PRACTICAL HAEMOGLOBINOPATHIES

By

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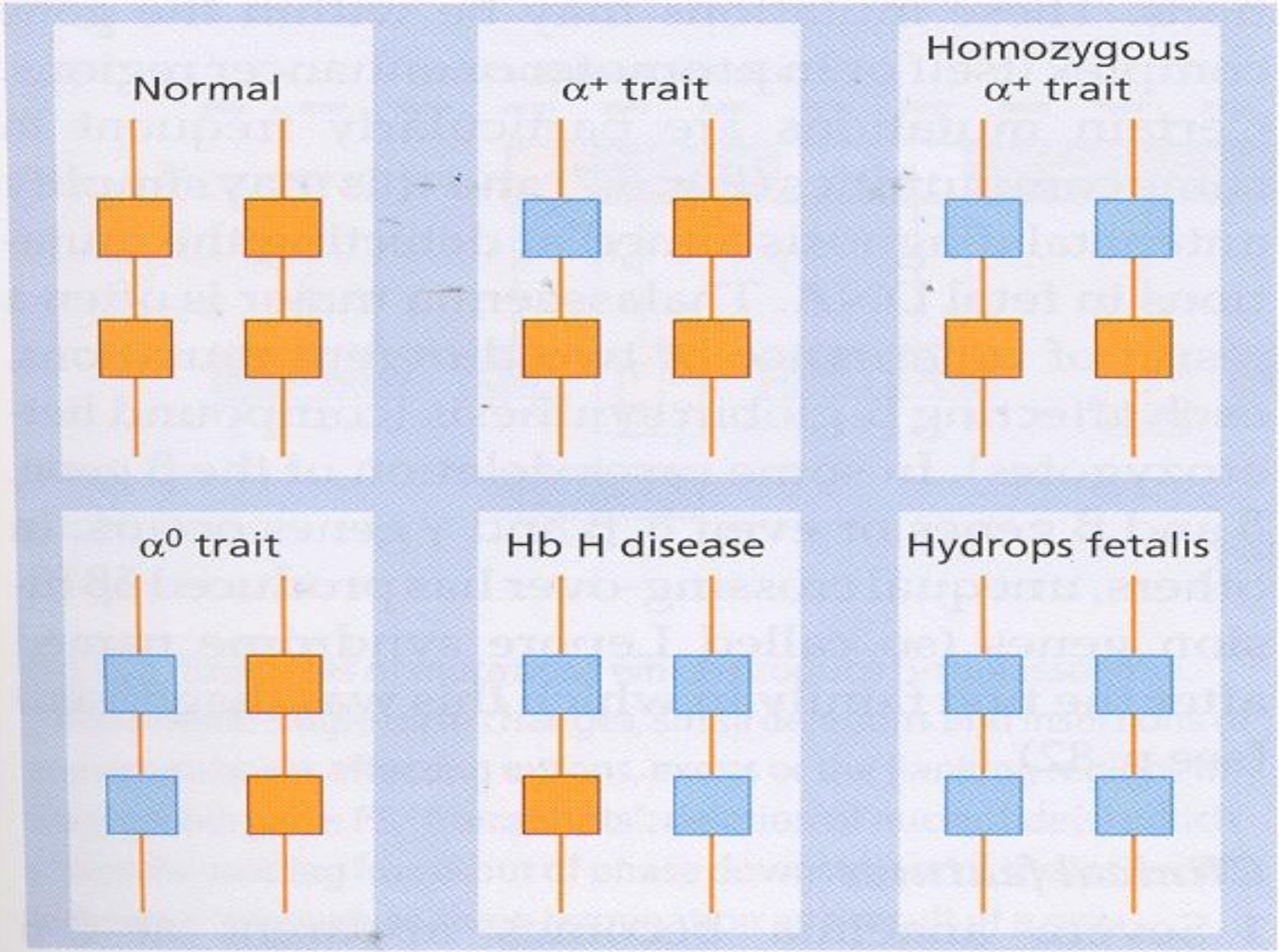
α - THALASSAEMIA

- **HETEROZYGOUS**
- **HOMOZYGOUS**


Normal
α+ trait
Homozygous α+ trait
α0 trait
Hb H disease
Hydrops fetalis

α^+-Thalassaemia trait (deletion of one or two α globin genes)

This is seen when an individual inherits the α^+-thalassaemia allele from one parent or two parents and a normal chromosome 16 from one or two parents (i.e. heterozygotes for the α^+ determinant or homozygous α^+ trait). Affected individuals are asymptomatic, although they have minor haematological changes such as slight reductions in mean cell volume (MCV) and mean cell haemoglobin (MCH).

α^0-Thalassaemia trait (deletion of both α-globin genes on one chromosome 16)

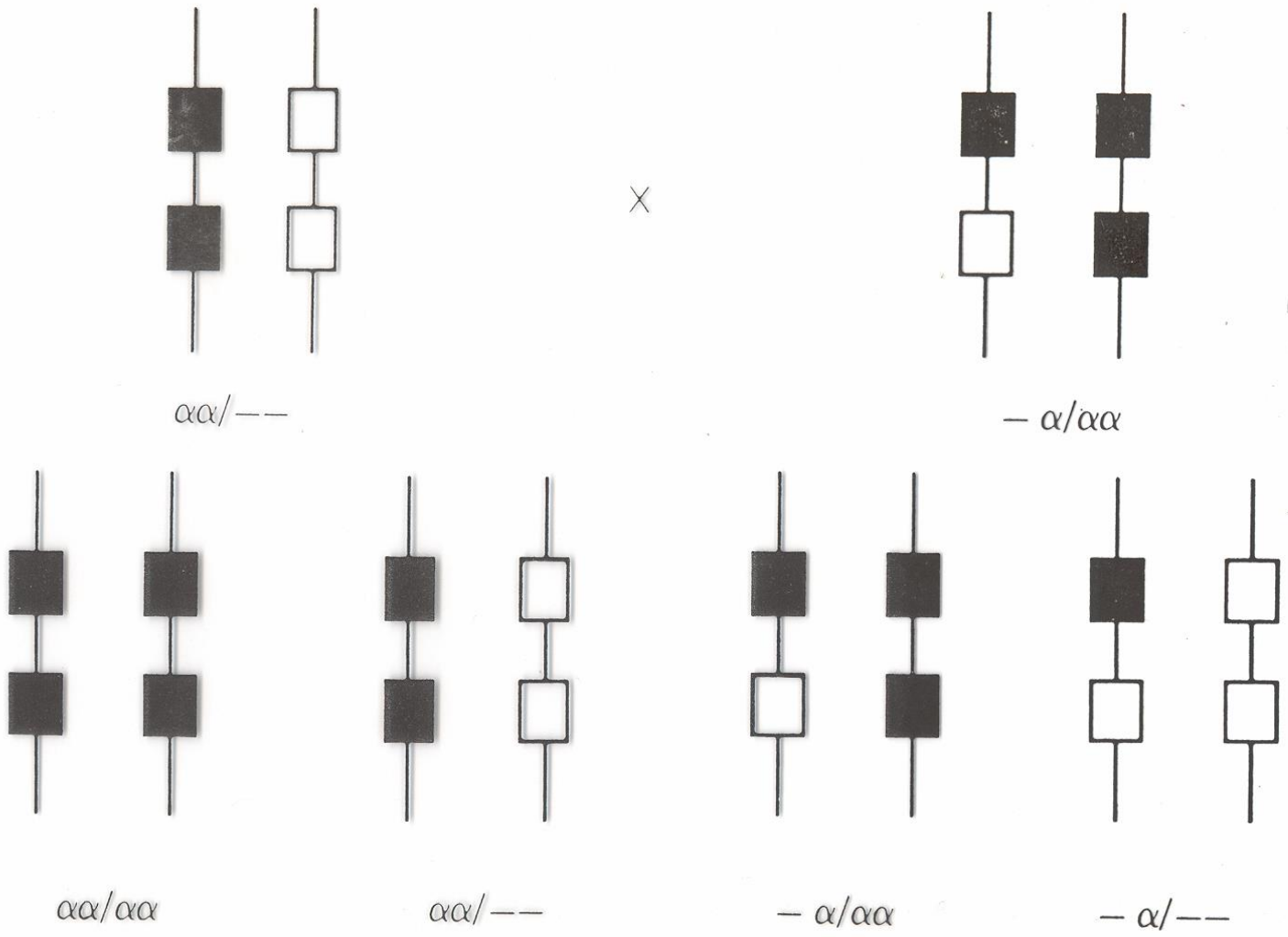
The Hb is either normal or slightly reduced and the MCV and MCH are low.

Haemoglobin H disease (deletion of three α-globin genes)

- This chronic haemolytic anaemia results from the inheritance of both the α^{+}- and α^{0}-thalassaemia alleles, leaving one functioning α-globin gene per cell. α-globin chains are produced at very low rates, leaving a considerable excess of β-chains, which combine to form tetramers (β_4). This tetramer is known as HbH.
- HbH is unstable and precipitates as the erythrocytes age, forming rigid membrane-bound inclusions that are removed during the passage of affected red cells through the spleen. The damage to the membrane brought about by this removal results in a shortened red cell lifespan.

cont'd…

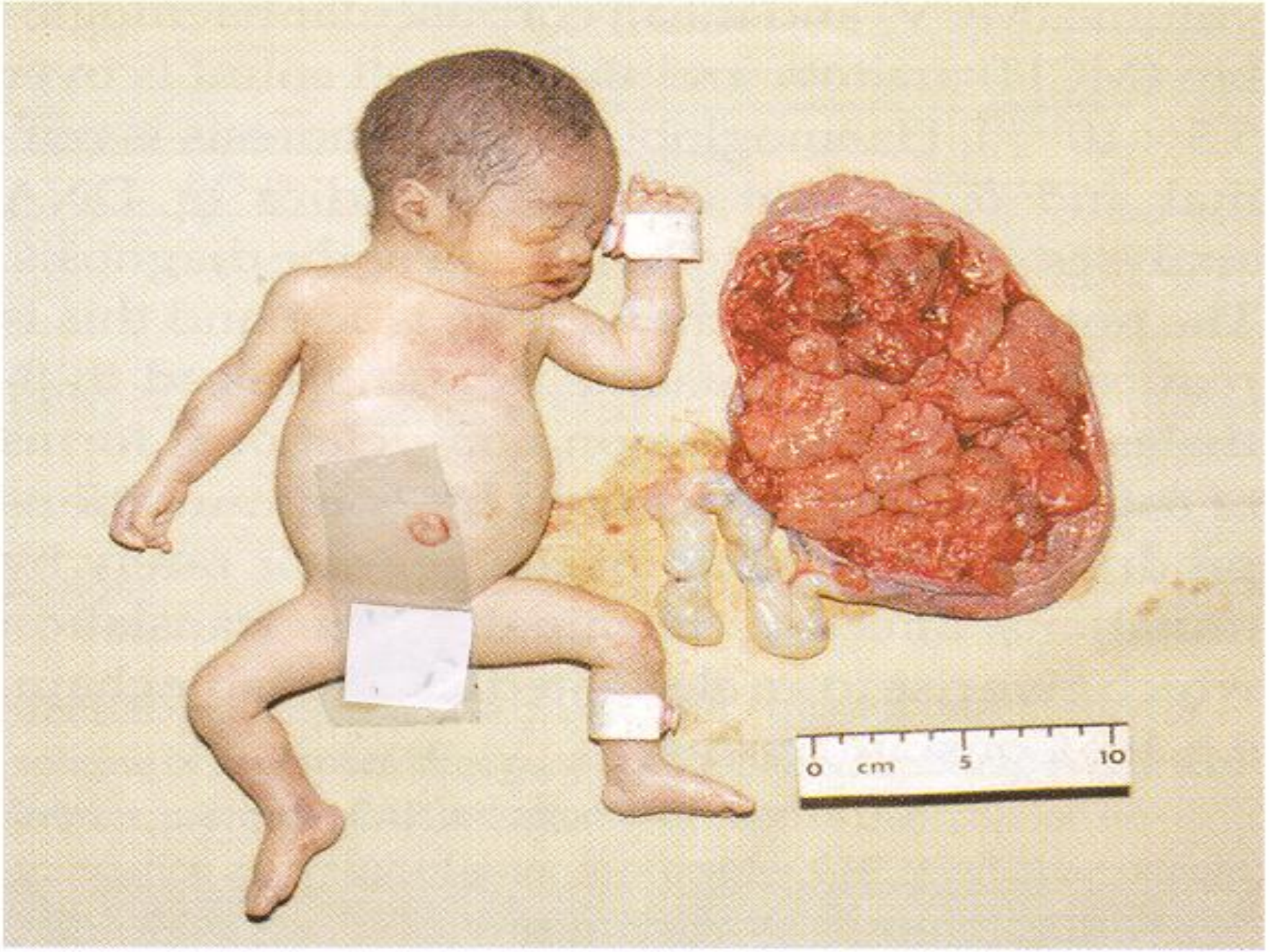
- Most patients are moderately affected, with anaemia of 7-11g/dl and markedly hypochromic, microcytic indices.
- Supravital staining of the blood film demonstrates cells with many HbH inclusions, giving a characteristic 'golf-ball' appearance.
- Most patients will be transfusion independent.
- Splenomegaly is seen in most patients.



Inheritance of HbH (−α/− −) disease. Normal α-globin genes are shown by closed boxes, and deleted or otherwise inactivated α-globin genes by open boxes.

Hb Bart's hydrops fetalis syndrome (deletion of all four α-globin genes)

No α-chains can be formed, and the fetal β-like chain γ-globin forms tetramers known as Hb Bart's. This haemoglobin is not useful for oxygen transport and, despite the persistence of the embryonic haemoglobin Hb Portland ($\zeta_2\gamma_2$), there is intrauterine or neonatal death due to hydrops.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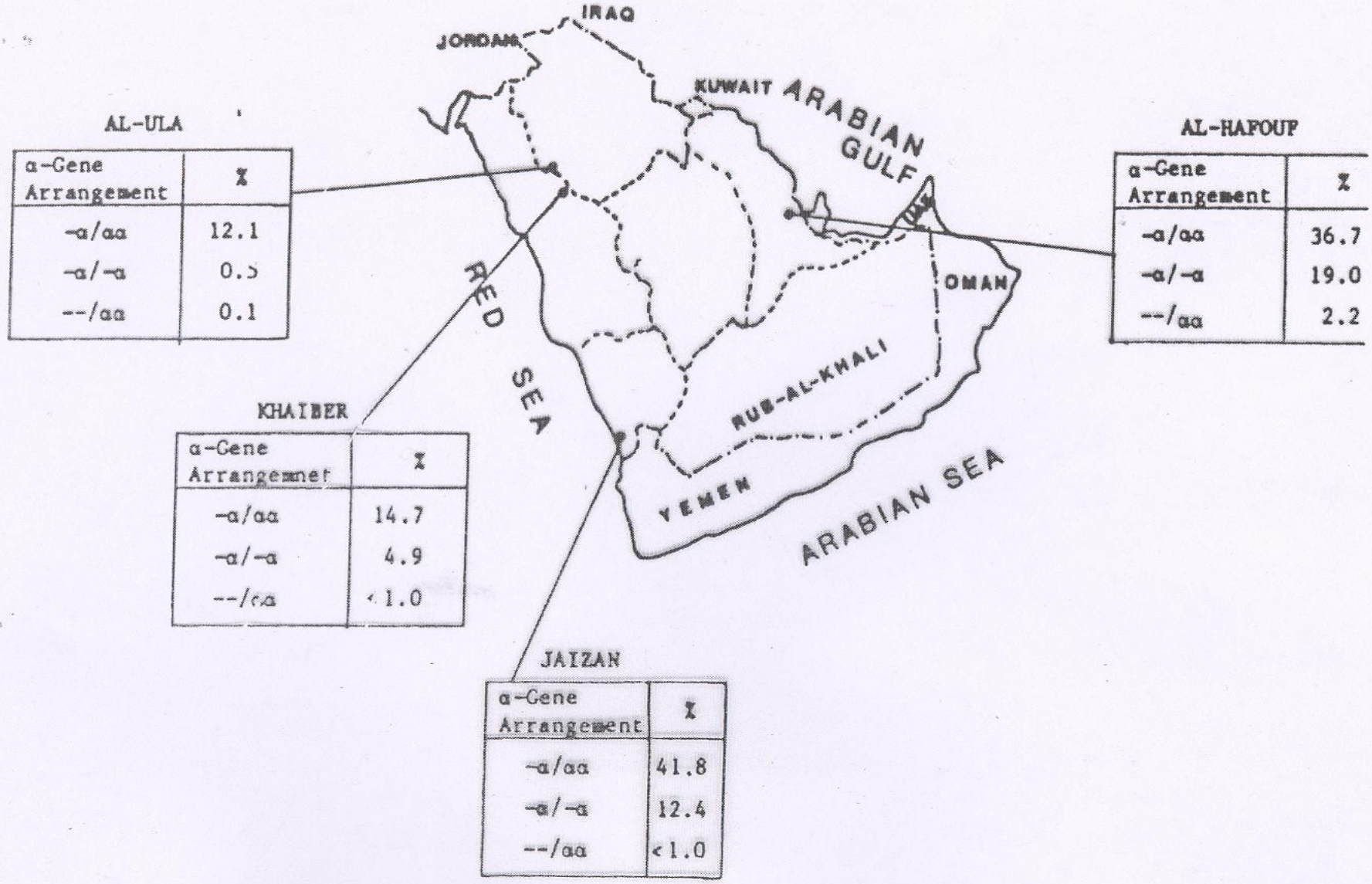

Frequency of α-thalassaemia due to α-gene deletion in different regions of Saudi Arabia (diagnosed using rest iction endonuclease Bam HI).

LABORATORY DIAGNOSIS OF ALPHA THALASSEMIA SYNDROME

- High red cell count in the trait
- Hypochromic microcytic red cells & target cells
- Normal serum iron or low in children
- Normal total iron binding capacity or high in children
- Positive Hb H inclusion bodies in the blood film preparation & positive Heinz bodies with vital stains
- Hemoglobin electrophoresis show presence of hemoglobin H (Hb H disease)
- Hemoglobin electrophoresis show low Hb A2 level
- Genetic study to confirm the diagnosis

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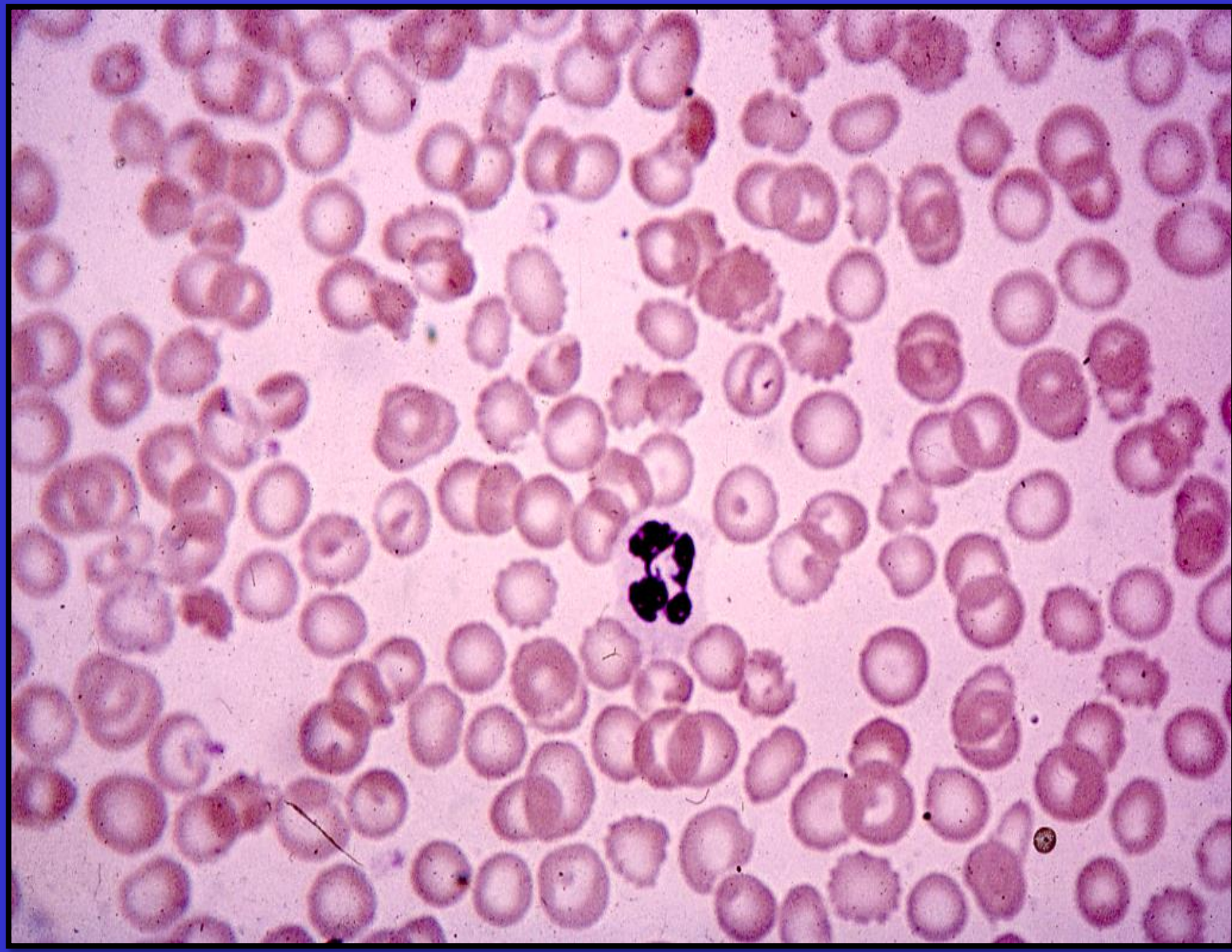
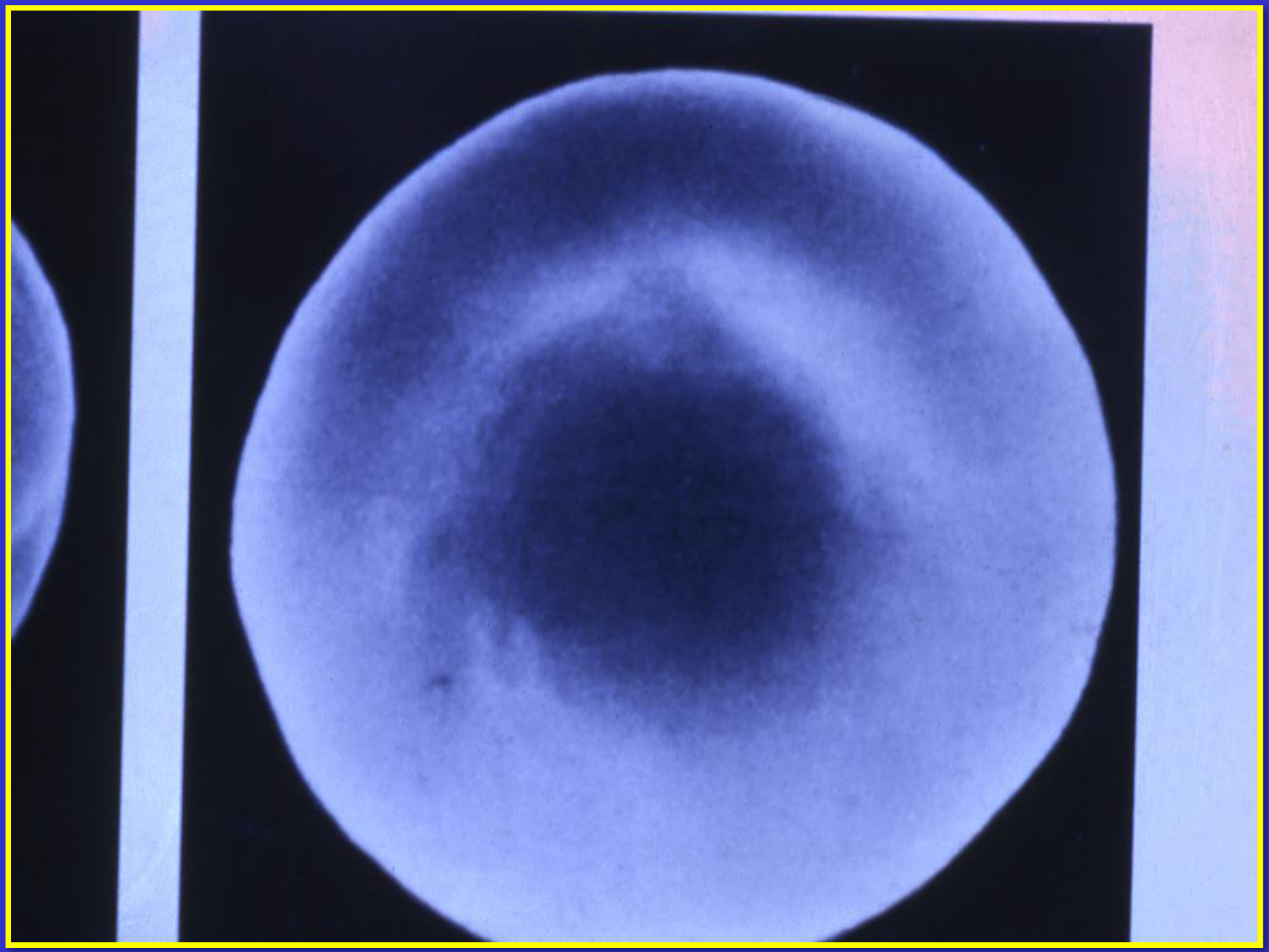
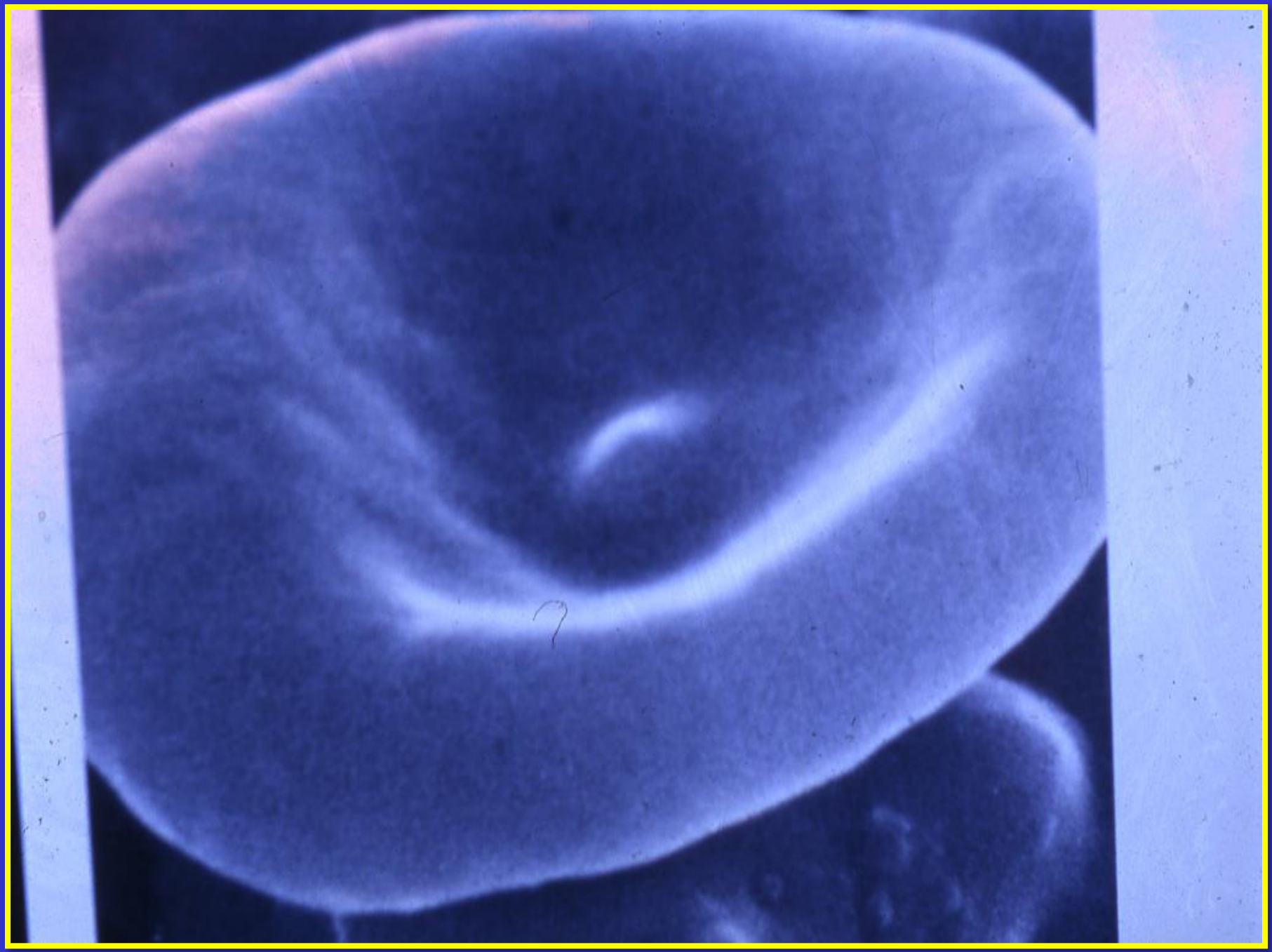
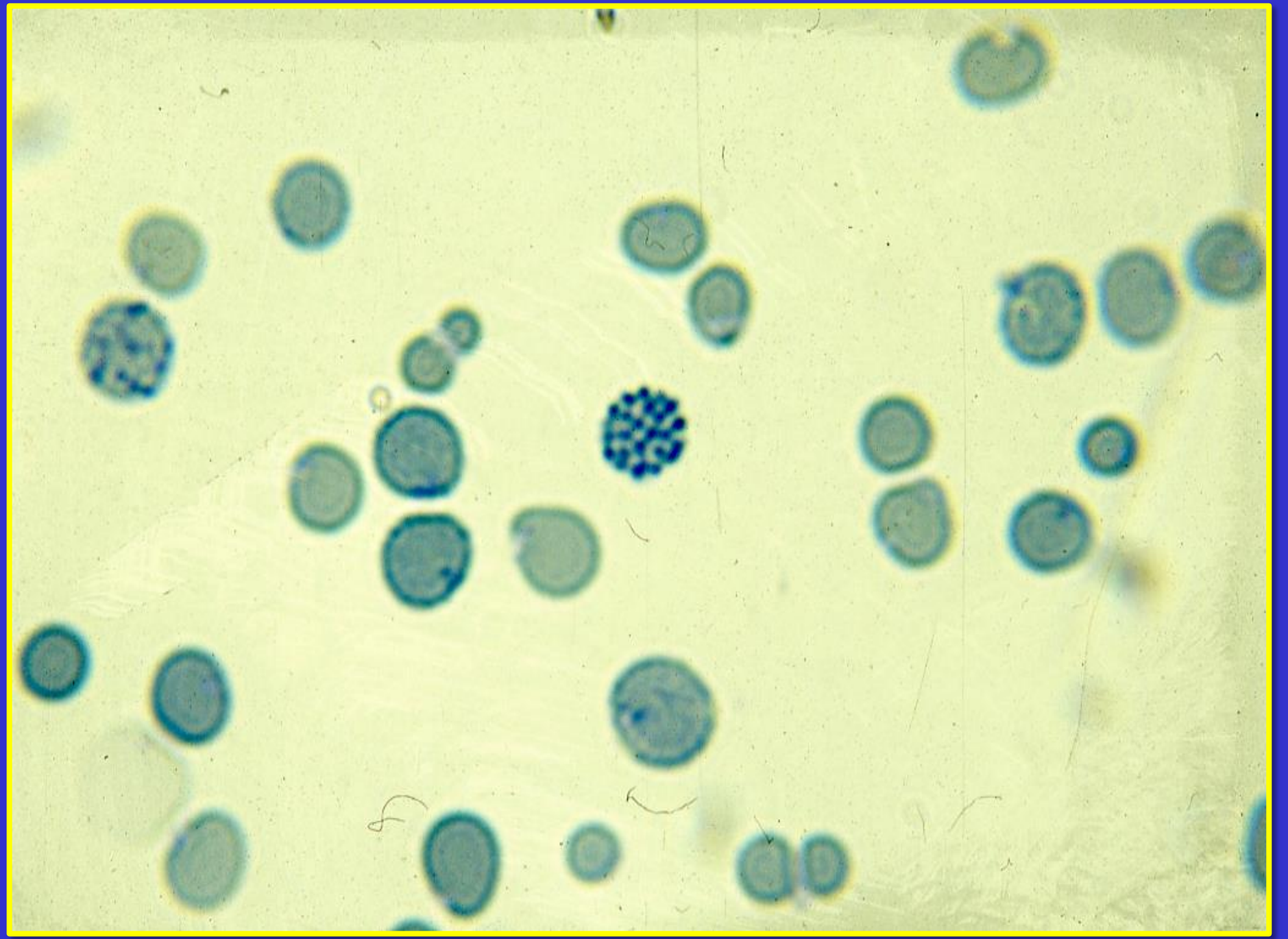
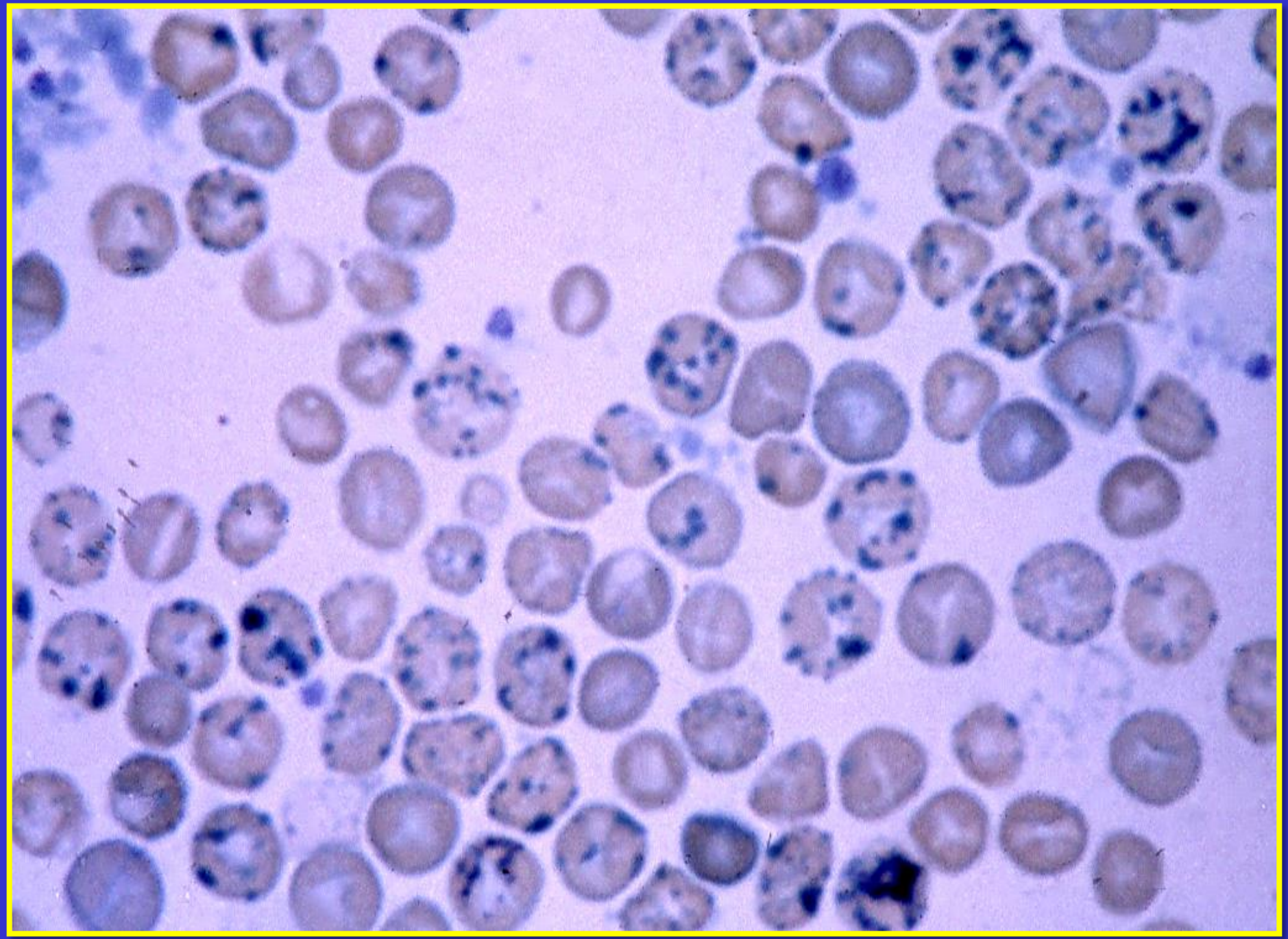
- Positive Hb H inclusion bodies in the blood film preparation & positive Heinz bodies with vital stains
- Hemoglobin electrophoresis show raised of hemoglobin H (Hb H disease)
- Hemoglobin electrophoresis show raised Hb Bart's in newborn babies and children below 1 year of age
- Hemoglobin electrophoresis show low Hb A2 level
- Genetic study to confirm the diagnosis

