Acquire Hemolytic Anemia

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Hemolytic anemia

- Anemia due to increased rate of RBC destruction
- Anemia occurs when rate of destruction exceeds production

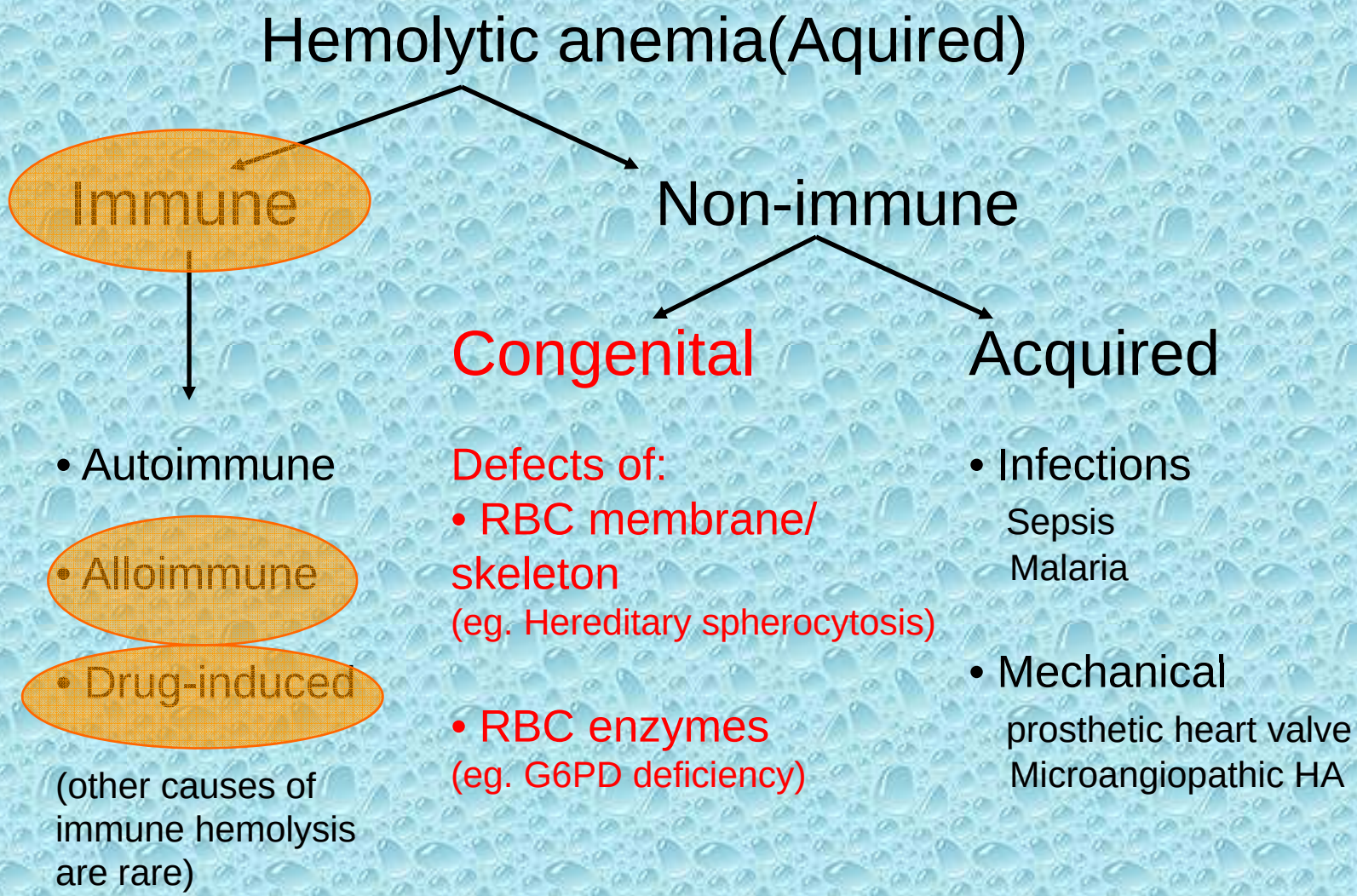
Normal red cell turnover

- Normal RBC survival of ~ 120 days
- Macrophages of the Reticuloendothelial (RE) system removes RBC's
  - Unclear what marks a red cell for removal
  - Spleen is major site of RBC clearance
- RE system is Extravascular
  - 90% of normal RBC destruction occurs without release of hemoglobin into circulation.

Is there hemolysis?

- Look for 3 lines of evidence:
  - 1. Damaged red cells on the blood film
    - Spherocytes (Immune hemolysis, HS)
    - Red cell fragments (Microangiopathic anemias)
  - 2. Marrow response to Hemolysis
    - Polychromasia on blood film
    - Reticulocytosis
    - Erythroid hyperplasia in marrow
  - 3. Biochemical evidence of RBC destruction
    - Increased unconjugated bilirubin
    - Increased lactate dehydrogenase
    - Decreased/absent haptoglobin

An approach to hemolytic anemia



Acquired hemolytic anemia

Immune mediated hemolytic anemia (Direct Coombs test is positive)

1- Autoimmune hemolytic anemia

Warm antibody autoimmune hemolytic anemia

- Idiopathic
- Systemic lupus erythematosus (SLE)
- Evans' syndrome (antiplatelet antibodies and hemolytic antibodies)

Cold antibody autoimmune hemolytic anemia

- Idiopathic cold hemagglutinin syndrome
- Infectious mononucleosis and mycoplasma (atypical) pneumonia
- Paroxysmal cold hemoglobinuria (rare)

2-Alloimmune hemolytic anemia

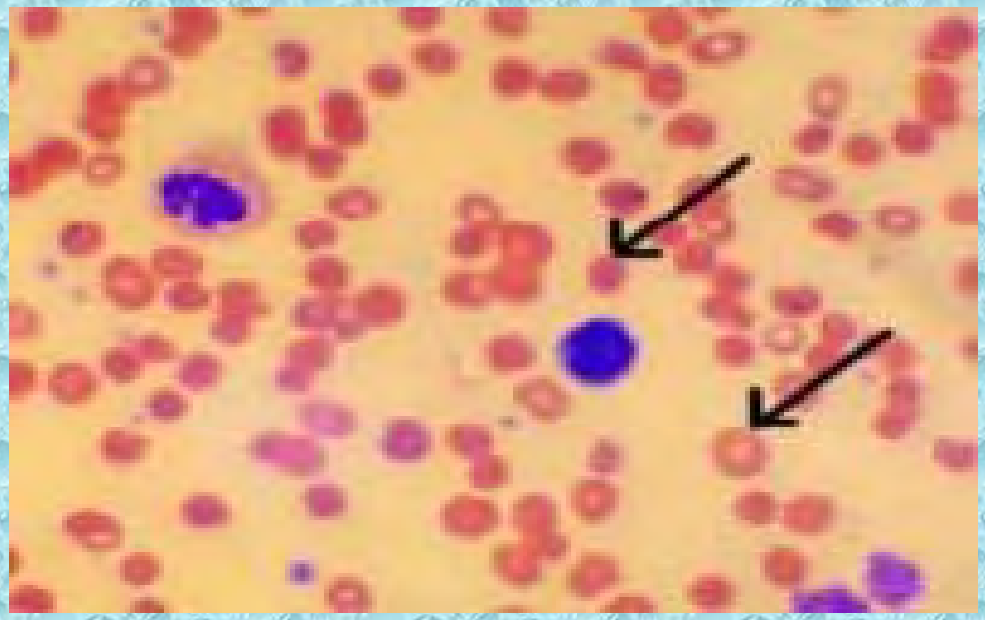
Haemolytic disease of the newborn (HDN)

- Rh disease (Rh D)
- ABO hemolytic disease of the newborn
- Anti-Kell hemolytic disease of the newborn
- Rhesus c hemolytic disease of the newborn
- Rhesus E hemolytic disease of the newborn
- Other blood group incompatibility (RhC, Rhe, Kidd, Duffy, MN, P and others)

Alloimmune hemolytic blood transfusion reactions (ie from a non-compatible blood type)

Drug-induced autoimmune hemolytic anemia Penicillin in high doses via the Hapten mechanism

Spherocytes. One arrow points to a spherocyte; the other, to a normal RBC with a central pallor.



