some contribute to normal RBC development and some play a role in human disease.

Ewald DR, Sumner SC.. *Wiley Interdiscip Rev Syst Biol Med*. 2016;8(6):517–535.

Blood Type Biochemistry and Human Disease

DISEASE RISK FACTOR		BLOOD GROUP/ANTIGENS
VASCULAR DISORDERS, VENOUS AND ARTERIAL THROMBOEMBOLISM, CORONARY HEART DISEASE, ISCHEMIC	STROKE, MYOCARDIAL INFARCTION REDUCED CLEARANCE OF VON WILLEBRAND FACTOR AND FVIII	GROUPS A > AB > B
PLAGUE, CHOLERA, TUBERCULOSIS, MUMPS		ANTIGEN PROFILE GROUP O
GONORRHEA, TUBERCULOSIS, S. PNEUMONIAE, E. COLI, SALMONELLA		ANTIGEN PROFILE GROUP B
SMALLPOX, E. COLI, SALMONELLA		ANTIGEN PROFILE GROUP AB
N. MENINGITIDES, H. INFLUENZA, C. ALBICANS, S. PNEUMONIAE, E. COLI URINARY TRACT INFECTIONS, S. PYOGENES, V. CHOLERA		ANTIGEN PROFILE NON-SECRETORS
H. PYLORI STRAIN-DEPENDENT GROUP A; 95% NON-O PEPTIC ULCERS, GASTRODUODENAL DISEASE		SECRETOR STATUS, H. PYLORI STRAIN
ALL NON-SECRETORS; GROUP O		
NOROVIRUS STRAIN-DEPENDENT SECRETORS; GROUPS O, A		
P. FALCIPARUM MALARIA RECEPTOR/ANTIGEN PROFILE KNOPS ANTIGENS; GROUPS A, B		

Disease risk is clearly multifactorial and causation is not implied by association, but blood group antigens may be one of the predisposing factors that contribute to

P. VIVAX MALARIA ANTIGEN PROFILE DUFFY FY ANTIGENS *or prevent disease processes.*

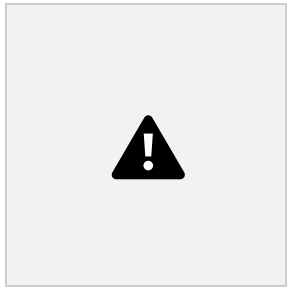
CHOLERA SEVERITY DIFFERS BY ANTIGEN PROFILE LEWIS ANTIGEN; NON- SECRETORS; NON-O GROUPS

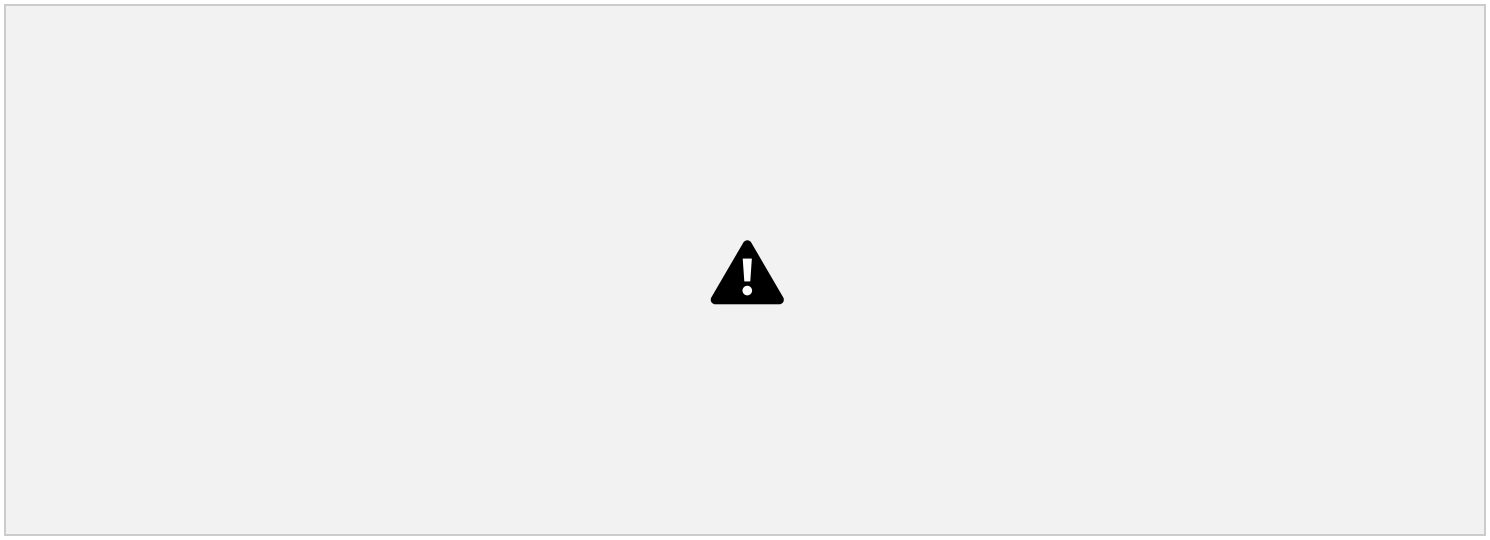
BACTERIAL MENINGITIS (N. MENINGITIDIS, H. INFLUENZA, S. PNEUMONIAE) *Med*. 2016;8(6):517–535.

ANTIGEN PROFILE NON-SECRETORS; A, AB, O BLOOD GROUPS

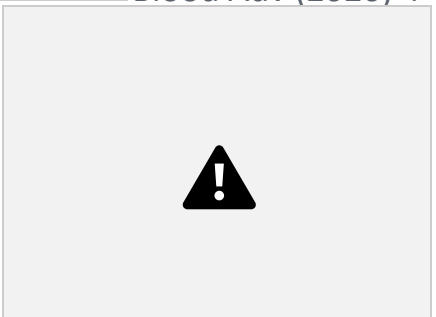
Ewald DR, Sumner SC.. *Wiley Interdiscip Rev Syst Biol*

COVID-19?





Blood Adv (2020) 4 (20): 4981–4989.



Blood Adv 2020 Oct 27;4(20):4990-4993.