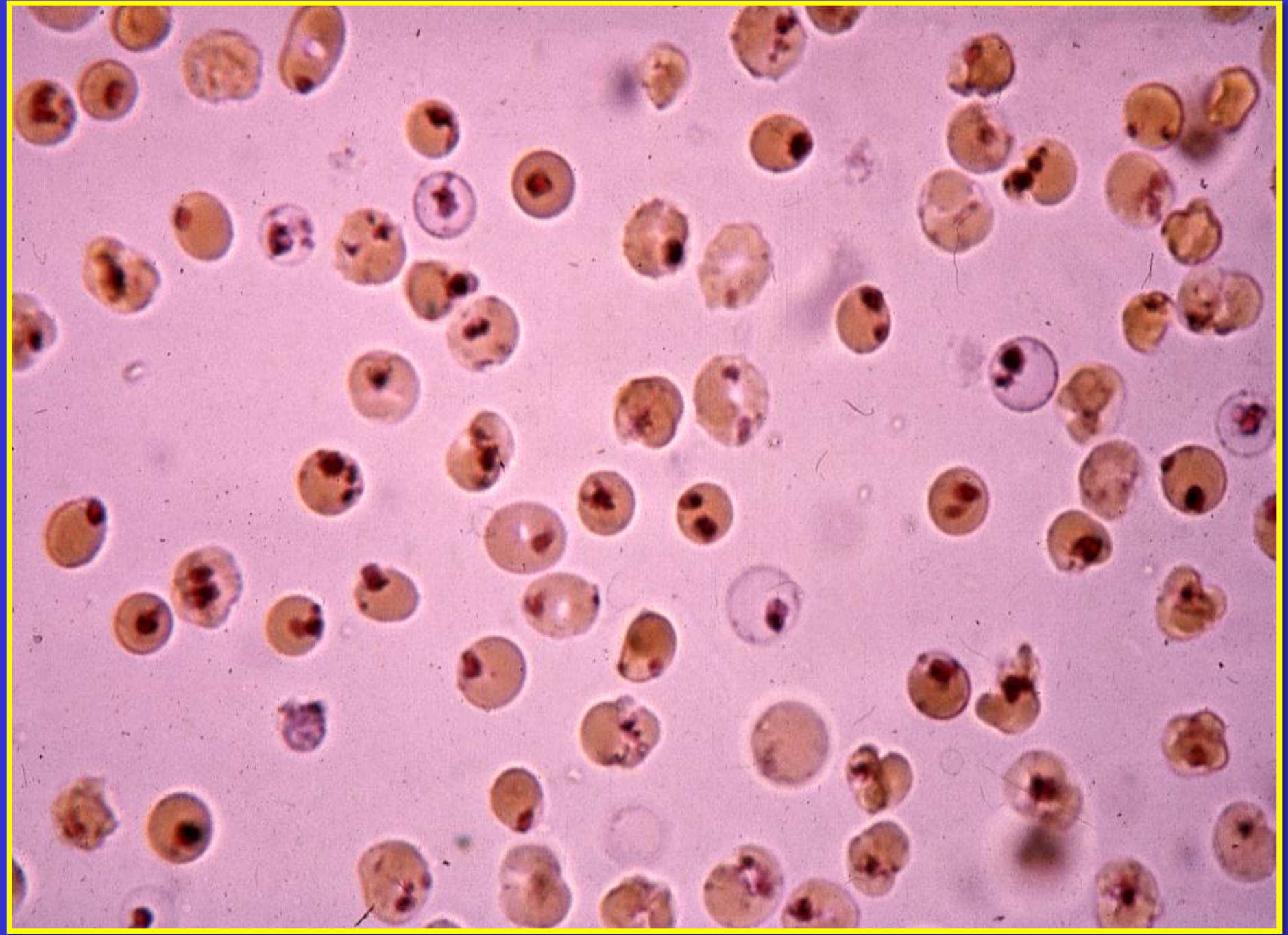


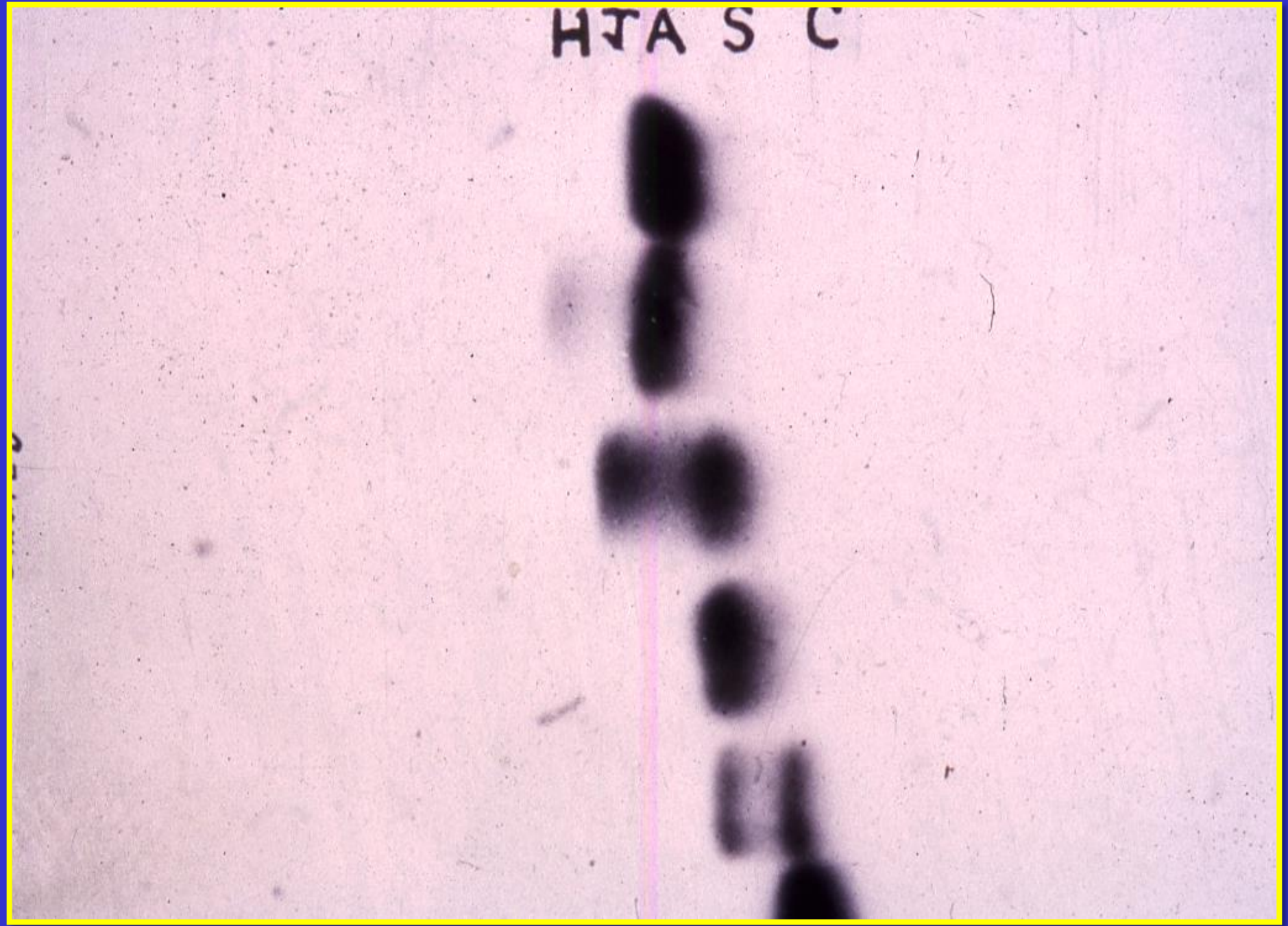






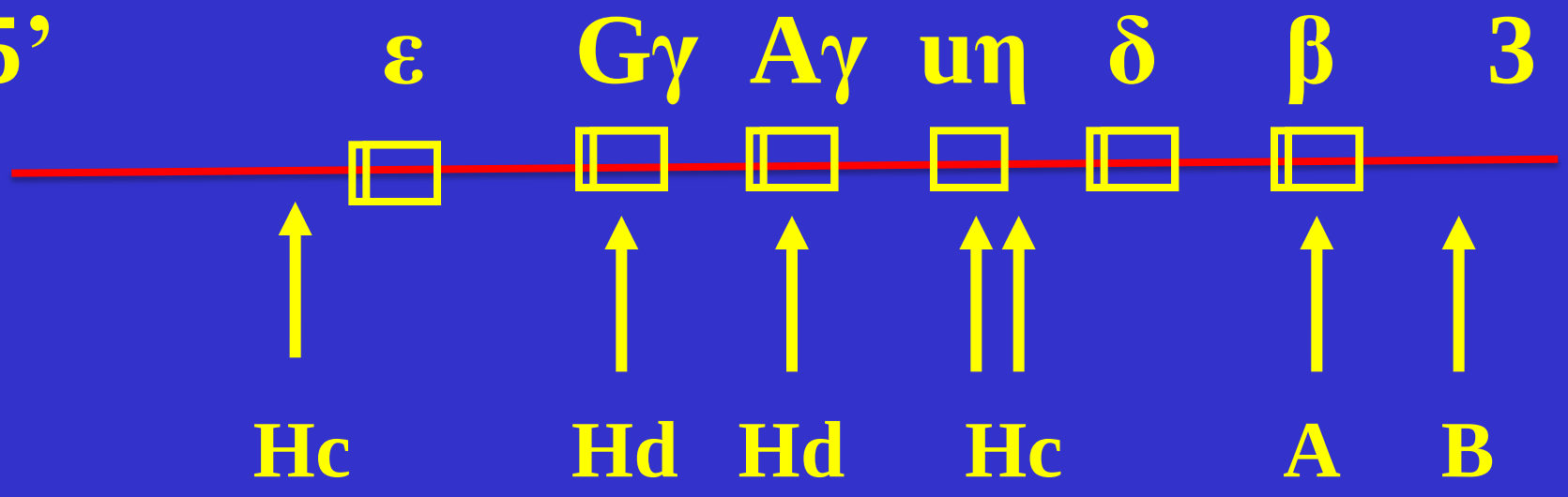





HTA S C

β-THALASSAEMIA

DR. SHIHAB AL-MASHHADANI



The –globin gene cluster showing the position of various common restriction endonuclease polymorphic sites. (Hc, Hinc II; Hd, Hind III; A, Ava II; B, Bam H1).

Molecular Defects in the β-Thalassaemia Syndrome

	β-Globin synthesis	β^-mRNA	β-Globin Gene	δ-Globin Synthesis	γ-Globin Synthesis
1. β^+-Thalassemia	Decreased	Decreased	Present	Present	Present
2. β^0-Thalassemia	Absent	Absent	Present	Present	Present
Ferrara Variant	Absent	Inactive	Present	Present	Present
Indian Variant	Absent	Absent	Partially Deleted	Present	Present
3. δβ-Thalassaemia	Absent	Absent	Deleted	Absent	Increased
4. HPFH	Absent	Absent	Deleted	Absent	increased

Hemoglobin Fractions in the Genotypic Variants of the β-Thalassaemia Syndromes

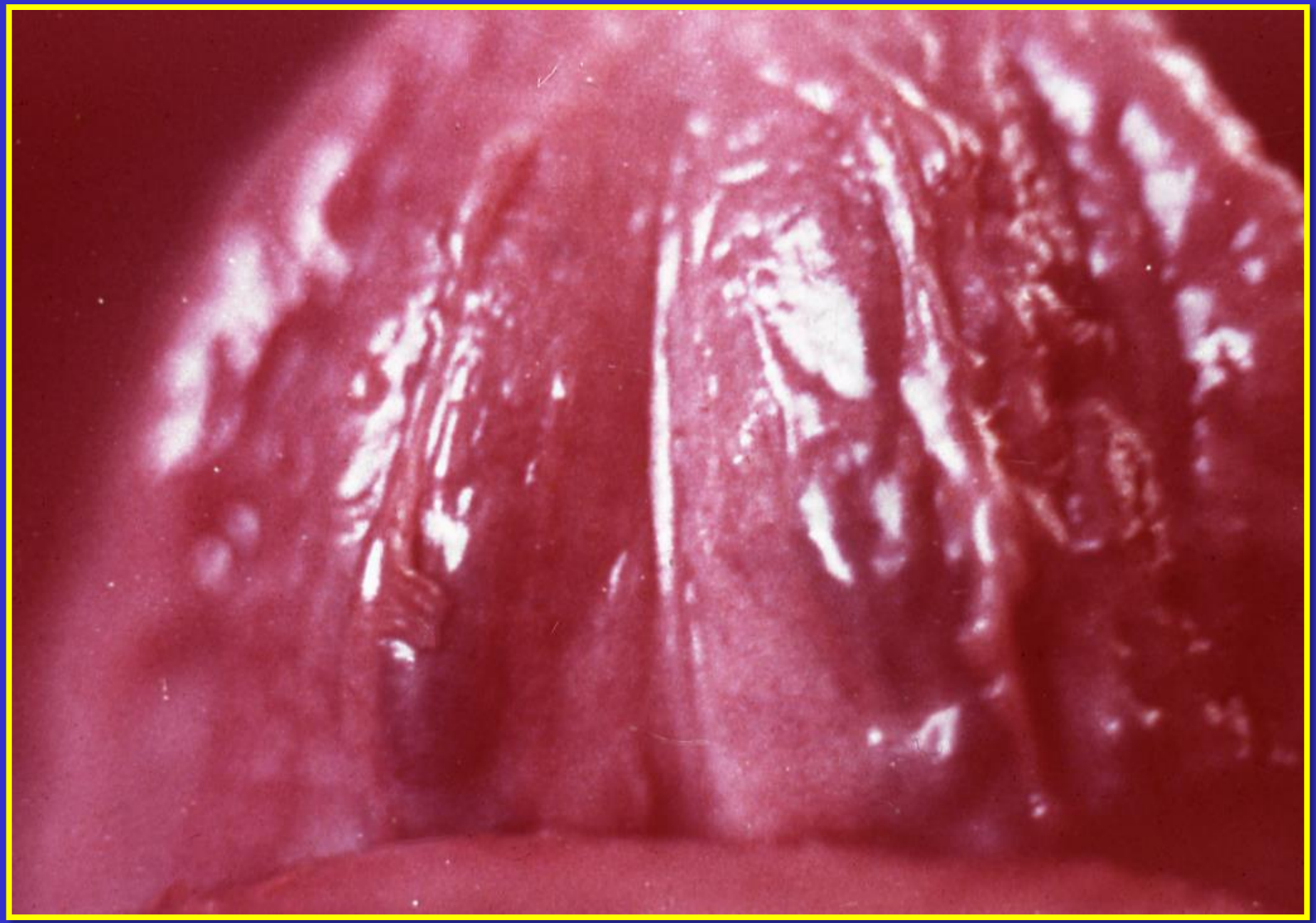
Genotype	HbA	HbA_2	HbF (%)	Other Hemoglobins
Normal				
β/β	97	2.5 – 3.2	<1	None
Thalassaemia major				
$β^0/β^0$	0	1.0 – 5.9	>94	Free α-chains
$β^+/β^+$ Mediterranean	Present	2.4 – 8.7	20 – 90	Free α-chains
$β^0/β^+$	Present	0.6 – 3.4	>75	None
$(δβ)$ Lepore/ $(δβ)$ Lepore	0	0	70 – 92	Hb Lepore (8-30%)
Thalassaemia intermedia				
$β^+/β^+$, black	Present	5.4 – 10.0	30 – 73	None
$β^0/ (δβ)^0$	0	0.3 – 2.4	60 – 99	None
$β^+/ (δβ)^0$	20 – 30	Decreased	Increased	None
$β^0/ (δβ)^0$ Lapore	0	Decreased	Increased	Hb Lepore (10%)
$β^+/ (δβ)^0$ Lepore	Present	Decreased	Increased	Hb Lepore (10%)
$β^0/β$	Present	>3.2	1.5 – 12	None
$(δβ)^0 / (δβ)^0$	0	0	100	None
$(δβ)^0/ (δβ)$Lepore	0	0	92	Hb Lepore (8%)
α/β	Present	Increased	Normal or increased	± Hb H

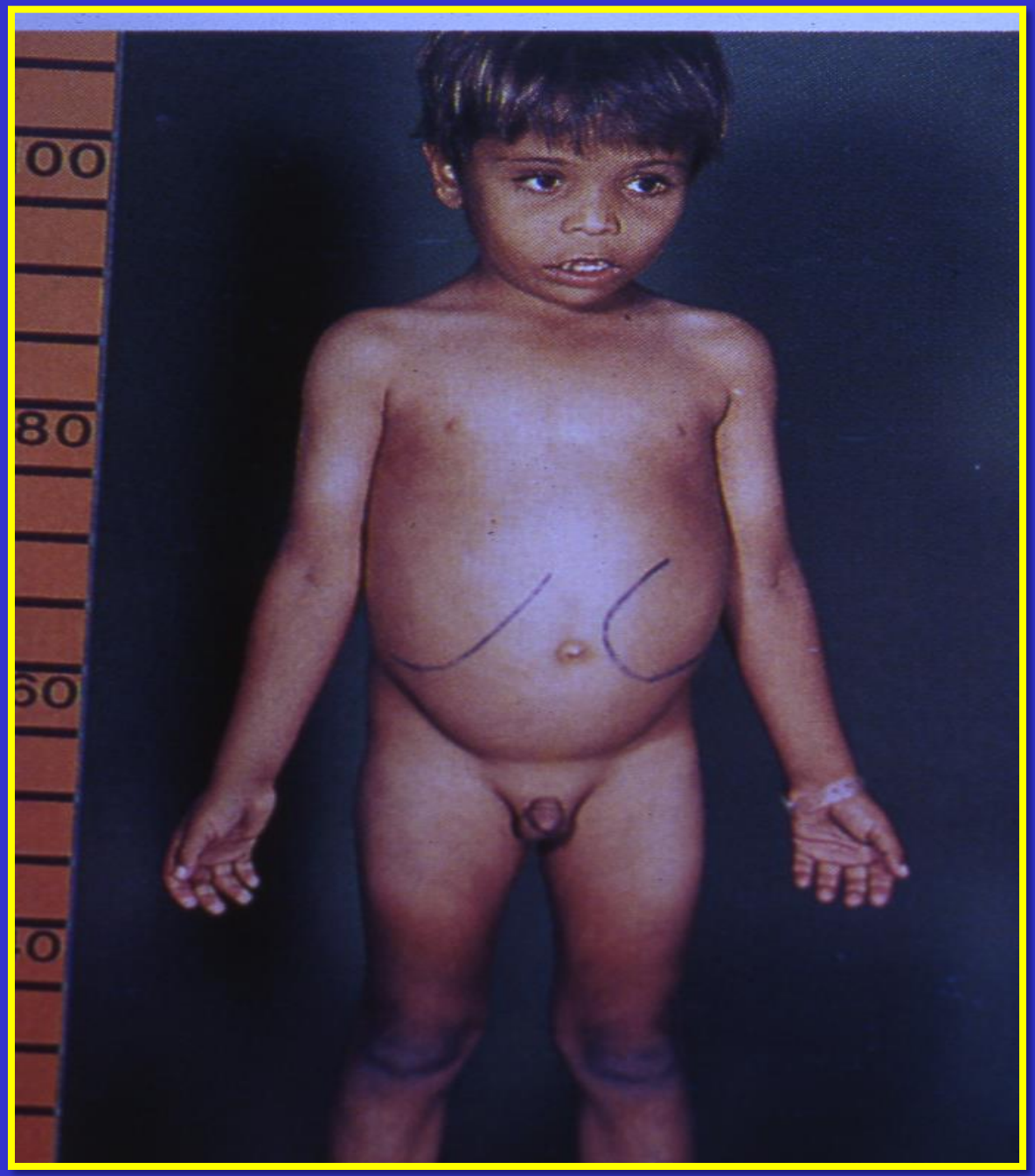
Hemoglobin Fractions in the Genotypic Variants of the β-Thalassaemia Syndromes (Continued)