Acquired hemolytic anemia

Non-immune mediated hemolytic anemia (Direct Coombs test is negative)

Drugs (i.e., some drugs and other ingested substances lead to haemolysis by direct action on RBCs, e.g. Ribavirin)
Toxins (e.g., Snake venom; plant poisons such as Aesculin)

Trauma

Mechanical (from Heart valves, extensive vascular surgery, microvascular disease, repeated mechanical vascular trauma)

Microangiopathic hemolytic anemia

Infections (Note: Direct Coombs test is sometimes positive in hemolytic anemia due to infection)

Malaria
Babesiosis
Septicaemia

Membrane disorders

Paroxysmal nocturnal hemoglobinuria (rare acquired clonal disorder of red blood cell surface proteins)
Liver disease

Diagnosis of immune hemolytic anemia

- 1. Direct Antiglobulin Test (DAT or direct Coomb's test)
  - Detects IgG or complement **on patient's red cells**
  - the vast majority of patients with active immune hemolysis will have a positive DAT.
- 2. Indirect Antiglobulin Test (IAT, indirect Coomb's test)
  - Detects antibody **in patient's serum** against red cell antigens
  - A positive IAT does not necessarily mean hemolysis is occurring - It may simply mean allo-immunization due to previous exposure to "foreign" red cell antigens (past pregnancy or transfusion).
- 3. Peripheral Blood Film: spherocytes

IMMUNE HEMOLYTIC ANEMIA

General Principles

- All require antigen-antibody reactions
- Types of reactions dependent on:
  - Class of Antibody
  - Number & Spacing of antigenic sites on cell
  - Availability of complement
  - Environmental Temperature
  - Functional status of reticuloendothelial system
- Manifestations
  - Intravascular hemolysis
  - Extravascular hemolysis

Laboratory feature	Intravascular hemolysis	Extravascular hemolysis
LDH	Grossly elevated	Elevated
Haptoglobin	Reduced or absent	Normal or reduced
Methemalbumin	Present	Absent
Hemosidinuria	Present	Absent
Splenomegaly	Absent	Present
Blood film	Schistocytes, helmet cells, fragmented red cells	Spherocytes, erythrophagocytosis
Hemoglobinemia	Present	Absent/present in severe cases
Hemoglobinuria	Present	Absent/present in severe cases

IMMUNE HEMOLYTIC ANEMIA

General Principles - 2

- Antibodies combine with RBC, & either
  1. Activate complement cascade, &/or
  2. Opsonize RBC for immune system
- If 1, if all of complement cascade is fixed to red cell, intravascular cell lysis occurs
- If 2, &/or if complement is only partially fixed, macrophages recognize Fc receptor of Ig &/or C3b of complement & phagocytize RBC, causing extravascular RBC destruction

IMMUNE HEMOLYTIC ANEMIA
Coombs Test - Direct

- Looks for immunoglobulin &/or complement of surface of red blood cell (normally neither found on RBC surface)
- Coombs reagent - combination of anti-human immunoglobulin & anti-human complement
- Mixed with patient's red cells; if immunoglobulin or complement are on surface, Coombs reagent will link cells together and cause agglutination of RBCs

IMMUNE HEMOLYTIC ANEMIA
Coombs Test - Indirect

- Looks for anti-red blood cell antibodies in the patient's serum, using a panel of red cells with known surface antigens
- Combine patient's serum with cells from a panel of RBC's with known antigens
- Add Coombs' reagent to this mixture
- If anti-RBC antigens are in serum, agglutination occurs

IMMUNE HEMOLYSIS

Drug-Related

- Immune Complex Mechanism
  - Quinidine, Quinine, Isoniazid
- "Haptenic" Immune Mechanism
  - Penicillins, Cephalosporins
- True Autoimmune Mechanism
  - Methyldopa, L-DOPA, Procaineamide, Ibuprofen

DRUG-INDUCED HEMOLYSIS

Immune Complex Mechanism

- Drug & antibody bind in the plasma
- Immune complexes either
  - Activate complement in the plasma, or
  - Sit on red blood cell
- Antigen-antibody complex recognized by RE system
- Red cells lysed as “innocent bystander” of destruction of immune complex

DRUG-INDUCED HEMOLYSIS
Haptenic Mechanism

- Drug binds to & reacts with red cell surface proteins
- Antibodies recognize altered protein, ± drug, as foreign
- Antibodies bind to altered protein & initiate process leading to hemolysis

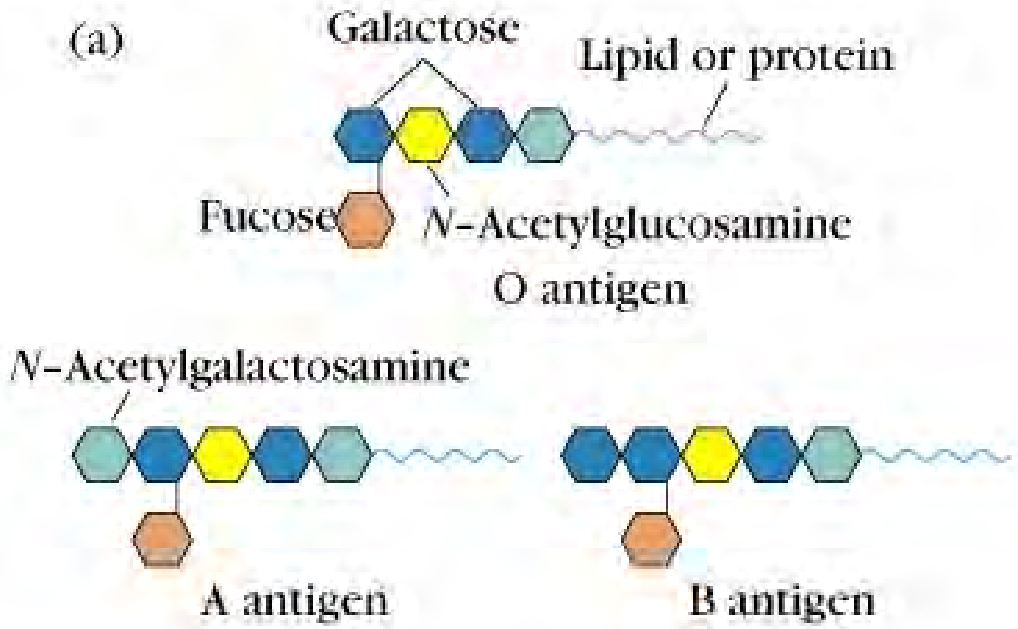
DRUG-INDUCED HEMOLYSIS
True Autoantibody Formation

- Certain drugs appear to cause antibodies that react with antigens normally found on RBC surface, and do so even in the absence of the drug

Transfusion reactions

- Antibodies to the A, B, and O antigens on red blood cells are usually IgM class
- An individual with blood group A has antibodies against B in their blood
- If a type A individual is accidentally transfused with blood containing type B cells, the anti-B antibodies will bind to the B blood cells and mediate their destruction by means of complement-mediated lysis

(a)



(b)

Genotype	Blood-group phenotype	Antigens on erythrocytes (*agglutinins*)	Serum antibodies (*isohemagglutinins*)
AA or AO	A	A	Anti-B
BB or BO	B	B	Anti-A
AB	AB	A and B	None
OO	O	None	Anti-A and anti-B