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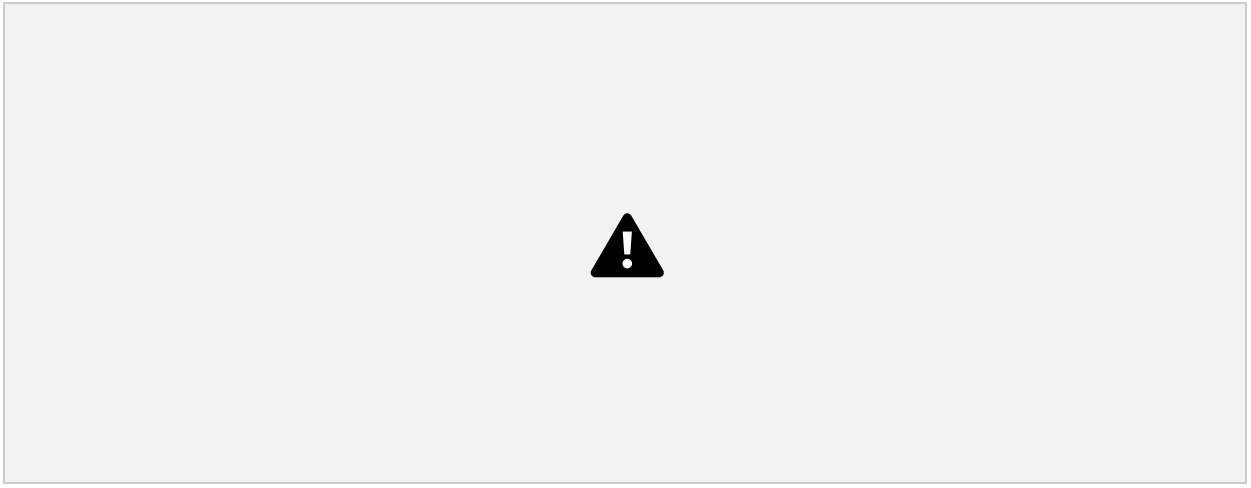
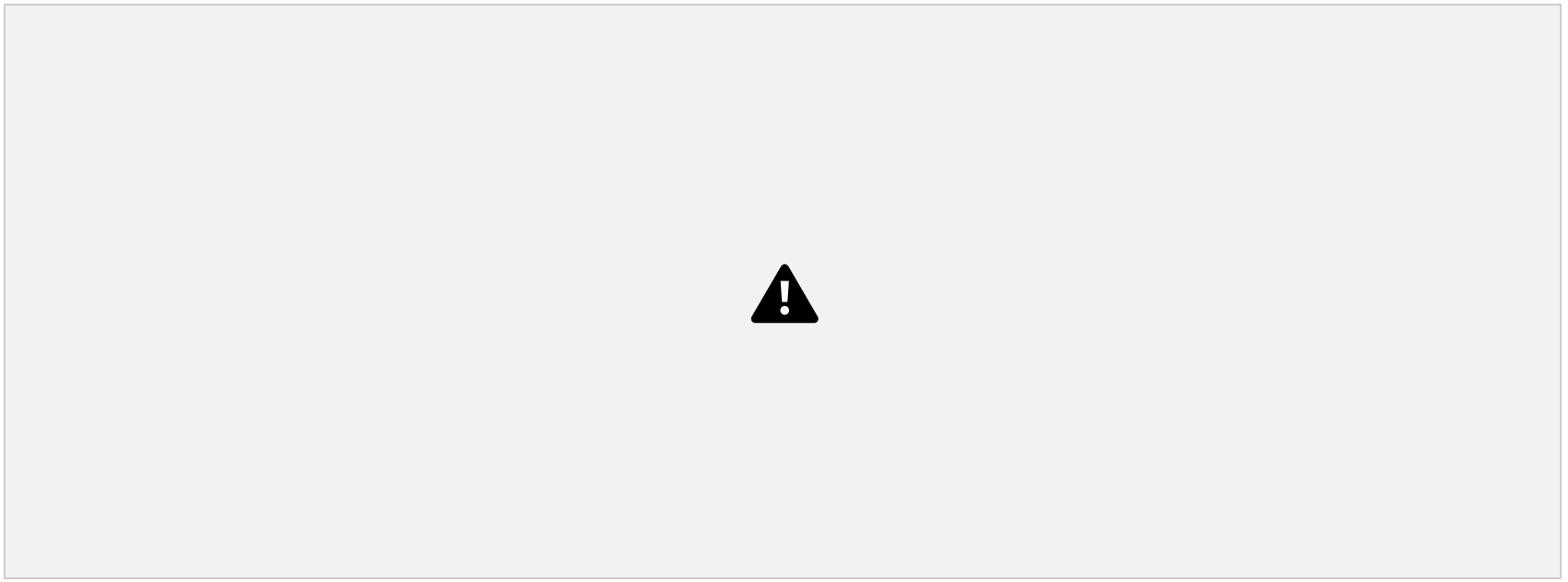
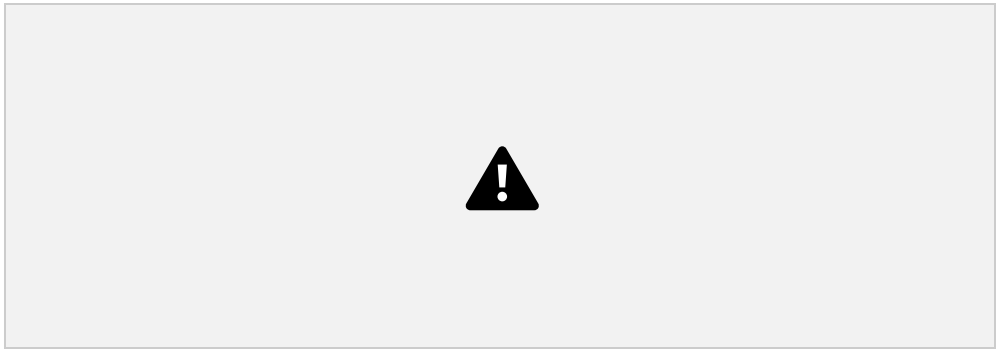
COVID-19?



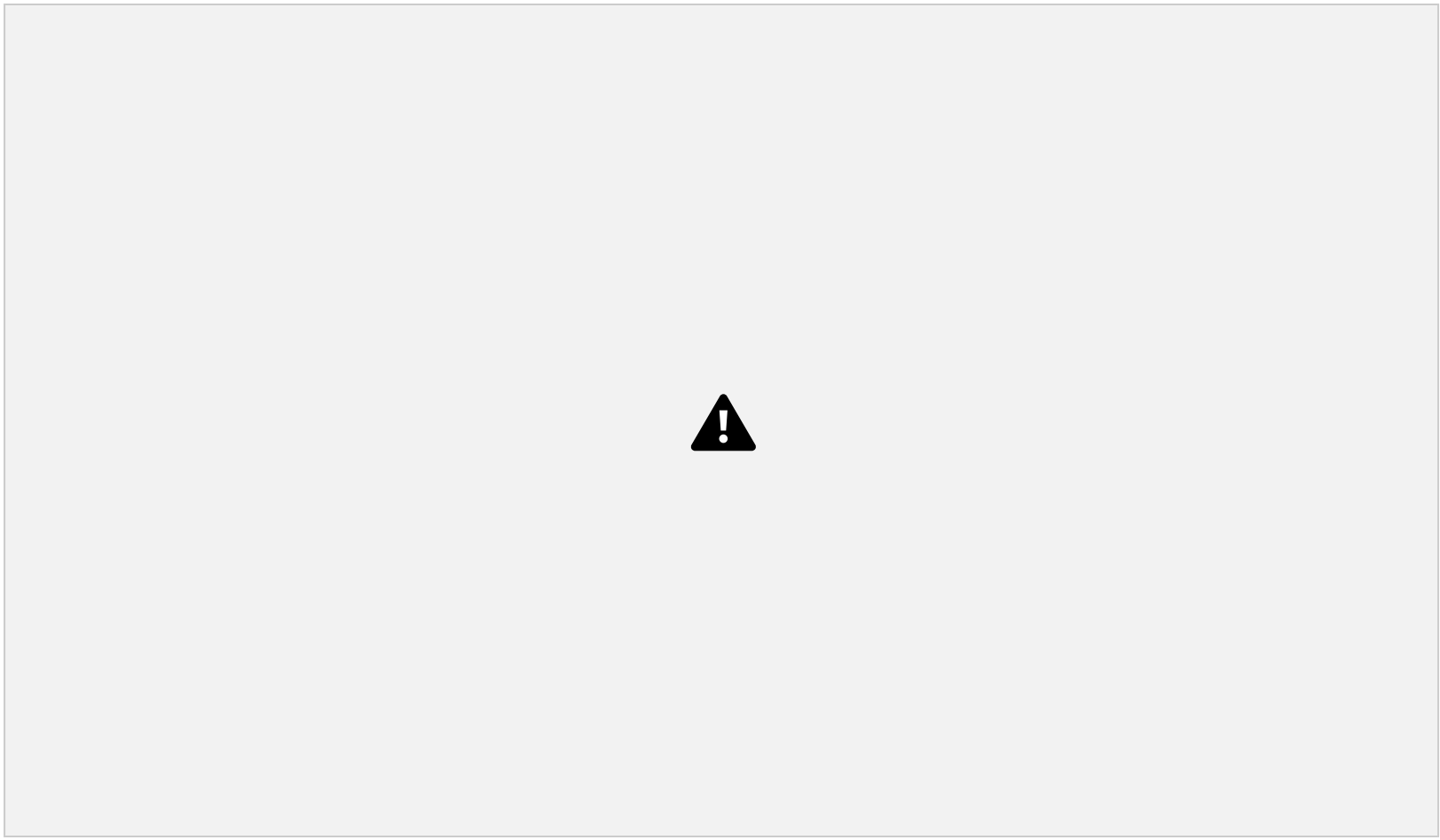


…The new studies that are coming will help us to better clarify this and many other aspects of the ABO involvement in the SARS-CoV

2 infection and the COVID-19 progression. ..