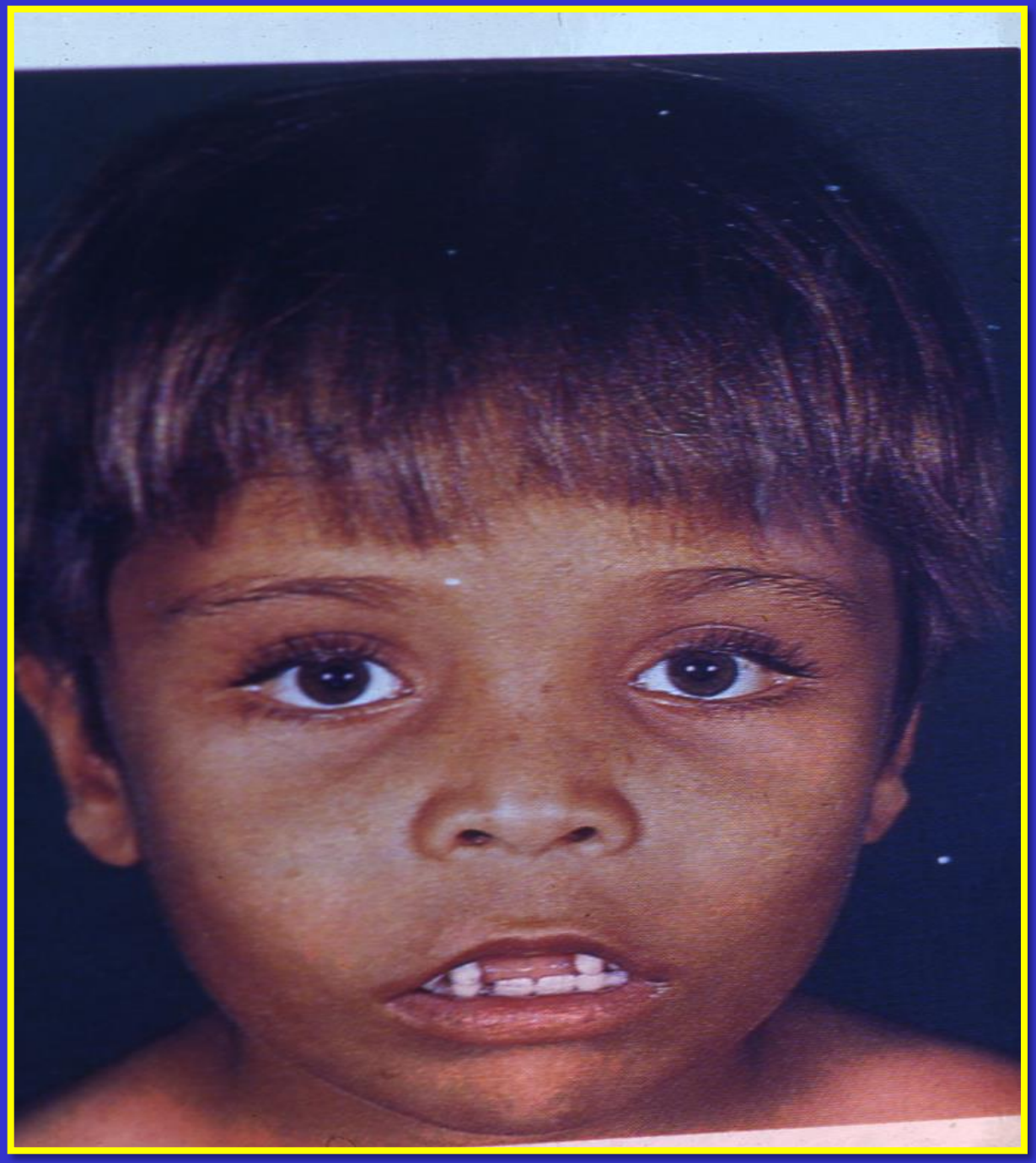
Genotype	HbA	HbA_2	HbF (%)	Other Hemoglobins
Thalassaemia minor				
β^{+}/β	>90	3.5 – 8.0	1 – 2	None
β^{0}/β	>90	3.5 – 8.0	1 – 2	None
$(\delta\beta)^{0}/\beta$	>90	2.5 – 8.0	5 – 20	None
$(\delta\beta)_{Lepore}/\beta$	Present	1.2 – 2.6	1 – 3	Hb Lepore (5 – 15%)
$(\gamma\delta\beta)^{0}/\beta$	Present	2.5 – 3.2	< 1 – 2	None
Thalassaemia minima				
β^{silent}/β	97	<3.2	<1	None

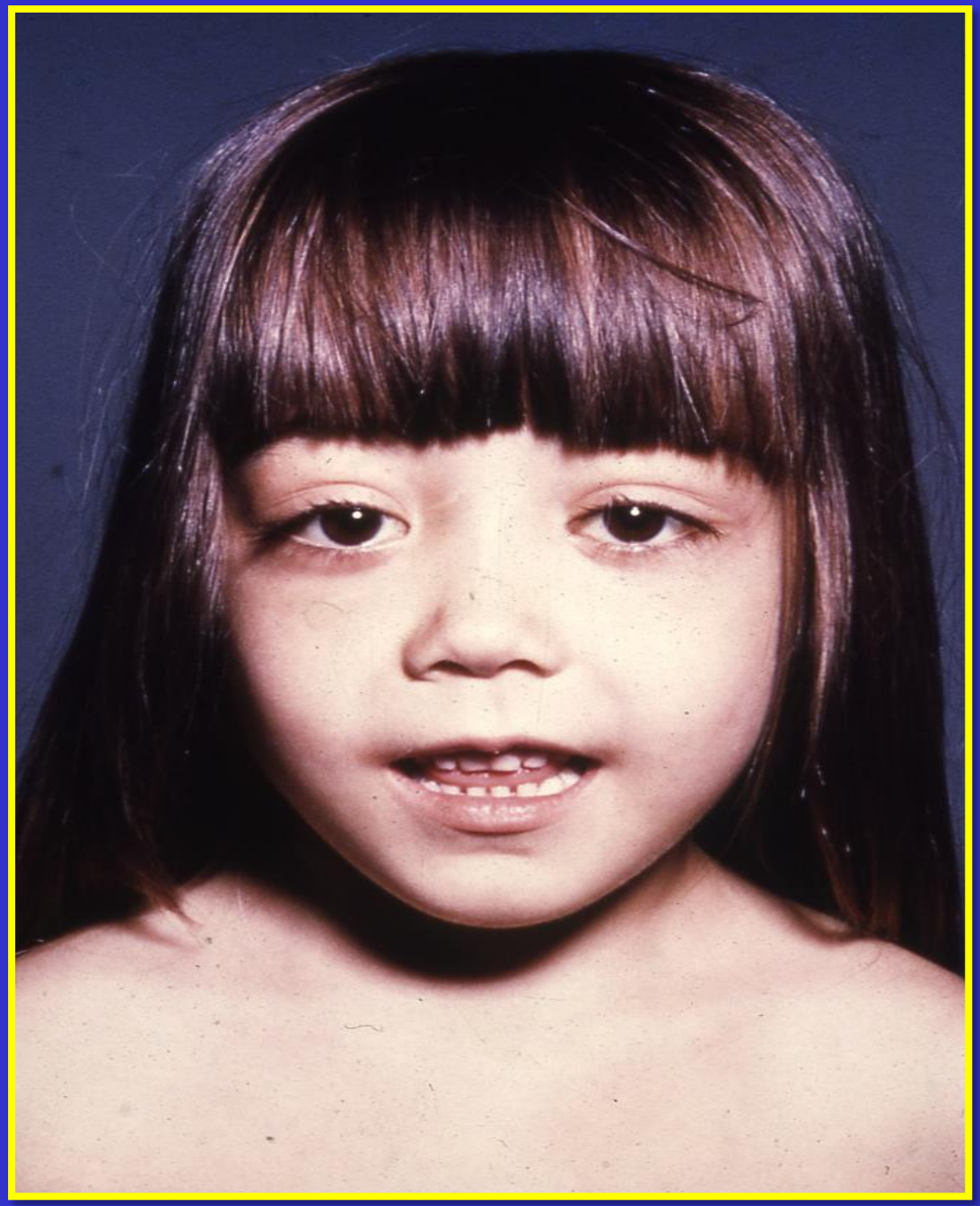
Clinical Manifestations in Thalassaemias

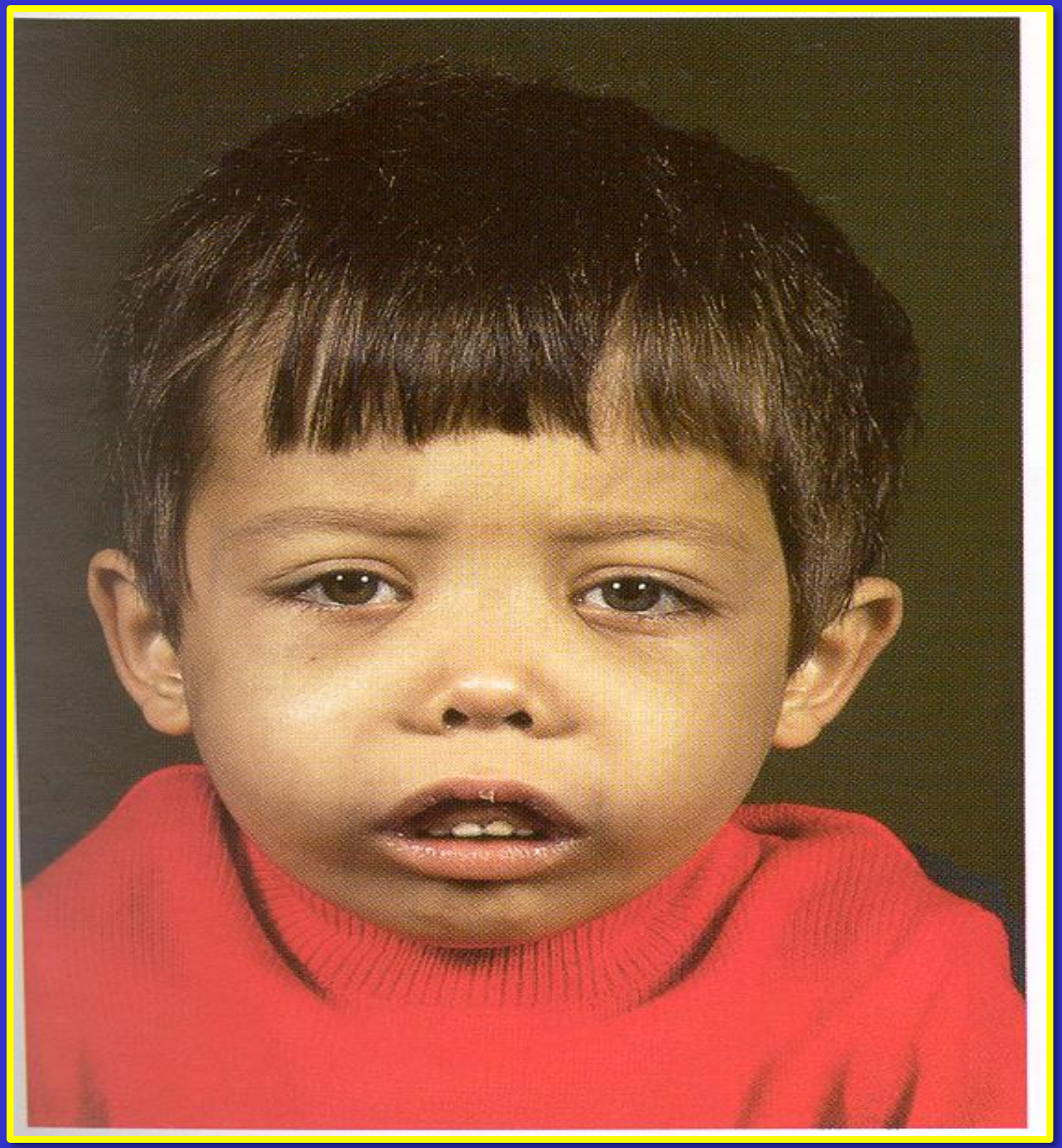
- **Pallor**
- **Jaundice**
- **Apathy and Anorexia**
- **Failure to Thrive**
- **Hepato-splenomegaly**
- **Skeletal Deformity**
- **Iron Overload mainfestations**

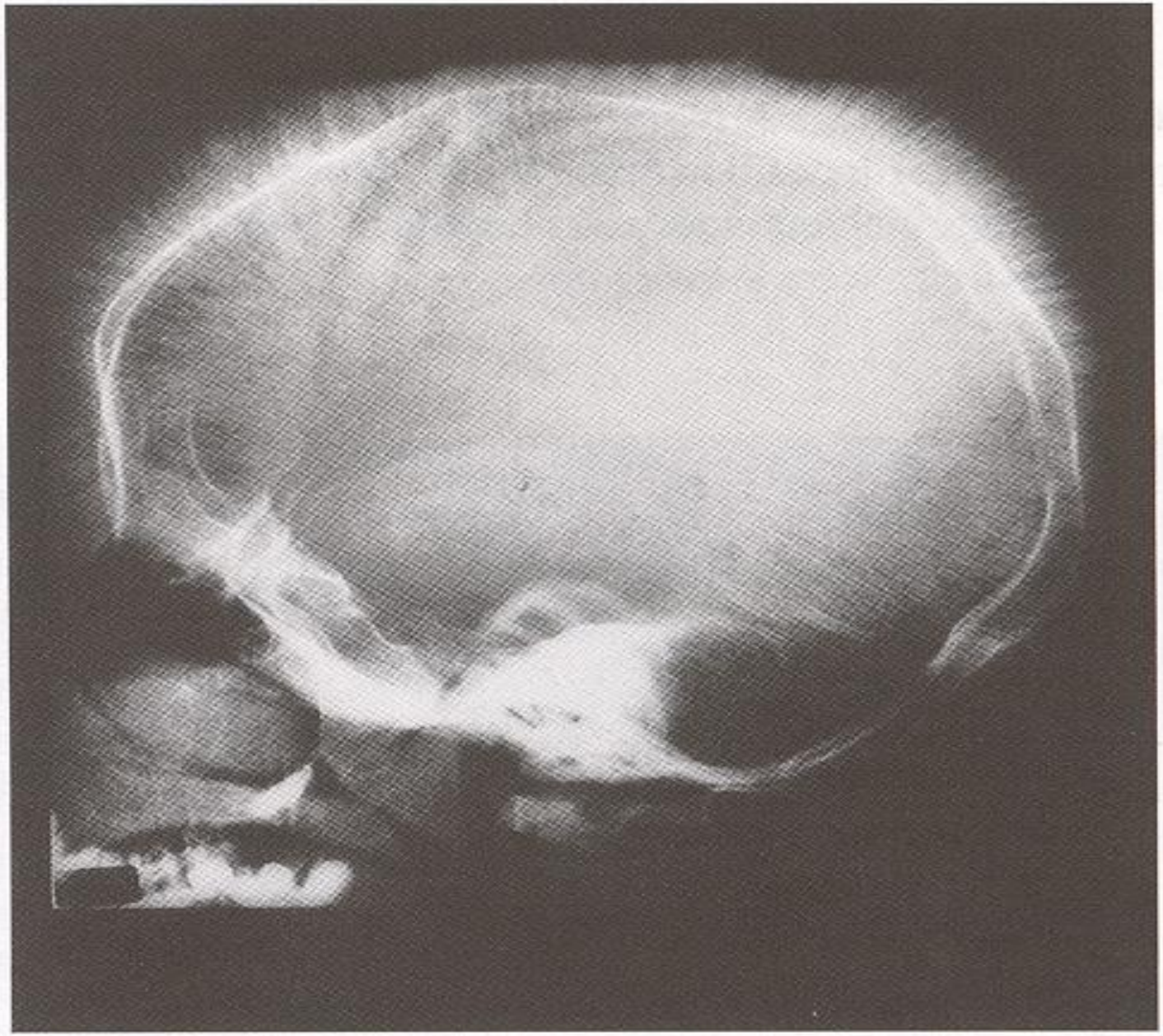


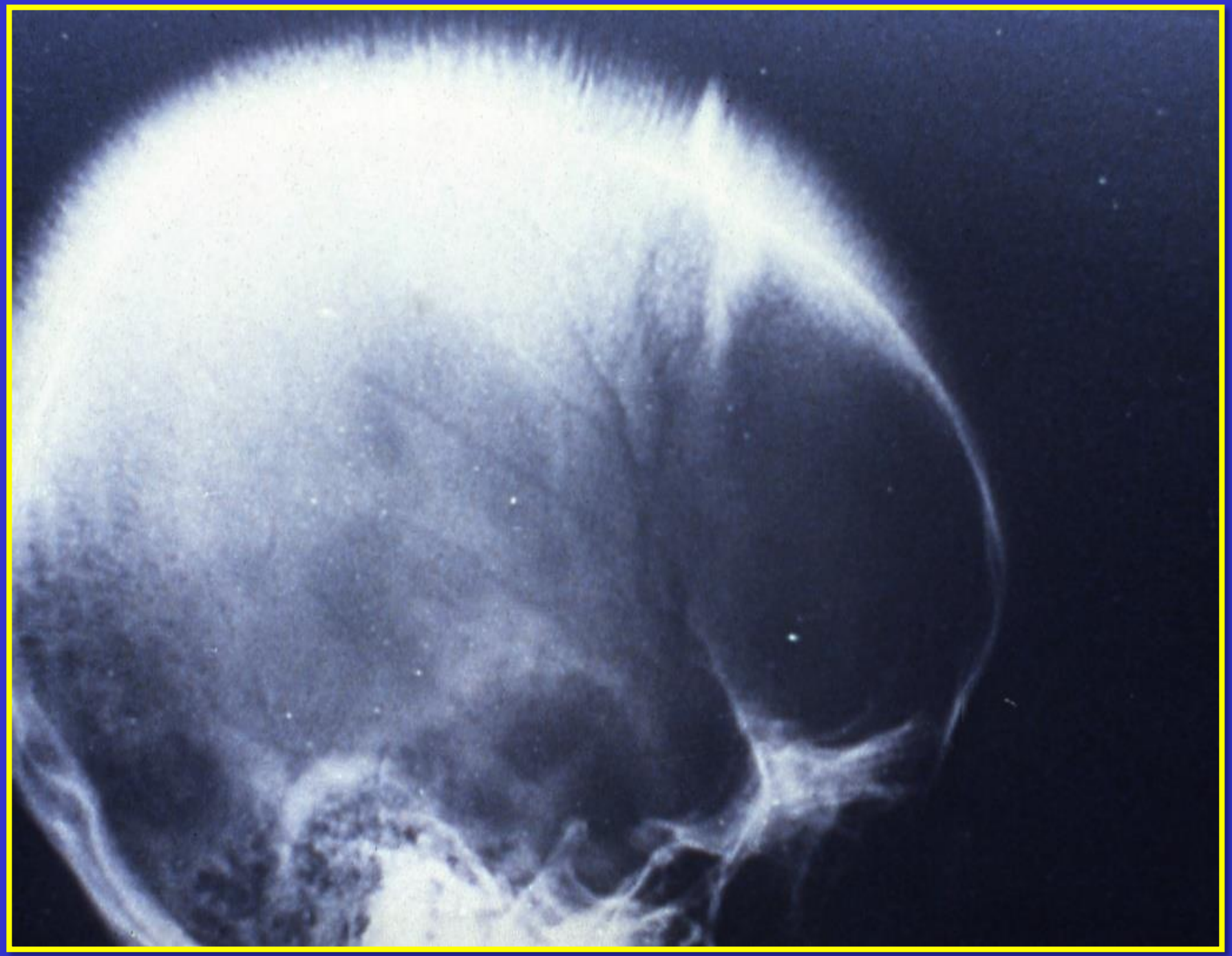


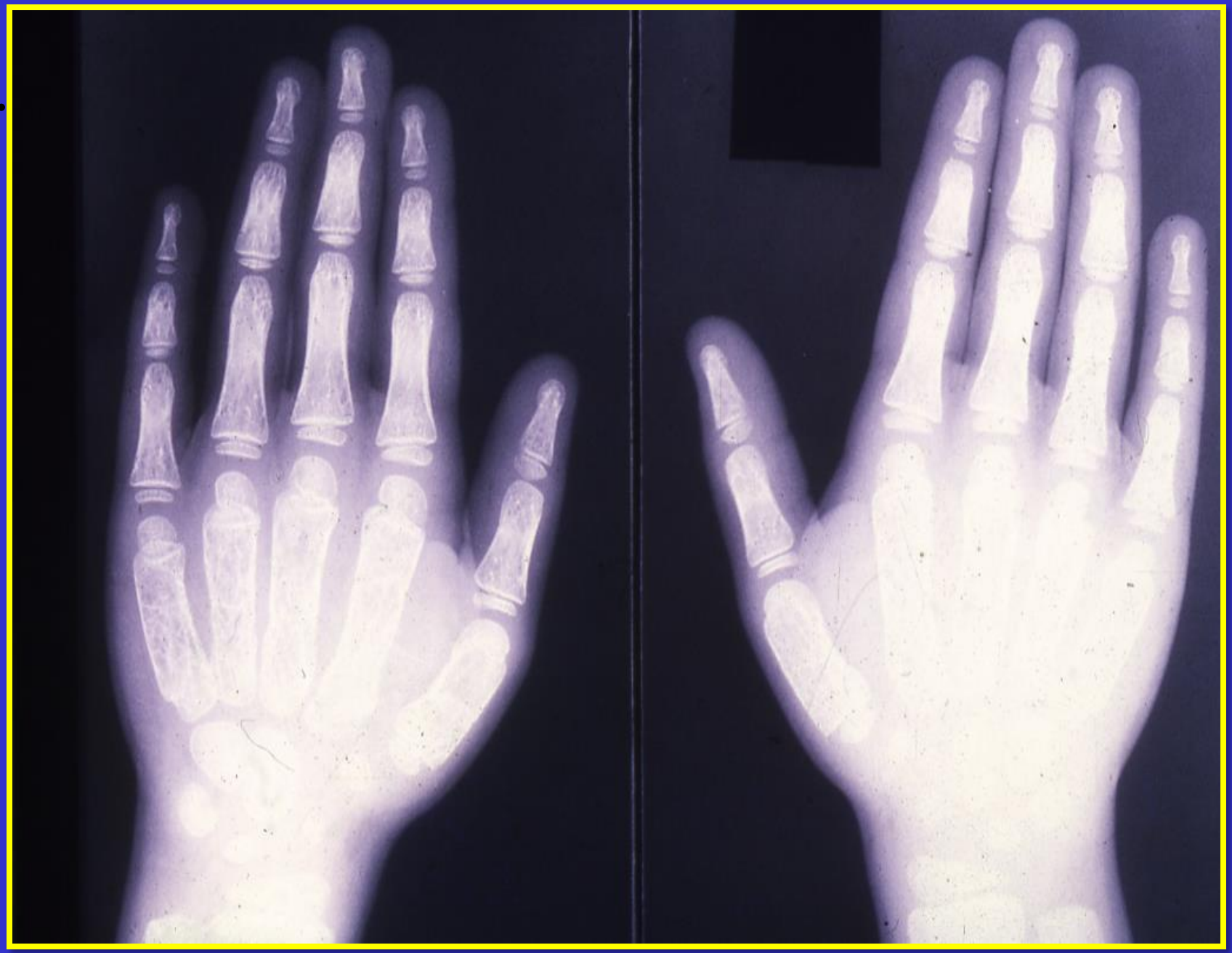


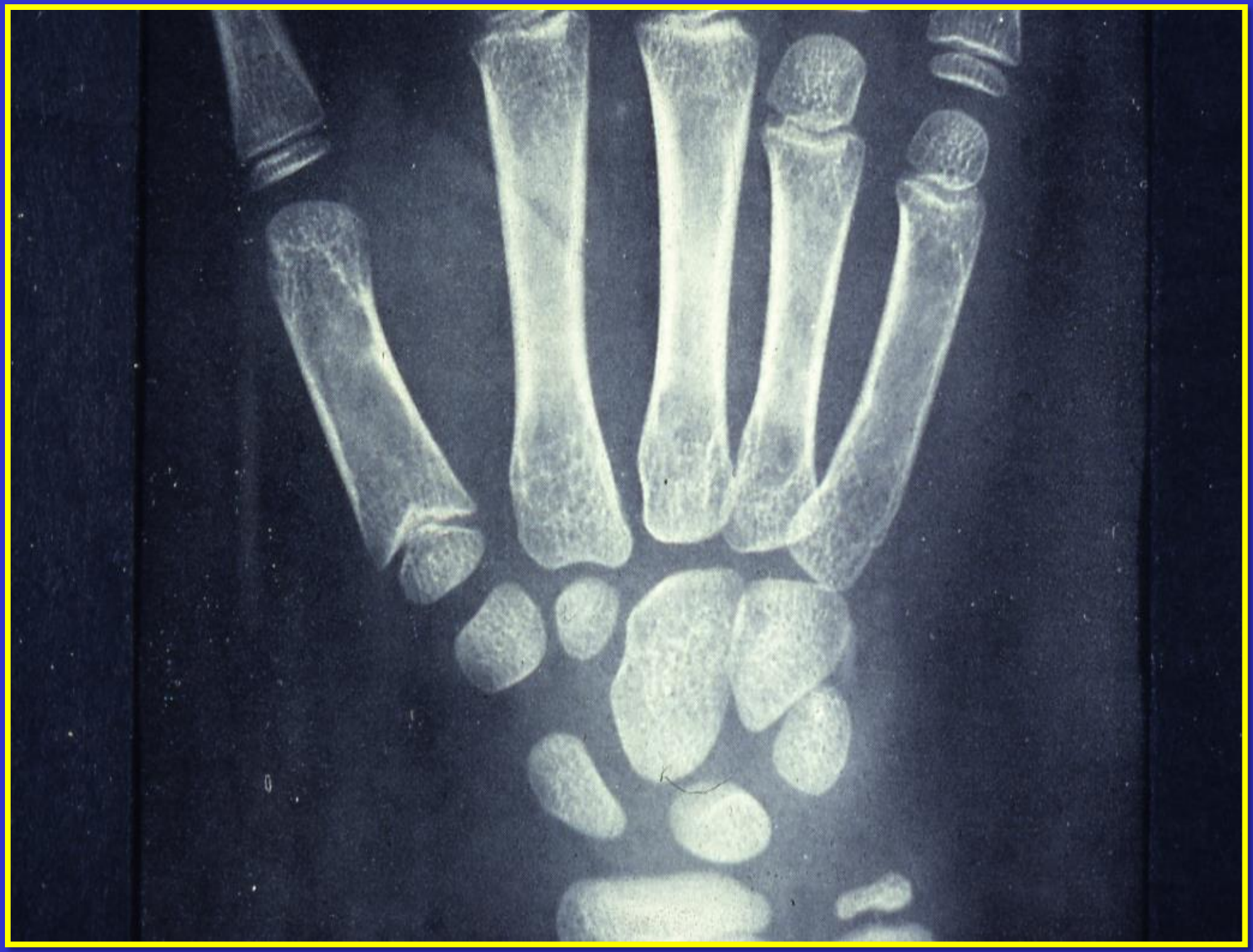












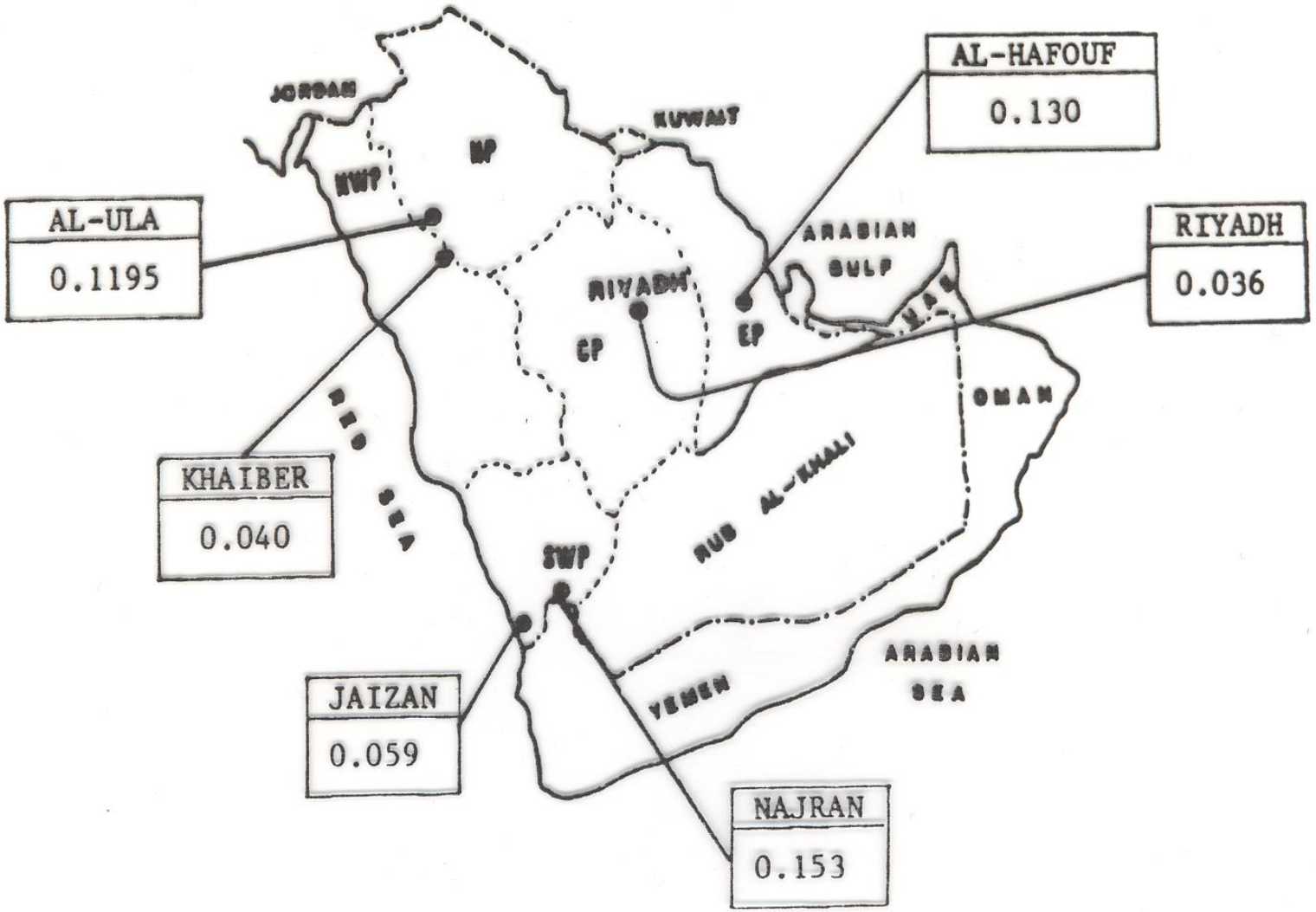
Clinical and Hematological Features of the β-Thalassemia Syndrome