ALLOIMUNE HEMOLYSIS

Hemolytic Transfusion Reaction

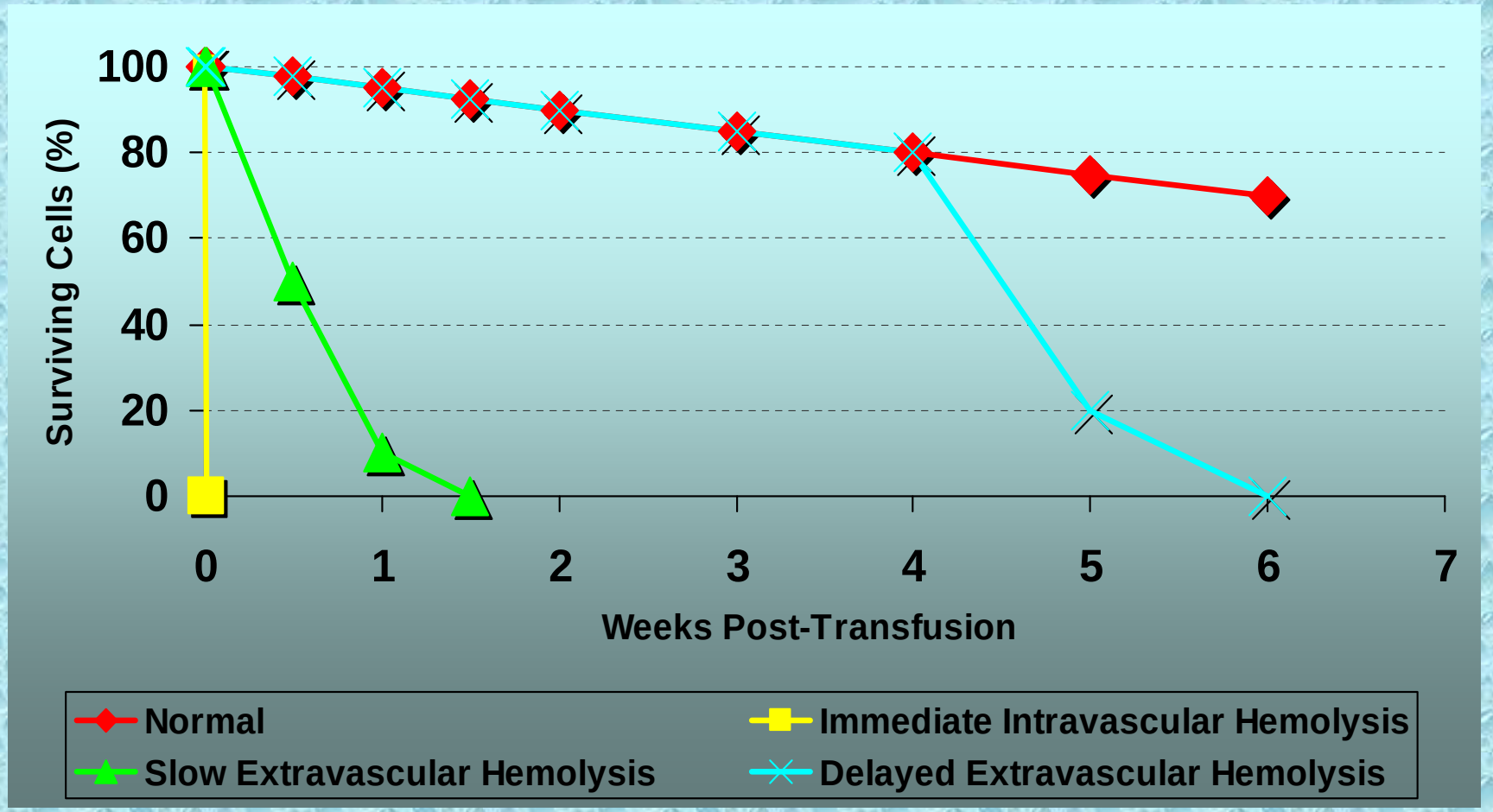
- Caused by recognition of foreign antigens on transfused blood cells
- Several types
  - Immediate Intravascular Hemolysis (Minutes) - Due to preformed antibodies; life-threatening
  - Slow extravascular hemolysis (Days) - Usually due to repeat exposure to a foreign antigen to which there was a previous exposure; usually only mild symptoms
  - Delayed sensitization - (Weeks) - Usually due to 1st exposure to foreign antigen; asymptomatic

Delayed hemolytic reaction

- Occurs in individuals who have received repeated transfusions of ABO-compatible blood that is incompatible for other blood groups
- The reaction develops within 2-6 days after transfusion
- The transfused blood induces clonal selection and production of IgG against a variety of receptors
- Blood group antigens that cause this: Rh, Kidd, Kell, and Duffy
- Symptoms: fever, low hemoglobin, increased bilirubin, jaundice and anemia

INCOMPATIBLE RBC TRANSFUSION

Rate of Hemolysis



Type II hypersensitivity (Antibody-mediated cytotoxic)

- Involves antibody-mediated destruction of cells
- This type is exemplified by blood transfucion reactions
- Host antibodies react with foreign antigens on the incompatible transfused blood cells and mediate destruction of those cells
- Antibodies mediate cell destruction by activating the complement system or though antibody-dependent cell-mediated cytotoxicity (ADCC) (cytotoxic cells bind to the Fc region of antibodies on target cells)

THE TIMES

February 4 1997 BRITAIN

Patient died after getting wrong blood transfusion

BY PAUL WILKINSON

A HEART surgery patient died after he was given a pint of blood intended for the person in the next bed, an inquest jury was told yesterday.

Two nurses were suspended after the death of Norman Lynes but were later exonerated by a hospital inquiry. Relatives of Mr Lynes, 73, a retired stock controller, have taken legal advice over action against Castle Hill Hospital at Cottingham, near Hull.

Mr Lynes died from kidney and bowel failure last June seven days after undergoing heart bypass surgery. The inquest in Hull was told that he had been given a pint of the A-positive blood group instead of O-positive during a post-operation transfusion as he lay in intensive care on a ventilator.

Staff nurse Nicola Wild discovered the error three hours later when she opened the bedside fridge for a second pint of blood. Inside, she found all of Mr Lynes's blood units still there.

Police carried out an investigation into possible criminal negligence but the Crown Prosecution Service decided to take no action. Despite suffering two heart attacks the previous year, Mr Lynes had been given a 95 per cent chance of surviving the operation.

The inquest continues today.

HAZARDS REPORTED

Serious Hazards of Transfusion **SHOT**

- Incorrect blood/component transfused (IBCT)
- Acute transfusion reaction (including anaphylaxis)(ATR)
- Delayed transfusion reaction (DHTR)
- Transfusion-associated graft-versus-host-disease (TA-GVHD)
- Transfusion-related acute lung injury (TRALI)
- Post-transfusion purpura (PTP)
- Transfusion transmitted infection, including bacterial contamination (TTI)
- Autologous pre-donation incidents

Serious Hazards of Transfusion **SHOT**

TOTAL ISSUES OF BLOOD COMPONENTS FROM THE TRANSFUSION SERVICES OF THE UK 1999/2000

Red Cells	2,737,572
Platelets	249,622
Fresh Frozen Plasma	365,547
Cryoprecipitate	94,114
TOTAL	**3,446,855**

