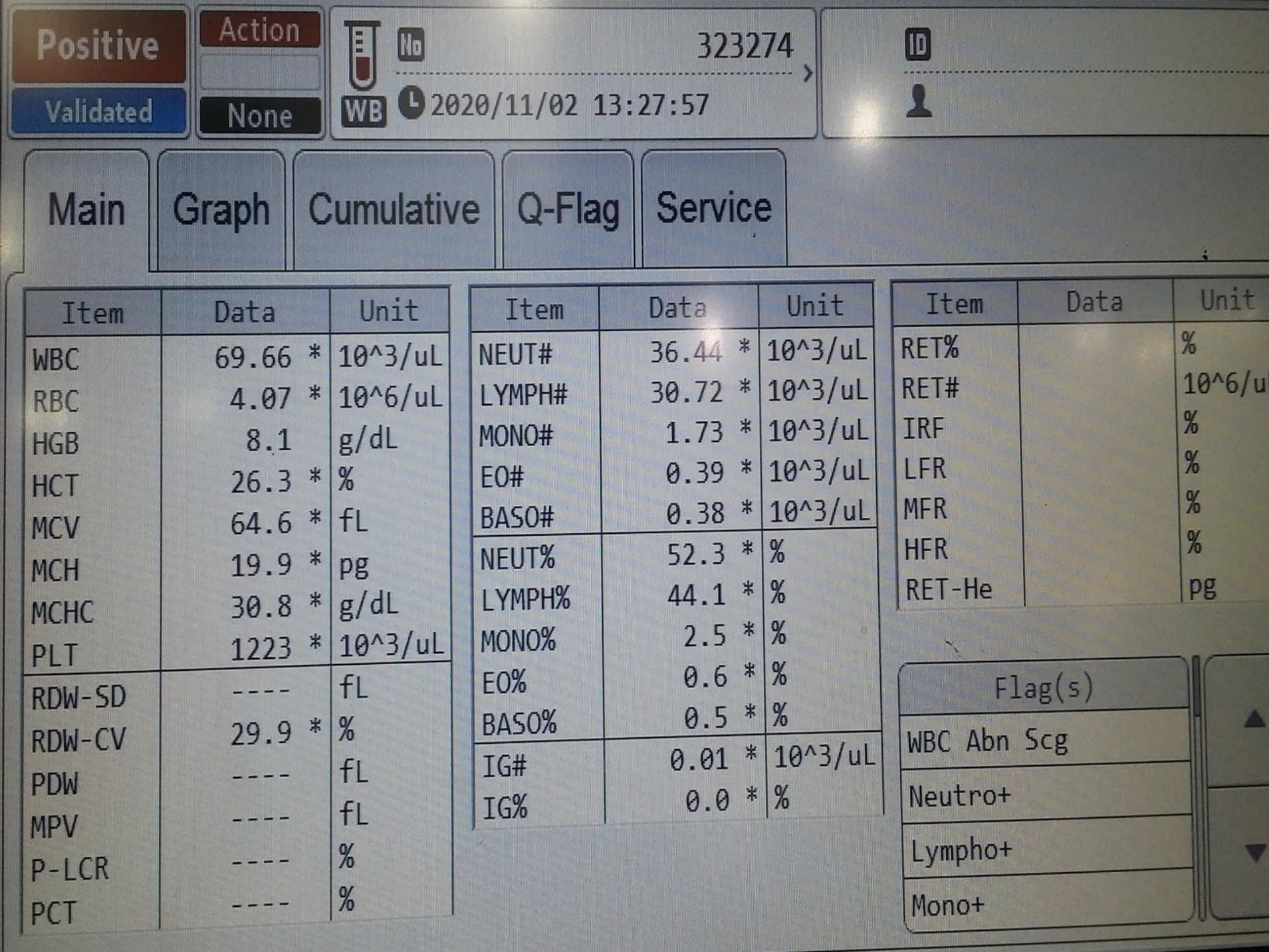
Diyala University – collage of medicine
Hematology -5th stage
Lec 7

Acquired Hemolytic Anemia

By:
Dr.zahraa najah alzuhairi



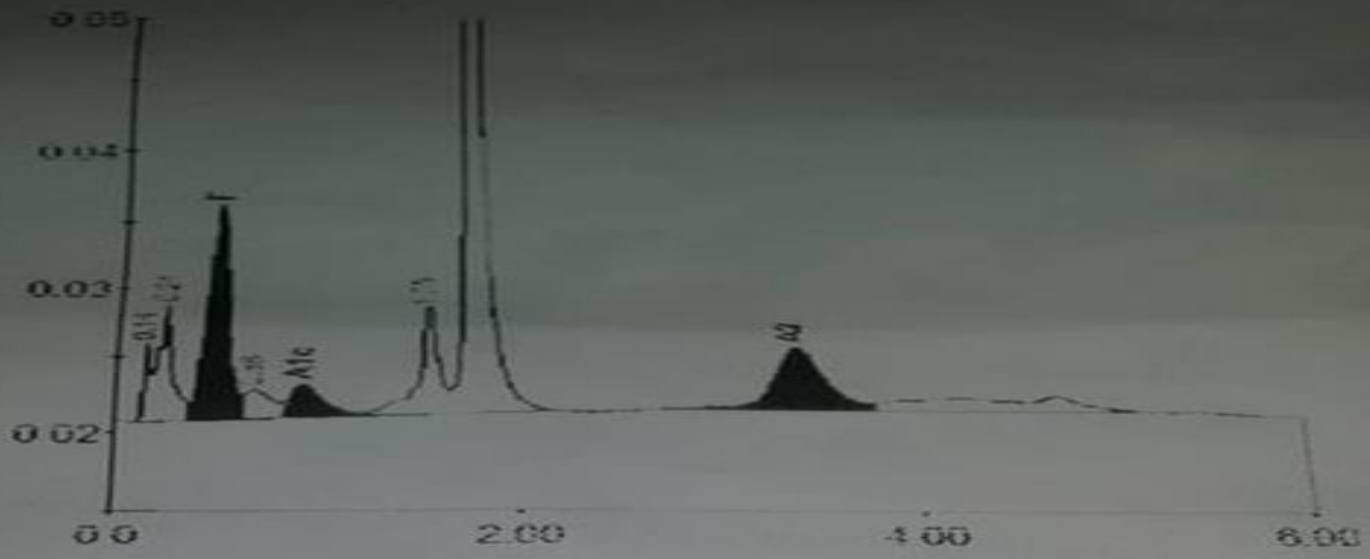
Item	Data	Unit
WBC	69.66 *	10^3/uL
RBC	4.07 *	10^6/uL
HGB	8.1	g/dL
HCT	26.3 *	%
MCV	64.6 *	fL
MCH	19.9 *	pg
MCHC	30.8 *	g/dL
PLT	1223 *	10^3/uL
RDW-SD	----	fL
RDW-CV	29.9 *	%
PDW	----	fL
MPV	----	fL
P-LCR	----	%
PCT	----	%

Item	Data	Unit
NEUT#	36.44 *	10^3/uL
LYMPH#	30.72 *	10^3/uL
MONO#	1.73 *	10^3/uL
EO#	0.39 *	10^3/uL
BASO#	0.38 *	10^3/uL
NEUT%	52.3 *	%
LYMPH%	44.1 *	%
MONO%	2.5 *	%
EO%	0.6 *	%
BASO%	0.5 *	%
IG#	0.01 *	10^3/uL
IG%	0.0 *	%