	Major	Intermedia	Minor	Minima
Severity of mainfestations	++++	++	+, ±	±, 0
Genetics	Homozygotes, double heterozygotes	Homozygotes, double heterozygotes, rarely heterozygotes	Heterozygotes	Heterozygotes
Splenomegaly	++++	++,+++	+,0	0
Jaundice	+++	++,+	0	0
Skeletal changes	++++,++	+,0	+,0	0
Anemia (Hb, g/dl)	<7	7 – 10	>10	Normal

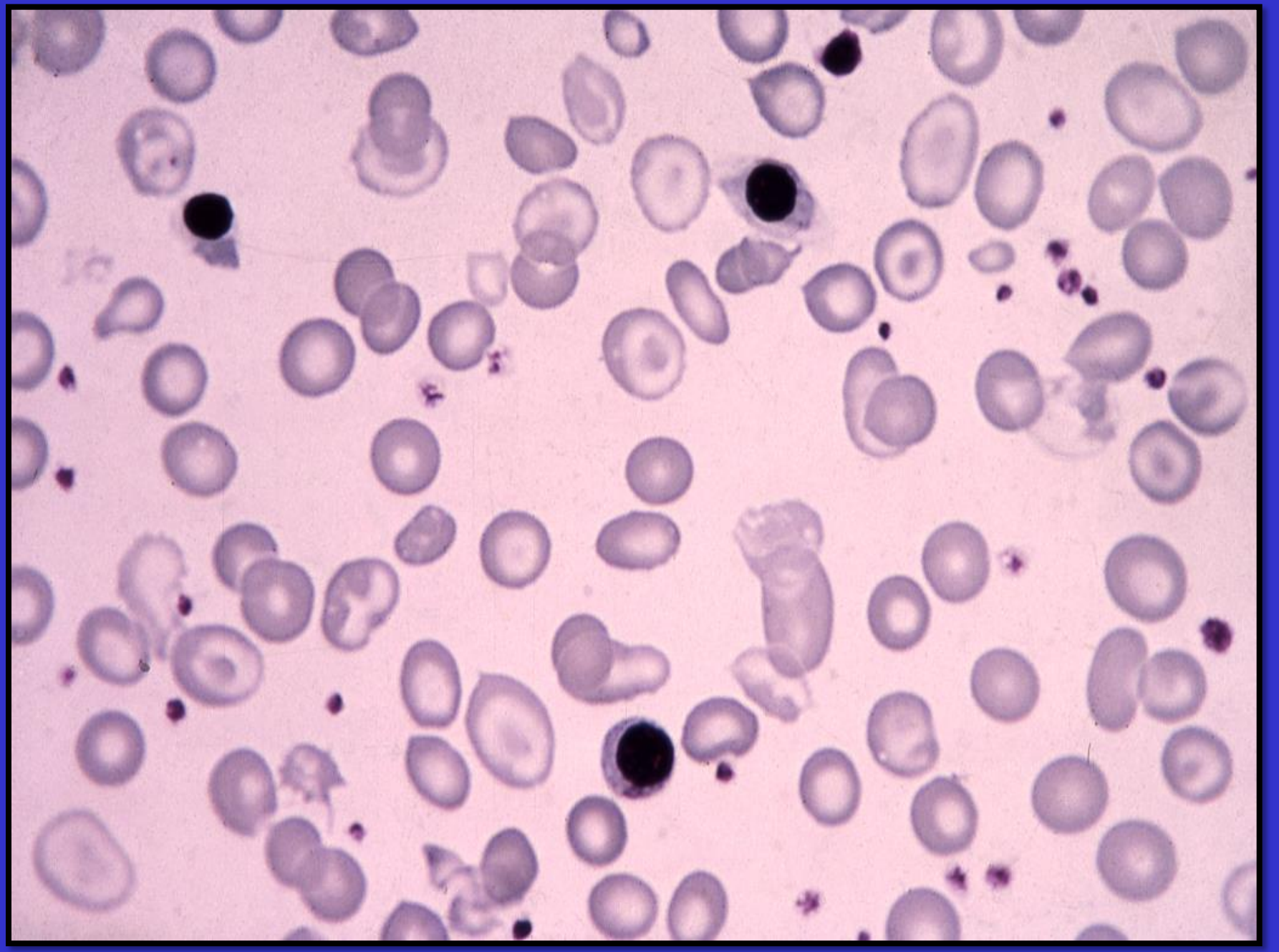
±, little or no abnormality; +, mild abnormality; ++++, prominent abnormality

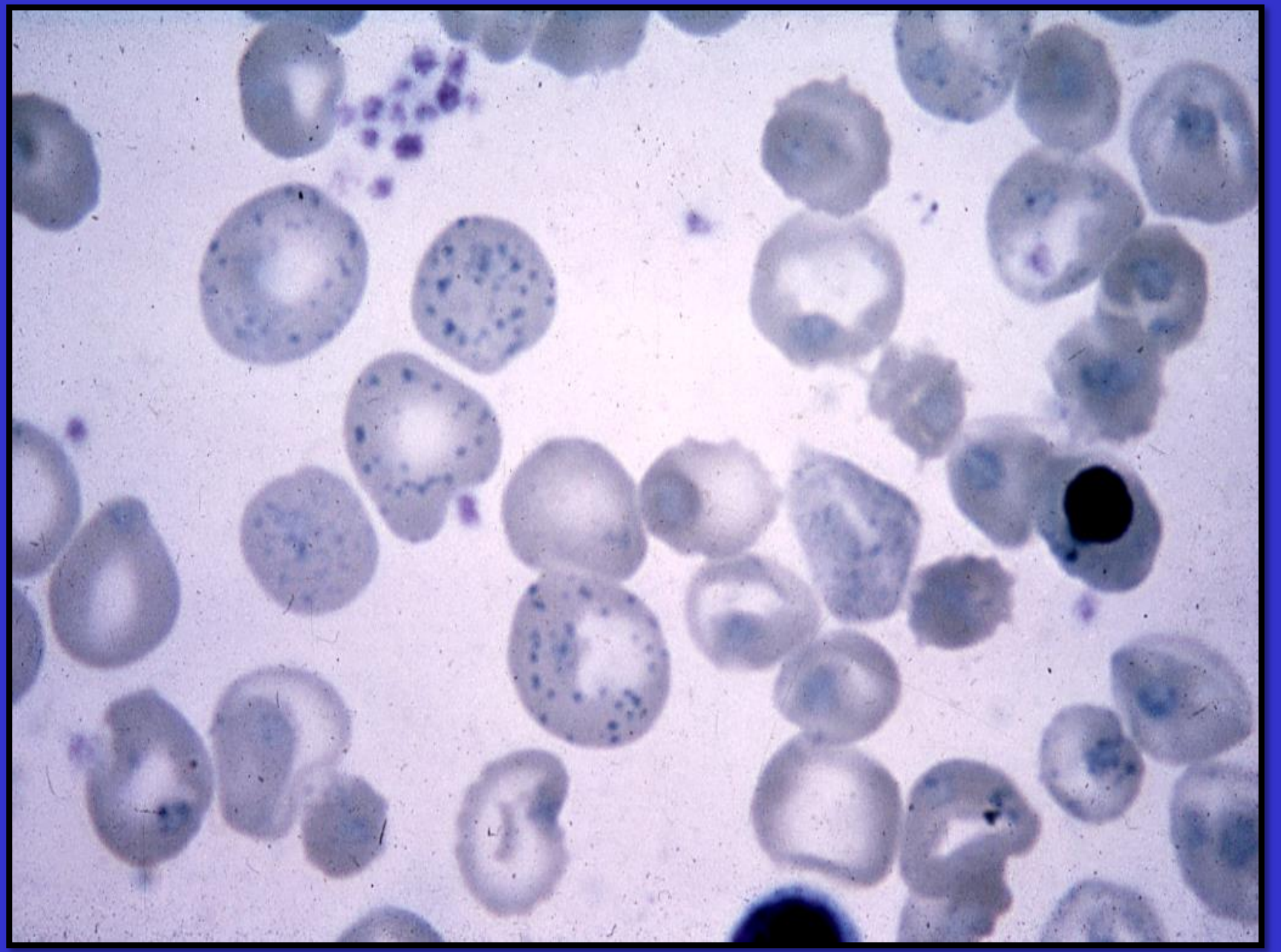
Clinical and Hematologic Features of the β-Thalassemia Syndrome (Continued)

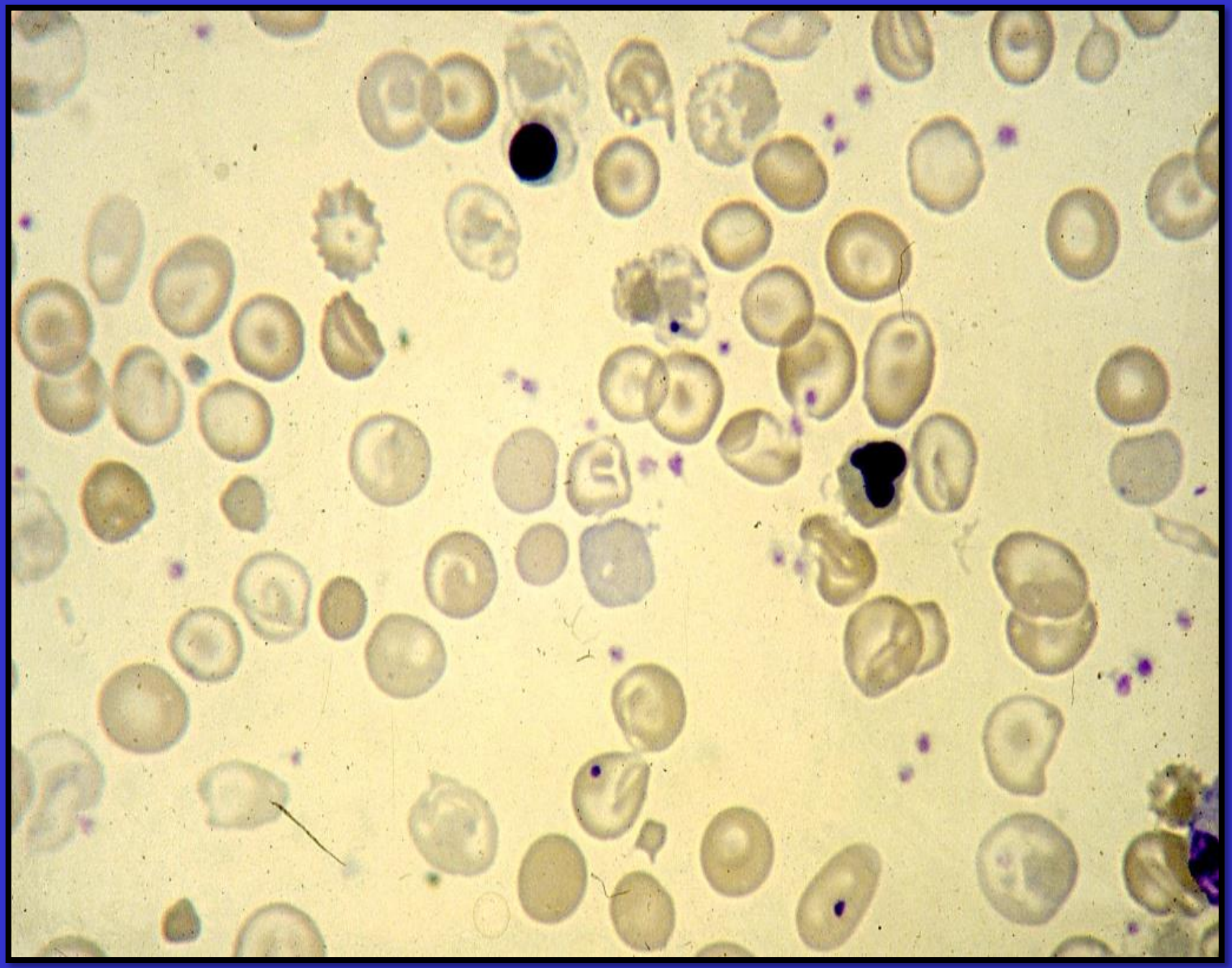
	Major	Intermedia	Minor	Minima
Hypochromia	++++	+++	++	+
Microcytosis	+++	++	+	0
Target cells	10 – 35%	++	+	±
Basophilic stippling	++	+	+	0, +
Reticulocytes (%)	5 – 15	3 – 10	2 – 5	1 – 2
Nucleated red cells	+++	+, 0	0	0
±, little or no abnormality; +, mild abnormality; ++++, prominent abnormality				

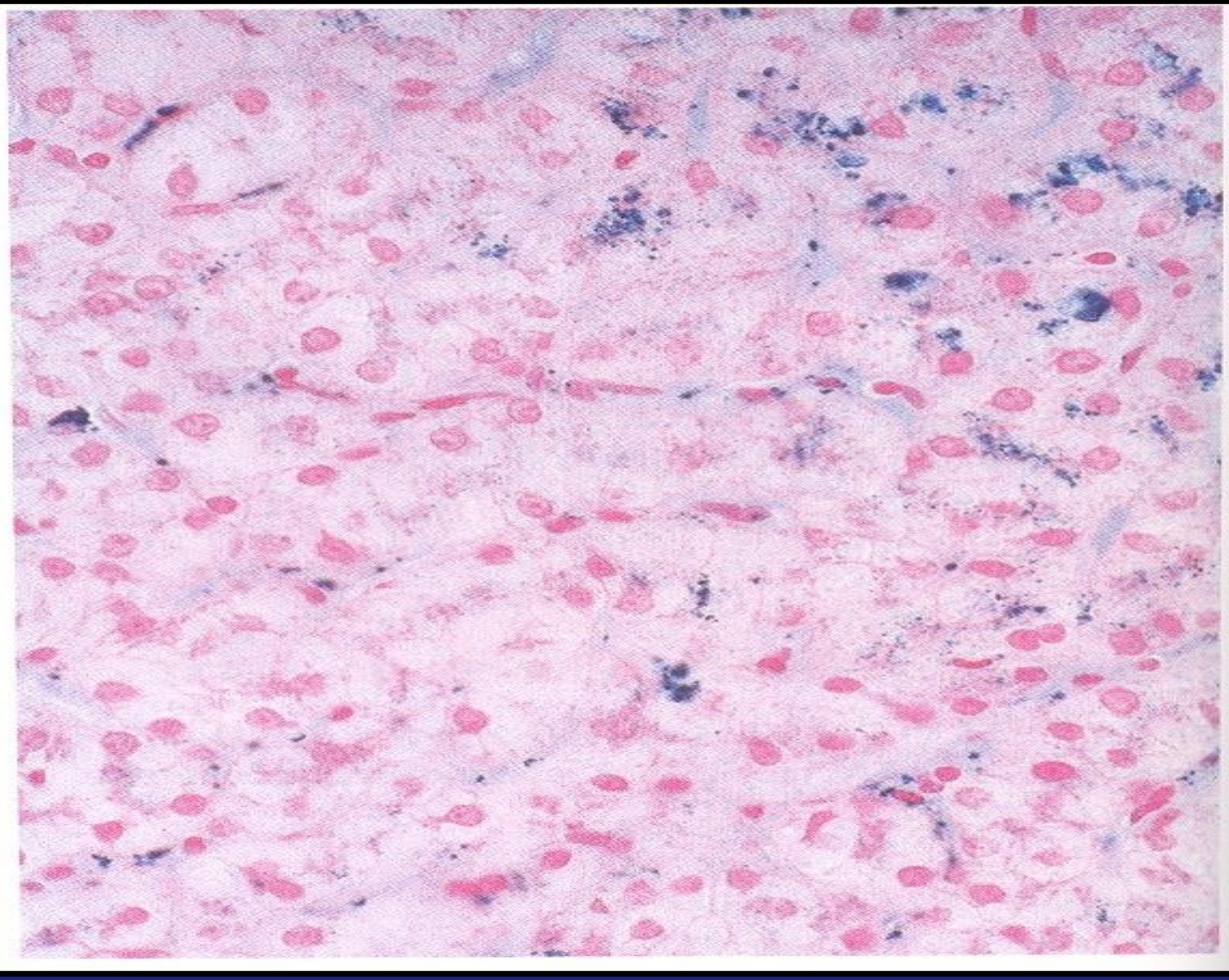


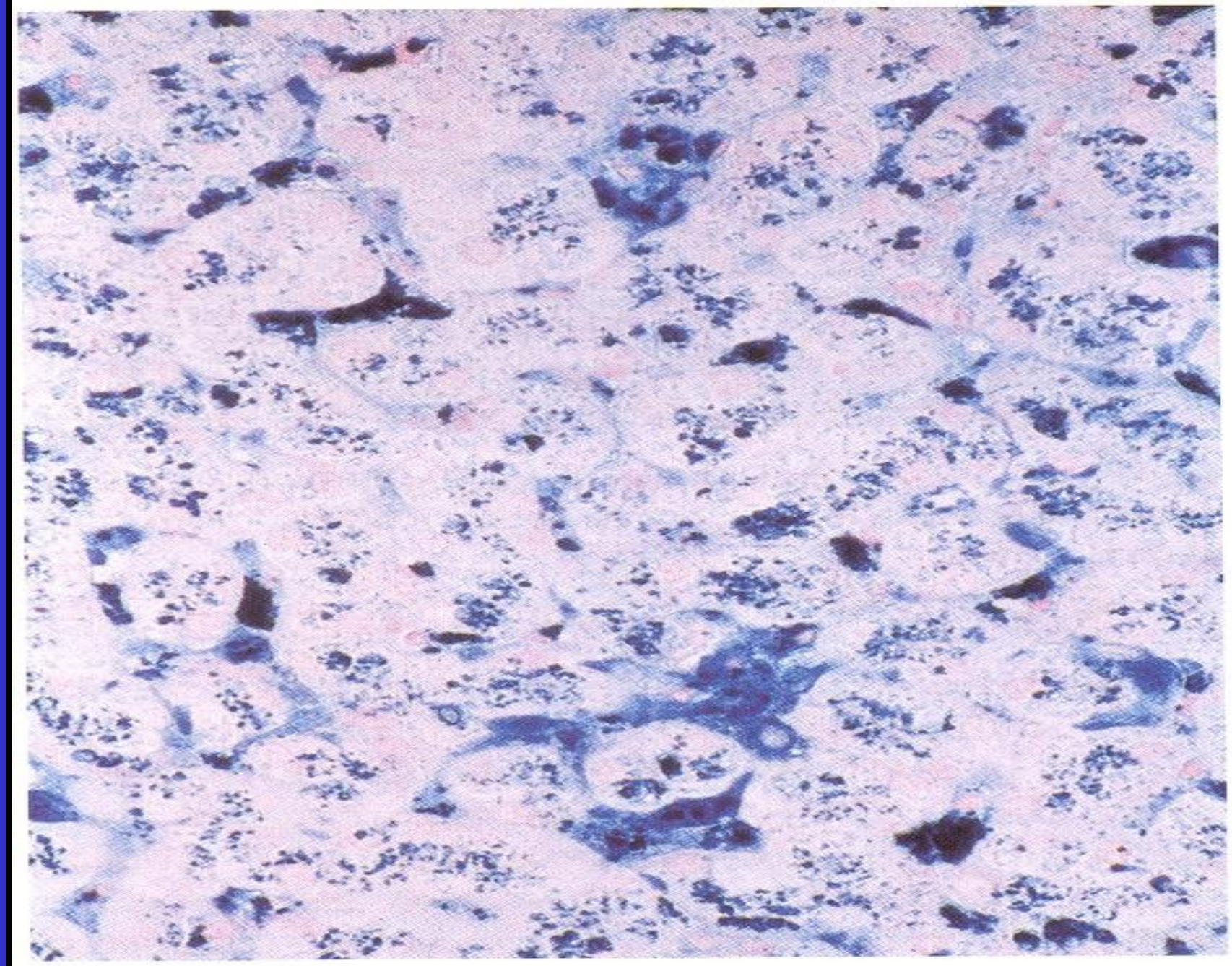
Frequency of β-thalassaemia in different regions of Saudi Arabia. (From Ref. No. 20.) (No. investigated: Al-Hafouf 300; Riyadh 250; Al-Ula 427; Khaiber 500; Jizan 1271; Najran 301.) $f = 8.8353$; $df = 10$; $p < 0.01$





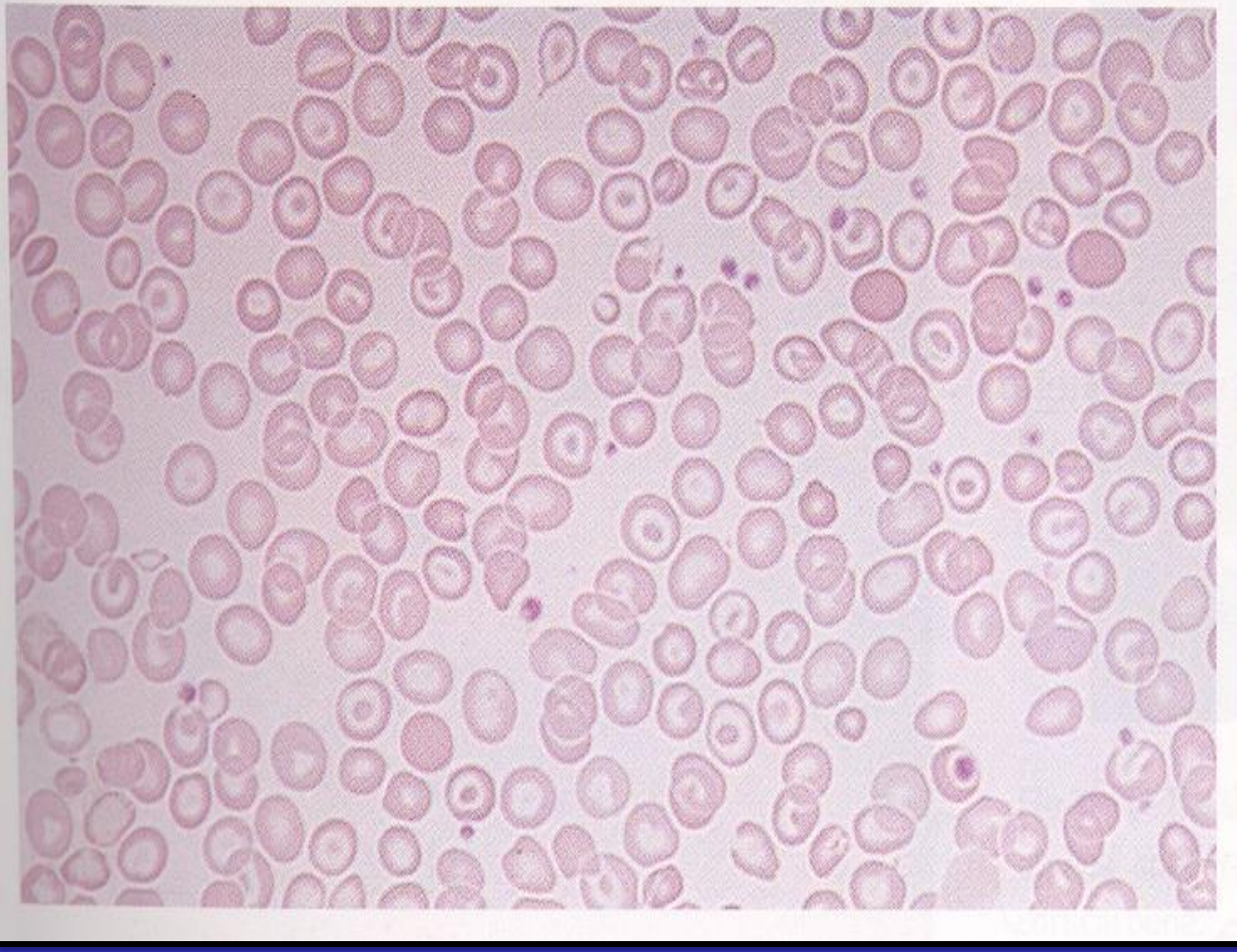






Laboratory Features of Beta Thalassemia Trait

- Mild hypochromic microcytic anemia with target red cells in the blood film.
- Raised red cells count.
- Raised Hb A2 level.
- Normal serum iron or low in children.
- Normal TIBC or raised in children.
- Normal red cell distribution width (RDW)
- Genetic study is required in difficult cases



ING KHALID HOSP.
O BOX 7805 RIYADH

HEMATOLOGY UNIT

Page No.:1

Pat.No
Name:
Hospital:KING KHALID UNIVERSITY HOSPITA DOB:
Location: (PCF01) PCC (Female)
Doctor:UNKNOWN *

ref:
eq No.:H02022419 Date Coll.:04/01/23(18/03/02) Date Recd.:04/01/23(18/03/02)
rinted:09/01/1423(23/03/02)08:32 Time Recd.:10:30

DTA Whole Blood

	Full Blood Count				
[*]	WBC	5.60		4 - 11	x10.e9/L
[ ]>	RBC	5.67	H	4.2 - 5.5	x10.e12/L
<[ ]	HGB	98	L	120 - 160	g/L
<[ ]	HCT	31.0	L	37 - 47	%
<[ ]	MCV	54.6	L	80 - 94	fl
<[ ]	MCH	17.3	L	27 - 32	pg
<[ ]	MCHC	315	L	320 - 360	g/L
[ ]>	RDW	15.6	H	11.5 - 14.5	%
[*]	PLT	426		140 - 450	x10.e9/L
[*]	MPV	7.9		7.2 - 11.1	fl
<[ ]	PDW	15.6	L	20 - 70	%
[ ]>	PCT	0.339	H	0.150 - 0.32	%
	Differential				
[*]	%NEUT	74		40 - 75	%
<[ ]	%LYMP	19	L	20 - 45	%
<[ ]	%MONO	2	L	3 - 9	%
[*]	%EOS	5		0 - 6	%
[*]	#NEUT	4.14		2 - 7.5	x10.e9/L
[*]	#LYMP	1.06		1 - 5	x10.e9/L
<[ ]	#MONO	0.11	L	0.2 - 0.8	x10.e9/L
[*]	#EOS	0.28		0.0 - 0.8	x10.e9/L
	Morophology				
	Flag Comments	3+ ,3+			
	Flag Comment 1				
	ANISO				
	MICRO	MK			
	MACRO				
	POIKILO				
	HYPO	MK			
	Polychromasia				
	LSHIFT				
	TARGET CELLS	SL			
	Ovalocytes	SL			
[*]	Retic Count	1.4		0.2 - 2.0	%
[ ]>	ESR	35	H	3 - 9	mm/hr

NG KHALID HOSPITAL | DEPARTMENT OF SPECIAL BIOCHEMISTRY
BOX 7805 RIYADH |==========

|Hosp No. Page No.:1
|Patient: AA
|Hosp Srce:KING KHALID UNIVERSITY HOSPIT DOB: 1
|Location: (EHC) Employee Health Clinic
ef: |Doctor:UNKNOWN *
No.:S0202265 Date Coll.:04/01/23(18/03/02) Date Recd.:04/01/23(18/03/02)
inted:09/01/1423(23/03/02)08:34 Time Recd.:10:51

ious Blood

Hemoglobin Electrophoresis

95 - 99	%	<[ ]	Hemoglobin A	93.5	L
0 - 2.0	%	[*]	Hemoglobin F	2.0	
2.0 - 3.5	%	[ ]>	Hemoglobin A2	4.5	H
			Hemoglobin S	0.0	
			Hemoglobin E	0.0	
			Hemoglobin C	0.0	
	%		Hemoglobin O	0.0	

PREMARITAL SCREENING

- CBC and Differential count
- Reticulocytes count
- Sickle cell solubility test
- Hb electrophoresis
- Virology study for hepatitis B, C, HIV by PCR

Kingdom of Saudi Arabia
Ministry of Higher Education
King Saud University
College of Medicine
& king Khalid University Hospital

المملكة العربية السعودية
وزارة التعليم العالي
جامعة الملك سعود
كلية الطب
ومستشفى الملك خالد الجامعي

نموذج فحص ما قبل الزواج

تاريخ سحب العينة : / / ١٤هـ رقم الملف الطبي: ..... رقم المختبر .....