Serious Hazards of Transfusion SHOT

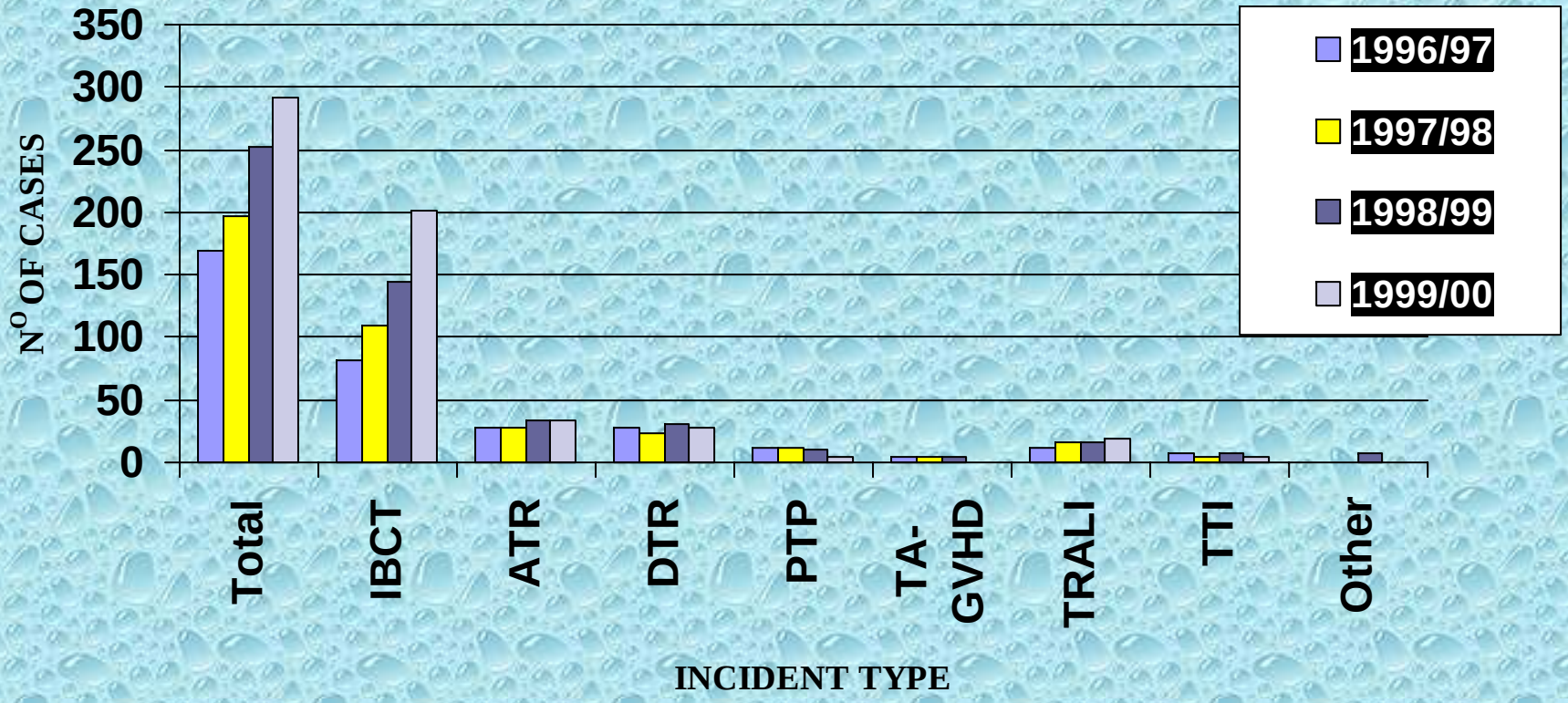
INCORRECT BLOOD COMPONENT TRANSFUSED

Episodes where a patient was transfused with a blood component or plasma product which did not meet appropriate requirements or which was intended for another patient

Serious Hazards of Transfusion

SHOT

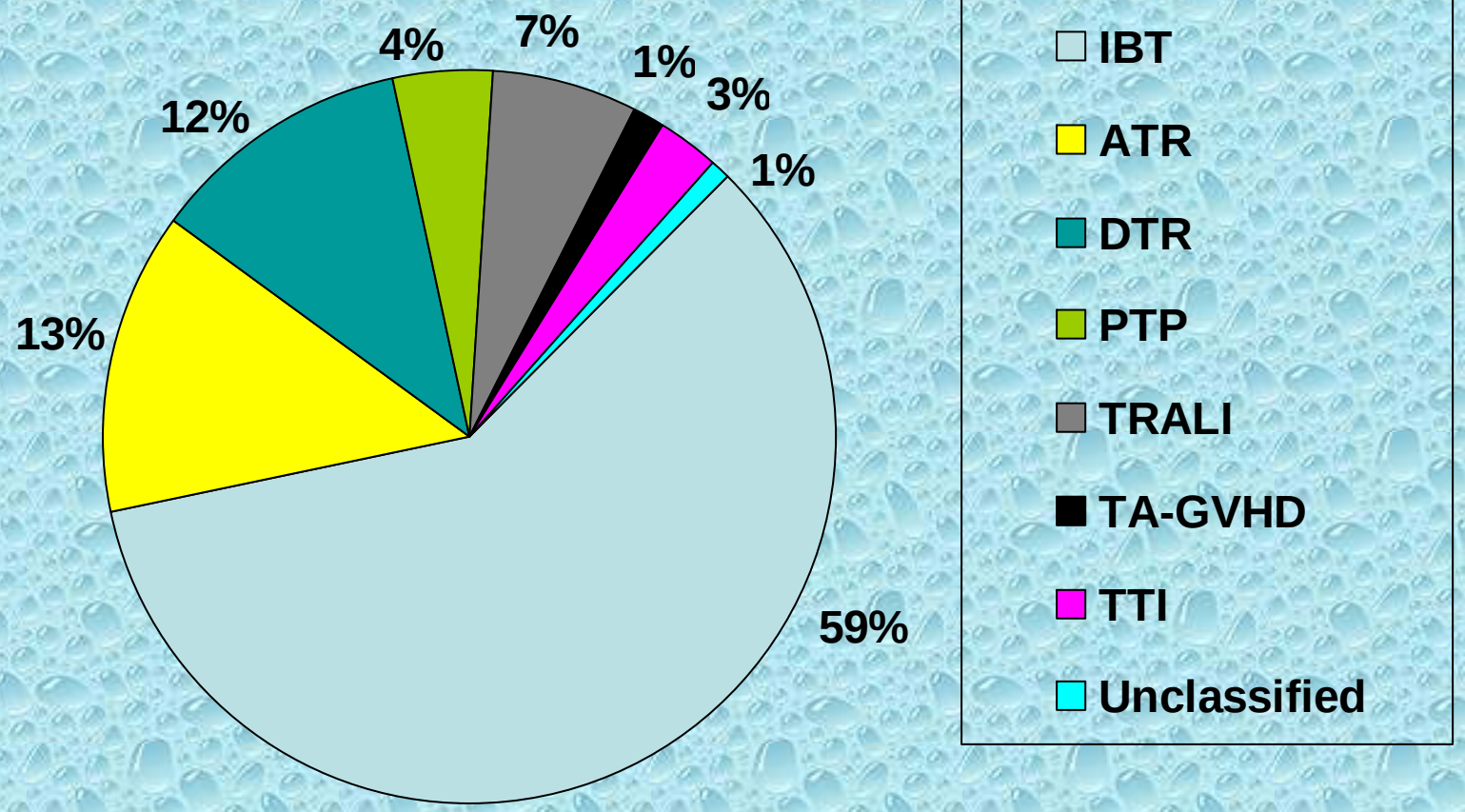
COMPARISON OF INCIDENTS REPORTED 1996 TO 2000



Serious Hazards of Transfusion **SHOT**

QUESTIONNAIRES BY INCIDENT

1996/97 - 1999/00 (n=862)



Serious Hazards of Transfusion SHOT

IBCT OUTCOME OF CASES 1996/97 - 1999/00 (n=509)

Outcome	No. of cases
Death definitely related to transfusion	5
Death probably related to transfusion	1
Death possibly related to transfusion	2
Death unrelated to transfusion	37
Major morbidity	54
Survived – no ill effects	406
Unknown	4
Total	**509**

Transfusion errors in New York State: an analysis of 10 years' experience. Linden et al, Transfusion 2000;40:1207-1213

- 36% possibility that a random unit will be incompatible
- Likelihood of fatal outcome of ABO incompatible unit is <10%

TABLE 1. Frequency of erroneous administration of RBCs in New York State, 1990 through 1999*

	Number	Frequency
ABO-incompatible	237	1/38,000
ABO-compatible	221	1/41,000
Total†	462	1/19,000
Adjusted total‡	659	1/14,000
Fatal reaction	5	1/1,800,000

* 9,000,000 transfusions were performed during this period.