Item	Data	Unit
RET%		%
RET#		10^6/u
IRF		%
LFR		%
MFR		%
HFR		%
RET-He		pg

Flag(s)
WBC Abn Scg
Neutro+
Lympho+
Mono+

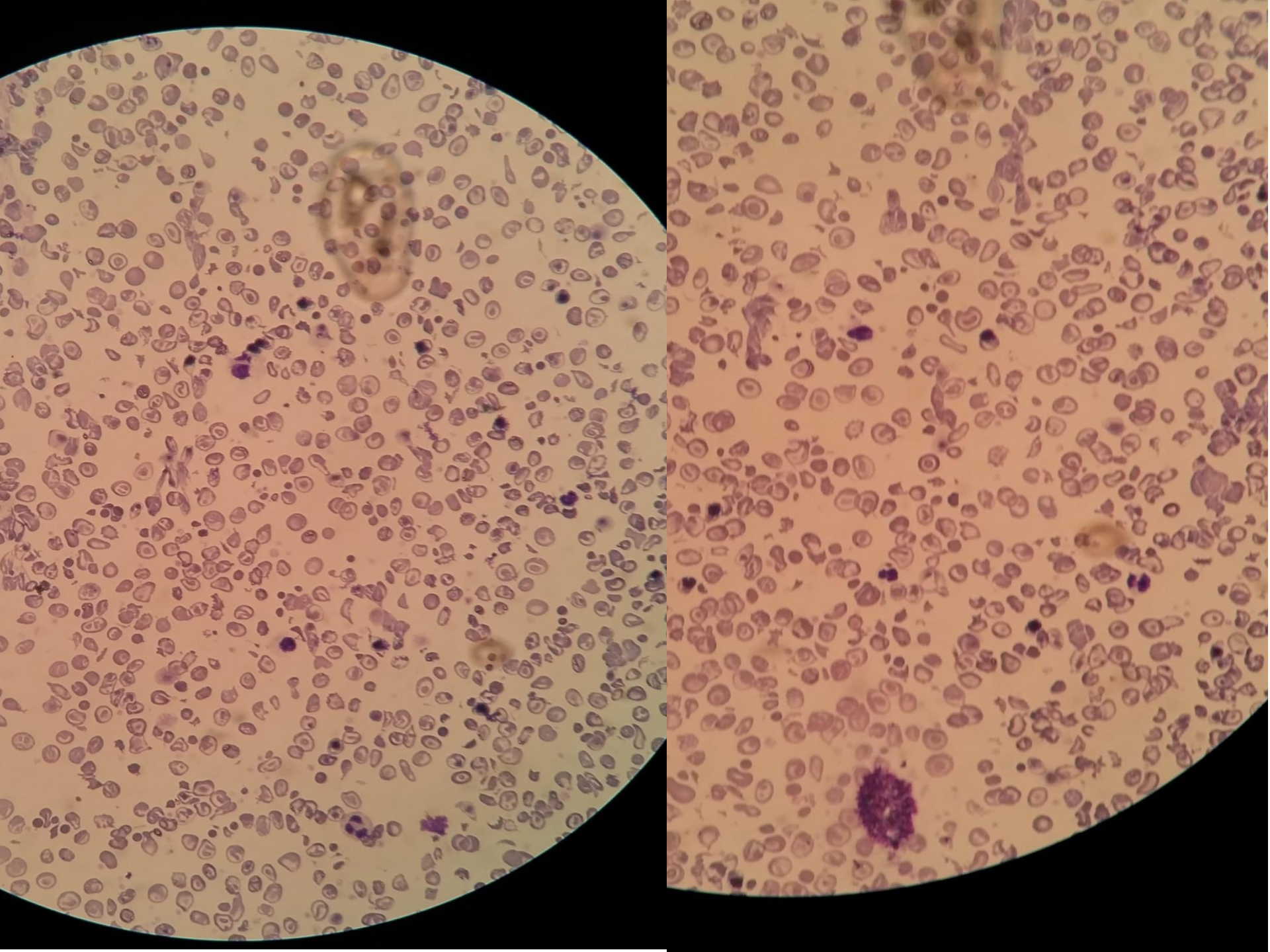
Sample ID: 323274
Injection date: 11/02/2020 09:21 AM
Injection #: 2
Method: HbA2/F
Rack #: ---
Rack position: 2



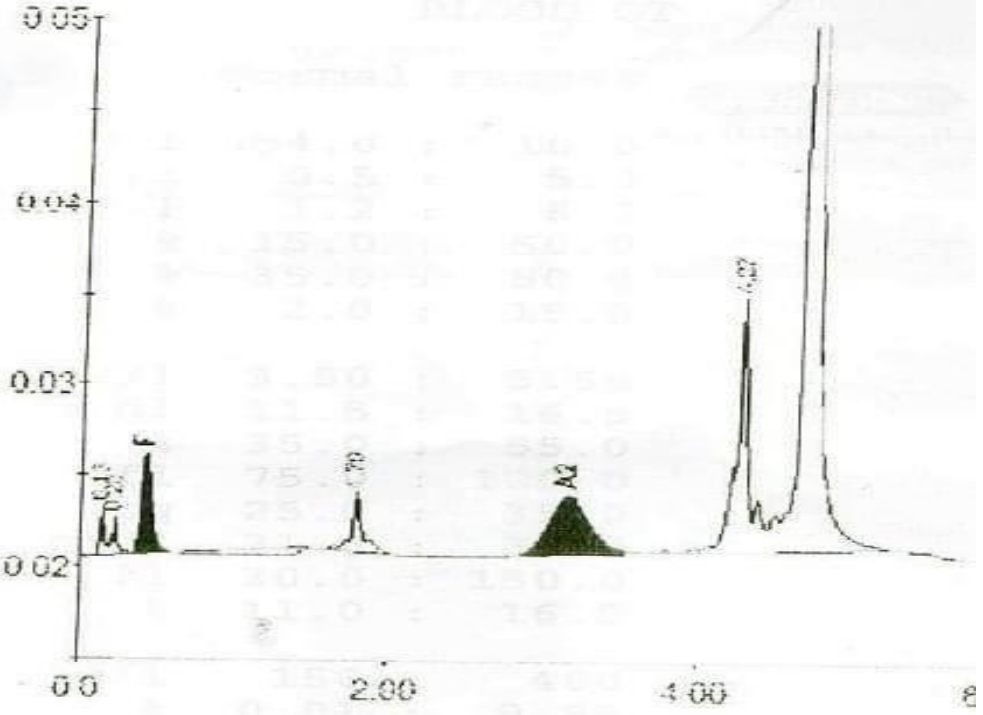
Peak table – ID: 323274

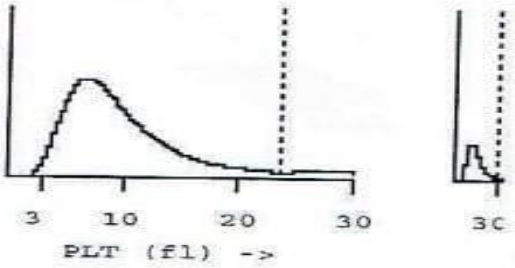
Peak	R. time	Height	Area	Area %
Unknown	0.14	5274	11922	1.1
A1a	0.24	8146	38829	3.7
F	0.48	15254	100560	10.7
LA1c/CHb-1	0.68	1892	17032	1.6
A1c	0.92	2135	23852	4.4
P3	1.55	7572	50599	4.9
A0	1.76	184709	721902	69.4
A2	3.41	4192	75405	8.8
Total Area:			1040100	

Concentration:	%
F	10.7
A1c	4.4
A2	8.8



WBC	H	10.7
LYM		1.9
GRAN	H	8.3
LYM%		18.1
GRA%		77.8
MID%		4.1
RBC		4.81
HGB		12.3
HCT		36.9
MCV		76.6
MCH		25.6
MCHC		33.5
RDWa		63.7
RDW%		15.5
PLT		328
PCT		0.30
MPV		9.3
LPCR		24.1
PDW		13.2