اسم الطبيب المعالج: ..... رقم التحويلة/النداء ..... العيادة .....

البيانات الشخصية:

الاسم ..... الجنسية: ..... العمر: ( ) الجنس : ☐ ذكر ☐ أنثى.

رقم السجل المدني/الإقامة: ..... العنوان: ..... الهاتف: .....

الفحوصات المطلوبة:

١- تعداد الدم الكامل (CBC). ٢- اختبار الخلايا المنجلية (Sickling).

٣- الرحلان الكهربي لخضاب الدم (Hb Electrophoresis). ٤- اختبارات أخرى (Other Tests).

LABORATORY RESULT

TEST	NORMAL RANGE	RESULT	REMARKS
RBCX10^{12}/L	M:4.7 – 6.1 ….F:4.2-5.5		
HBg/dL	M:13 –18 ….F:12-16		
Het%	M:42 – 52 ….F:37- 47%		
MCV fL	80 – 94		
MCH pg	27 – 32		
MCHCg/dL	32 – 36		
RDW	11.5 – 14.5%		
Retic	0.5 – 2%		
Sicking Test	Positive or Negative		
Hb A	95 – 97%		
Hb A2	2.0 – 3.5%		
Hb F	<1.5%		
Abnormal Hemoglobin			
TEST	PATIENT RESULT	HEMOGLOBIN	PATIENT RESULT
Hb S		Hb J	
Hb C		Hb O – Arab	
Hb D		Hb H	
Hb E		Hb Barts	
Hb G		Other Test	
Other Hb			

المشرف الفني بالوحدة: .....

ملاحظات: ..... COMMENTS:.....

.....

.....

استشاري أمراض الدم بالمختبر: ..... التوقيع: .....

✱ **ملاحظة هامة:** هذه الشهادة تبين نتيجة الفحص المخبري لمرضي الأنيميا المنجلية والثلاسيميا فقط، ولا تشمل أي أمراض وراثية أخرى للطرفين المعنيين.

Kingdom of Saudi Arabia
Ministry of Higher Education
King Saud University
College of Medicine
& king Khalid University Hospital

المملكة العربية السعودية
وزارة التعليم العالي
جامعة الملك سعود
كلية الطب
ومستشفى الملك خالد الجامعي

نموذج فحص ما قبل الزواج

تاريخ سحب العينة : / / ١٤ هـ رقم الملف الطبي: رقم المختبر .....

اسم الطبيب المعالج: .. رقم التحويلة/النداء ..... العيادة .....

البيانات الشخصية:

الاسم .. الجنس .. العمر: ( ) الجنس : ذكر

رقم السجل المدني/الإقامة: ..... العنوان: ..... الهاتف: .....

الفحوصات المطلوبة:

١ – تعداد الدم الكامل (CBC). ٢ – اختبار الخلايا المنجلية (Sickling).

٣ – الرحلان الكهربي لخضاب الدم (Hb Electrophoresis). ٤ – اختبارات أخرى (Other Tests).

LABORATORY RESULT

TEST	NORMAL RANGE	RESULT	REMARKS
RBCX10^{12}/L	M:4.7 – 6.1 ....F:4.2-5.5	4.5	
HBg/dL	M:13 –18 ....F:12-16	12.9	
Het%	M:42 – 52 ....F:37- 47%	37.8	
MCV fL	80 – 94	83.9	
MCH pg	27 – 32	28.6	
MCHCg/dL	32 – 36	34.1	
RDW	11.5 – 14.5%	13.6	
Retic	0.5 – 2%	–	
Sicking Test	Positive or Negative	Negative	
Hb A	95 – 97%	96.9	
Hb A2	2.0 – 3.5%	2.6	
Hb F	<1.5%	<0.5	
Abnormal Hemoglobin			
TEST	PATIENT RESULT	HEMOGLOBIN	PATIENT RESULT
Hb S		Hb J	
Hb C		Hb O – Arab	
Hb D		Hb H	
Hb E		Hb Barts	
Hb G		Other Test	
Other Hb			

المشرف الفني بالوحدة: .....

ملاحظات: سالمة من أمراض الدم الوراثية

COMMENTS: .....

استشاري أمراض الدم بالمختبر: ..... التوقيع: 6-2-26

ملاحظة هامة: هذه الشهادة تبين نتيجة الفحص المخبري لمرضي الأنيميا المنجلية والثلاسيميا فقط، ولا تشمل أي أمراض وراثية أخرى للطرفين المعنيين.

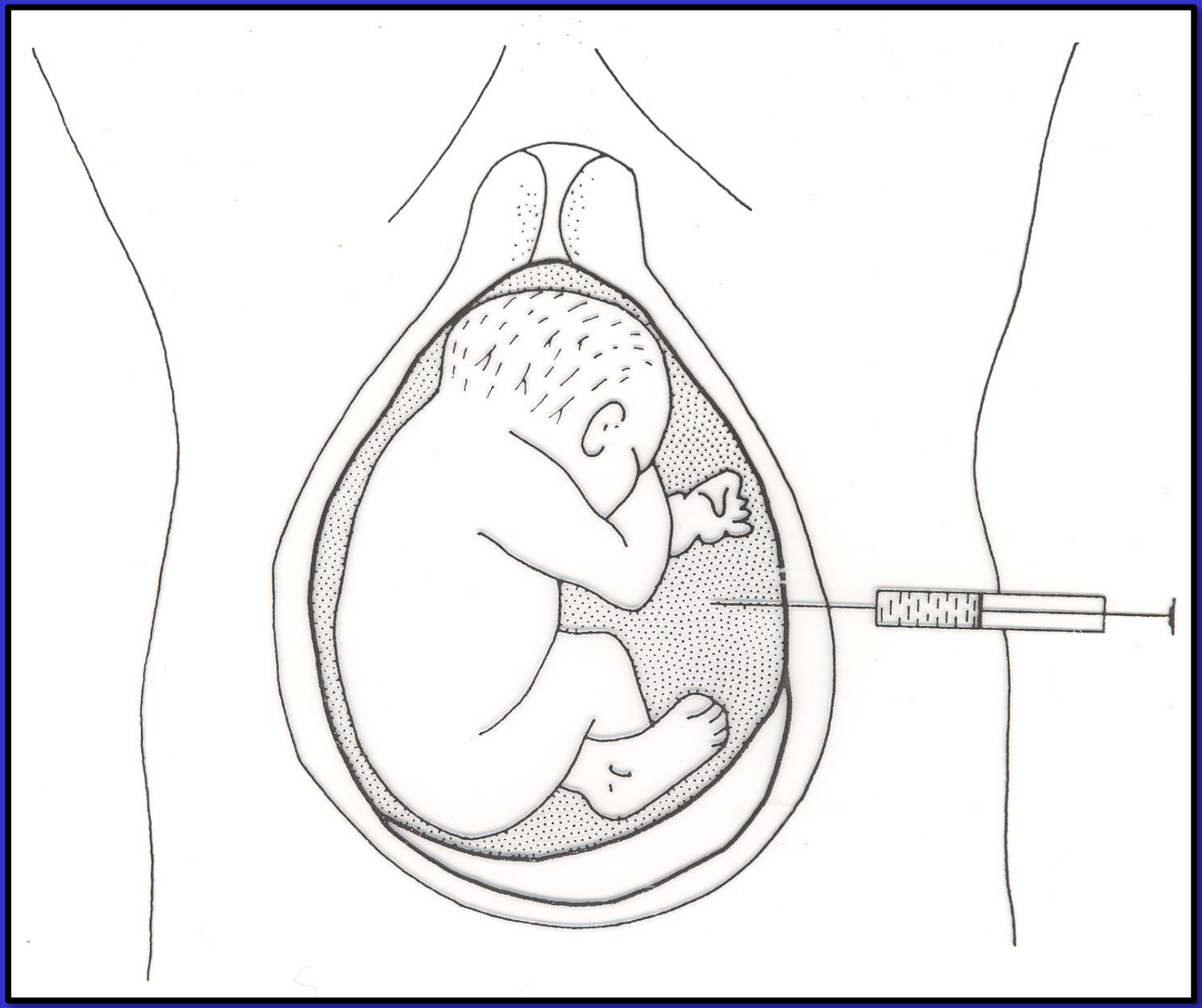
Prenatal diagnosis of the haemoglobinopathies (Including thalassaemia)

DNA Analysis

A. Chorionic villus sampling
   - Transcervical approach (9 – 11 weeks of pregnancy)
   - Transabdominal approach (up to 15 weeks of pregnancy)

B. Amniotic fluid cell analysis (16 – 20 weeks gestation)

C. Fetal blood sampling (> 20 weeks gestation)
   - DNA analysis
   - Haematological parameters
   - Biochemical analysis
   - Globin chain synthesis
     - α/β Ratio
     - α/γ Ratio
     - α/δ Ratio



DNA ANALYSIS

1. Gene mapping
2. RFLPs linkage analysis
   (Restriction fragment length polymorphisms)
3. Oligonucleotide probes
   (Using short gene probes 17 – 19 Nucleotide)
4. Gene amplification
   (Enzymatic amplification of DNA sequences)

   DNA polymerase chain reaction technique.

MANAGEMENT OF THE THALASSEMIAS

- **Blood Transfusion**
- **Iron chelation therapy**
- **Splenectomy**
- **Hormone replacement**
- **Bone marrow transplantation**
- **Gene therapy**

SUMMARY OF RECOMMENDATIONS FOR THE TREATMENT OF THALASSEMIA MAJOR

TRANSFUSION

Transfusion, in the absence of cardiopathy:

- **Blood-type the patient completely;**
- **Vaccinate hepatitis B negative patients against hepatitis;**
- **Transfuse when the Hb remains consistently below 8 g/dL, or earlier if there are other indications;**
- **Keep the pretransfusion Hb between 10.5 and 11 g/dL;**
- **Give 10-15 mL/kg of blood preparation in 2 h;**
- **Do not raise the posttransfusion Hb above 16 g/dL;**
- **Choose a 3-4 week transfusion interval.**

SUMMARY OF RECOMMENDATIONS FOR THE TREATMENT OF THALASSEMIA MAJOR (Continued)

TRANSFUSION

Transfusion in the presence of cardiopathy, or when the Hb is less than 5 g/dL:

- **Inject furosemide 1-2 mg/kg;**
- **Preferably use fresh blood;**
- **Do not transfuse more than 5 mL/kg of blood;**
- **Do not transfuse faster than 2 mL/kg, or for more than 4 h;**
- **If necessary, divide the blood among 2 or more bags;**
- **Use very short intertransfusion intervals.**

IRON CHELATION THERAPY

1) Desferrioxamine S.C. 20-60 mg/kg/day in 8 h (average 40 mg/kg/day, or 280 mg/kg/ week).
2) In selected subjects, give desferrioxamine i.v. in high dose, maximum 100 mg/kg over 8 h, only on the days of transfusion.

SPLENECTOMY

1) Is indicated when the blood consumption is more than 1.5 times normal.
2) Give anti-pneumococcal vaccine to children more than 2 years old prior to splenectomy.
3) Inform the patients and their family doctors of increased risk of serious infections.
4) Give prophylactic penicillin, and a platelet anti-aggregant when there is thrombocytosis.

INVESTIGATIONS

Prior to treatment:	**Study the case, and do complete red cell typing.**
Before each transfusion:	**Hb, cross-match and red cell antibody detection, serum transminases (in areas with a high incidence of hepatitis). Record the date of transfusion, net weight and mean hematocrit of the blood preparation, and the Hb of the patient**
After each transfusion:	**Measure the posttansfusion Hb.**
Every 3 months:	**Measure height and weight**
Every 6 months:	**Ferritin estimation.**
Every year:	**Evaluate growth and development.**
	Calculate the transfusion indices.
	Evaluate iron balance.
	Complete evaluation of the case.
Variable intervals:	**Cardiac and endocrinological investigations according to the clinical state of the patient.**

Diagnosis of Haemoglobinopathies including Thalassaemias

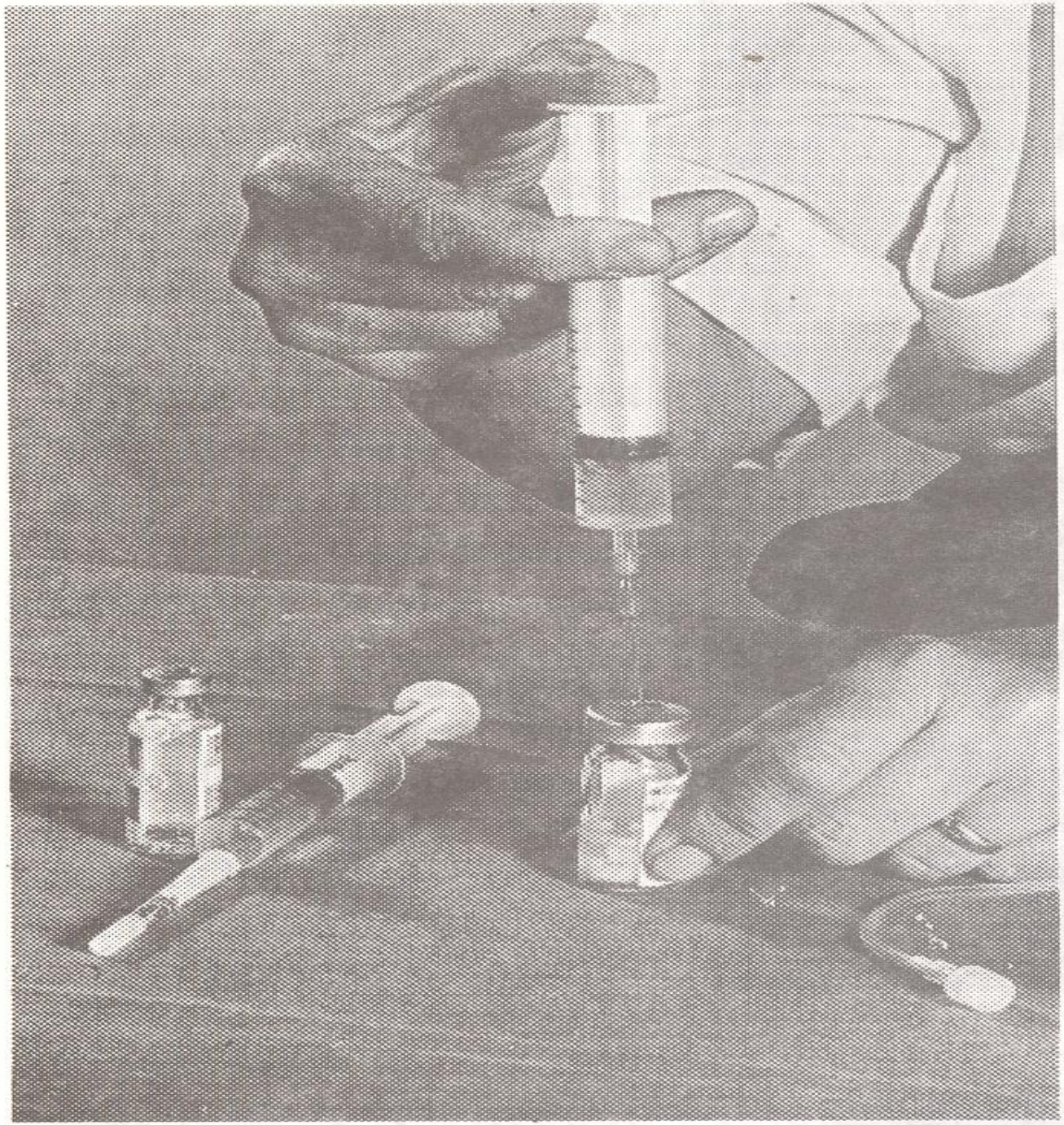
A. **Personal & Family History**

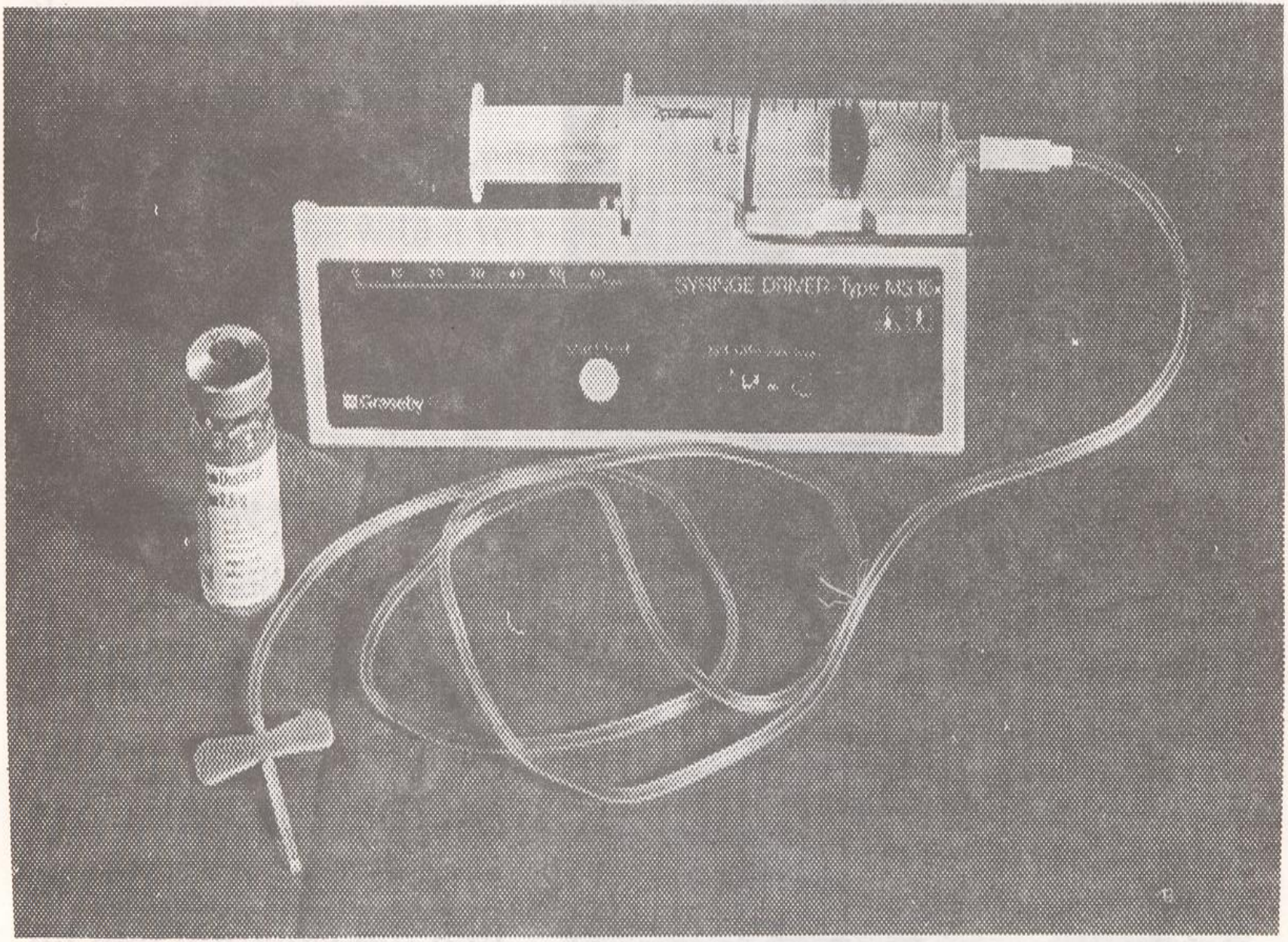
B. **Physical Examination**

C. **Laboratory Investigation**

1. Haematological Tests – CBC, Red cell indices, blood film Morphology, reticulocyte count.
2. Sickling Tests – Sickle cell test, Sickle cell solubility test.
3. Hb Electrophoresis at alkaline/acidic pH and quantitation.
4. Quantitation of HbA2 and HbF
5. Osmotic fragility test
6. Serum iron total iron binding capacity and ferritin level
7. Biochemical tests:

   Liver functions tests, renal function tests, blood gases and acid-base status, bone profile and urine analysis.
8. Special Tests
   - A. Family studies (Laboratory Investigations)
   - B. Measurement of Alpha/Non-Alpha chain ratio
   - C. Gene Studies





ORAL IRON CHELATION THERAPY

- **Deferiprone [Ferriprox]**
- **Oral Tablet or Syrup 5 to 10 mg /kg /day divided in 3 doses.**
- **More effective than desferoxamine in chelating cardiac iron.**
- **Total iron excretion with deferiprone is less than with desferoxamine.**
- **Major adverse effect especially in children include - Gastrointestinal symptoms, joint pain, liver disfunction, neuropenia in 27% of patients.**

ORAL IRON CHELATION THERAPY (*cont'd…*)

- ✓ **Deferasirox (EXJADE, NOVARTIS)**
- ✓ **The dose is 20-30 mg/kg/day once daily.**
- ✓ **Approved by FDA.**
- ✓ **Reduction of liver iron to 50%, reduction of serum ferritin to 70% after 1 year treatment.**

Side effects:

- **Nausea, vomiting, diarrhea, abdominal pain, skin rash.**
- **Mid increase in serum cratinine in 30% of patients as with Desferoxamine ocular and auditory disturbance have been reported.**
- **Increase in serum transaminases in 10% of patients.**
- **Reduction of the dose in steps 5-10mg/kg/day every 3-6 months depending on serum ferritin level.**

Assessment of Iron Stores

- Serum ferritin
- Serum iron and percentage saturation of transferrin (iron-binding capacity)
- Bone marrow biopsy (Perl's stain) for reticuloendothelial stores
- DNA test for mutation resulting in Cys282 Tyr in the HFE gene
- Liver biopsy (parenchymal and reticuloendothelial stores)
- Liver CT scan or MRI
- Cardiac MRI
- Desferrioxamine iron excretion test (chelatable iron)
- Repeated phelobotomy until iron deficiency occurs

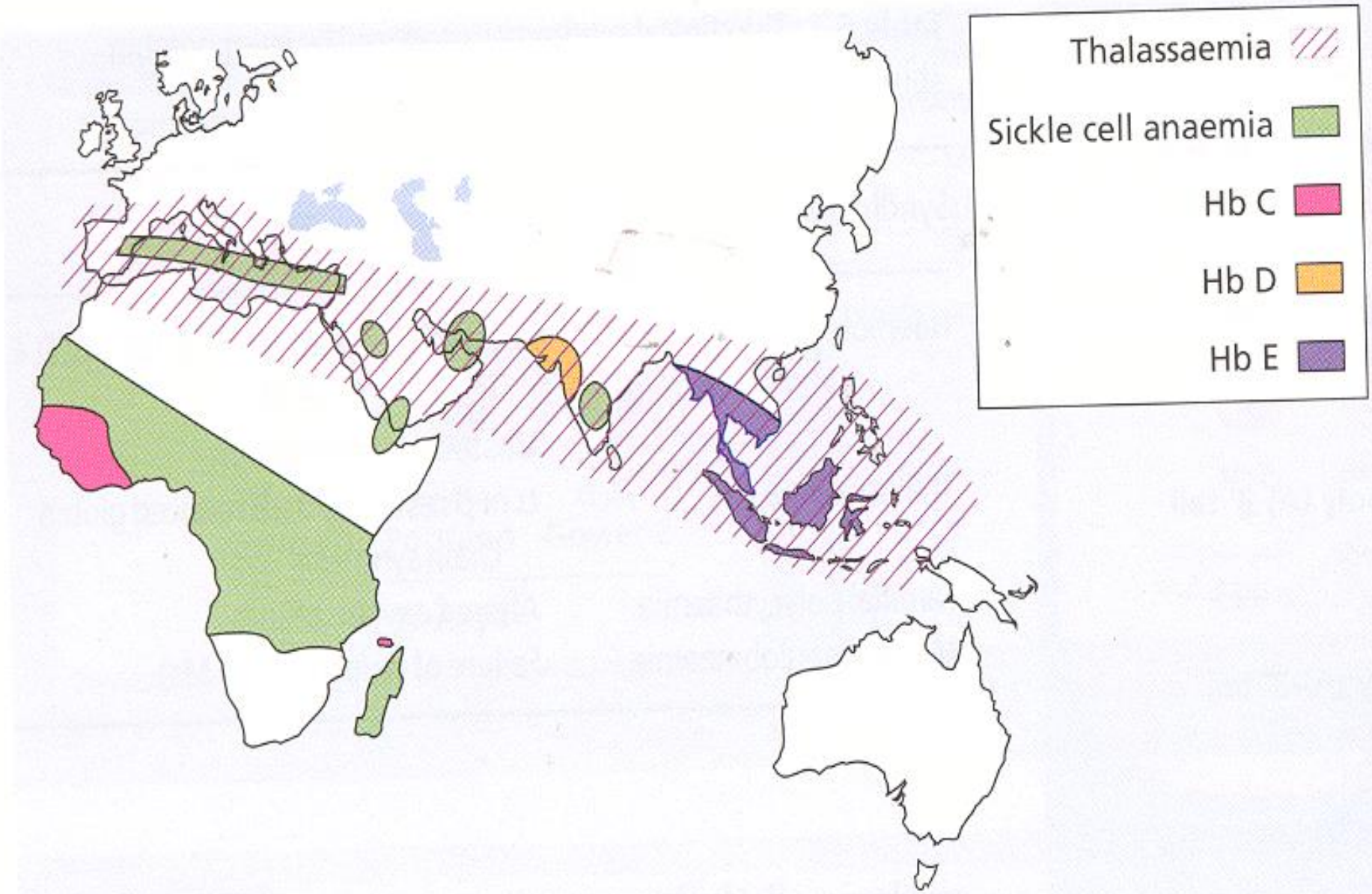
Assessment of tissue damage caused by iron overload

Cardiac	**Clinical; chest X-ray; ECG; 24-h monitor; echocardiography; radionuclide (MUGA scan to check left ventricular ejection fraction at rest and with stress**
Liver	**Liver function tests; liver biopsy; CT scan**
Endocrine	**Clinical examination (growth and sexual development) glucose tolerance test; pituitary gonadotrophin release tests; thyroid, parathyroid, gonadal, adrenal function, growth hormone assays; radiology for bone age; isotopic bone density study**

CT, computed tomography; ECG, electrocardiography; MRI, magnetic resonance imaging; MUGA, multiple gated acquisition.