† Includes 4 cases in which ABO compatibility was not reported.

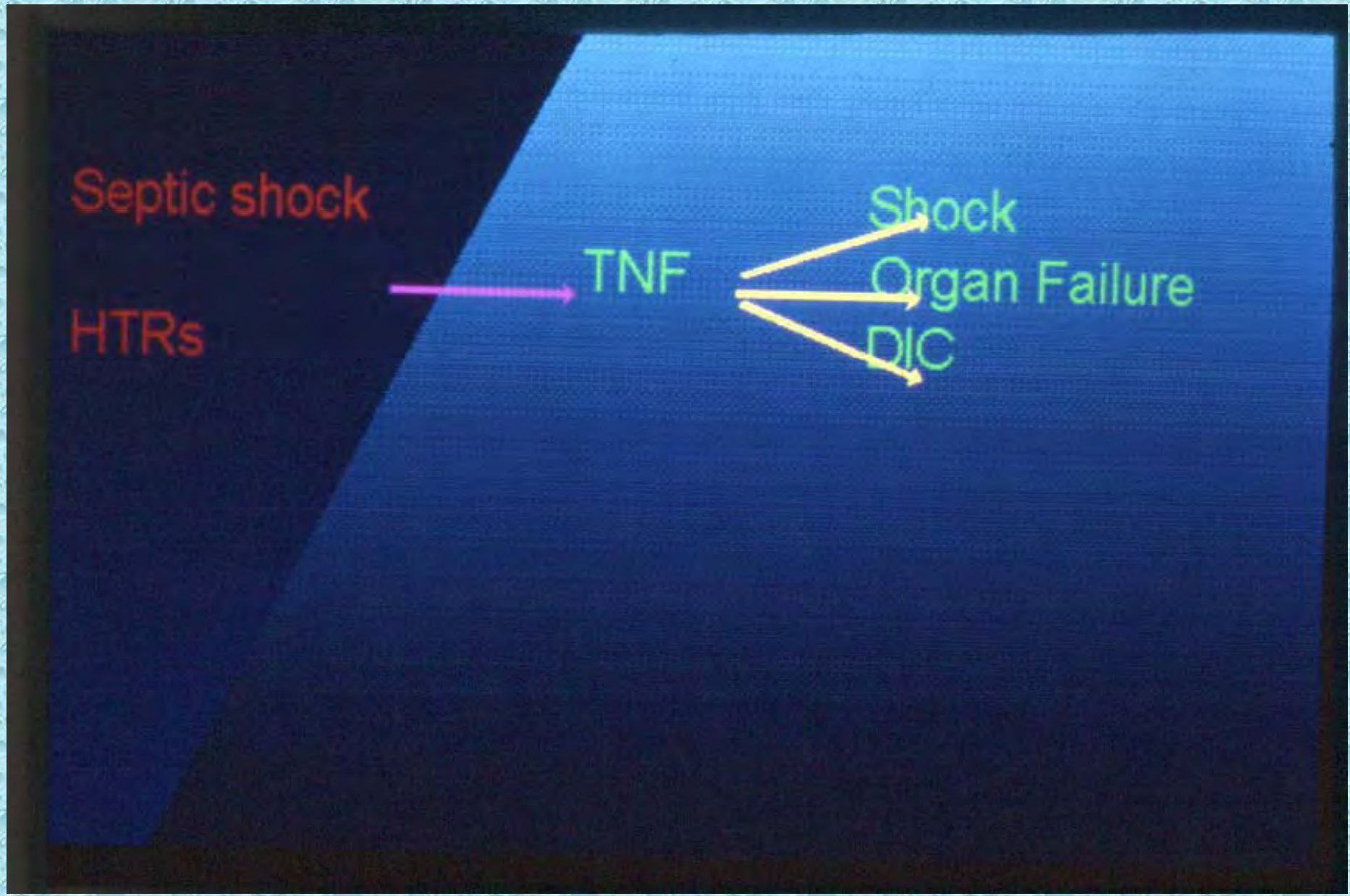
‡ Adjusted to correct for estimated underreported/undetected ABO-compatible erroneous transfusions. A compatible-to-incompatible ratio of 1.78 was used.

ALLOIMMUNE HEMOLYSIS

Testing Pre-transfusion

- ABO & Rh Type of both donor & recipient
- Antibody Screen of Donor & Recipient, including indirect Coombs
- Major cross-match by same procedure (recipient serum & donor red cells)

- **Error Management**

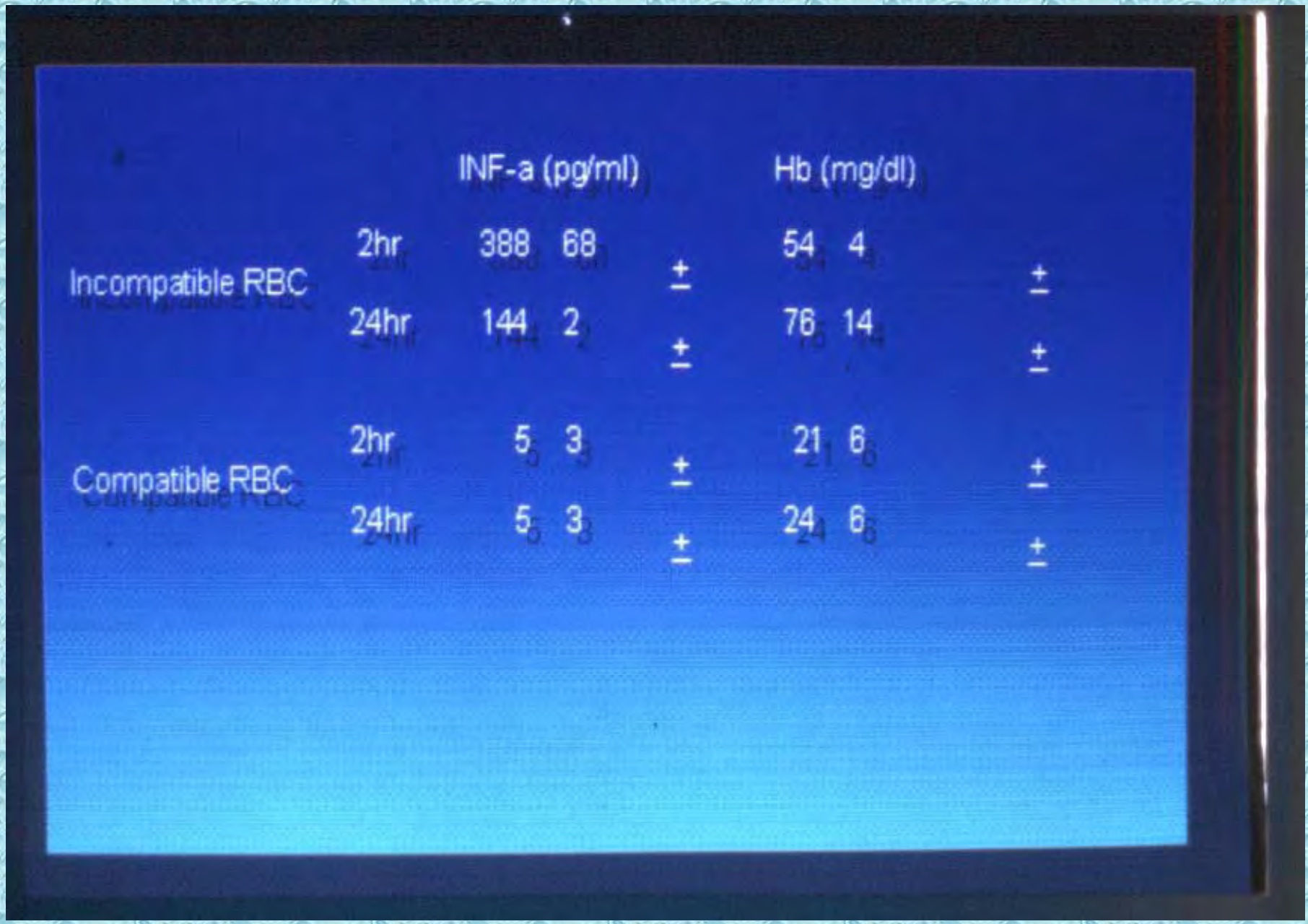

Septic shock
HTRs
TNF
Shock
Organ Failure
DIC

In vitro model of the hemolytic transfusion reactions

"Davenport"