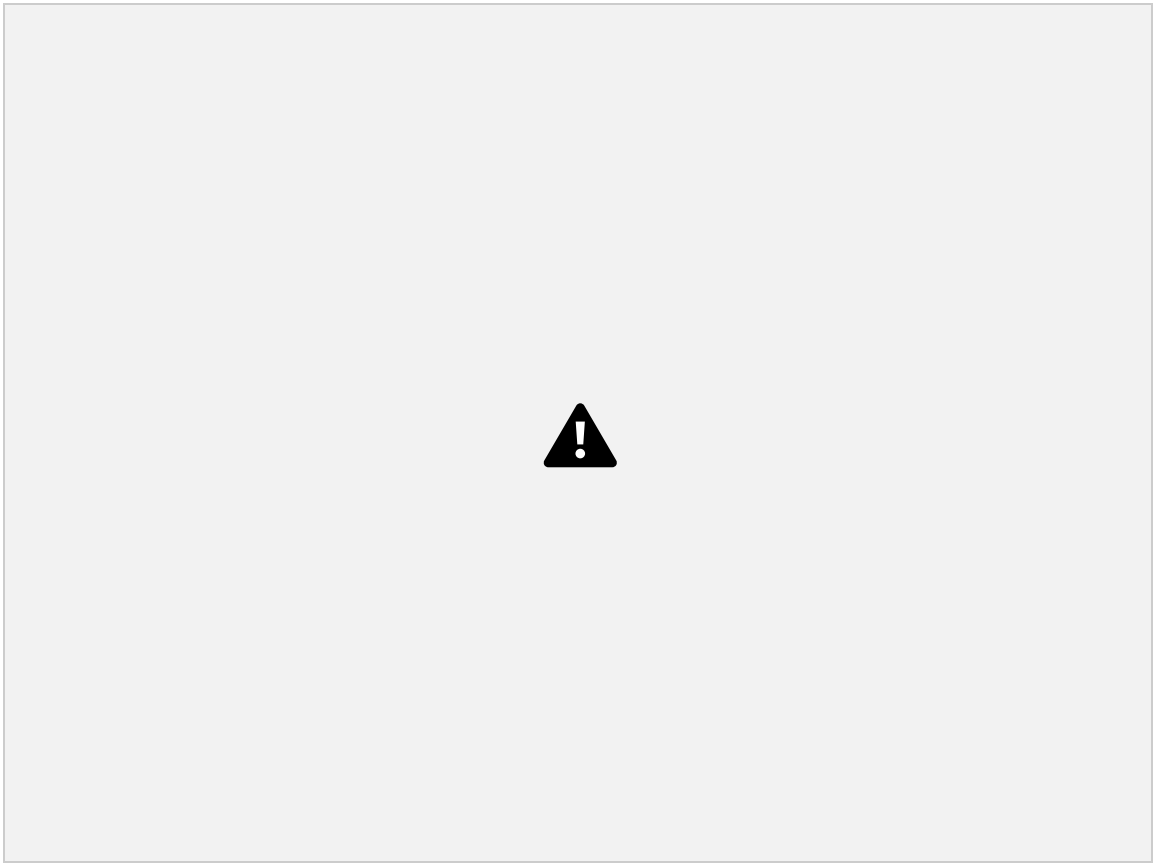


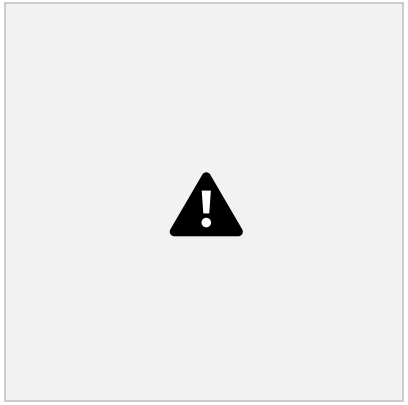
Curiosità: ABO e COVID-19?



Vox Sang 2021

ABO e COVID-19?





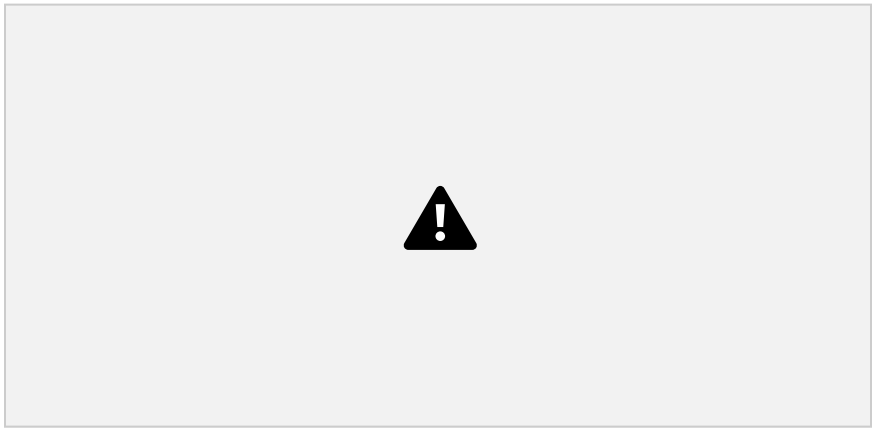
Taken together, these studies suggest that the risk of infection with SARS-CoV-2 and the risk of severe COVID-19 disease may be lower in group O individuals than non-group O individuals. Nonetheless, these results are not definitive and further studies are warranted. *Vox Sang 2021*

Subgroups of A (A^1 and A^2)

- Subgroups of A are phenotypes that differ from others of the same ABO group with respect to the amount of A antigen carried on RBCs
- Variant gene produces a weaker than normal red cell antigen

Subgroups of A (A^1 and A^2)

- Different levels of expression of A on RBCs are classified into ***subgroups***
  - 80% of group A individuals are A1
  - Approximately 20% are A2
- **Transferase produced by A2 gene differs from that produced by A1, less efficient in converting H chains to A**



Difference between A^1 and A^2

- **A^1 has more A and less H antigen on the cell.**
- **A^2 has less A and more H antigen**
- Cannot be detected serologically
- **A^2 can produce anti- A^1**
- In most cases, anti-A1 is of no clinical significance, reacting well below body temperature, and is merely a laboratory nuisance causing ABO discrepancies.
- Anti-A1 is considered clinically significant when it reacts at 37 °C.
- The presence of anti-A1 may cause discrepancies in forward and reverse grouping.

Subgroups of A

- Subgroups of A weaker than A2 (**Ael, Aint, A3, Ax, Am, etc**) are seen only infrequently (**less then 1%**) and are characterized by decreasing numbers of A antigens

Subgroups of B

- Less common than subgroups of A
- Criteria resembles that used for A subgroups

AB's have a wide variety, as they can inherit all the possibilities of the A and B group

ABO ANTIBODIES

… following the Landsteiner law

IgM

NATURAL

“COLD” (REACT AT 20-24°C)