Abnormal Haemoglobins (Haemoglobinopathies)

HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION



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 1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
VAL-HIS-LEU-THR-PRO-GLU-GLU-LYS-SER-ALA-VAL-THR-ALA-LEU-TRY

 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
GLY-LYS-VAL-ASN-VAL-ASP-GLU-VAL-GLY-GLY-GLU-ALA-LEU-GLY-ARG

 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
LEU-LEU-VAL-VAL-TYR-PRO-TRY-THR-GLN-ARG-PHE-PHE-GLU-SER-PHE

 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
GLY-ASP-LEU-SER-THR-PRO-ASP-ALA-VAL-MET-GLY-ASN-PRO-LYS-VAL

 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
LYS-ALA-HIS-GLY-LYS-LYS-VAL-LEU-GLY-ALA-PHE-SER-ASP-GLY-LEU

 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
ALA-HIS-LEU-ASP-ASN-LEU-LYS-GLY-THR-PHE-ALA-THR-LEU-SER-GLU

 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
LEU-HIS-CYS-ASP-LYS-LEU-HIS-VAL-ASP-PRO-GLU-ASN-PHE-ARG-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
LEU-GLY-ASN-VAL-LEU-VAL-CYS-VAL-LEU-ALA-HIS-HIS-PHE-GLY-LYS

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
GLU-PHE-THR-PRO-PRO-VAL-GLN-ALA-ALA-TYR-GLN-LYS-VAL-VAL-ALA

136 137 138 139 140 141 142 143 144 145 146
GLY-VAL-ALA-ASN-ALA-LEU-ALA-HIS-LYS-TYR-HIS
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 1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
VAL-LEU-SER-PRO-ALA-ASP-LYS-THR-ASN-VAL-LYS-ALA-ALA-TRY-GLY