Group O blood + Group A blood → Complement mediated lysis

IgG-coated RBCs + Mononuclear cells from fresh whole blood → IgG mediated HTRS


INF-a (pg/ml)
Hb (mg/dl)
Incompatible RBC
2hr 388 68 ±
24hr 144 2 ±
54 4 ±
76 14 ±
Compatible RBC
2hr 5 3 ±
24hr 5 3 ±
21 6 ±
24 6 ±

Leukocyte phenotypic changes in an in vitro model of ABO HTRs

icoll gradient separated MN cells from group O whole
lood resuspended in autologous plasma (5 x 10 cell/ml)
ml of this suspension + 6ul of group A RBCs
plasma studies : TNF , IL8
cellular studies :phenotypic markers

TABLE 1. WBC phenotypic markers

Specificity	Antigen/protein	Cellular expression
CD25	Tac antigen (interleukin-2 receptor)	Activated B- and T-lymphocytes; macrophages
CDw108	JMH protein	Activated polymorphonuclear cells; T-, B-, and natural killer-lymphocytes
CD109	Glycosylphosphatidylinositol-linked protein	Activated T-lymphocytes
CD14	Lipopolysaccharide receptor	Monocytes (decreased with activation by lipopolysaccharide)
CD44	Hyaluronic acid receptor	All WBCs

Table 1. *Various mediators of the systemic inflammatory response and their biologic effects*

Mediator	Hemodynamic effects	Coagulation effects	Other effects
TNF	Hypotension	Increase tissue factor activity and decrease thrombomodulin expression by endothelial cells	Fever, capillary leak
IL-1	Hypotension Endothelin release	Increase tissue factor activity and decrease thrombomodulin expression by endothelial cells	Fever, leukocytosis; stimulate other cytokine production
IL-6			Stimulate T-cell proliferation and immunoglobulin production
IL-8			Neutrophil chemoattraction
MCP-1			Monocyte chemoattraction
Complement		Thromboplastin release by WBCs	Activate phagocytes, stimulate cytokine production
Nitric oxide	Vasodilation		
Endothelin	Vasoconstriction		
Leukocyte adhesion molecules			Attract WBCs

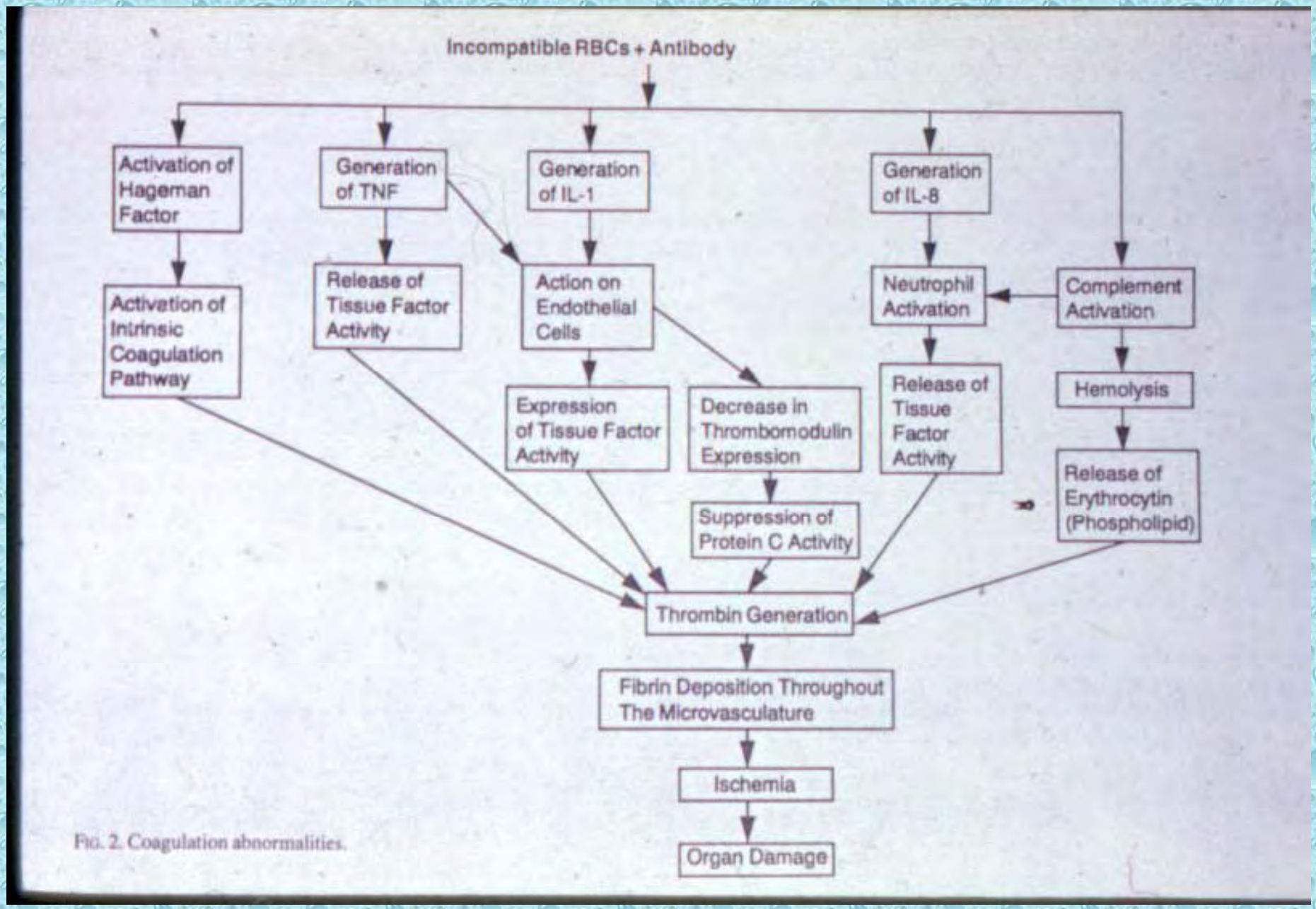


FIG. 2. Coagulation abnormalities.