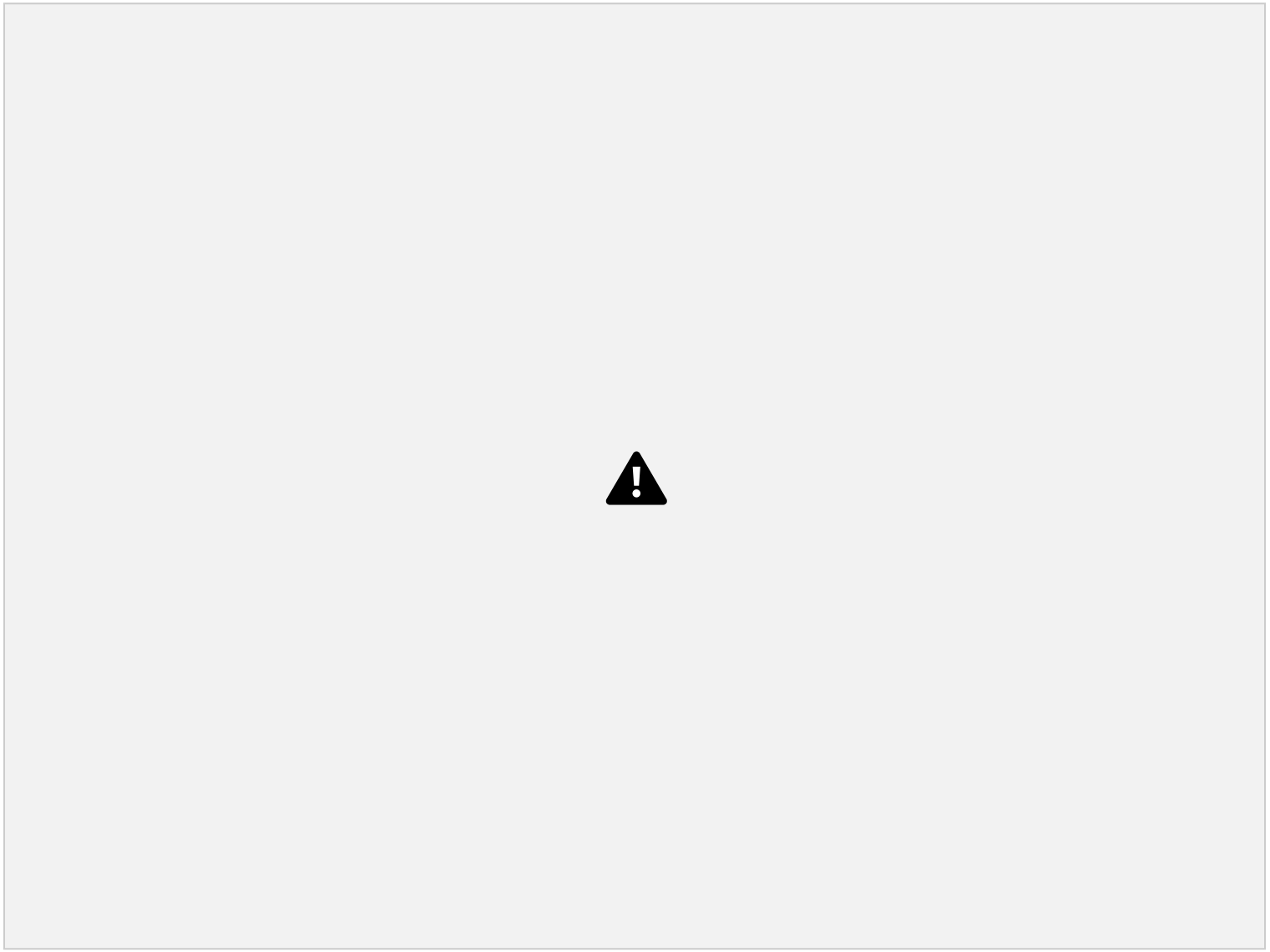
IgG

IMMUNE

“WARM” (REACT AT 37°C)

Whether they are IgG or IgM, ABO antibodies can activate complement readily !!!
INCOMPATIBILITIES CAN CAUSE LIFE THREATENING TRANSFUSION REACTIONS!!!

HYPERSENSITIVITY



HYPERSENSITIVITY

TYPE II HYPERSENSITIVITY (CYTOTOXIC) IS MEDIATED BY ANTIBODIES DIRECTED TOWARD ANTIGENS PRESENT ON THE SURFACE OF CELLS OR OTHER TISSUE COMPONENTS.

✔ **Type II Hypersensitivity**

✔ Type II hypersensitivity is an antibody-dependent process in which specific antibodies bind to antigens, resulting in tissue damage or destruction.

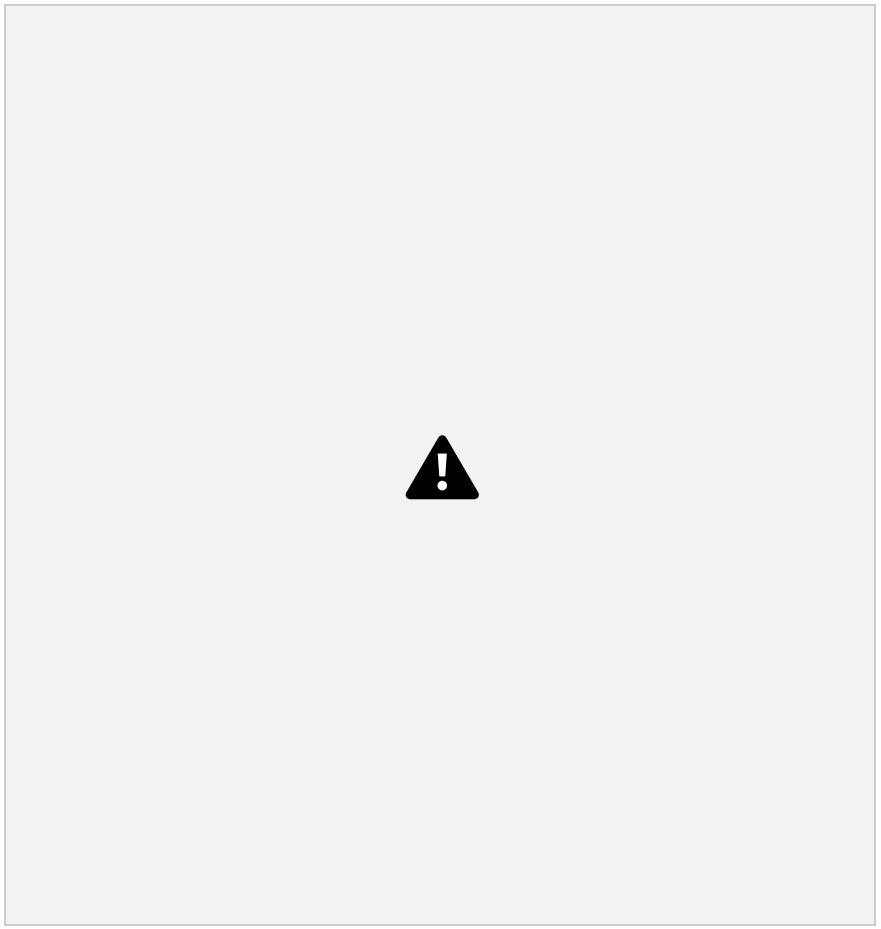
✔ If the antigen is present on cell surfaces, antibody binding can result in cell lysis through the in situ fixation of complement.

✔ IgM antibodies (multimeric) are often more effective in fixing complement than are than IgG antibodies (monomeric).

✔ **Type II hypersensitivity is typified by a transfusion reaction in which mismatched red blood cells are rapidly destroyed by specific preformed antibodies (anti-ABO or -Rh) and complement.**

✔ Although fixation of complement can result in direct cell lysis, opsonization and recruitment of inflammatory cells is often a more important cause of cell injury.

Hemolytic Transfusion Reaction



Immunologic incompatibility between donor and recipient cell types is the most common cause of clinically significant hemolytic transfusion reactions.

Acute reactions (i.e., those occurring within 24 hours after transfusion) develop in response to red cells transfused in patients with preexisting antibodies.

Incompatible A and B blood-group antigens interact with preexisting IgM antibodies and less commonly with hemolytic **IgG antibodies, both of which fix and activate complement.**

N Engl J Med 2019;381:150-62.

meccanismi di eritrodistruzione

IgG e C3b *IgG*

C3b

Legame al recettore Fc

•Hb plasma
Hemoglobinemia
• riduzione aptoglobina

Hemoglobinuria
↓ Haptoglobin
•emoglobinuria
Aderenza alle **C3b**

opsonization
cellule fagocitiche,
phagocytosis
minima fagocitosi

Chemiotassi
Chemotaxis

Macrophages **receptors binding**

Adherence Aderenza macrofagi

normale

•**Aumento bilirubina**

↑ **Bilirubin**

•**Frammentazione**

GR **Fragmentation** of **RBC**

Conversione a C3d **C3d conversion**

Sopravvivenza

AABB, Technical Manual,

Hemolytic Transfusion Reactions I - Intravascular Hemolysis

Pathophysiological features of

ACUTE HEMOLYTIC TRANSFUSION REACTIONS.

Foreign blood-group antigen recognition and binding by **circulating IgM** → activation of **TERMINAL COMPLEMENT** → formation of the **membrane attack complex (MAC).**

The **MAC destroys red-cell membranes,** releasing **free hemoglobin (Hb**) into the intravascular space → **end-organ damage (acute tubular necrosis and renal failure)**

Early complement components → endothelial damage, increased capillary permeability through activation of mast cells, polymorphonuclear cells, monocytes, and endothelial cells → release of cytokines and interleukins , DIC (**disseminated intravascular coagulation**) and ↑ TNF-α tumor necrosis factor α.

N Engl J Med 2019;381:150-62.

Hemolytic Transfusion Reactions II- Extravascular Hemolysis

Pathophysiological features of

DELAYED HEMOLYTIC TRANSFUSION REACTIONS.

Incomplete complement activation (IgG and C3b opsonization) → splenic and hepatic erythrophagocytosis, resulting in **spherocytes and microspherocytes**.

Lysis of red cells → unconjugated bilirubin, which is **transported to the liver**.
Hepatic conjugated bilirubin is excreted as urobilinogen and stercobilinogen.