 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
LYS-VAL-GLY-ALA-HIS-ALA-GLY-GLU-TYR-GLY-ALA-GLU-ALA-LEU-GLU

 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
ARG-MET-PHE-LEU-SER-PHE-PRO-THR-THR-LYS-THR-TYR-PHE-PRO-HIS

 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL-LYS-GLY-HIS-GLY-LYS

 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
LYS-VAL-ALA-ASP-ALA-LEU-THR-ASN-ALA-VAL-ALA-HIS-VAL-ASP-ASP

 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
MET-PRO-ASN-ALA-LEU-SER-ALA-LEU-SER-ASP-LEU-HIS-ALA-HIS-LYS

 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
LEU-ARG-VAL-ASP-PRO-VAL-ASN-PHE-LYS-LEU-LEU-SER-HIS-CYS-LEU

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
LEU-VAL-THR-LEU-ALA-ALA-HIS-LEU-PRO-ALA-GLU-PHE-THR-PRO-ALA

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
VAL-HIS-ALA-SER-LEU-ASP-LYS-PHE-LEU-ALA-SER-VAL-SER-THR-VAL

136 137 138 139 140 141
LEU-THR-SER-LYS-TYR-ARG
```

Some Known Haemoglobin Mutants

NAME	SUBSTITUTION
Hb. S	α2 β2 6 GLU → VAL
Hb. C	α2 β2 6 GLU → LYS
Hb. E	α2 β2 26 GLU → LYS
Hb. O ARAB	α2 β2 121 GLU → LYS
Hb. D PUNJAB	α2 β2 121 GLU →GLN
Hb RIYADH	α2 β2 120 LYS → ASN
Hb. HAMMERSMITH	α2 β2 42 PHE → SER
Hb. N. BALTIMORE	α2 β2 95 LYS → GLU
Hb. KORLE-BU	α2 β2 73 ASP → ASN
Hb. K. WOOLWICH	α2 β2 132 LYS → GLN
Hb. K. IBADAN	α2 β2 46 GLY → GLU
Hb. KÖ LN	α2 β2 98 VAL → MET
Hb. J. BALTIMORE	α2 β2 16 GLY → ASP

Some Known Haemoglobin Mutants

NAME	SUBSTITUTION
Hb. G. PHILADELPHIA	α2 68 ASN → LYS β2
Hb. ZAMBIA	α2 60 LYS → ASN β2
Hb. G. CHINESE	α2 30 GLU → GLN β2
Hb. HASHARON	α2 47 ASP → HIS β2
Hb. J. TONGARIKI	α2 115 ALA → ASP β2
Hb. J. OXFORD	α2 15 GLY → ASP β2
Hb. NORFOLK	α2 57 GLY → ASP β2

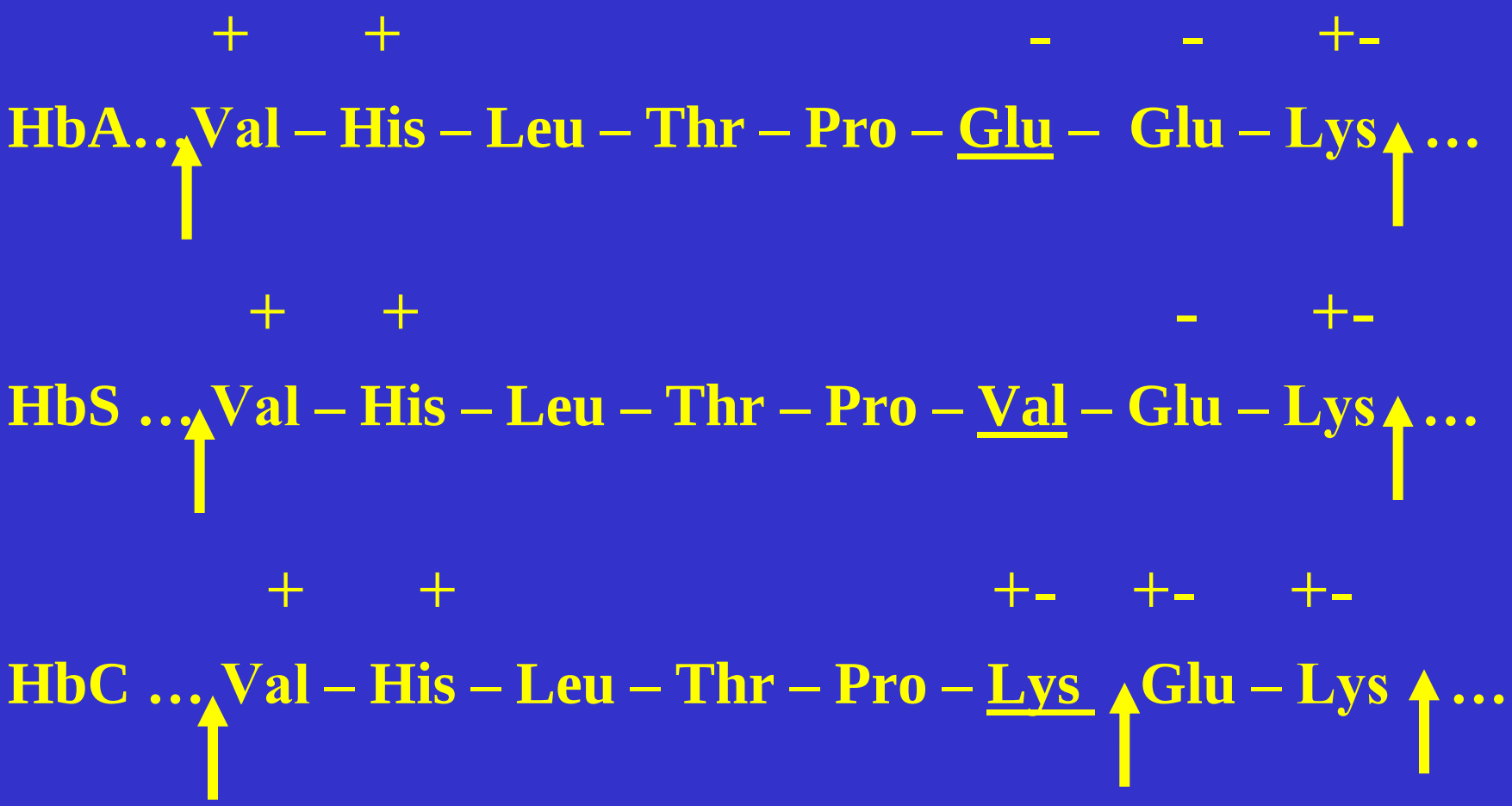
DNA Coding for the Amino-Acid in the sixth position in the β-chain

Normal

	5	6	7
Amino Acid	pro	glu	glu
DNA Base Composition	CCT	G A G	G A G

Sickle

DNA Base composition	CCT	G T G	G A G
Amino Acid	pro	val	glu
	5	6	7



Amino acid sequences of the peptides 4 in haemoglobins A, S and C.

CONGENITAL HAEMOLYTIC ANAEMIAS

Haemolysis due to defects of the red cell membrane

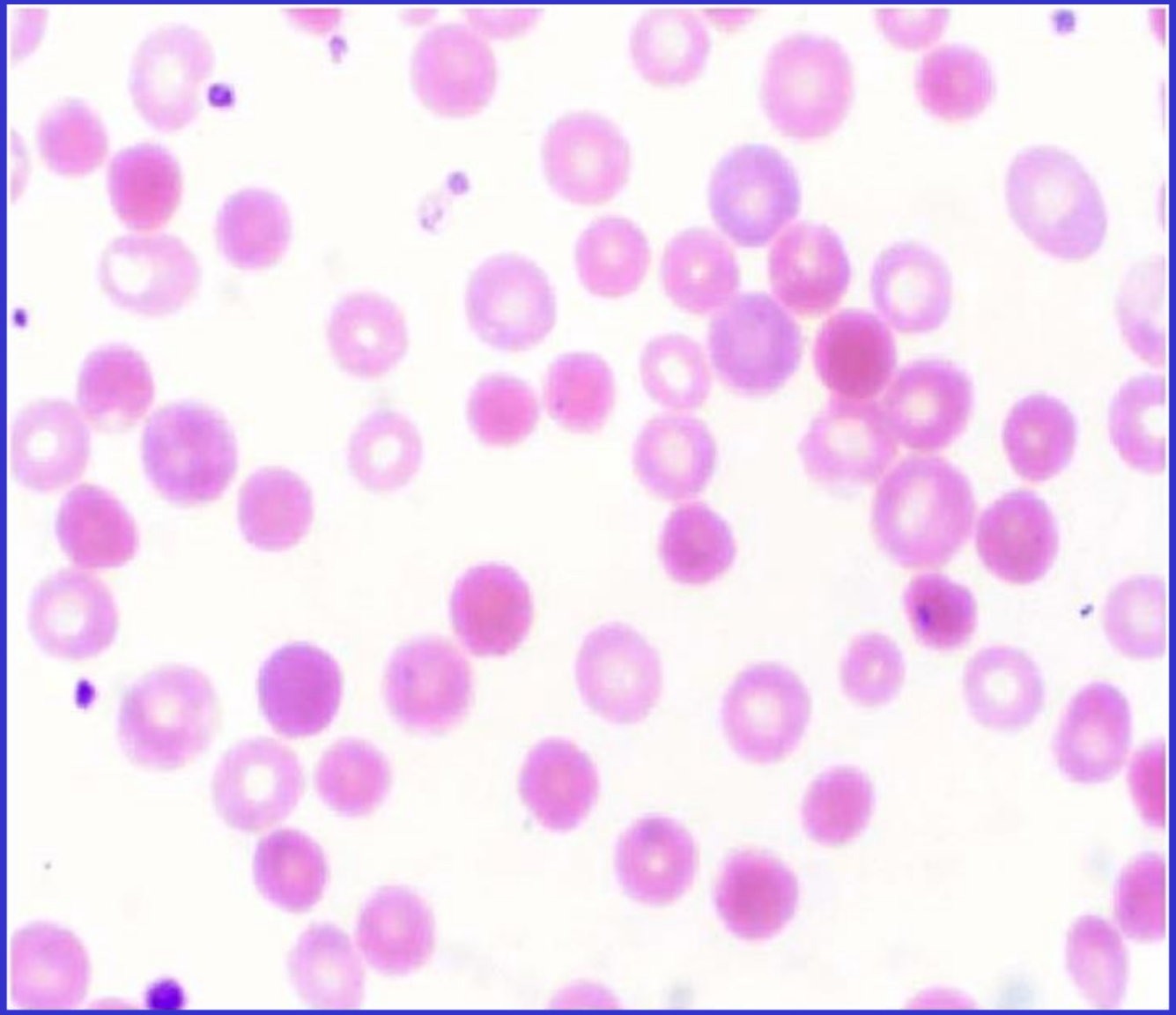
- Since the diameter of a normal red cell is similar to that of the smallest capillary lumen, it is essential for the red cell to be able to undergo significant deformations while traversing the circulation.
- A flexible red cell cytoskeleton, which interacts with red cell phospholipid membrane.
- Key components of the cytoskeleton include α and β spectrin, actin and protein 4.1, while connections linking the cytoskeleton to the overlying red cell phospholipid bilayer include band 3, Rh-associated glycoprotein and glycophorin C.
- Defects in any of these proteins can jeopardize the integrity of the red cell and shorten its lifespan.

Hereditary spherocytosis (HS)

- The most common haemolytic anaemia due to a membrane defect is hereditary spherocytosis (HS) in Caucasian
- About 60% of patients have mutations affecting the Ankyrin gene
- Loss of Ankyrin then leads to secondary reductions in spectrin and protein 4.1
- This leads to a loss of membrane surface area, with cells adopting a spheroid rather than biconcave shape
- Less deformable than normal red cells
- Destroyed by splenic macrophages, leading to a reduction in red cell survival by extravascular haemolysis

cont'd…

- Around 20% of all HS patients have mild disease with well-compensated haemolysis
- The majority of patients have moderate disease characterized by a Hb concentration of 8-11g/dl, while a small percentage have severe disease requiring intermittent or even regular transfusions
- Complications of the chronic haemolysis in HS include the development of pigment gallstones
- Aplastic crises may occur secondary to parvovirus B19 infection
- Megaloblastic anaemia due to folate deficiency is also occasionally found



A blood film from a patient with HS showing many spherocytes.

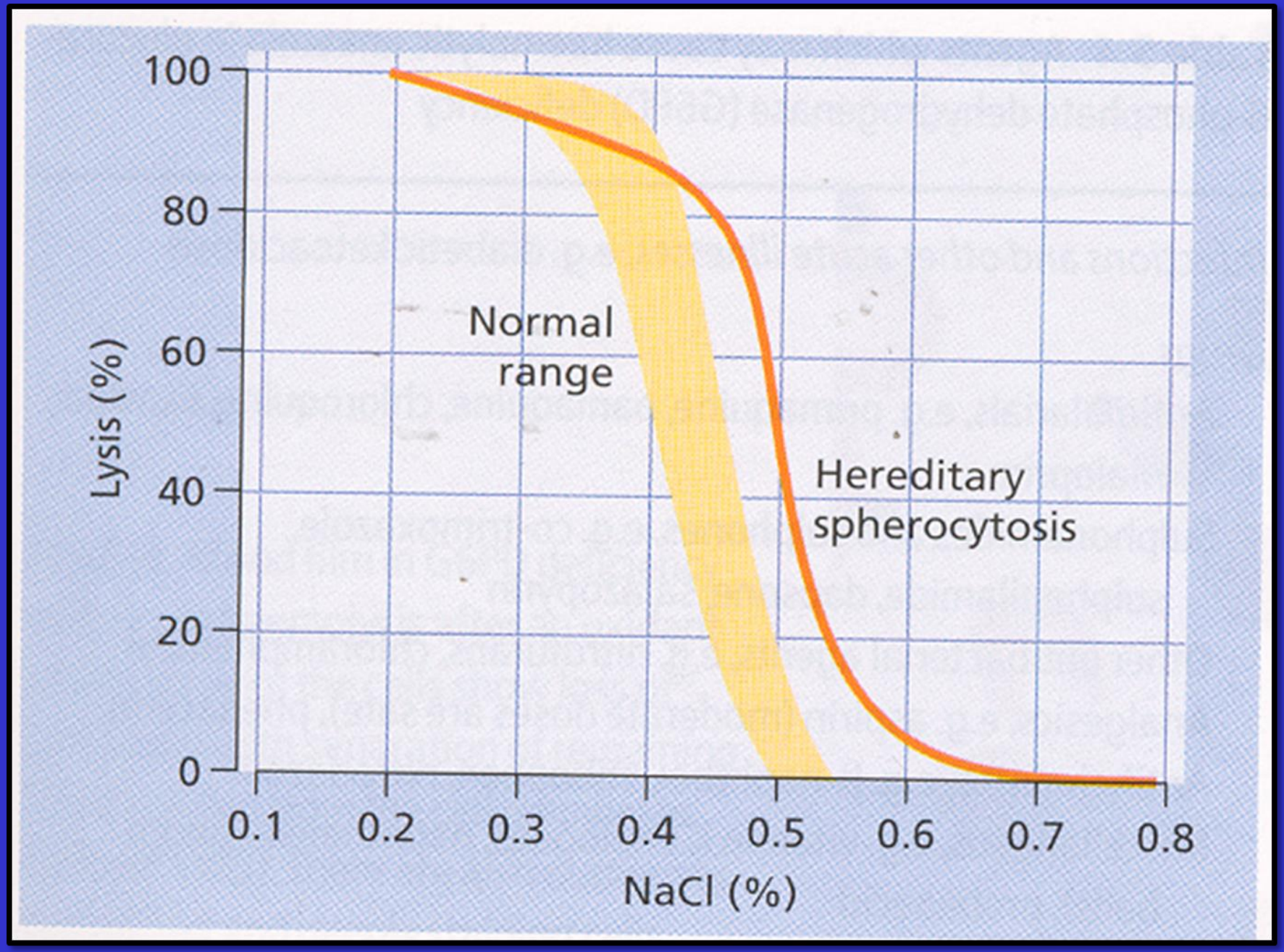
Diagnosis and management

- Family history
- Mild jaundice
- Pallor and splenomegaly
- Laboratory findings (anaemia, reticulocytosis and elevated plasma bilirubin
- Presence of spherocytes on the peripheral blood film
- The eosin-5-maleamide (EMA) binding test (may be used if more definitive evidence for the diagnosis is needed)
- The red cell membrane proteins study
- Electrophoresis on a denaturing polyacrylamide gel

cont'd…

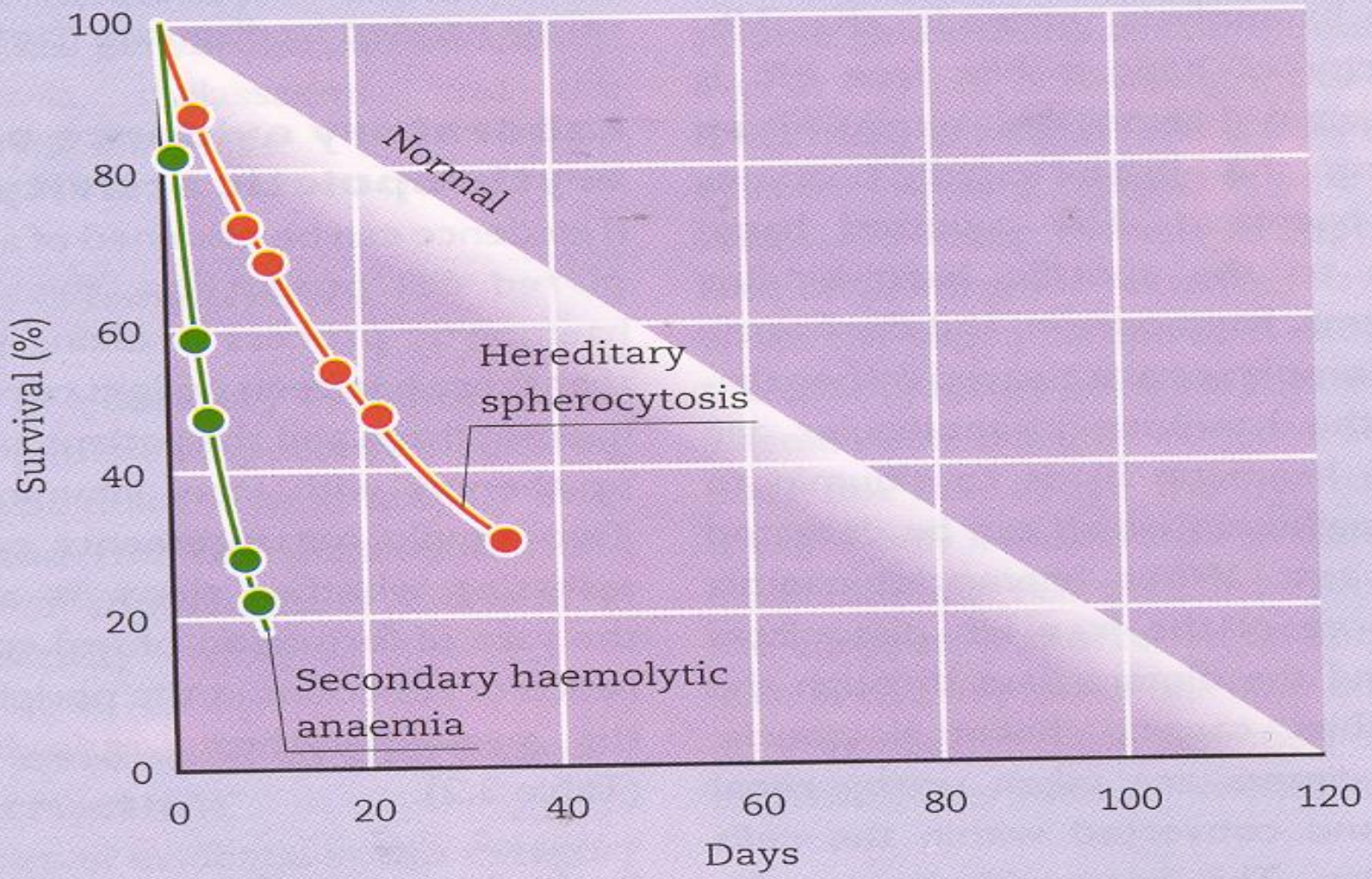
The treatment of HS:

- Folic acid supplementation
- Splenectomy (children with severe disease). Splenectomy will, however, increase the risk of significant infection, particularly from encapsulated organisms. This risk is especially marked in children under the age of 5, delayed until age 5-10 years.
- Administration of pneumococcal and meningococcal vaccine and *Haemophilus influenzae* type b vaccine (splenectomy preoperative preparation)
- Prophylactic penicillin V is advised lifelong (post splenectomy)



OSMOTIC FRAGILITY TEST

RED CELL SURVIVAL MEASUREMENTS



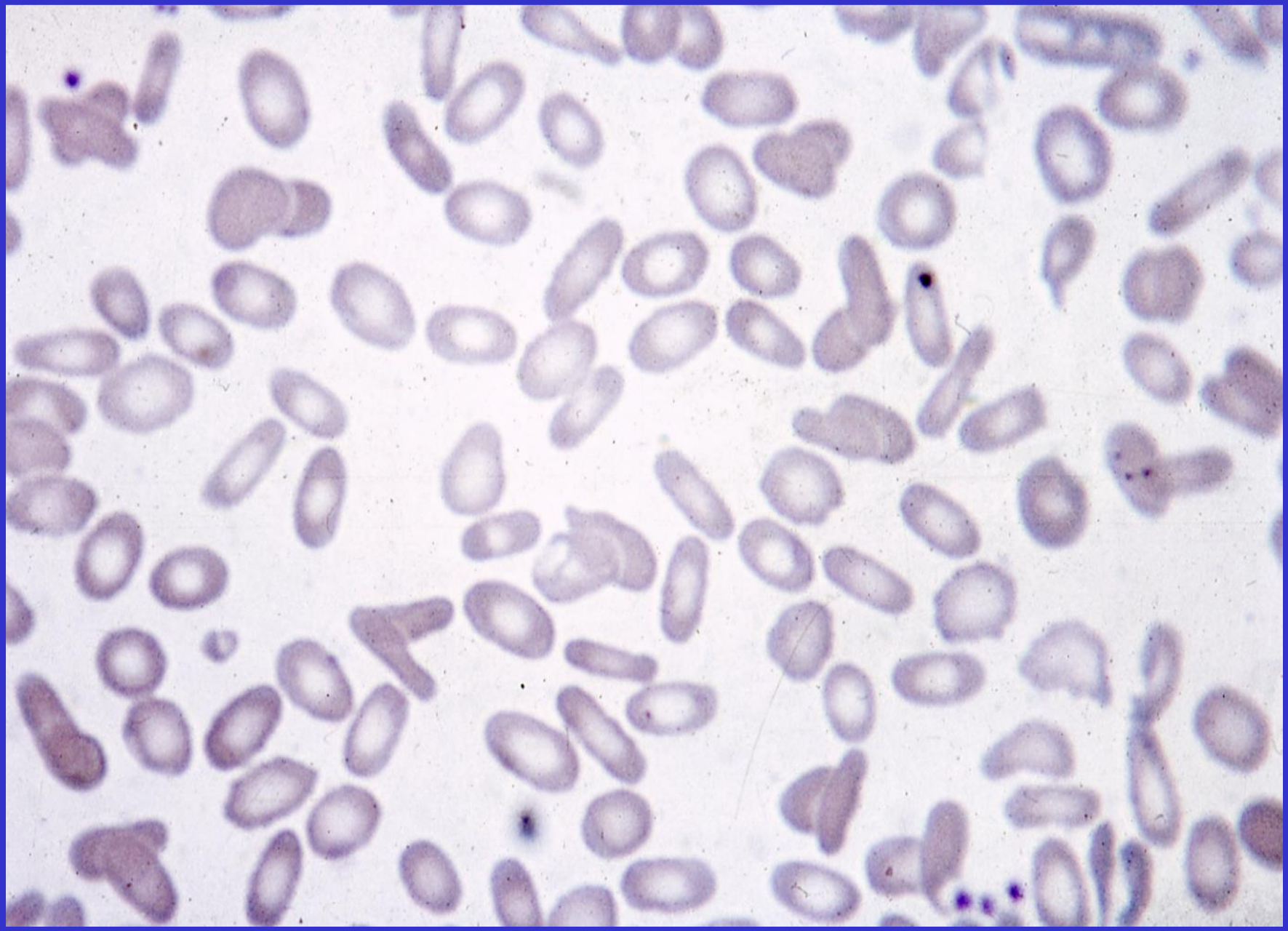
Hereditary elliptocytosis

- Hereditary elliptocytosis (HE) is also a relatively common condition
- Defects in α spectrin
- Most patients are clinically asymptomatic, some will have a chronic symptomatic haemolytic anaemia.
- All show the very characteristic red cell shape on peripheral blood films.

Hereditary pyropoikilocytosis

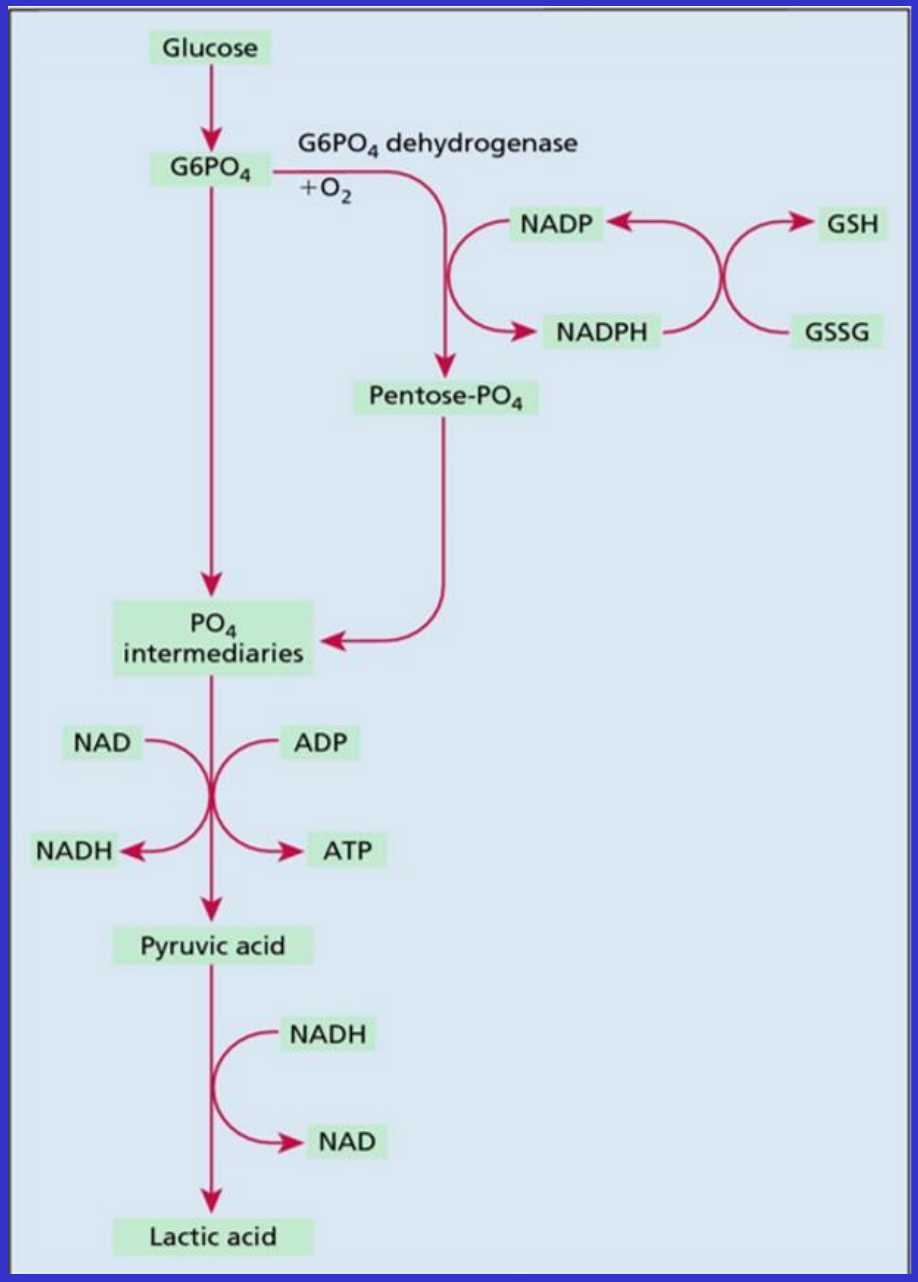
- Severe disturbance of the multimerization of spectrin
- Severe haemolytic anaemia from infancy
- Bizarre peripheral blood morphology, including microspherocytes and poikilocytes. Such patients are described as having hereditary pyropoikilocytosis.

ELLIPTOCYTOSIS

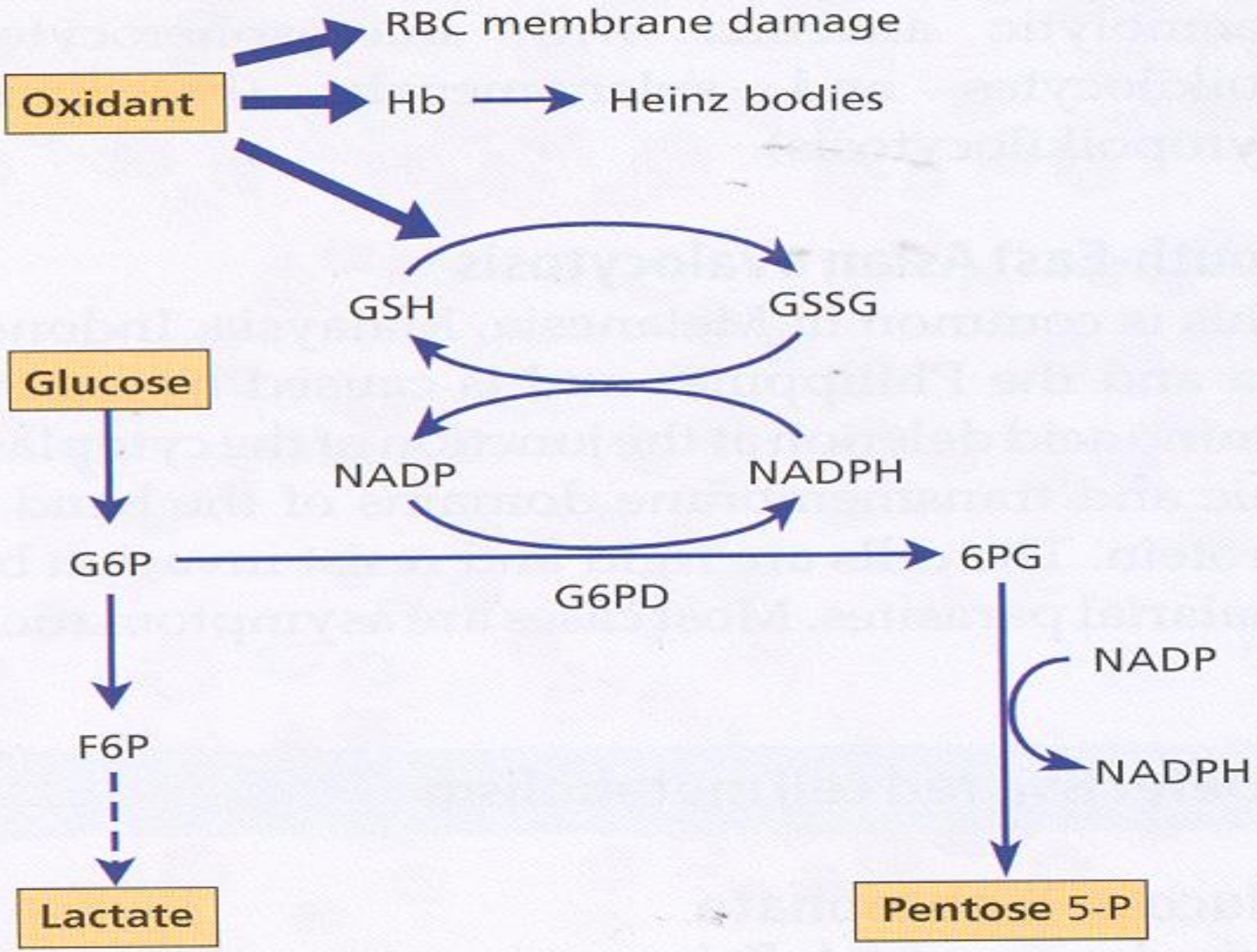


Haemolysis due to abnormalities of red cell enzymes

Haemolytic anaemias may also result from congenital abnormalities of the enzymes required for energy transfer in glucose metabolism. The red cell needs a continuous supply of energy for the maintenance of membrane flexibility and cell shape, the regulation of sodium and potassium pumps, and the maintenance of Hb in the reduced ferrous form.



A schematic diagram of the pathway of glucose metabolism in the red cell, to show the important role of G6PD. A decreased activity of the enzyme leads to a deficiency of the reducing compounds NADPH and GSH.


RBC membrane damage
Oxidant
Hb
Heinz bodies
GSH
GSSG
Glucose
NADP
NADPH
G6P
6PG
G6PD
NADP
NADPH
F6P
Lactate
Pentose 5-P

Glucose-6-phosphate dehydrogenase deficiency

- ✓ Deficiency of glucose-6-phosphate dehydrogenase (G6PD), the first enzyme of the pentose-phosphate shunt, will prevent the normal generation of NADPH, with subsequent erythrocyte sensitivity to oxidative stress.
- ✓ Various point mutations in the G6PD gene on the X chromosome resulting in enzymes with altered activity.
- ✓ The normal G6PD enzyme is designated type B and is the prevalent form worldwide; G6PD type A is a normal variant found in approximately 20% of healthy individuals of African ancestry.
- ✓ Defective forms of G6PD include the A- African variant and the Mediterranean variant.
- ✓ Since the gene for G6PD is found on the X chromosome, affected individuals are male.

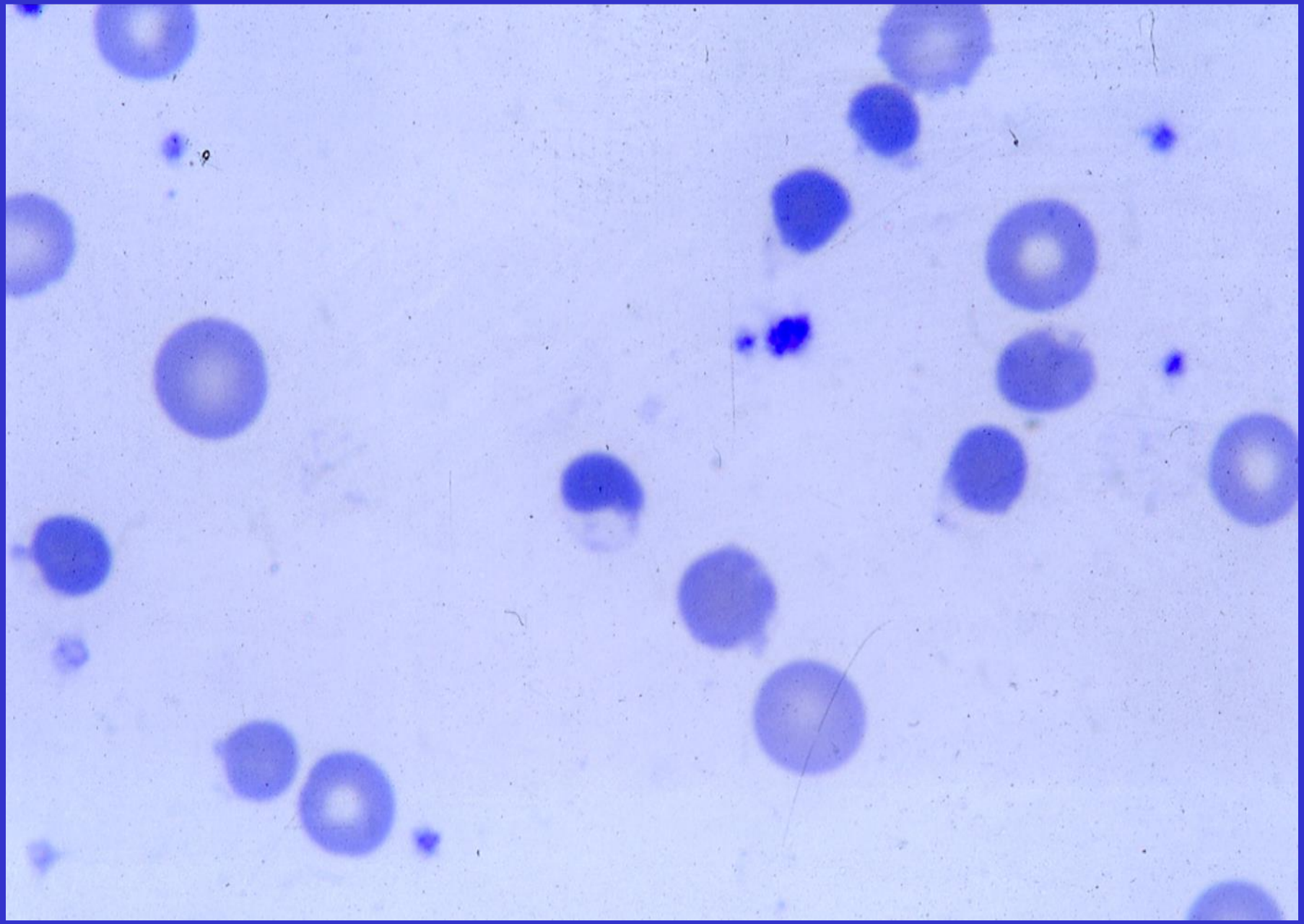
cont'd…

- ✓ Patients with low levels of the enzyme are poorly protected against oxidative challenge - with some medications and even foods resulting in marked oxidative damage to the red cell.
- ✓ When the red cell is exposed to oxidants, haemoglobin is converted to methaemoglobin and denatured.
- ✓ Denatured haemoglobin then precipitates forming inclusions in the red cell (termed Heinz bodies and detected by supravital staining, as in Figure 3.7).
- ✓ Heinz bodies, and the portion of the red cell membrane to which they become attached, are removed by splenic macrophages as the red cells pass through the spleen; the resulting inclusion-free cells display unstained areas at their periphery ('bite' cells, seen in Figure 3.8).

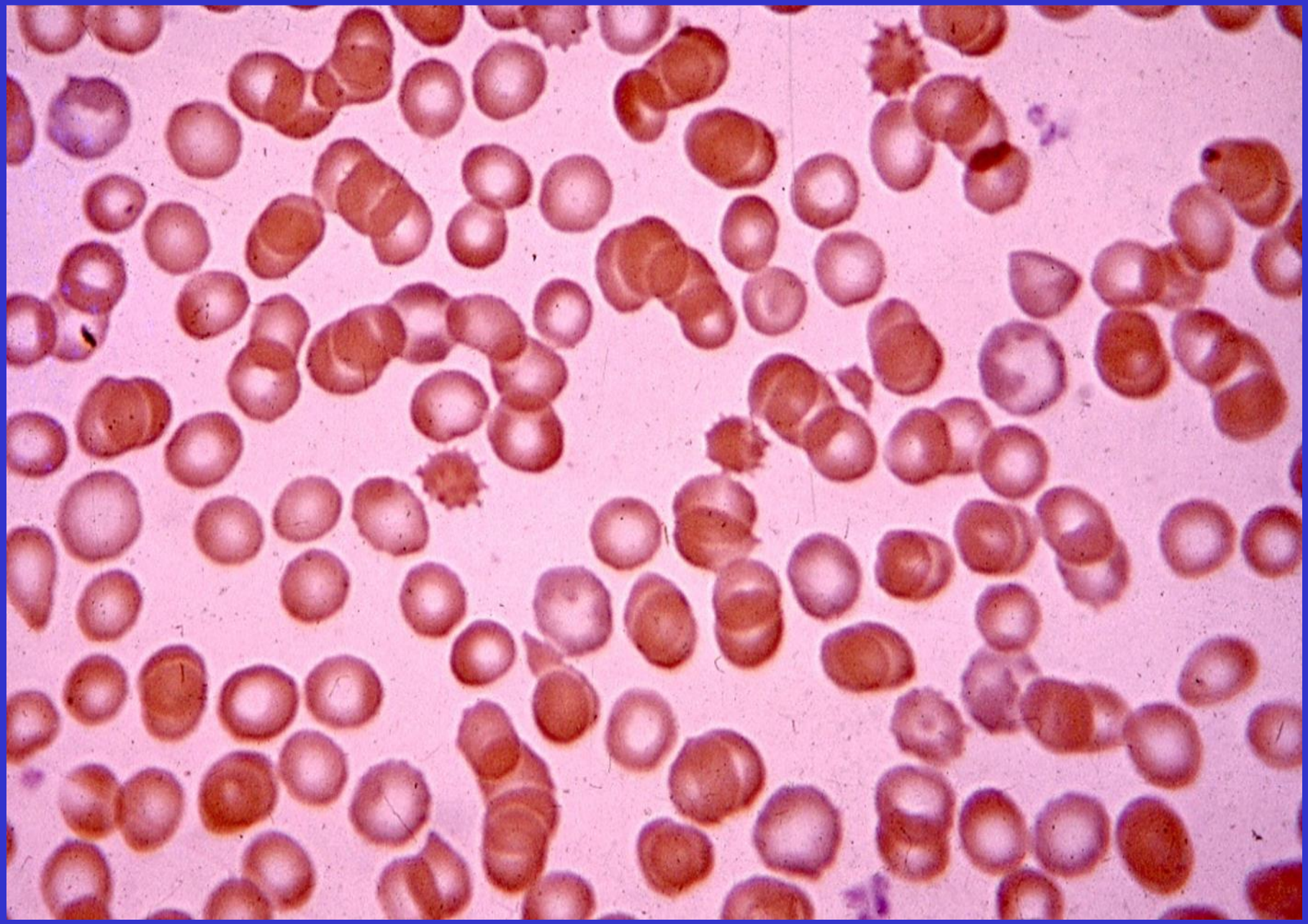
cont'd…

- ✓ Screening tests and assays for detecting G6PD deficiency are available.
- ✓ Haemolysis typically begins 1-3 days following exposure to the oxidative stressor, with anaemia being maximal about 7-10 days after exposure.
- ✓ Patient may report dark urine due to haemoglobinuria.
- ✓ ***Favism*** **-** a syndrome in which an acute haemolytic anaemia occurs after the ingestion of the broad bean (*Vicia fava)* in individuals with a deficiency of G6PD (commonly of the Mediterranean type). Favism usually affects children; severe anaemia develops rapidly and is often accompanied by haemoglobinuria.

G6PD DEFICIENCY



PYRUVATE KINASE DEFICIENCY



Treatment of G6PD deficiency

- Treatment generally focuses on the avoidance of oxidative precipitants to haemolysis. In many cases, haemolysis is self limiting.
- Packed red cell transfusion may be required in cases of severe haemolysis.