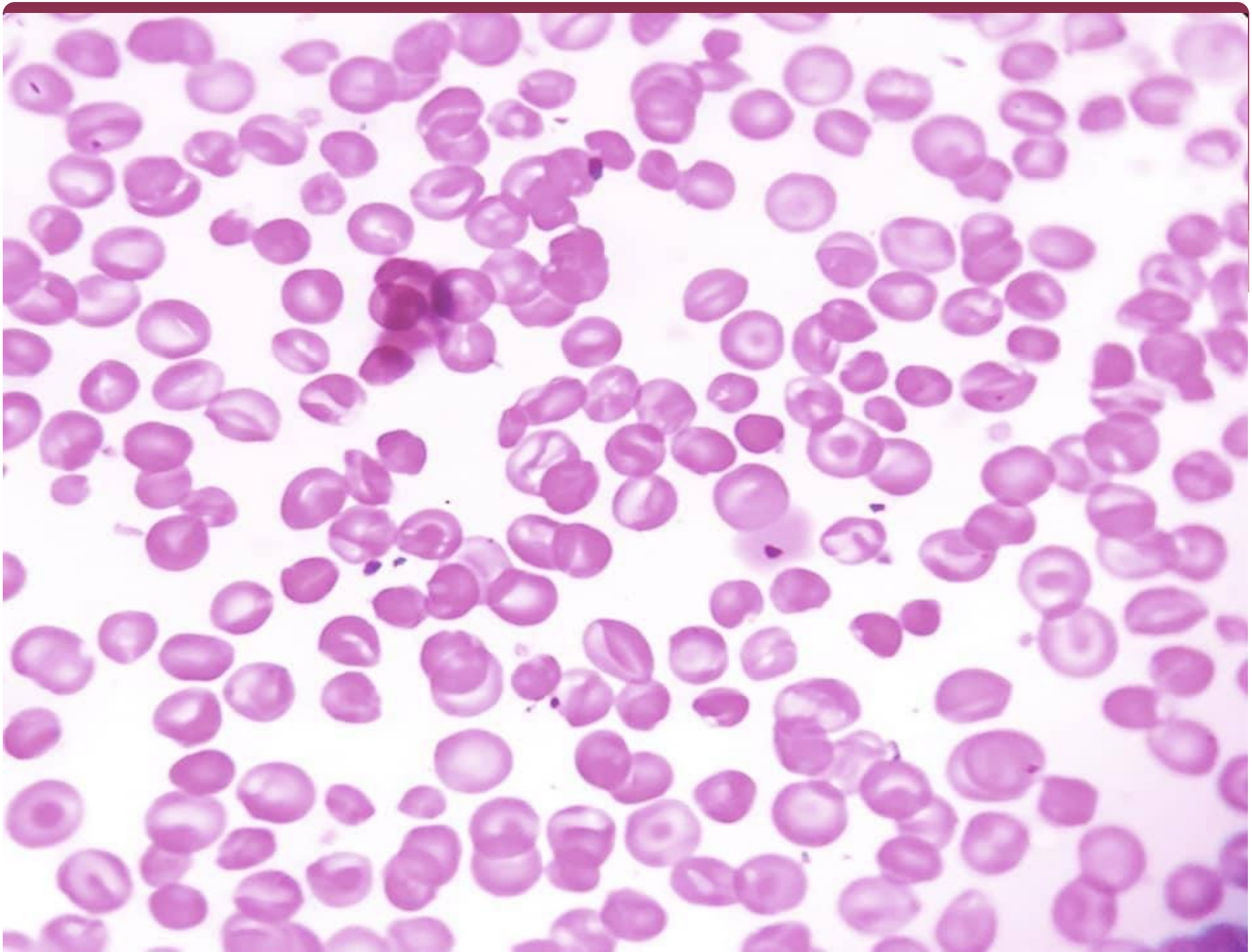


Peak table - ID: 2531

Peak	R.time	Height	Area	Area %
Unknown	0.13	2742	5096	0.2
A1a	0.23	2080	5488	0.3
F	0.42	5553	25628	1.0
A0	1.79	3272	15751	0.7
A2	3.15	3226	59545	2.8
S-Window	4.27	14275	78595	3.6
C-Window	4.71	945065	1966181	91.2

Total Area: 2156284

Concentration:	%
F	1.0
A2	2.8



Bio-Rad Variant II HPLC

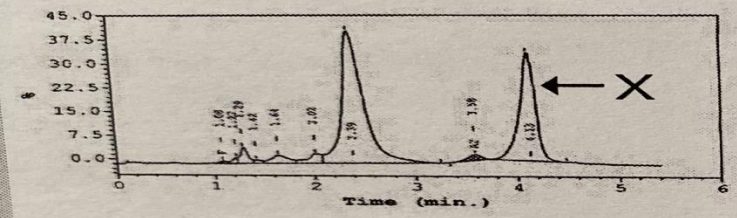
Peak Name	Area %	Area %	Retention Time (min)	Peak Area
F	0.4	---	1.08	8197
Unknown	---	0.6	1.22	12856
E2	---	2.4	1.29	48079
Unknown	---	0.5	1.42	10433
E3	---	2.7	1.64	54183
Unknown	---	2.7	2.02	54463
Ao	---	56.3	2.39	1117410
A2	1.9*	---	3.58	33040
D-window	---	32.6	4.13	647306

Total Area: 1985968

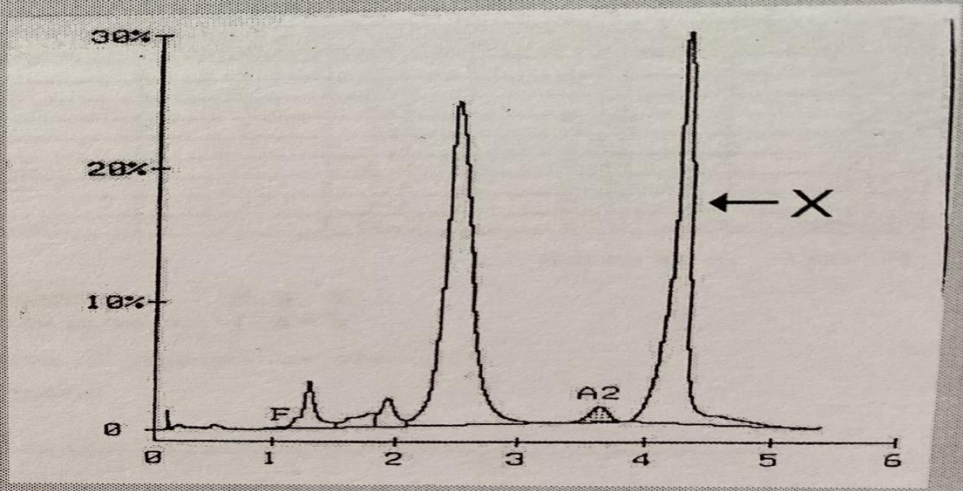
F Concentration = 0.4 %
A2 Concentration = 1.9* %

*Values outside of expected ranges

Analysis comments:



Bio-Rad Variant HPLC



Notes

X = Haemoglobin D-Punjab, also known as haemoglobin D-Los Angeles

The mobility of haemoglobin D-Punjab on acid agarose is sometimes slightly anodal (above) that of haemoglobin A but this is not apparent on this gel

Causes sickle cell disease when co-inherited with haemoglobin S

Heterozygotes are asymptomatic

heterozygote.

Bio-Rad Variant II HPLC

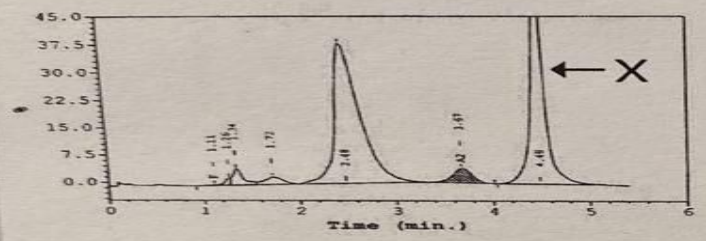
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.1*	---	1.11	1575
Unknown	---	0.7	1.26	14888
P2	---	2.4	1.34	55203
P3	---	2.0	1.72	46445
Ao	---	51.0	2.48	1165434
A2	4.0*	---	3.67	91721
S-window	---	39.8	4.48	908775

Total Area: 2284041

F Concentration = 0.1* %
A2 Concentration = 4.0* %

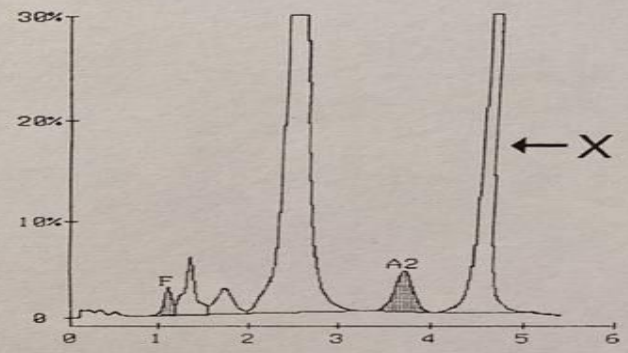
*Values outside of expected ranges

Analysis comments:



Bio-Rad Variant HPLC

ANALYTE ID	%	TIME	AREA
F	1.4	1.10	36317
P2	3.8	1.35	103436
P3	2.6	1.73	71630
Ao	54.5	2.40	1501659
A2	4.9	3.68	116843
S-WINDOW	33.1	4.53	910459
	TOTAL AREA		2740344
F	1.4%	A2	4.9%



Notes X = Haemoglobin S Z1, Z2 and Z3 = δ chain variants
N = N-Baltimore

The apparent increase in A_2 is partly due to the co-elution of altered S

Haemoglobin S is clinically significant since it tends to polymerize at low oxygen pressures, in some circumstances even in heterozygotes leading, for example, to haematuria

Heterozygotes also have an increased incidence of the rare condition, medullary carcinoma of the kidney

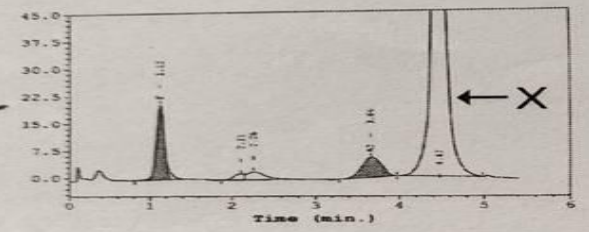
ygote.