Anemia from red-cell destruction and **jaundice** from excess unconjugated and conjugated bilirubin are the primary clinical **manifestations of delayed hemolytic transfusion reactions.**

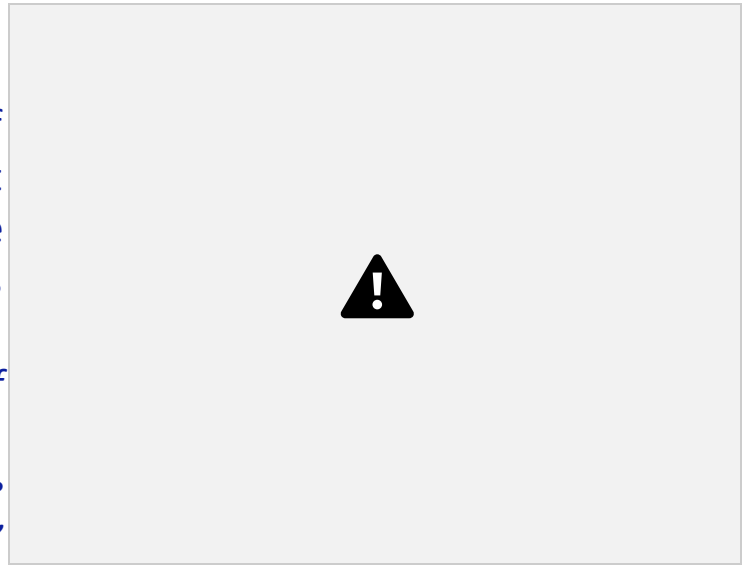
N Engl J Med 2019;381:150-62.

Hemolytic Transfusion Reactions

Hemolytic Transfusion Reaction

The earliest description of an incompatible hemolytic transfusion reaction dates to the experimental start of transfusion therapy in the mid-17th century.

Jean-Baptiste Denis described what has become the classic reaction:

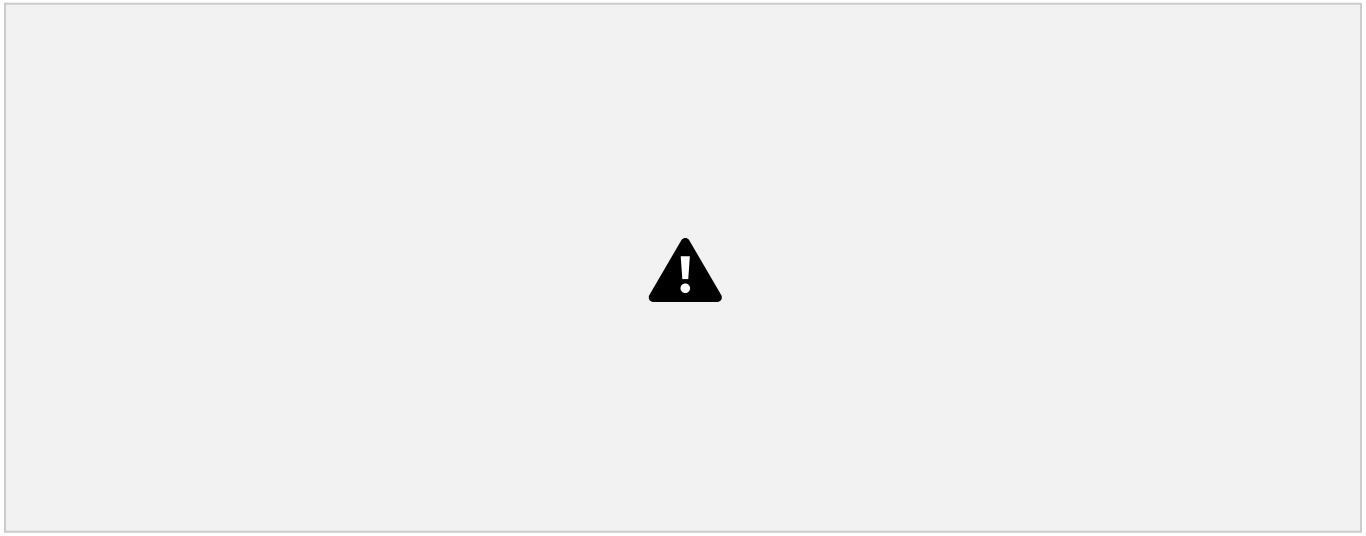


The patient was transfused with 5-6 ounces of calves' blood. During the procedure, the patient complained that the vein in his right arm became quite painful. The procedure was repeated 2 days later; a larger transfusion was given. Following the transfusion, however, *the patient complained of pain in the arm vein; his pulse rose, he vomited, and he had a severe nosebleed, pain over the kidney, and an "oppressive sensation in the chest." The next day, he "made a great glass of urine with*

a color as black as if it had been mixed with the soot of a chimney."

Hemolytic Transfusion Reactions

in the last 15 years there was an overall decline in deaths related to hemolytic transfusion reactions, with persistently low numbers of reported deaths in more recent years.
to date, wrong blood in a tube, determined by the misidentification of the patient to be transfused at pre-transfusion test, still represent a big problem



Annual Reported Deaths in the United States from Hemolytic Transfusion Reactions. The data, reported by the Food and Drug Administration for fiscal years 2005 through

2016,show **an overall decline in deaths related to hemolytic transfusion reactions, with persistently low numbers of reported deaths in more recent years.**

Hemolytic Transfusion Reactions

- If an ABO-incompatible transfusion occurs or is suspected, the transfusion should be stopped immediately, the venous line should be kept open with normal saline, and supportive care administered as needed.
- Close surveillance of the patient's vital signs for the first 30 minutes of transfusion should help identify most incompatible transfusions early.

Death occurs in 15% of cases of ABO incompatibility

and may result from as little as 30 mL of transfused ABO incompatible blood

Janatpour KA, Kalmin ND, Jensen HM, et al. Clinical outcomes of ABO

ansfusions. Am J Clin Pathol 2008;129:276–81.

ON REQUEST

The request for blood products should be in writing form, including:

- Patient identification (full name, age or birth date).
- Diagnosis and indication for transfusion.
- Requested product(s) and number of units.
- Date and time of request and desired delivery.
- Name, signature of the prescribing physician.

The following information must be mentioned:

- ABO blood group and RhD.
- Did the patient receive transfusion earlier?
- If YES when was the last?
- Has the patient been pregnant?

- Are previous transfusion reactions known?
- Have red cell antibodies ever been detected?

BLOOD TRANSFUSION REQUEST

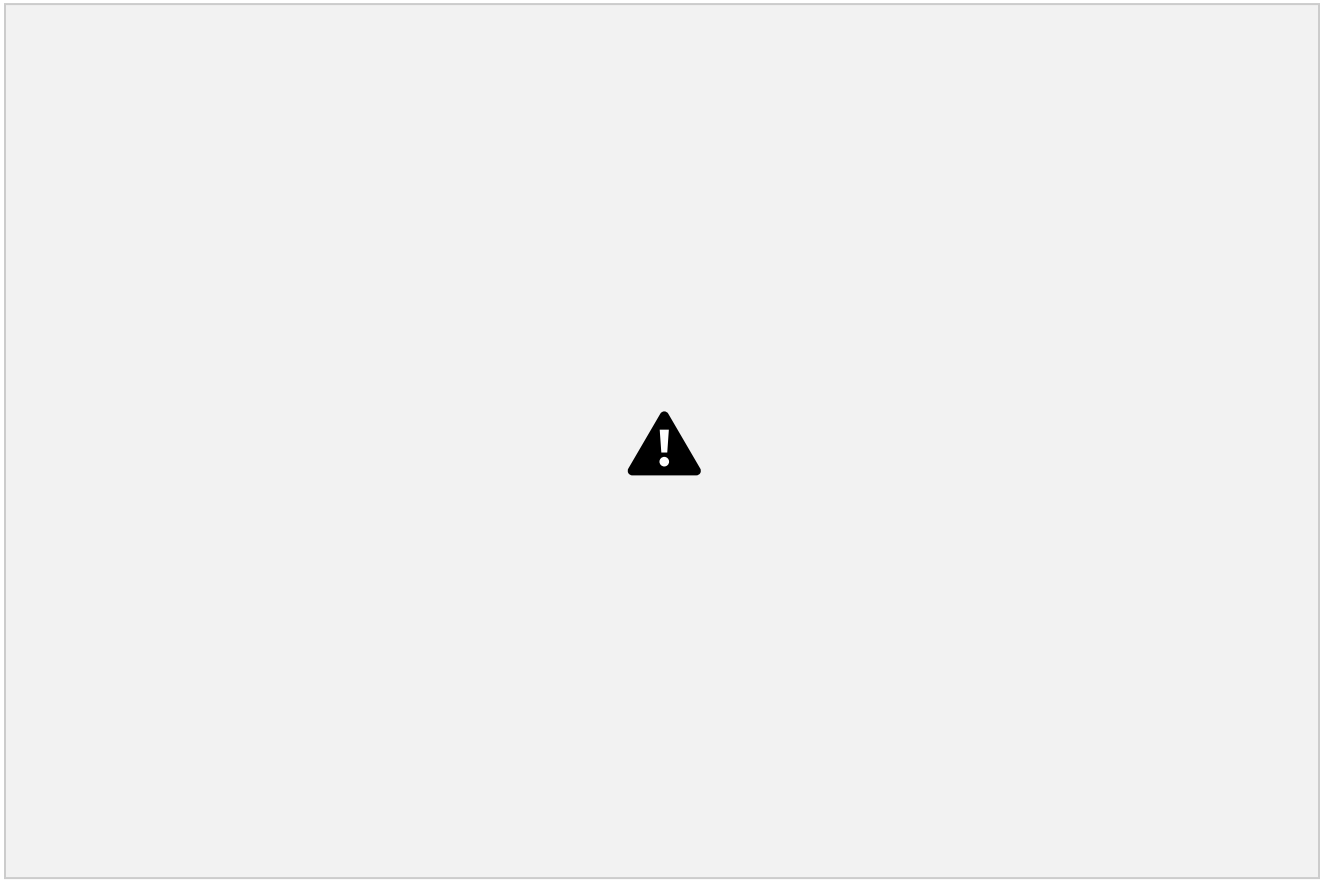
To ensure patient safety, the transfusion laboratory requires the patient to have been tested on more than one occasion prior to deliver red blood cells.