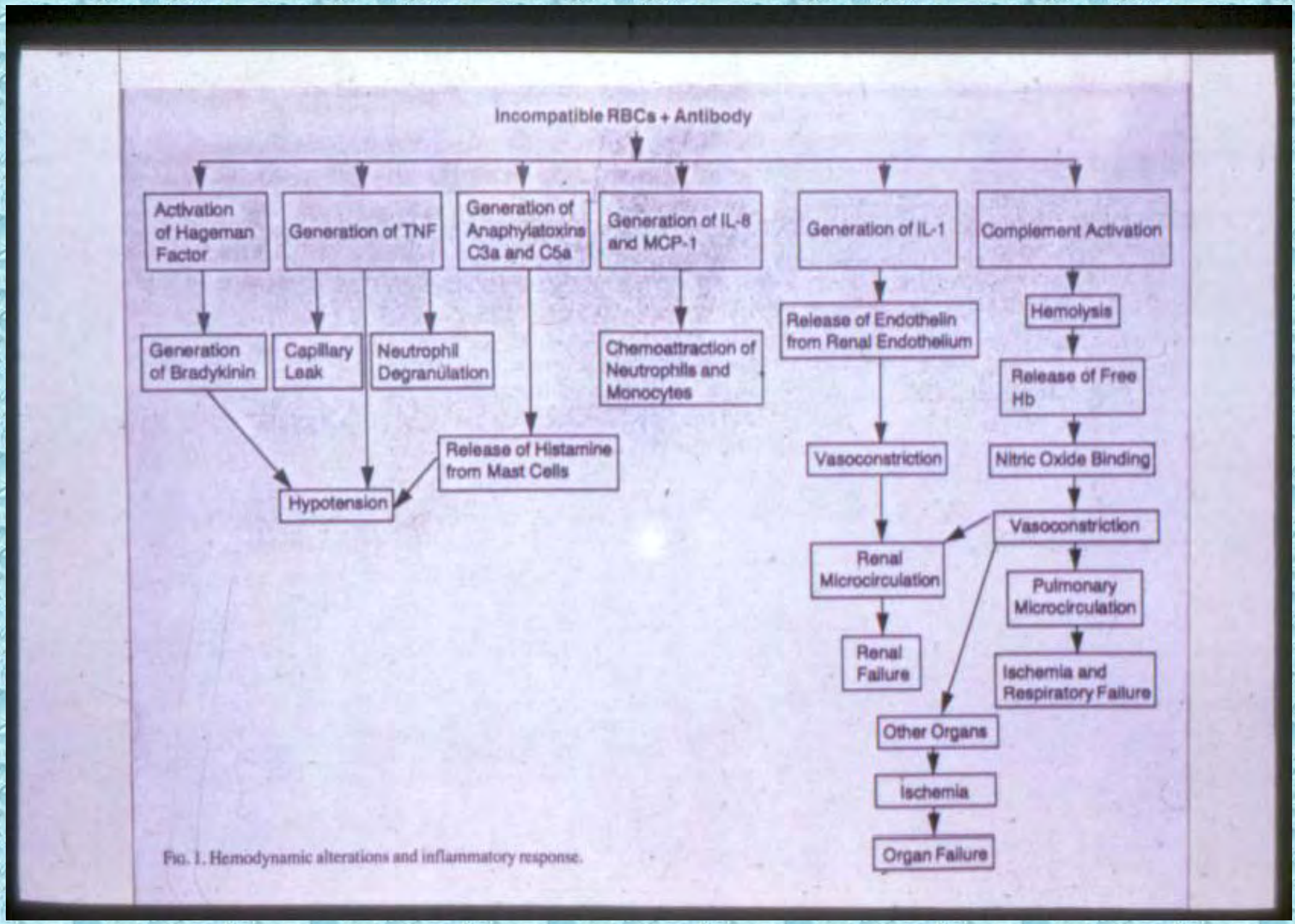


FIG. 1. Hemodynamic alterations and inflammatory response.

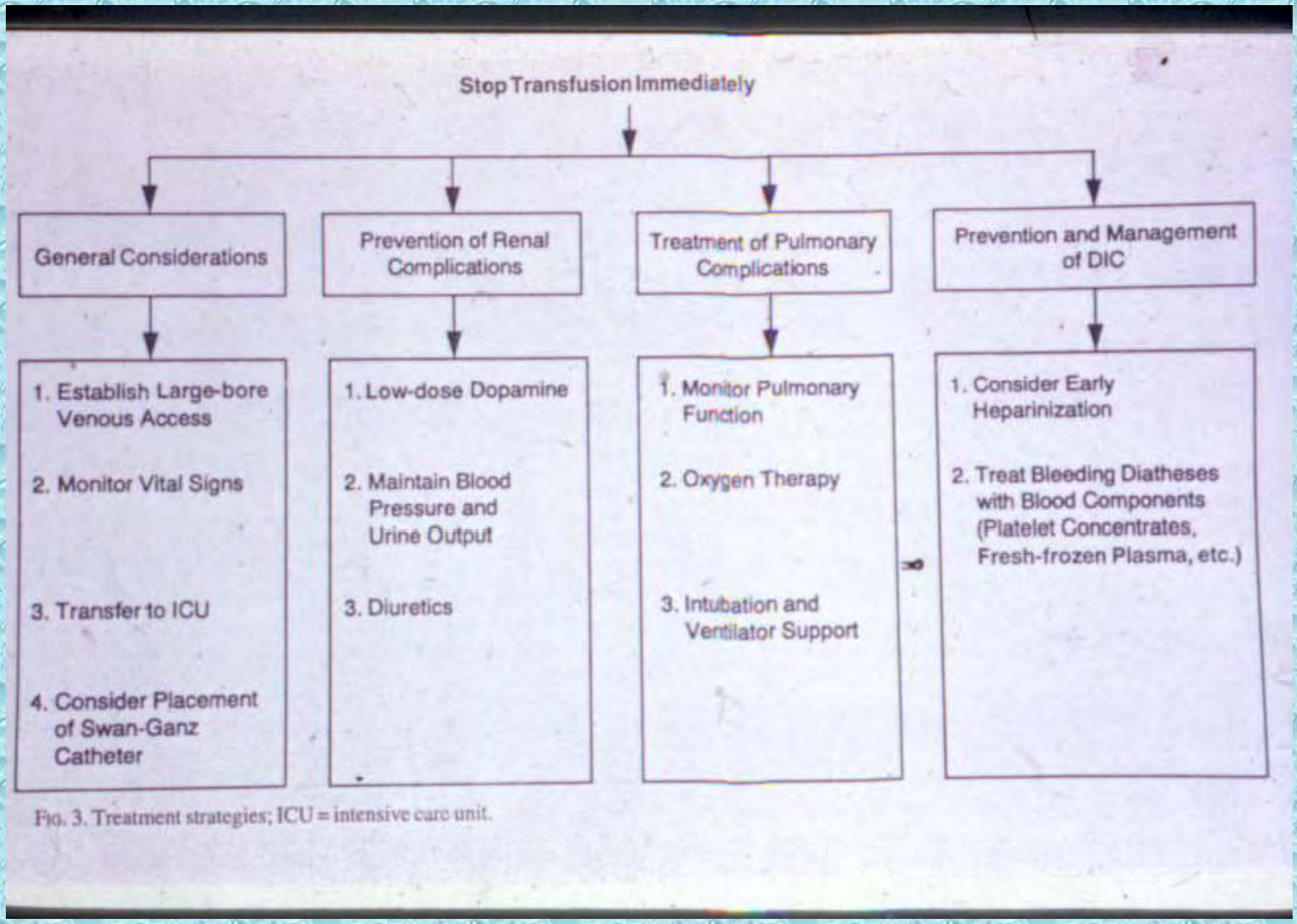


Fig. 3. Treatment strategies; ICU = intensive care unit.