Bio-Rad Variant II HPLC

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	7.9*	---	1.12	167376
Unknown	---	0.8	2.11	18054
Ao	---	1.9	2.26	44737
A2	5.5*	---	3.66	118672
S-window	---	84.8	4.47	1944780

Total Area: 2294419

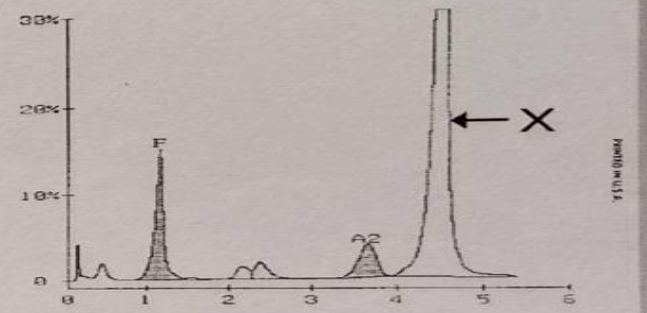
F Concentration = 7.9* %
A2 Concentration = 5.5* %
*Values outside of expected ranges
Analysis comments:



Bio-Rad Variant HPLC

ANALYTE ID	%	TIME	AREA
F	7.6	1.16	153037
P3	0.2	1.54	4185
Unknown 1	1.2	2.18	22100
Ao	1.7	2.38	32180
A2	4.2	3.65	74286
S-WINDOW	84.5	4.54	1614957
TOTAL AREA			1900665

F 7.6% A2 4.2%



Notes X = Haemoglobin S Y = an ageing band
Z1, Z2 and Z3 = δ chain variants, G = G-Philadelphia, N = N-Baltimore

The double peak in the position of Ao is glycated haemoglobin S

The apparent increase in A2 is partly due to the co-elution of altered S

Haemoglobin S is clinically significant since it polymerizes at low oxygen pressures

Haemoglobin S interacts with β thalassaemia and a number of variant haemoglobins (e.g. C, D-Punjab, O-Arab) leading to sickle cell disease

Bio-Rad Variant II HPLC

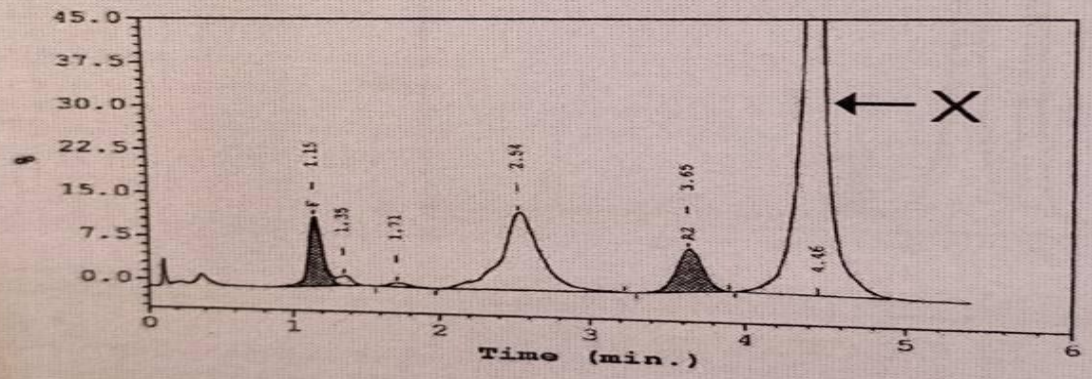
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	6.1*	---	1.15	50892
P2	---	1.0	1.35	8344
P3	---	0.5	1.71	4649
A0	---	19.7	2.54	169055
A2	7.1*	---	3.65	58889
S-window	---	66.1	4.46	568405

Total Area: 860235*

F Concentration = 6.1* %
A2 Concentration = 7.1* %

*Values outside of expected ranges

Analysis comments:



Classification

1. Immune Hemolytic anemia: Anemia result from Ab directed against patient RBC. It includes Autoimmune, Alloimmune and anemia induces by Drugs.
2. Non Immune Hemolytic anemia: Hemolysis produced by mechanisms other than antibodies.

Table 9.1 Classification of immune haemolytic anaemias.

Antigen type	Antibody	Diseases	Associations
Autoimmune	Warm antibody	Primary	Idiopathic
		Secondary	Autoimmune diseases (ITP, SLE, Rheumatoid arthritis)
			Lymphoproliferative disorders
			Infections (EBV)
			Ovarian cysts
			Ovarian carcinoma and some other cancers
			Drugs
	Cold antibody	Cold haemagglutinin disease (CHAD)	
		Cold antibody syndromes	Infections (*M. pneumoniae*), lymphoproliferative disorders
	Donath–Landsteiner antibody	Paroxysmal cold haemoglobinuria (PCH)	Post viral, syphilis
Alloimmune	Induced by red cell antigens	Haemolytic transfusion reactions	
		Haemolytic disease of the newborn (HDN)	
		Post-stem-cell allografts	
	Drug dependent	Antibody/macrophage mediated	
		Antibody/complement mediated	
		Membrane modification	
		Autoimmune	

WARM Autoimmune HA:

* This AIHA in which the **auto-antibody** best reacts with red cells at 370C, is usually an **IgG** class, and is usually associated with **extravascular hemolysis.**
* **Pathogenesis**: RBC coated with Ig (usually IgG alone or Ig with complement or complement alone C3) taken up by RE MQ, part of coated membrane loss and become spherocytes then permanently destroy

Clinical features:

* Variable age, more in females.
* Insidious onset of pallor and jaundice with splenomegaly on examination.
* Feature of secondary causes.

Lab.findings

* Anemia, spherocytosis, Polychromasia, reticulocytosis, increase bilirubin.
* Sometimes associated with immune TCP (Evan syndrome).
* Most important is positive Direct Coombs test in all cases.
* Indirect Coombs test positive in 50%.

* Treatment: remove underlying causes, corticosteroid, splenectomy, immunosuppression and other lines.