The two separate samples for Group must have been taken at TWO

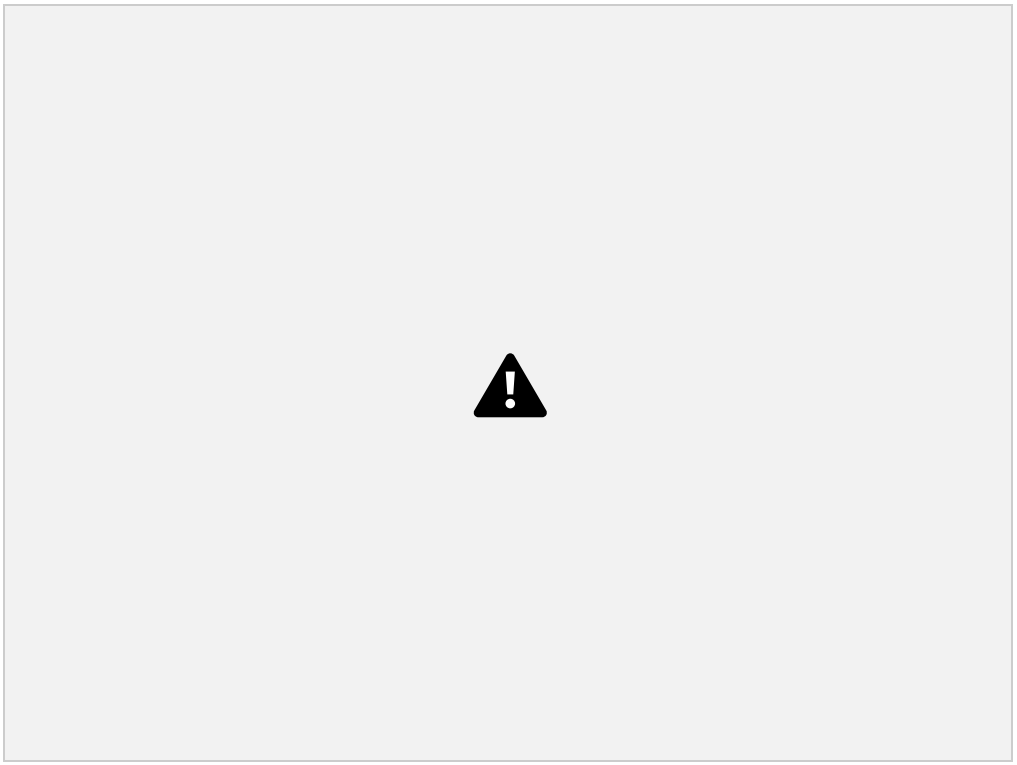
different times

Blood donation

Which blood types are your red blood cells compatible with?



Blood donation



Taking into account the absence of AB antibodies, a AB individuall should be considered a universal plasma donor

Platelets incompatibles transfusion could cause platelet refractoriness: platelets transfused are destroyed, so the transfusion is less efficient

DEVELOPMENT of anti-A and anti-B

- Antibody production begin slowly in first few months of life

- Babies cannot be reversed typed :
  - Antibodies present in baby derive from mum
  - Are not born with antibodies, detectable at 3 to 6 months of age
  - Reach maximum level at five years
- Once produced remain constant until elderly

HDFN
IgG can cross the placenta!!!
In case of ABO incompatibility beetween mother and fetus anti-A, anti-B or anti – A,B IgG can lead to HDFN

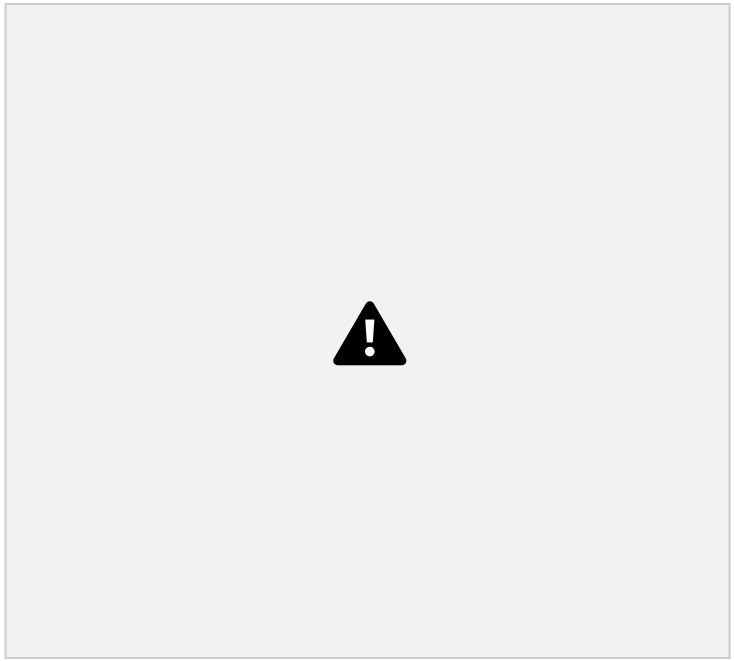
The most common presentation is jaundice!

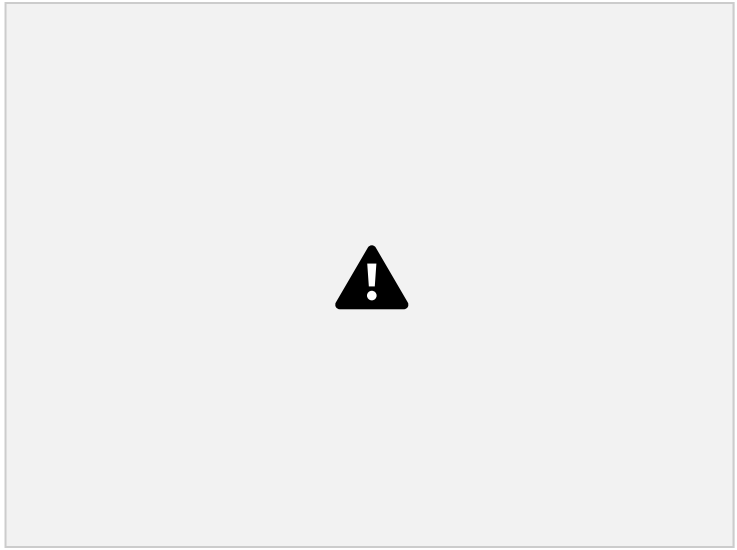
Agglutination of erytrocytes

AB0 typing

The blood grouping and Rh(D) typing procedure is based on the principle of agglutination.