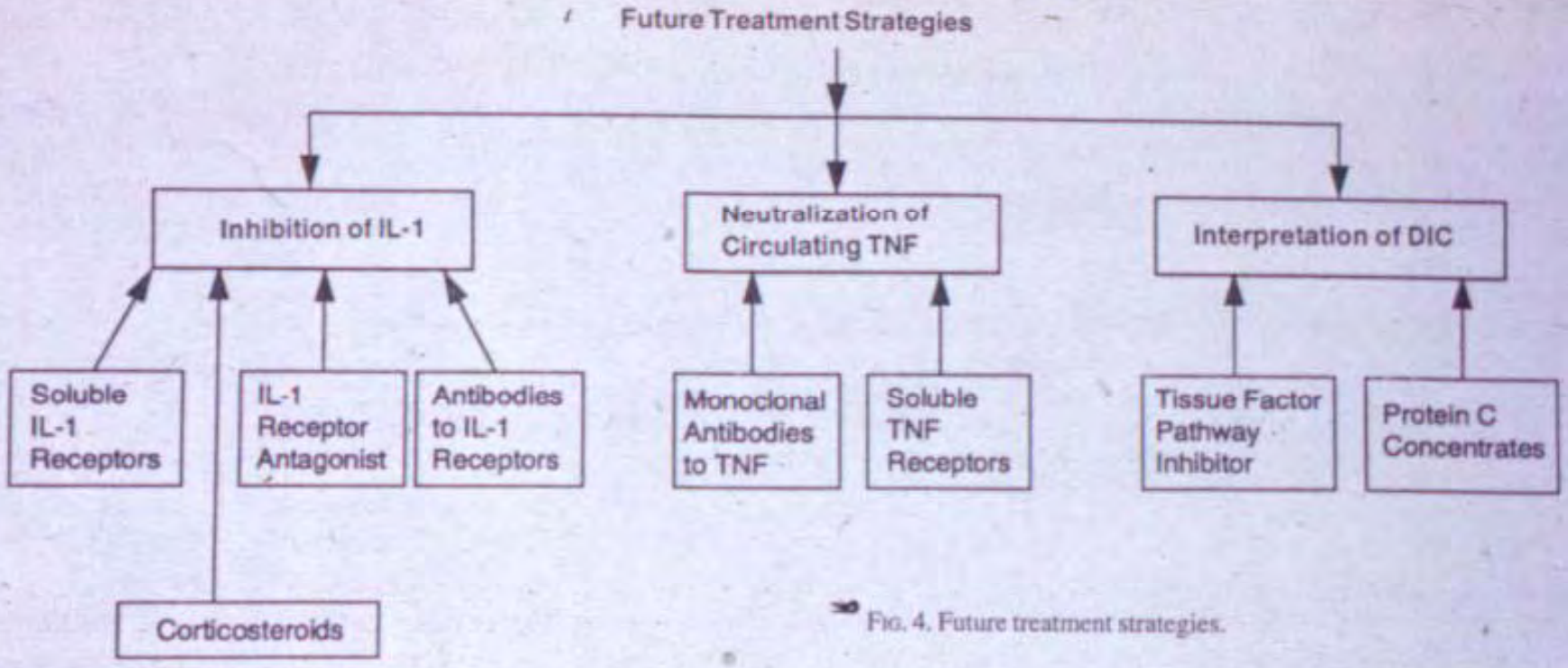


Fig. 4. Future treatment strategies.

ALLOIMMUNE HEMOLYSIS

Hemolytic Disease of the Newborn

- Due to incompatibility between mother negative for an antigen & fetus/father positive for that antigen. Rh incompatibility, ABO incompatibility most common causes
- Usually occurs with 2nd or later pregnancies
- Requires maternal IgG antibodies *vs.* RBC antigens in fetus

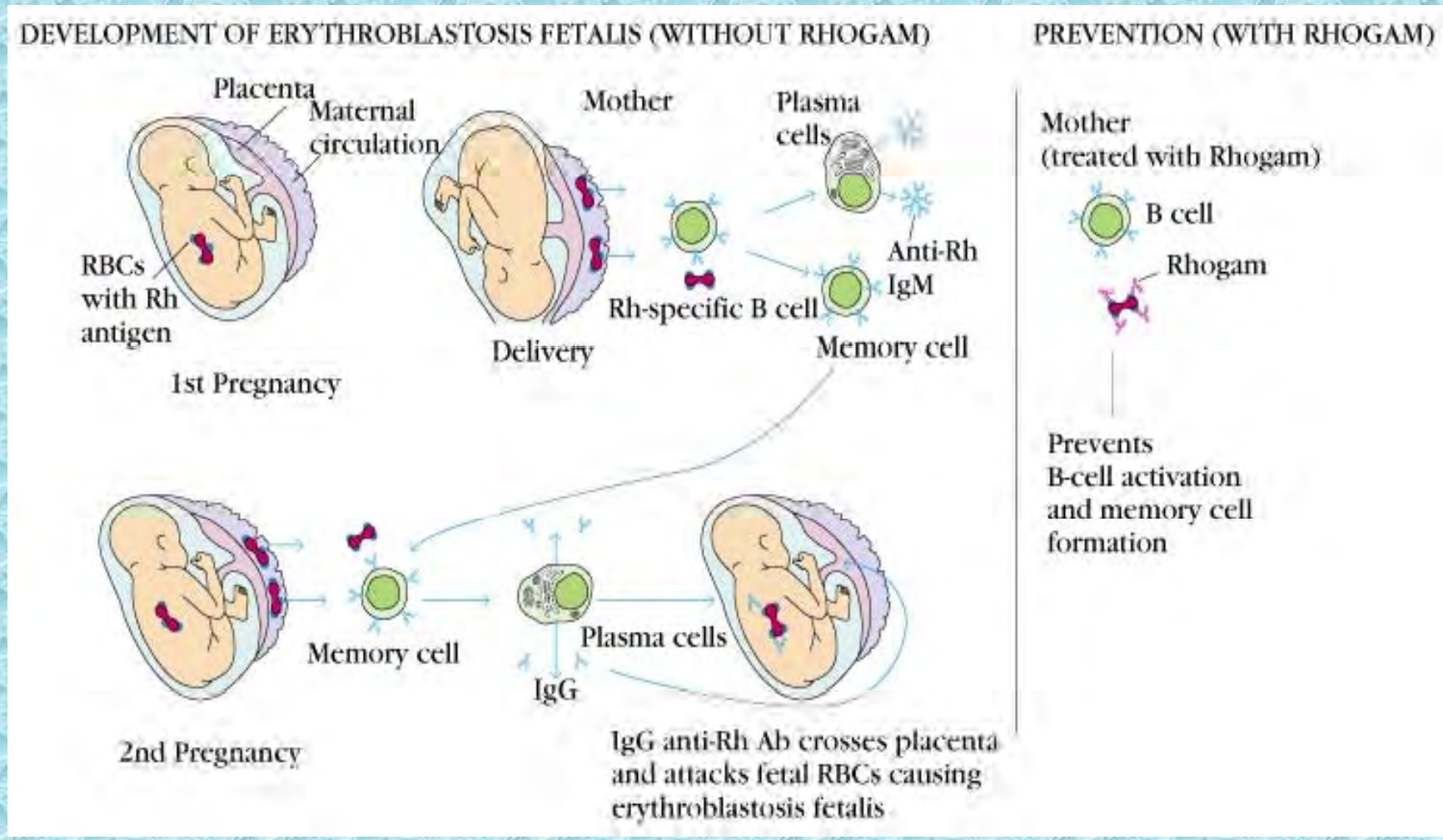
ALLOIMMUNE HEMOLYSIS

Hemolytic Disease of the Newborn - #2

- Can cause severe anemia in fetus, with erythroblastosis and heart failure
- Hyperbilirubinemia can lead to severe brain damage (kernicterus) if not promptly treated
- HDN due to Rh incompatibility can be almost totally prevented by administration of anti-Rh D to Rh negative mothers after each pregnancy

Prevention

- Hemolytic disease of the newborn caused by Rh incompatibility can be almost entirely prevented by administering antibodies against the Rh antigen to the mother within 24-48 hours after the first delivery
- These antibodies are called Rhogam
- They bind to fetal red blood cells that have entered the mother's circulation and facilitate their clearance before B-cell activation


DEVELOPMENT OF ERYTHROBLASTOSIS FETALIS (WITHOUT RHOGAM)
Placenta
Maternal circulation
RBCs with Rh antigen
1st Pregnancy
Mother
Delivery
Plasma cells
Anti-Rh IgM
Rh-specific B cell
Memory cell
Memory cell
Plasma cells
IgG
2nd Pregnancy
IgG anti-Rh Ab crosses placenta and attacks fetal RBCs causing erythroblastosis fetalis
PREVENTION (WITH RHOGAM)
Mother (treated with Rhogam)
B cell
Rhogam
Prevents B-cell activation and memory cell formation

The End