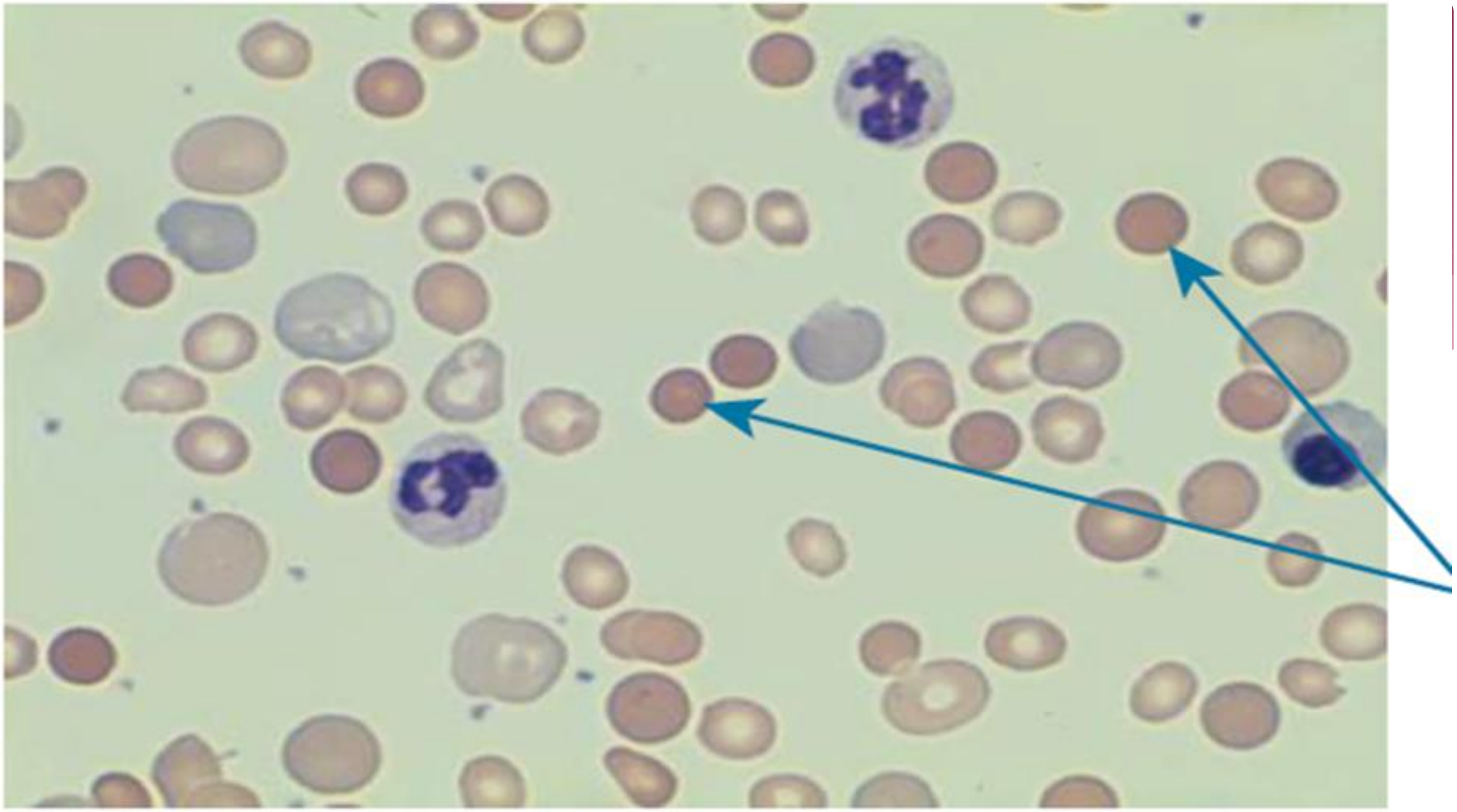
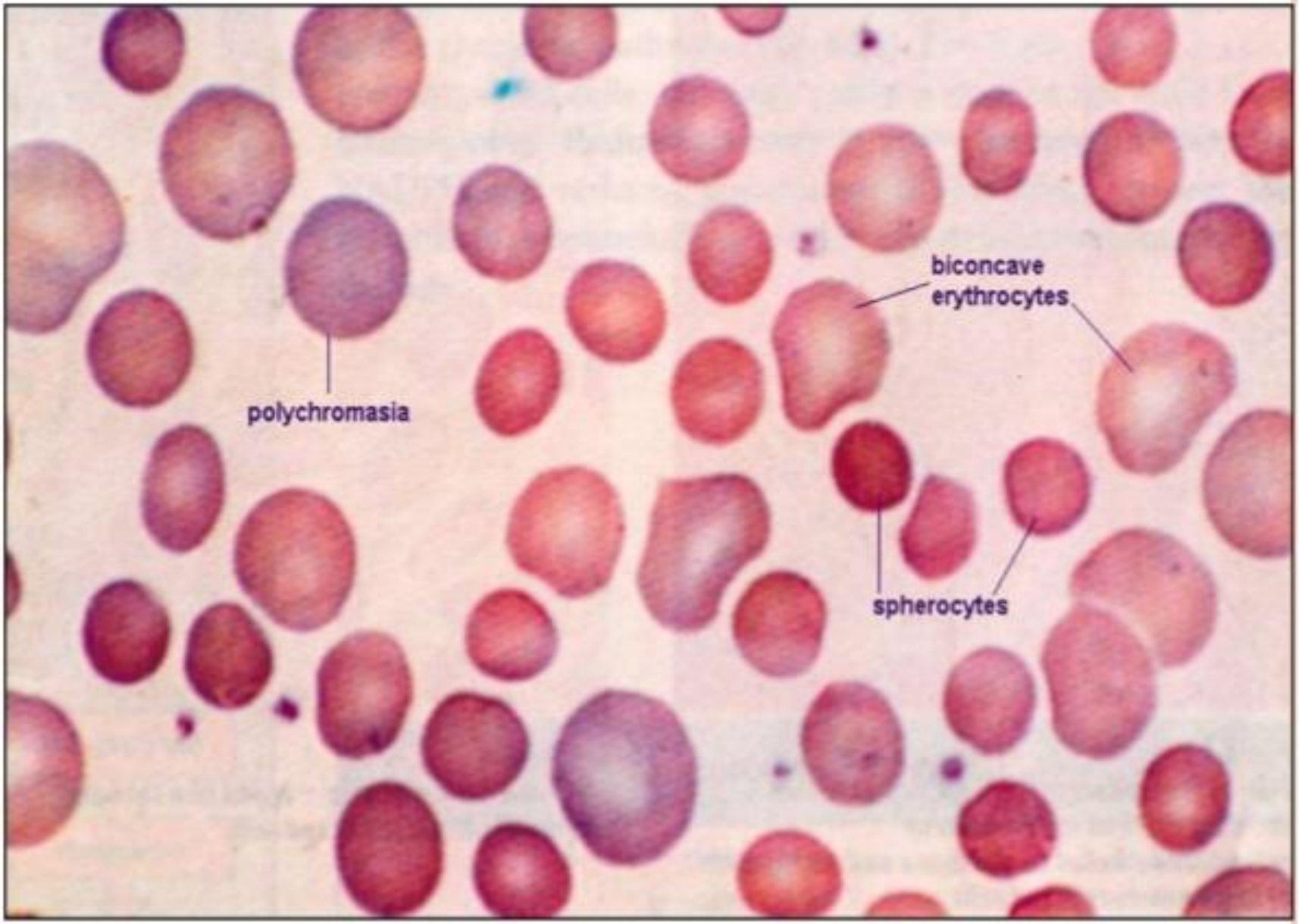
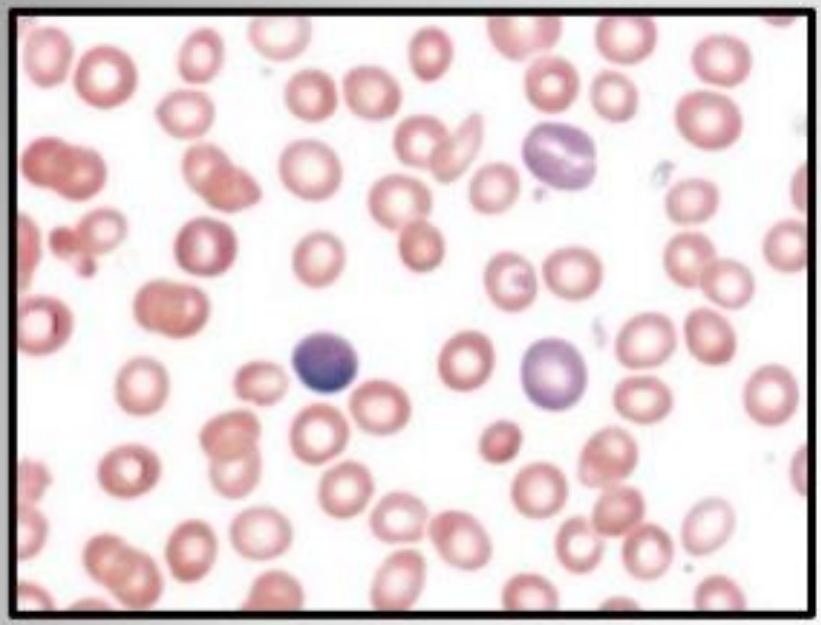
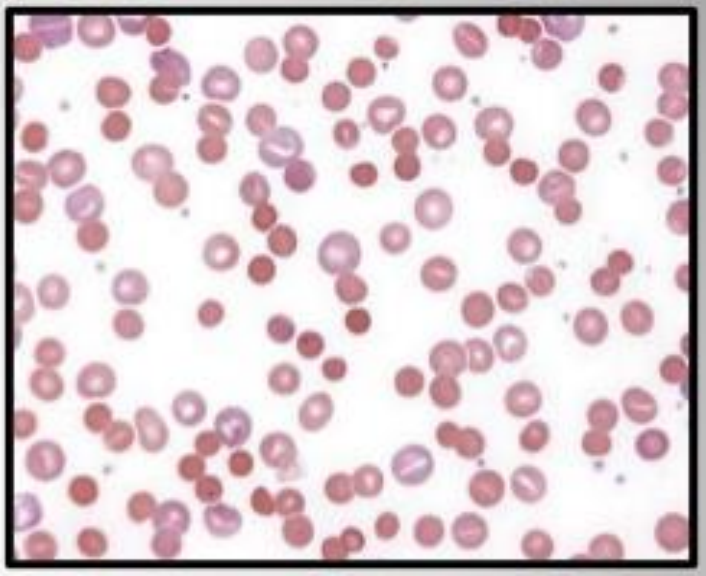


Figure 9.1 Warm autoimmune haemolytic anaemia. Blood film showing spherocytosis (arrows), polychromasia and a nucleated red blood cell (×40).


polychromasia
biconcave
erythrocytes
spherocytes



Peripheral blood film with Romanowsky stain demonstrating polychromatophilic cells. The polychromatophilic cells are basophilic because of increased RNA content. The cells are usually larger than normocytic red blood cells



Autoimmune hemolytic anemia. Numerous spherocytes, small round RBCs lacking central pallor, are shown in this blood smear from a case of Coombs-positive hemolytic anemia.

COLD Autoimmune HA

* Here the autoantibody reacts best with RBC in the cold at temp. < 370C, typically at 40C.
* **Pathogenesis:**