Normal red blood cells, possessing antigens, will agglutinate in the presence of antibodies directed toward those antigens. Commercial antisera are used to test patient and donor cells



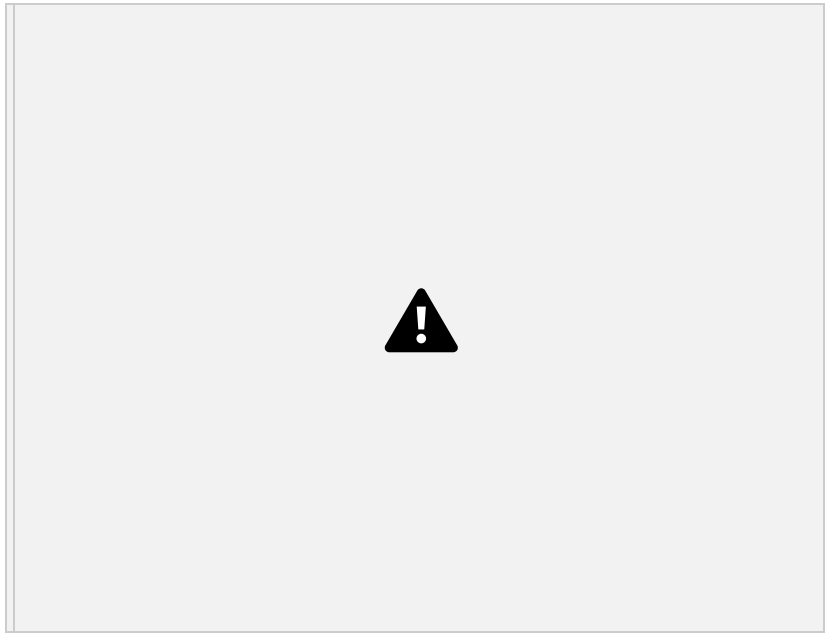


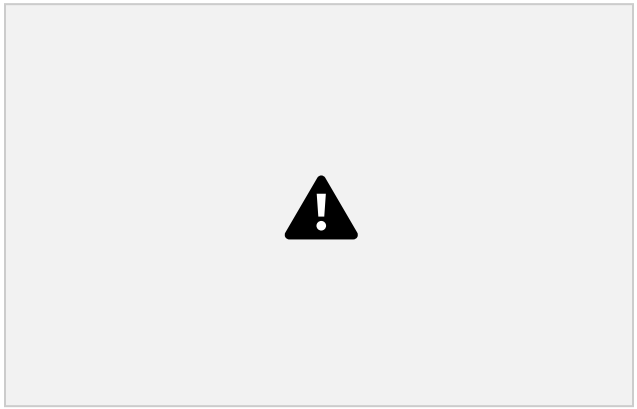
A

positive

AB0 direct grouping (we know the antibodies)

Anti –A	Anti-B
GROUP A pos	neg
GROUP B neg	pos
GROUP AB	pos pos
GROUP 0 neg	neg



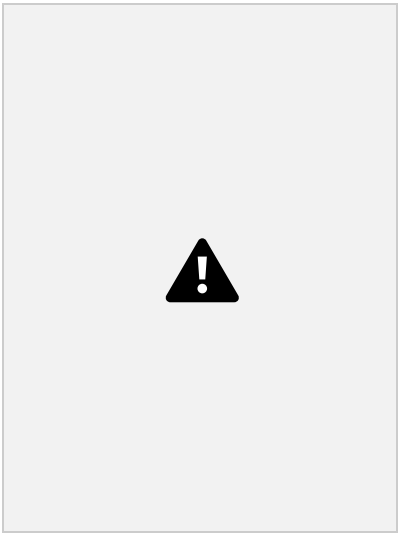


AB0 reverse grouping

(we know the antigens)

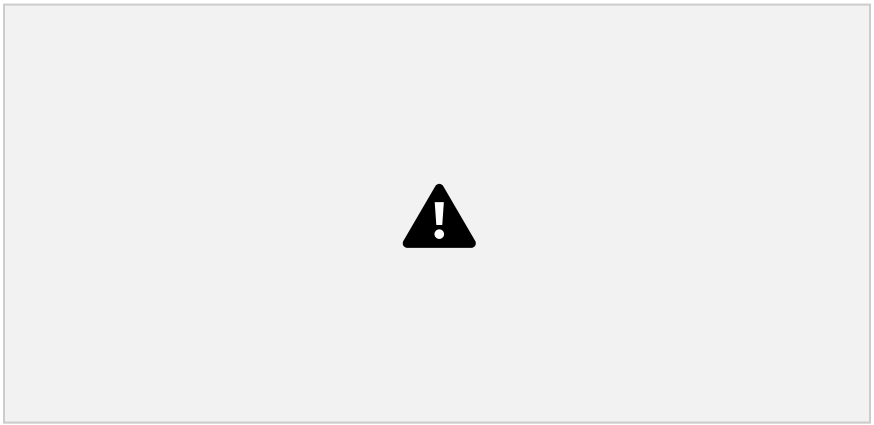
A cells B cells 0 cells

GROUP A neg pos neg GROUP B pos neg neg GROUP AB neg neg neg GROUP 0 pos pos neg



PCR-SSP

AB0 typing



0A genotype, A phenotype

The Rh System

- The most important blood group system after AB0 in

transfusion medicine

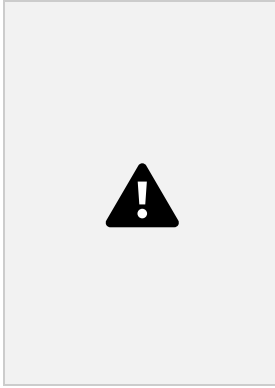
- 1937 Levine and Landsteiner
- A very important protein of blood groups

  - Highly immunogenic (90% possibility to became immunized) - Trasfusions
  - Pregnancies
  - Variability in populations
  - RhD+ 85% of caucasians, 94% Africans, 98% Asiatics - High genetic polymorphism

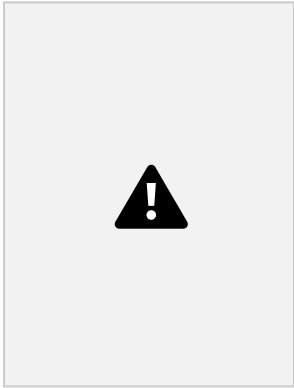
Discovered in 1937 by Levine and Landsteiner

- In **1939 by Levine and Stetson found an antibody directed at the D antigen in the serum of a woman whose fetus had fatal hemolytic disease of the newborn**.

• **The Rh system was identified by the work of Landsteiner and Wiener who found that human RBCs were agglutinated by an antibody, apparently common to all rhesus monkeys and 85% of humans. This factor was named the Rh factor.**



• **Landsteiner and Wiener immunized guinea pigs and rabbits with the RBCs of Rhesus monkeys, the antibody produced by these animals agglutinated 85% of human RBCs**. Later the antigens detected by the rhesus antibody and by the human antibody were established as dissimilar, but the system had already been named.



• **This contribution to medical science was the most significant event in blood group systems research since the discovery of the ABO system 40 years earlier.**

The Rh/D factor

The **Rh/D factor is** not the single entity as originally thought but **a complex system of antigens**.

There are actually two genes, **RHD** and **RHCE**, accounting for five main antigens: **D, C, c, E, e**.

The D antigen is the strongest of the Rh system and most potent antigenically and therefore the most important in haemolytic disease and in transfusion reactions.

Rh positive is D positive !

The Rh/D factor

Rh System

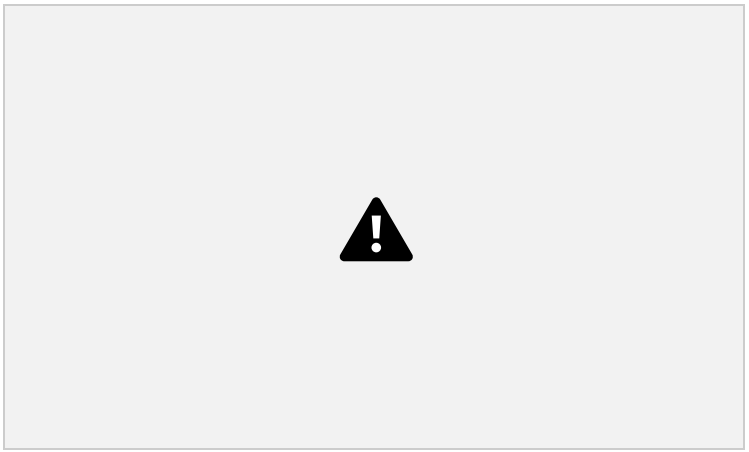
- ✔ Two genes (**RHD, RHCE**) in close proximity on **CHROMOSOME 1** encode the erythrocyte **Rh proteins, RhD and RhCE**; **one carries the D antigen**, and **the other carries CE antigens** in various combinations (ce, Ce, cE, or CE).
- ✔ The genes each have ten exons, are 97% identical.
- ✔ Individuals who lack RhD protein, “Rh or D negative”, most often have a complete deletion of the RHD gene

Rh+ Phenotypes

CcDee CCDee CcDEe
CCDEe CcDEE CCDEE
ccDee ccDEe ccDEE
You can see all the possible combination!!