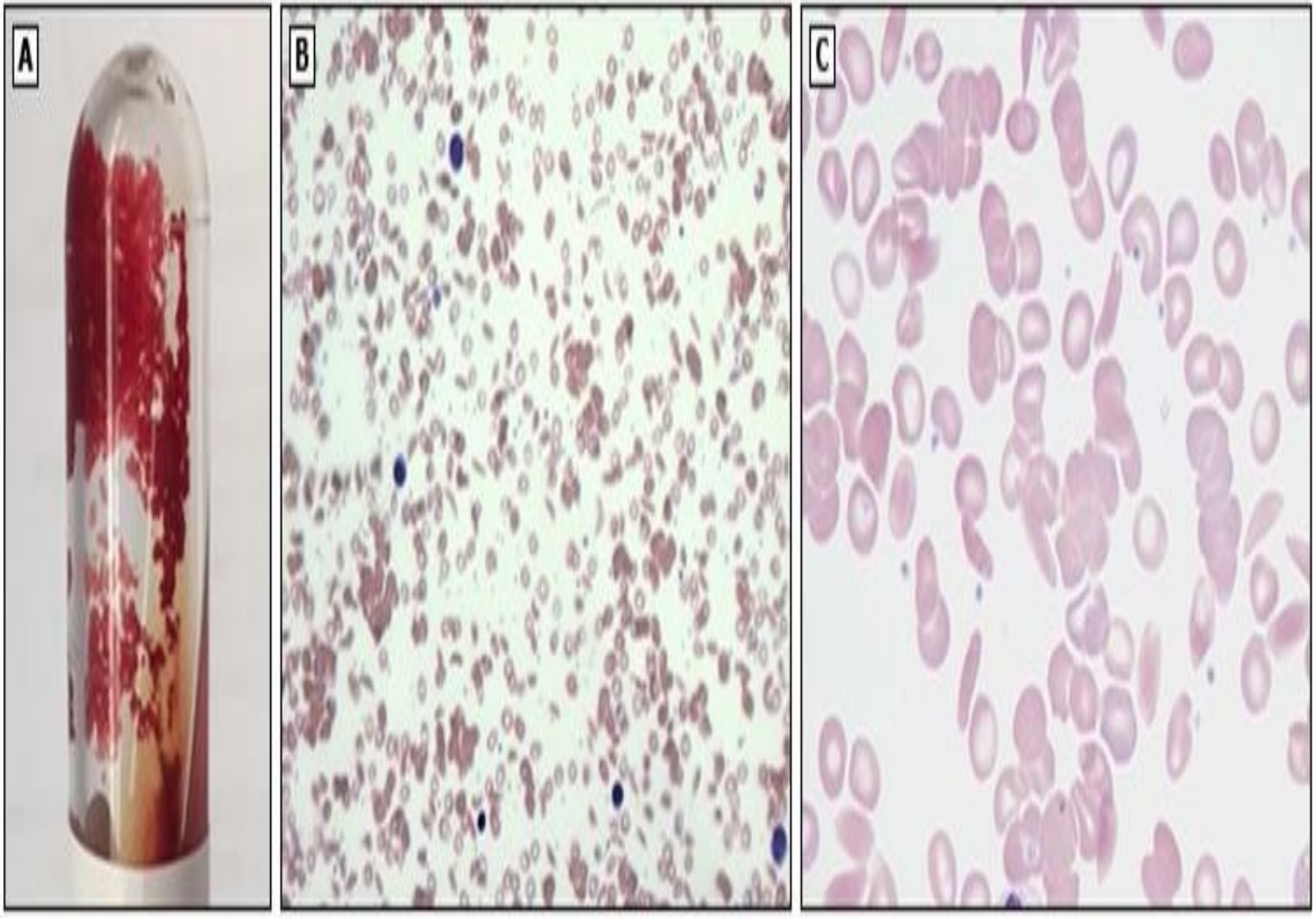
  Ab usually IgM either monoclonal (idiopathic, secondary to LPD), or polyclonal (infections), these Abs attach to RBC in peripheral circulation when the temperature is cooled. Complement fixation occurs and both EV and IV hemolysis can occur, but mainly with EVH (in the liver). Complement alone is usually detected on RBC.

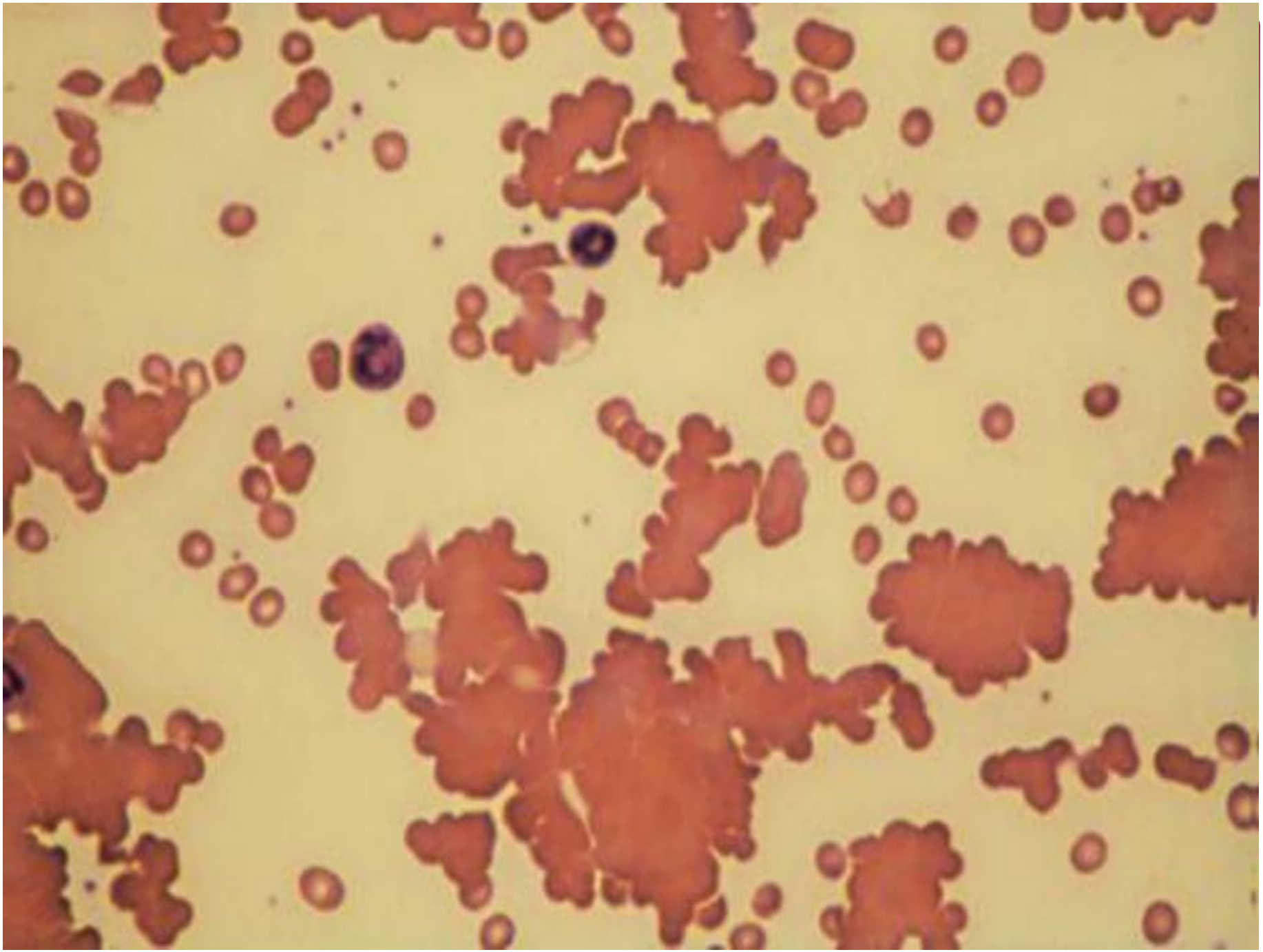
Clinical features:

* Patient may have chronic HA aggravated by cold.
* Mild jaundice and SM may be present.
* Patient may develop acrocyanosis.

Lab.findings:

* Anemia, red cells agglutination and features of associated disorders.
* Direct Coomb's test is classically positive, reveal complement alone.
* Detection of significant cold antibodies by the cold agglutinin titer tests


A
B
C



* **Treatment**: warming, treat underlying causes, Alkalating agent may be use, splenectomy not usually help

Alloimmune HA

- All immunization results from previous pregnancy, transfusion, transplantation.
- To detect occurrence of Alloimmunization :
  - Previous history of Tx, Pregnancy or Transplant may give a clue.
  - Screening patient's serum for Atypical RBC antibodies.

- Routs of immunization
  - Normal pregnancy.
  - Normal labor.
  - Abortion, ectopic pregnancy, obstetric trauma.
  - Previous history of transfusion.
  - Without history.
- Present in 2 types : (HDN & HTR)

Hemolytic disease of newborn (HDN)

- condition in which the life span of fetal or newborn RBc is shortened due to maternal alloAb against RC Ags inherited from father
- fetomaternal hemorrhage/ maternal Ab/ placental passage of alloAb/ Ab attach to fetal RBC Ags/ destruction of RBC.
- Abs can cause HDN: - Rh - ABO

RH_HDN

- more severe than ABO Ab
- Assessment of HDN
  - Maternal antenatal assessment of HDN:
    - ABO - Rh - Ab screen (indirect coomb's test)
  - Fetal antenatal assessment of HDN:
    -doppler flow velocity - Aminocentesis
    -Cordiocentesis - Aminocyte DNA

ABO-HDN

- Is extremely low,
- Less sever
- Occur in first pregnancy
- Usually the mother is group O and fetus is group A or B

❑ **Prevention** of HDN

- Using anti-D Ig.
- Prophylaxis reduce (90%) occurrence but not totally prevent due to:
  - early undetected abortion
  - clerical and administration errors
  - Primary immunization (0.8-1.5%) of Rh-ve women carrying Rh+ve fetus.

Hemolytic Transfusion Reaction (HTR)

- 2 types Immediate HTR & Delay HTR
- **Immediate HTR**: Is due to rapid destruction of donor red cells by Abs in the recipient's plasma.
- It is most commonly caused by Anti-A or Anti-B present in recipient plasma destroying A, B or AB donor cells
- Hemolysis either **IVH** or **EVH**

- **Delayed HTR:** Usually not predictable or preventable.
- Is due to previous sensitization of the recipient to one or more Ag by previous Transfusion, pregnancy or transplant.
- Antibody is not usually detectable by routine pre-transfusion screen.
- Transfusion of blood containing the appropriate Ag, will trigger a brisk anamnestic response.