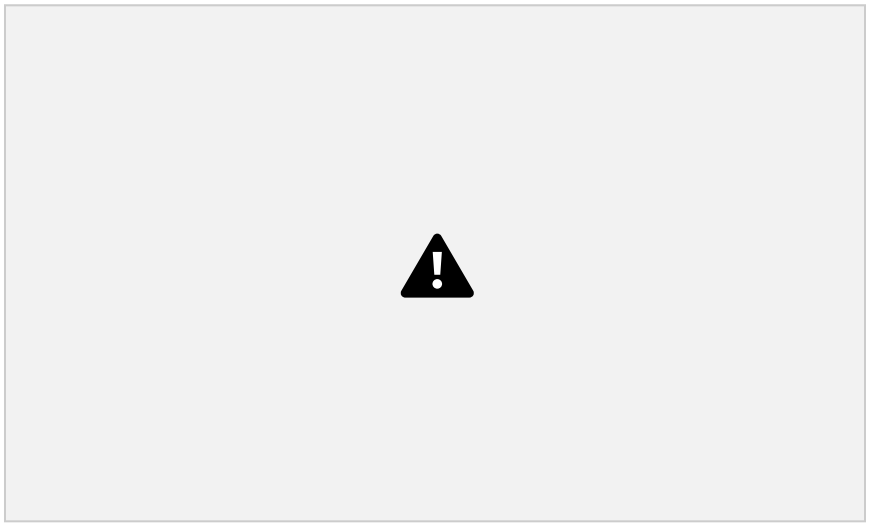
Ccdee
CcdEe
Ccdee
Ccdee
ccdEE
CcdEe
ccdee

Rh- Phenotypes

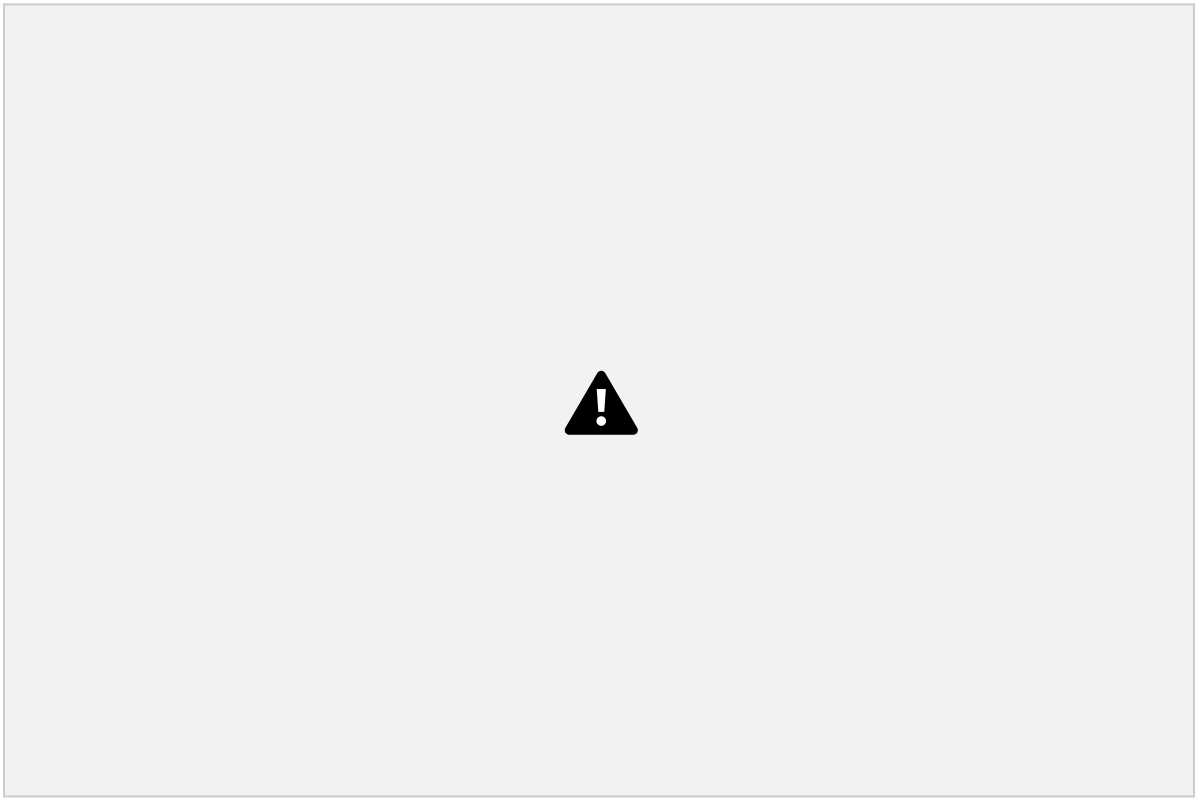


Rh system

In the Rh system there are no natural antibodies: it is necessary to have an immunization (through pregnancy or transfusion) to produce the specific antibody.



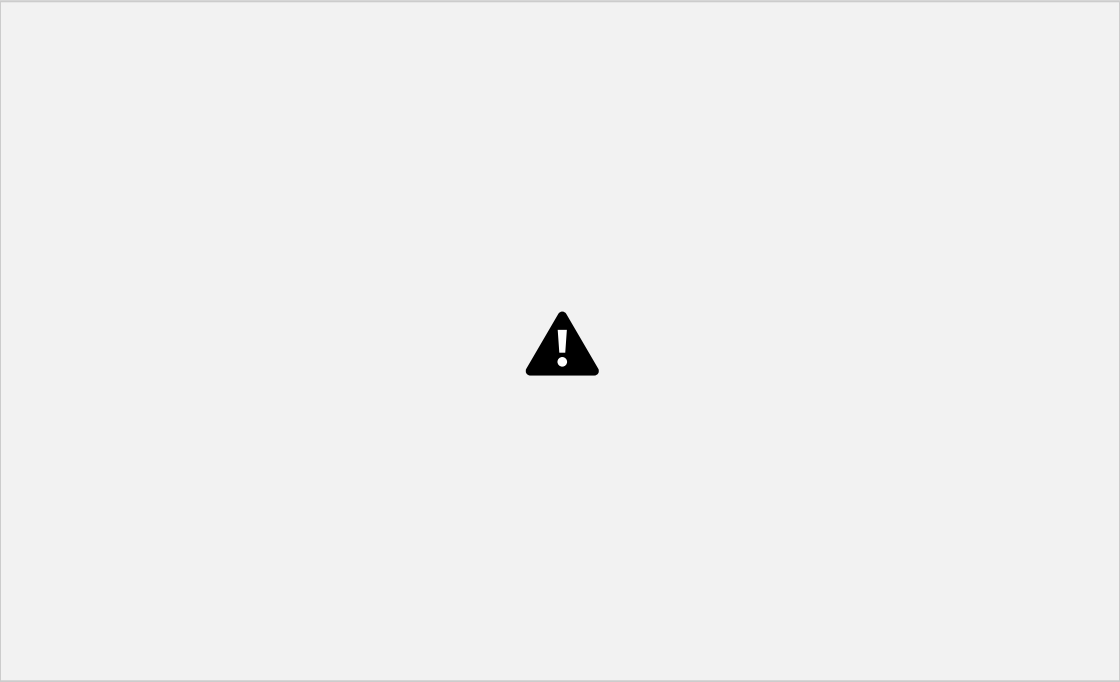
Blood donation



Now you are able to observe again this picture taking into account also the RhD system!

Remeber that antibodies are not natural and it is necessary to have immunizing events such as pregnancies or previous blood transfusions

HDFN RH



When an RhD negative mother is exposed to the RhD positive red cells (usually as transplacental haemorrhage), she develops allo-anti-D which crosses the placenta and then results in the destruction of fetal red cells. Clinical manifestations of RhD haemolytic disease (HDN) range from asymptomatic mild anaemia to hydrops fetalis or stillbirth associated with severe anaemia and jaundice.

HDFN RH

Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev. 2000

Prevention includes administration of anti-D immunoglobulin for any event associated with TPH during pregnancy, and at delivery of an RhD positive infant. Prophylactic routine administration of anti-D immunoglobulin at 28 (and 34) weeks gestation, in addition to the above, has reduced alloimmunisation to <1% of RhD negative women carrying an RhD positive fetus

Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev. 2000

D VARIANT

WEAK D PARTIAL D

D weak

First described by Stratton on 1946 as Du, Weak D expression results from single point mutations in RHD that encode amino acid changes predicted to be intracellular or in the transmembrane regions of RhD with reduced number of D antigen sites on the RBCs.

Over 99 different mutations, the most common being a Val270Gly designated Type 1, cause weak D expression . Mutations are catalogued on the Rhesus Base and blood group mutation websites and are updated regularly.

Occurs in an estimated **0.2%-1% of Caucasians**.