Drug induce immune HA

- Drug adsorption mechanism: Ab against drug-RC membrane complex e.g. penicillin.
- Immune complex mechanism: complement fixation on RC membrane due to drug-Ab complex e.g guinidine, rifampicin.
- Autoimmune mechanism: of unclear role of drug e.g. methyldopa

Table 9.3 Drug-induced immune haemolytic anaemias: clinical and serological features.

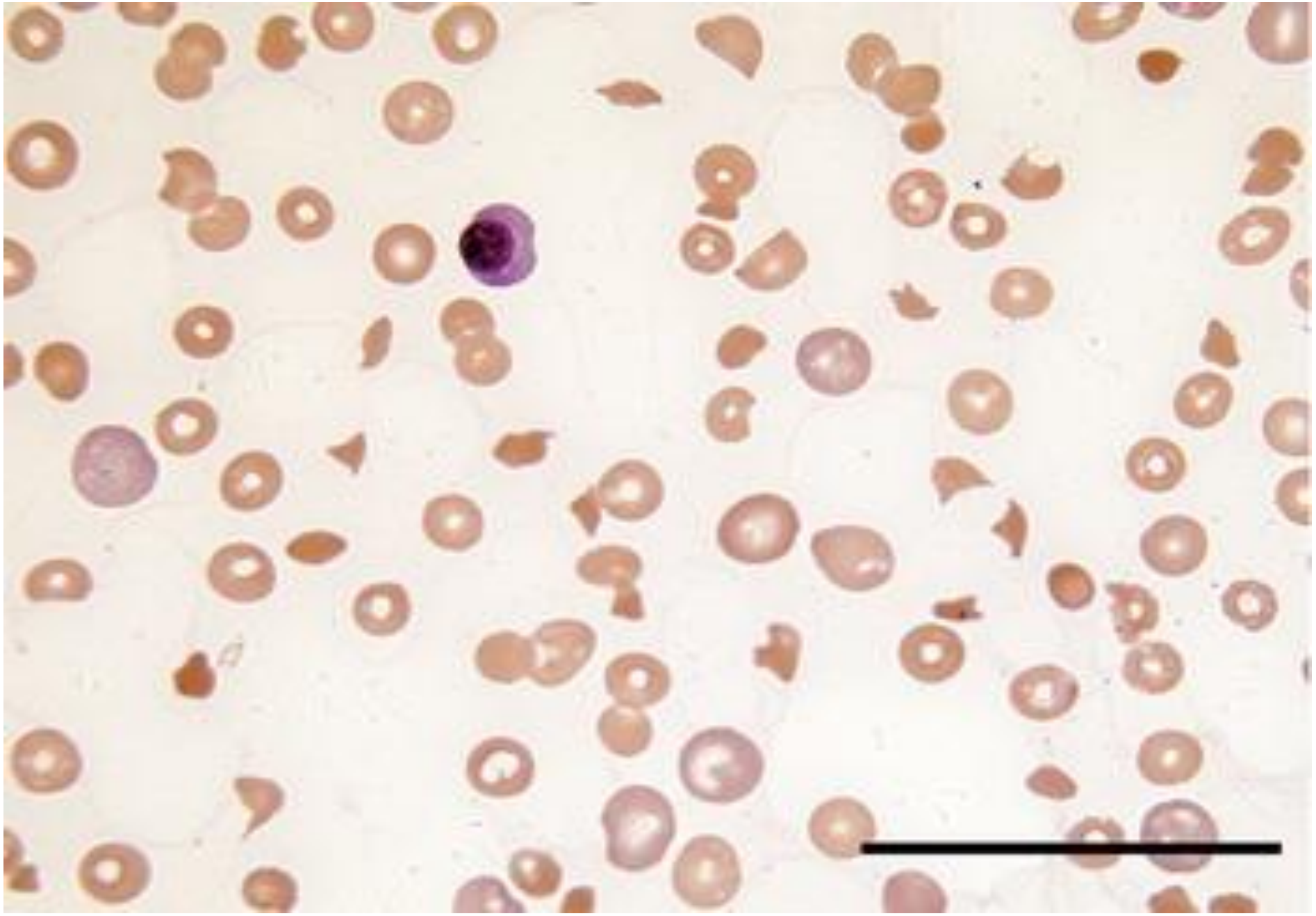
	Drug adsorption mechanism	Immune complex mechanism	Autoimmune mechanism	Membrane modification mechanism
Examples	Penicillin Cephalosporins	Third-generation cephalosporins Quinidine Diclofenac	Methyldopa Procainamide Mefenamic acid Fludarabine* Cladribine*	Cephalosporins Cisplatin Carboplatin
Dose/duration	Large therapeutic doses/prolonged	Very low dose on second or subsequent exposure/short	Therapeutic about 6 weeks	Therapeutic
Haemolysis	Extravascular Subacute	Intravascular Acute	Extravascular Mild/subacute	Rare
DAT	IgG ± C′3	C′3 only	IgG only	IgG
Serum reaction	To drug-treated cells	Only in presence of drug or metabolite	To normal cells	To drug-treated cells
Eluate reaction	To drug-treated cells	Non-reactive	To normal cells	To drug-treated cells

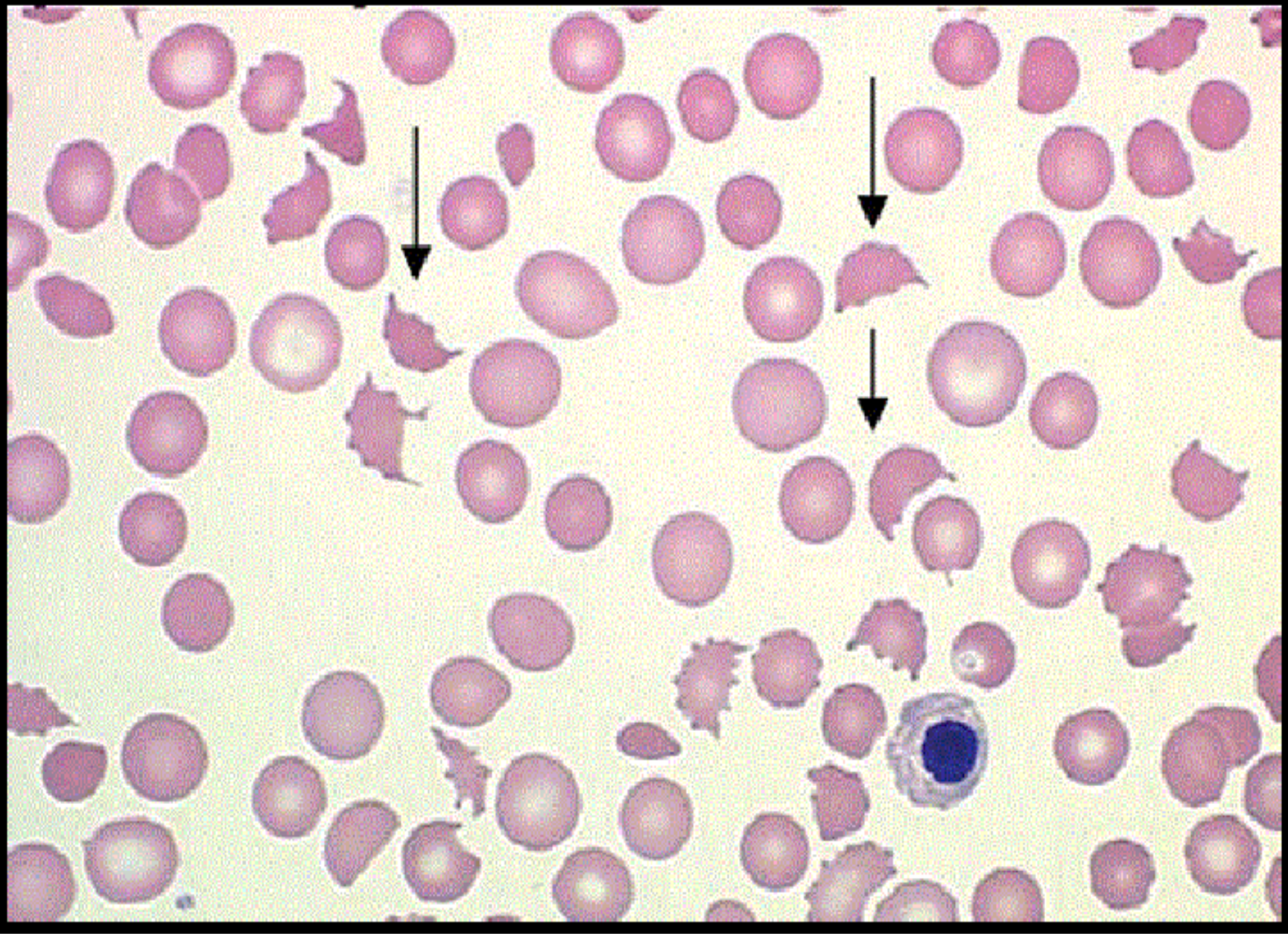
Non-immune acquired hemolytic anemias.

Cause	Mechanisms	Examples
Infections	Intracellular organisms	*Falciparum* malaria Babesiosis *Bartonella*
	Endotoxin-induced DIC	Meningococcal sepsis Pneumococcal sepsis Gram-negative sepsis
	Haemophagocytic syndromes	Atypical mycobacterial infections HIV Viruses
	Enzyme toxins	*Clostridium perfringens* Snake, spider bites
Chemical and physical agents	Oxidative damage	Drugs Industrial/domestic substances
	Heat	Burns
	Osmotic lysis (fresh water), dehydration of red cells (salt water)	Drowning
	Enzyme inhibition	Lead poisoning Copper (Wilson's disease)
Fragmentation (mechanical)	Lysis on prosthetic surfaces	Cardiac haemolysis Perivalvular leak
	Vasculitis, endothelial cell swelling, fibrin shear	Microangiopathic haemolytic anaemia March haemoglobinuria
Acquired membrane disorders	Lipid or cholesterol changes	Liver disease
	Somatic mutation	Paroxysmal nocturnal haemoglobinuria (PNH)

Table 9.6 Causes of microangiopathic haemolytic anaemia.

Disease	Microangiopathy
Haemolytic–uraemic syndrome	Endothelial cell swelling, microthrombi in renal vessels
Thrombotic thrombocytopenic purpura	Platelet plugs, microaneurysms, small-vessel thrombi
Renal cortical necrosis	Necrotizing arteritis
Acute glomerular nephritis	
Malignant hypertension	
Pre-eclampsia	Fibrinoid necrosis
HELLP	
Polyarteritis nodosa	Vasculitis
Wegener granulomatosis	
Systemic lupus erythematosus	
Homograft rejection	Microthrombi in transplanted organ
Mitomycin C	Uncertain
Ciclosporin	Renal vessel anomalies
Carcinomatosis	Abnormal tumour vessels, intravascular coagulation (disseminated or localized)
Primary pulmonary hypertension	Abnormal vasculature
Cavernous haemangioma (Kasabach–Merritt)	Local vascular changes, thrombosis

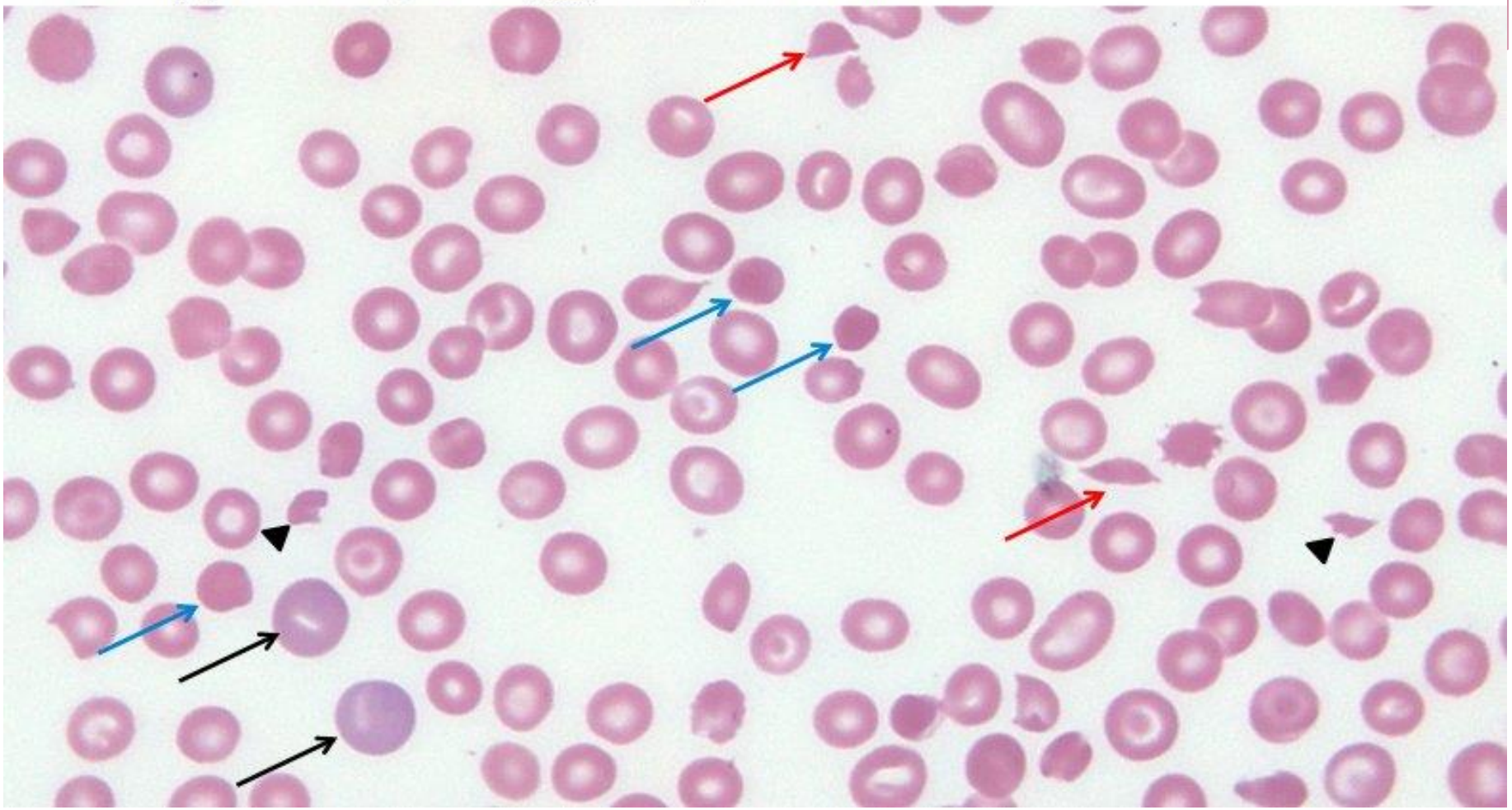




Microangiopathic hemolytic anemia is characterized by an increase in:

- spherocytes (blue arrow)
- schistocytes (red arrow)
- non-specific red cell fragments (black arrowhead)
- polychromatophilic cells (black arrow)

Peripheral smear changes are insensitive for conditions such as disseminated intravascular coagulation and other testing (platelet count, D-dimer, etc) are required for evaluation.



Aplastic anemia and BM failure

i. Marrow infiltration or replacement.

ii. Hypoplasia/aplasia :

1. Aplastic anemia (**pancytopenia**) all 3 lines.
   - Congenital as Fanconi anemia, Dyskeratosis congenita.
   - Acquired AA.
2. **Single line** (anemia, neutropenia and TCP)
   - **Congenital** Anemia: Diamond-Blackfan anemia.
   - Neutropenia: Kostmann's syndron.
   - TCP: cong.amegakaryocytic TCP.
   - **Acquired** RCA, acquire neutropenia, acquire amegakaryocytic

Etiology of AA:

1. **Congenital**: as Fanconi anemia, Dyskeratosis congenita.
2. **Acquired** AA :
   - Idiopathic in 50 -75% of cases. most common type and suggested to be autoimmune T-cells mediated disorder
   - Irradiation; accidental or therapeutic.
   - Chemicals; benzene, insecticide.
   - Drugs as cytotoxic agents, Chloramphenicol, sulpha, gold.
   - Chloramphenicol (1:25000 – 1:40000 oral rout not by injection and reported with eye drops).
   - Infective agents as Hepatitis, HIV, EBV,ect.
   - SLE, pregnancy, GVHD.
   - Malignancy as ALL, AML, MDS

Clinical manifestations:

1. Any age but peak at 30 yrs.
2. Bleeding tendency.
3. Features of anemia as tiredness.
4. Infections.
5. No jaundice except post-hepatitis cases.
6. No organomegaly

Lab. Findings:

- ✓ Pancytopenia (reduction in HB, WBC, and Platelets.(
- ✓ Blood film: RC normochromic, usually macrocytic, with reduced retics.
- ✓ BMA: hypocellular, may be dry (cannot diagnosed alone.(
- ✓ BMB (required for diagnosis): hypocellular.
- ✓ Cytogenetic analysis for congenital types.
- ✓ Ix to distinguish AA from PNH e.g. Ham's test, CD55,59 assay .

Management

- ✓ Supportive: packed RBC, platelets concentrates, infection prophylaxis.
- ✓ Immunosuppressive agents as Anti-Lymphocyte Globulin and cyclosporin.
- ✓ Hemopoietic growth factors as GM-CSF, G-CSF.
- ✓ BMT is therapeutic option for younger patient.
- ✓ Androgen previously use as 1st line and may use in Fanconi anemia.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