Other red cell enzyme deficiencies causing haemolysis

- Pyruvate kinase deficiency is another relatively common example.
- There is usually a chronic haemolytic anaemia and some patients may benefit from splenectomy.

Haemolysis due to haemoglobin defects

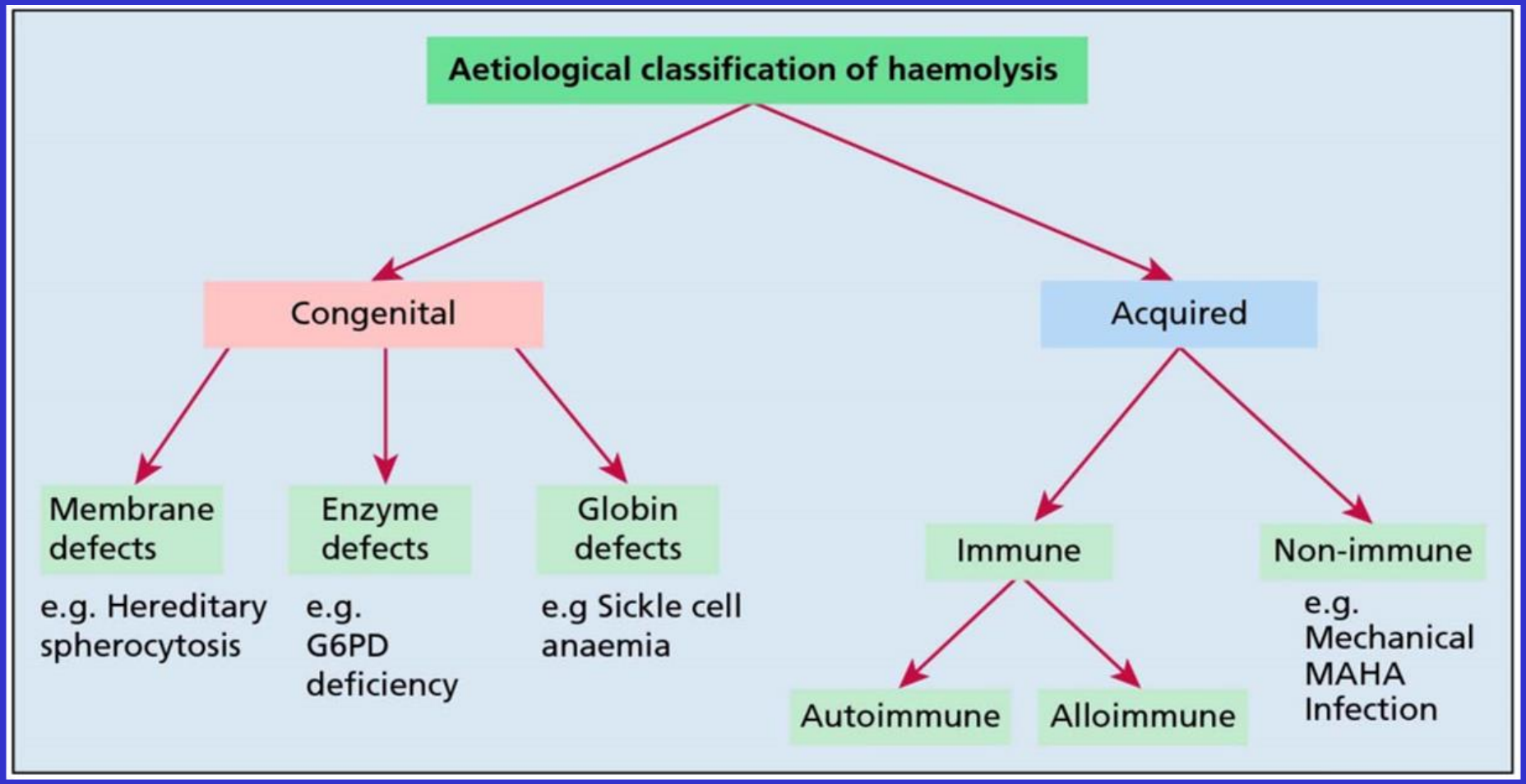
- Defects in the structure of haemoglobin.
- Structural variants of the globin chains may affect the lifespan of the red cell, with sickle cell anaemia being the best-described example.
- A tendency of the HbS variant to polymerize under conditions of low oxygen tension leads to distortion of the erythrocyte in the well-recognized sickle shape.

Acquired haemolytic anaemias

In the acquired haemolytic anaemias, red cells may be destroyed either by immunological or by non-immunological mechanisms.

Classification Of Haemolytic Anaemias

Hereditary	Acquired
Haemoglobin	Allografts, especially marrow transplantation
Abnormal (Hb S, Hb C, unstable)	drug associated
Thalassaemia	**Red cell fragmentation syndrome**
Membranopathy	Arterial grafts, cardiac valves
Enzymopathy	Microangiopathic
	Thrombotic thrombocytopenic purpura
	Haemolytic uraemic syndrome
	Meningococcal sepsis
	Pre-eclampsia
	Disseminated intravascular coagulation
	March haemoglobinuria
	Infections
	Malaria, clostridia
	Chemical and physical agents
	Especially drugs, inductrial/domestic substances, burns
	Secondary
	Liver and renal disease
	Paroxysmal nocturnal haemoglobinuria



A classification of haemolytic anaemia by aetiology. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; MAHA, microangiopathic haemolytic anaemia.

Classification of AIHAs

Caused by warm-reactive antibodies

Idiopathic

Secondary (chronic lymphocytic leukaemia, Lymphoma, systemic lupus erythematosus (SLE), some drugs)

Caused by cold-reactive antibodies

Cold haemagglutinin disease

Idiopathic

Secondary (*Mycoplasma pneumoniae* infection, infectious mononucleosis, lymphomas)

Paroxysmal cold haemoglobinuria

Idiopathic

Secondary (some viral infections, congenital and tertiary syphilis)

Immune haemolytic anaemias

- In these conditions, antigens on the surface of red cells react with antibodies sometimes with complement activation. IgG-coated red cells interact with the Fc receptors on macrophages in the spleen, and are then either completely or partially phagocytosed. When the phagocytosis is partial, the damaged cell will return to the circulation as a spherocyte. Red cells that are also coated with the activated complement component C3 may interact with C3 receptors on macrophages and are usually completely phagocytosed. In most instances where complement is activated, the cascade sequence only proceeds further and permits deposition of the membrane attack complex (C5-C9) with resultant intravascular haemolysis.

cont'd…

- The immune haemolytic anaemias may be due to autoantibodies; that is, antibodies formed against one or more antigenic constituents of the individual's own tissues. These include autoimmune haemolytic anaemia (AIHA) and some drug-related haemolytic anaemias. It is also possible to develop alloimmune haemolytic anaemia, consequent on the production of antibodies against red cells from another individual, as in haemolytic transfusion reactions and haemolytic disease of the newborn.

Autoimmune haemolytic anaemias

‘Warm’ autoantibodies react best with the red cell antigen at 37°C and are usually of IgG subtype.

‘Cold’ antibodies react best at temperatures below 32ºC (usually below 15ºC) and, since they are usually of IgM subtype, are capable of agglutinating red cells.

Classification of AIHAs

Caused by warm-reactive antibodies

Idiopathic

Secondary (chronic lymphocytic leukaemia, Lymphoma, systemic lupus erythematosus (SLE), some drugs)

Caused by cold-reactive antibodies

Cold haemagglutinin disease

Idiopathic

Secondary (*Mycoplasma pneumoniae* infection, infectious mononucleosis, lymphomas)

Paroxysmal cold haemoglobinuria

Idiopathic

Secondary (some viral infections, congenital and tertiary syphilis)

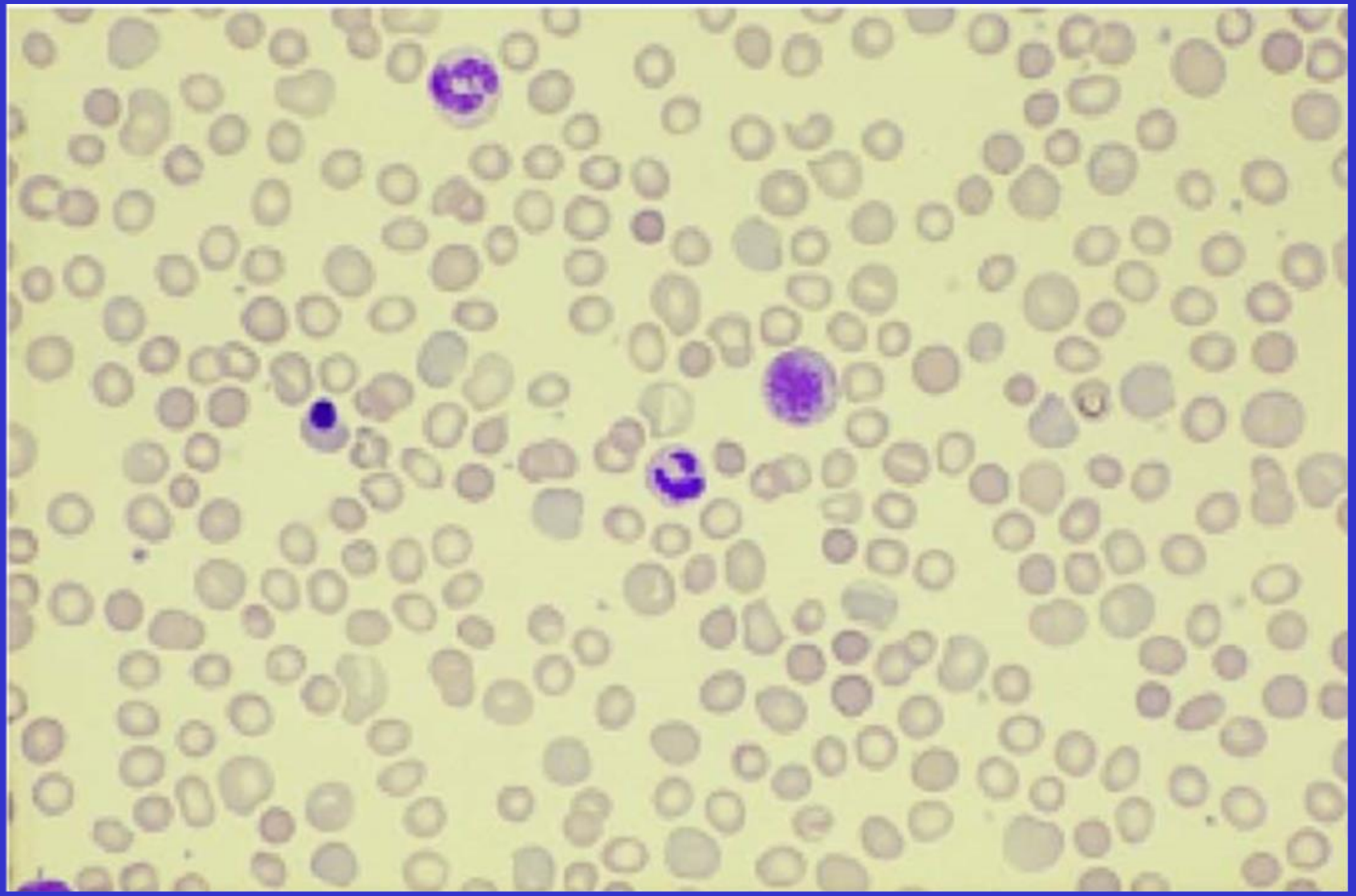
Warm AIHA

1) In idiopathic warm AIHA, haemolysis dominates the clinical picture and no evidence can be found of any other disease.
2) In secondary AIHA, the haemolysis is associated with a primary disease such as chronic lymphocytic leukaemia or systemic lupus erythematosus (SLE).

- The antibody-coated red cells undergo partial or complete phagocytosis in the spleen and by the Kupffer cells of the liver. There may be partial activation of the complement cascade.

cont'd…

- Haematological findings include anaemia, spherocytosis, reticulocytosis and occasional nucleated red cells in the peripheral blood. The critical diagnostic investigation is the direct antiglobulin test.
- Haemolysis can be limited by treatment with prednisolone.
- If reduction in haemolysis is not maintained when the dose of steroids is lowered, splenectomy or alternative immunosuppressive therapy should be considered.
- The anti-CD20 monoclonal antibody rituximab, as well as immunosuppressants such as azathioprine or cyclophosphamide, may be beneficial in reducing autoantibody production.



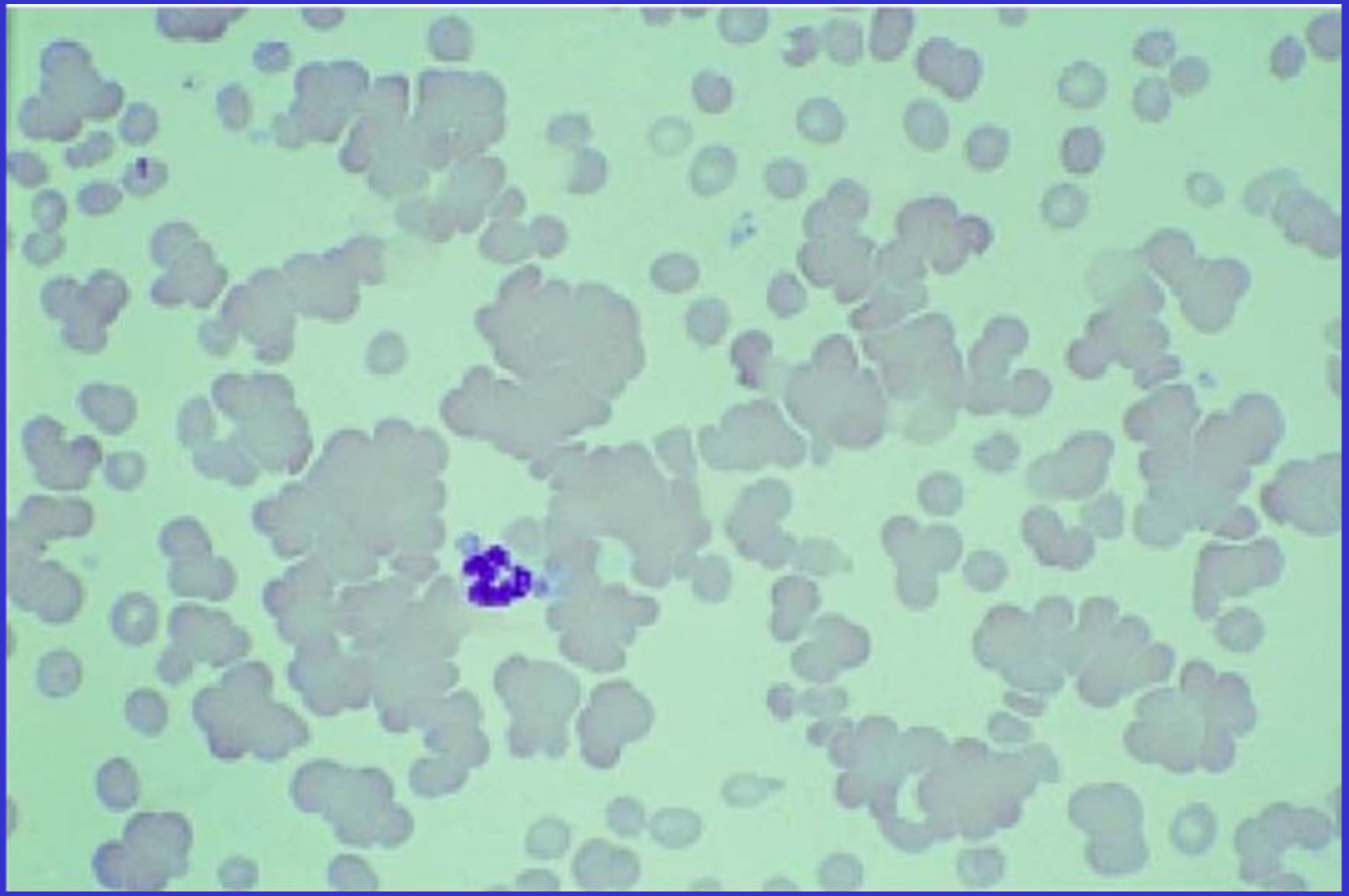
Blood film from a patient with idiopathic AIHA (warm-reactive antibody) showing prominent spherocytosis and polychromasia

Cold haemagglutinin disease (CHAD)

- Since cold antibodies react with red cells only at temperatures below about 32ºC, they typically bind to the red cell surface in the cooler superficial blood vessels of the peripheries.
- Since the cold antibodies are typically of the IgM subtype, their pentameric structure permits direct agglutination of red cells coated with antibody; they are therefore sometimes termed cold agglutinins.
- Symptoms due to cold AIHA are worse during cold weather. Exposure to cold provokes acrocyanosis, due to the formation of agglutinates of red cells in the vessels of the skin.

cont'd…

- The direct activation of the complement system leads to red cells lysis and, consequently, to haemoglobinaemia and haemoglobinuria.
- Chronic idiopathic CHAD is managed initially simply by keeping the patient warm.
- Treatment with rituximab may be effective.
- Other causes of haemolytic anaemia with an immune element include: 1) paroxysmal nocturnal haemoglobinuria; 2) paroxysmal cold haemoglobinuria; 3) drug-related haemolytic anaemias.

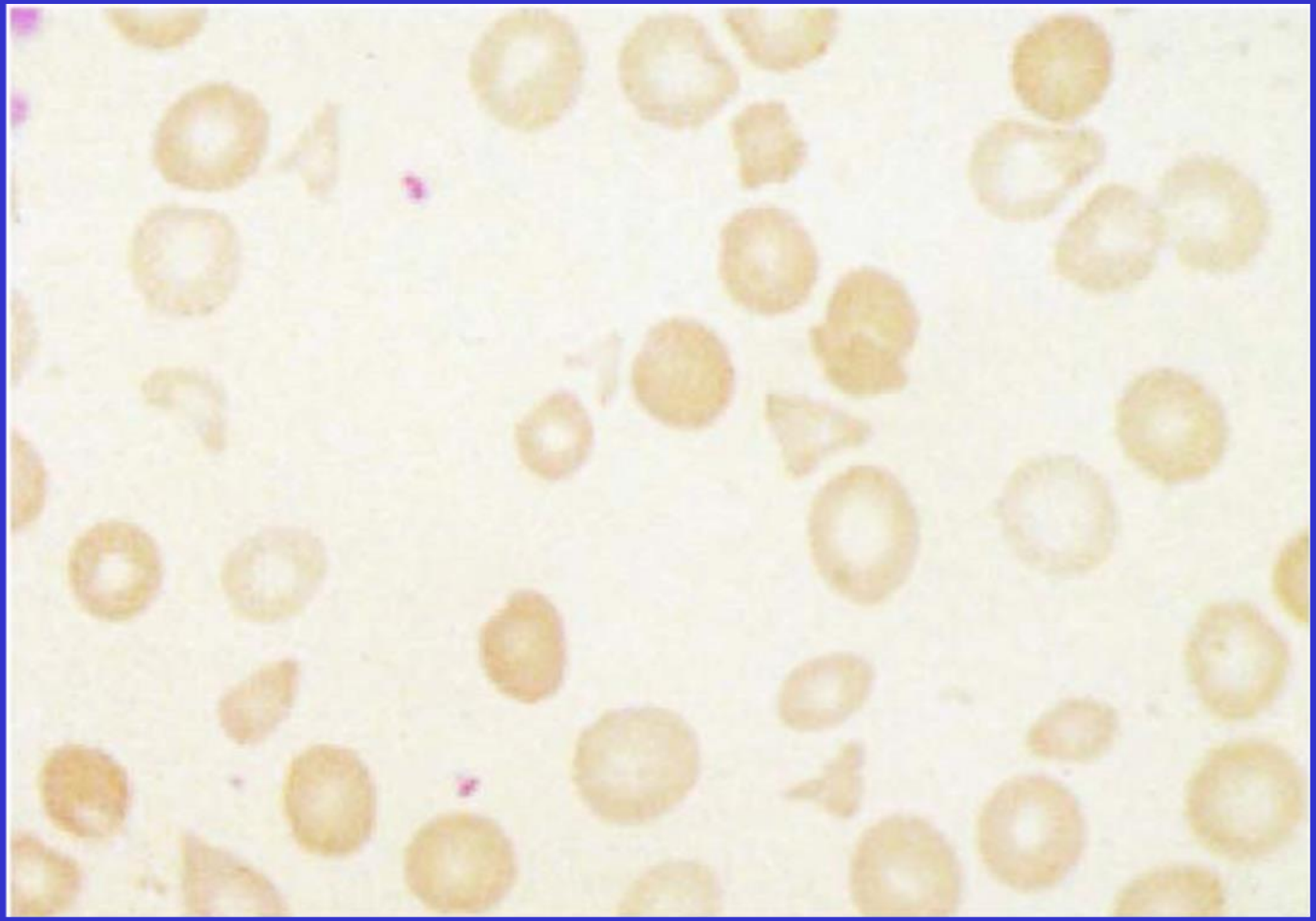


Numerous red cell agglutinates on a blood film from a patient with idiopathic CHAD.

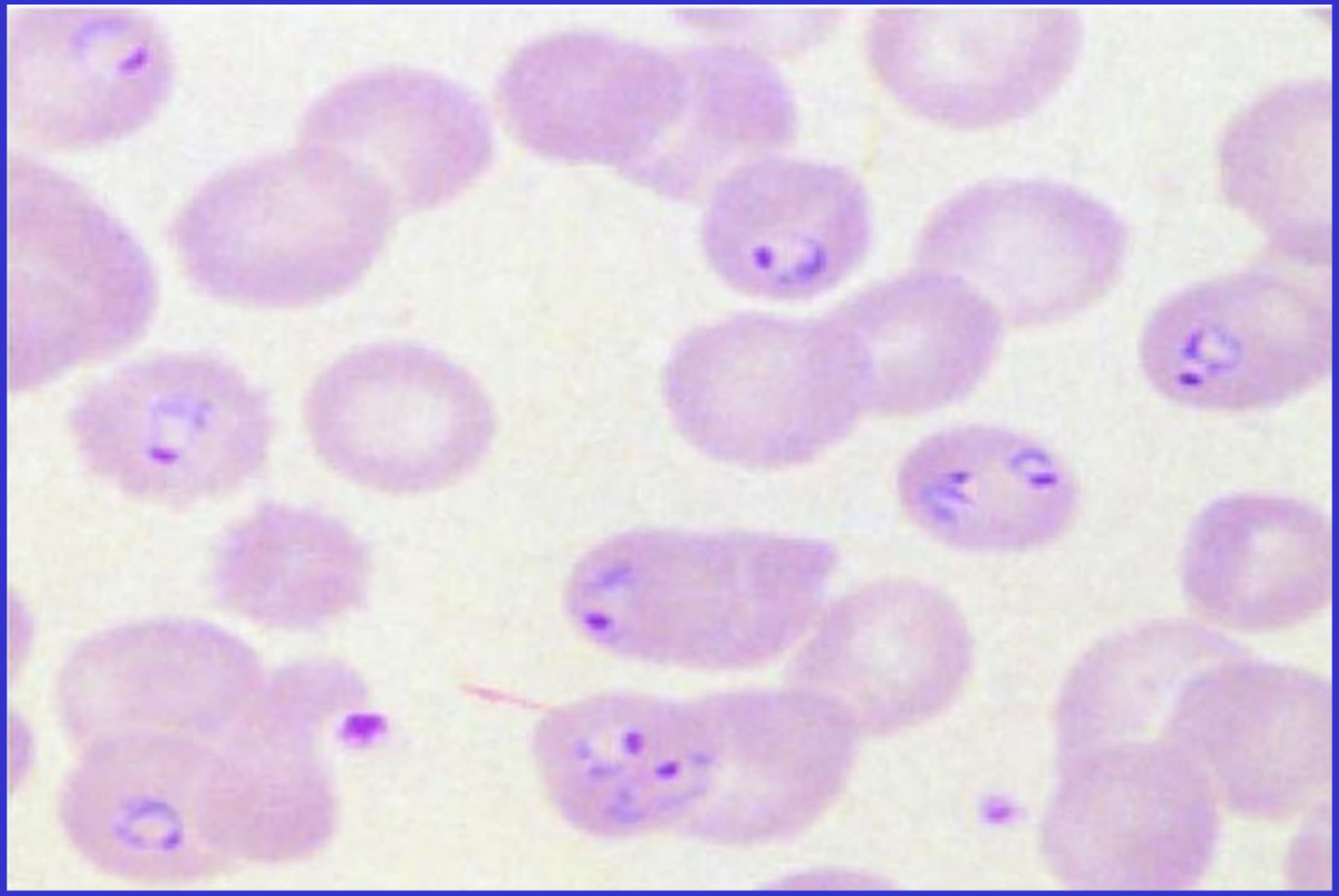
Non-immune haemolytic anaemias

Mechanical damage to red cells

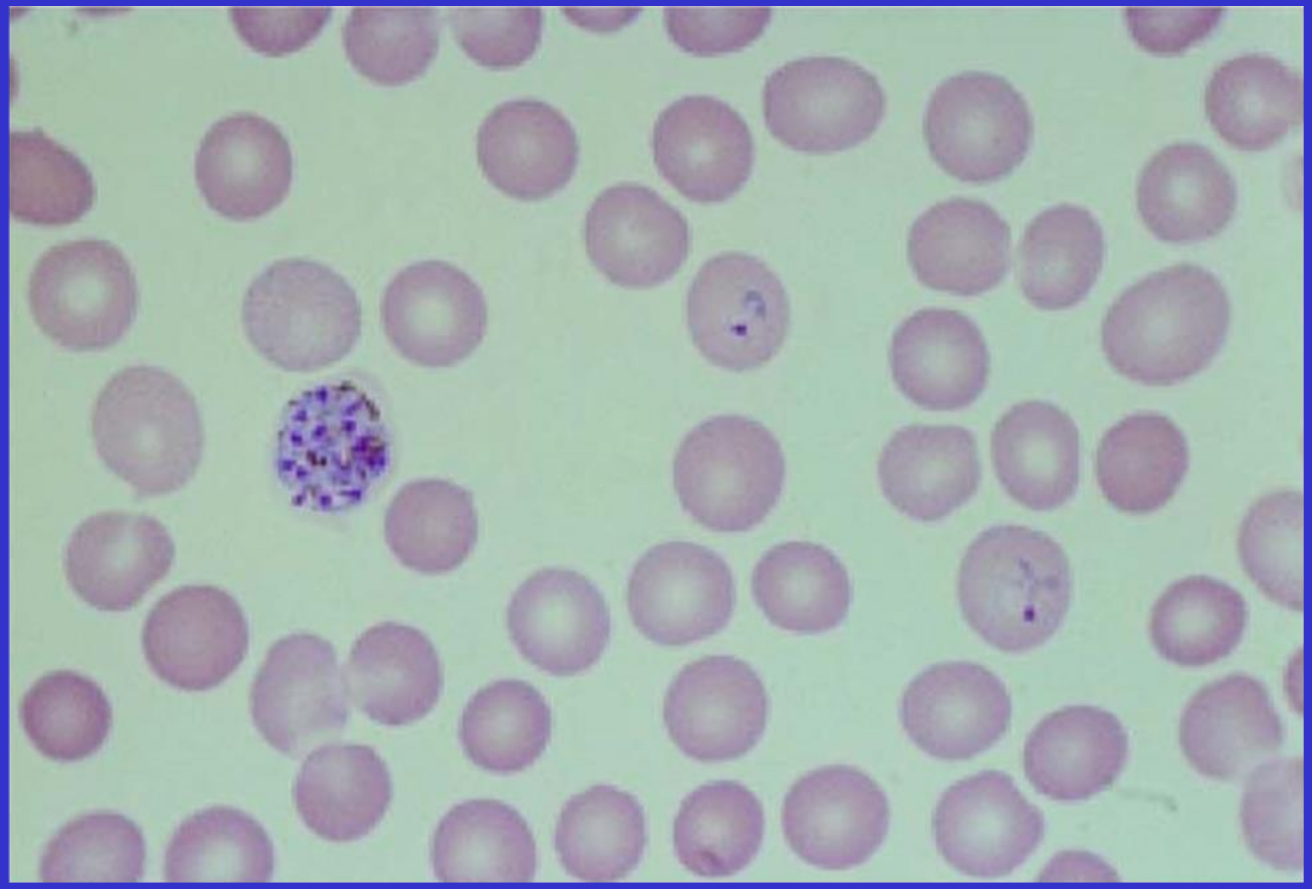
Several of the mechanical causes of acquired non-immune haemolytic anaemia are summarized in Table 3.3. Red cells are mechanically damaged when they impact upon abnormal surfaces. In disseminated intravascular coagulation inappropriate activation of the coagulation cascade produces fibrin strands which are thought to cause mechanical destruction of red cells. Such damage usually results in the presence of red cell fragments in the blood film.



Fragmented red cells (schistocytes) in the blood film of a patient with a malfunctioning aortic valve prosthesis.



Blood film from a patient with *Plasmodium falciparum* malaria showing several parasitized red cells. Red cells heavily parasitized with malaria may be subject to intravascular lysis.

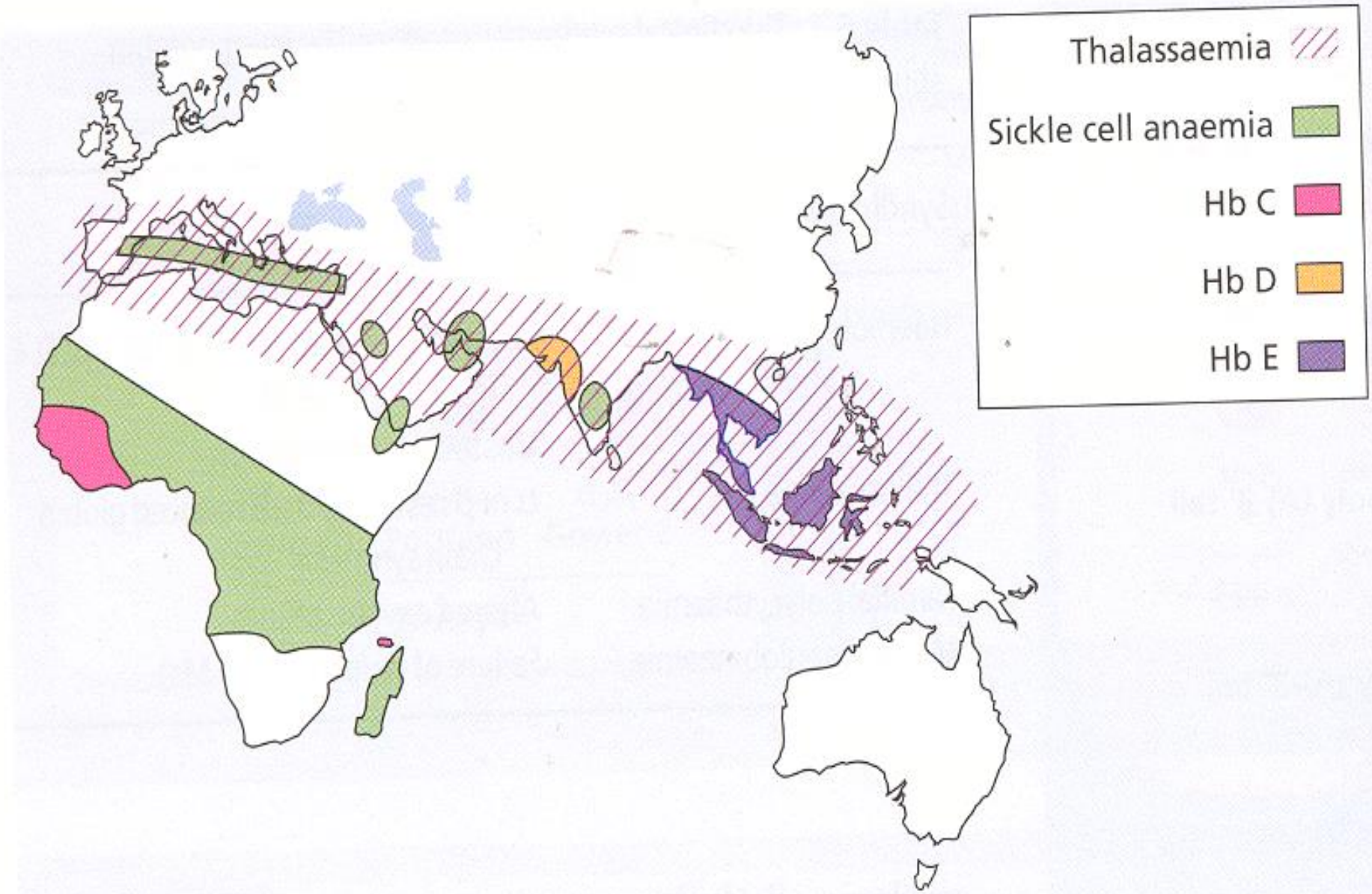


Blood film from a patient with *Plasmodium vivax* malaria showing two parasitized red cells, each containing a single parasite (ring form or early trophozoite and an ameboid late trophozoite). Another red cell contains a schizont. Some of the parasitized cells are slightly enlarged.

Hypersplenism

Hypersplenism describes the reduction in the lifespan of red cells, granulocytes and platelets that may be found in patients with splenomegaly due to any cause. The cytopenias found in patients with enlarged spleens are also partly caused by increased pooling of blood cells within the spleen.

HAEMOGLOBIN VARIANTS: GENE DISTRIBUTION



Abnormal Haemoglobin Variants

Hb C:-

- Is due to replacement of glutamic acid in position 6 of the beta chain by lysine ($\alpha_2\beta_2$ 6-GLU→ LYS).

- About 7-22% of people of West Africa are hetrozygotes especially Nigeria and North Ghana

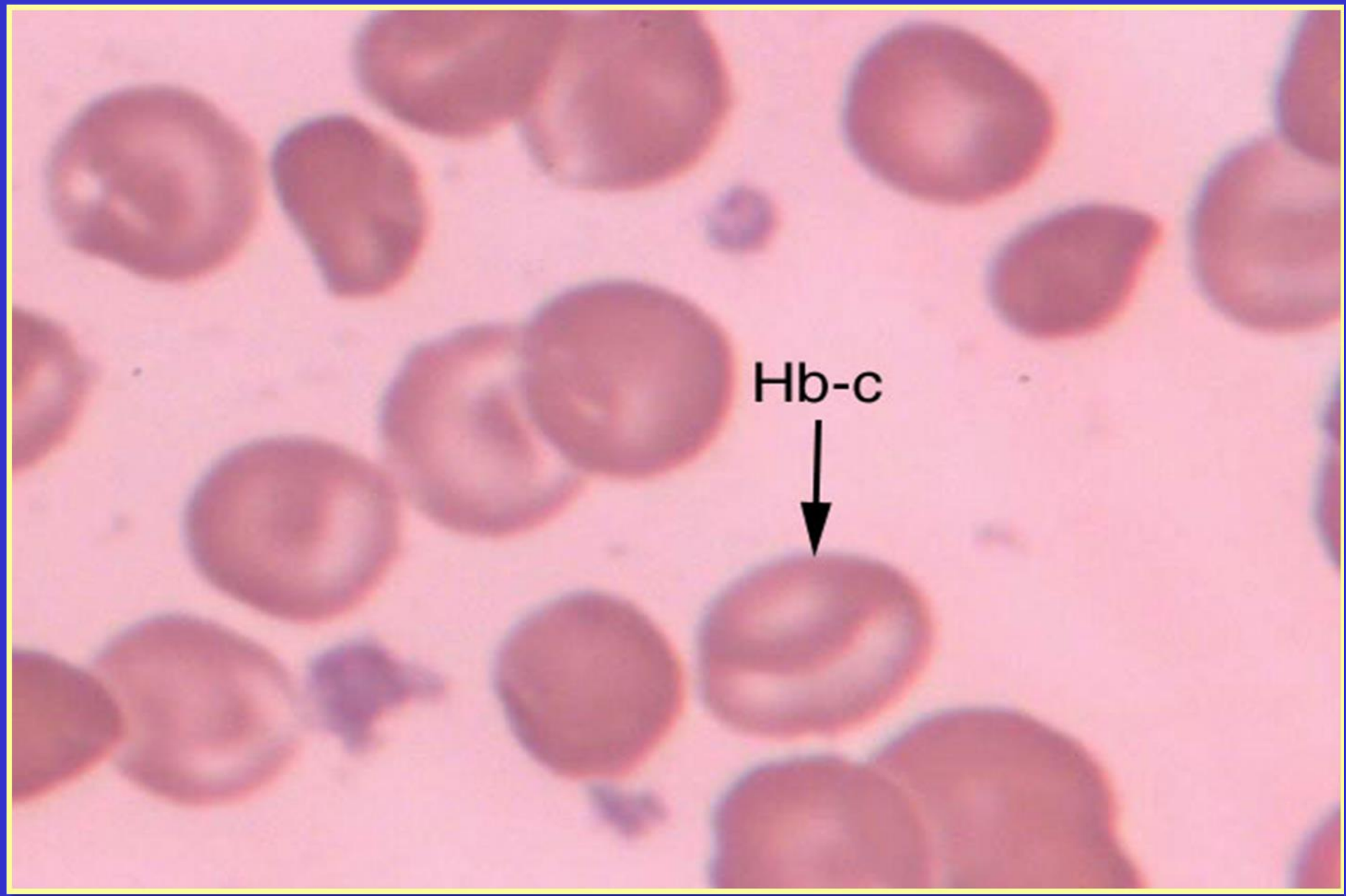
- Homozygotes are rare and have mild to moderate hemolytic anaemia with many thick target RBCs in the blood film and mild to moderate splenomegaly.

- The chronic hemolytic anaemia is due to reduced red cell deformability on deoxygenation.

  Deoxygenated HbC is less soluble than deoxygenated HbA.

- Double heterozygotes with sickle Hb S/C give moderate to severe anaemia with symptoms of sickle cell disease.

HAEMOGLOBIN C DISEASE



Hb D Punjab

($\alpha_2\beta_2$-121 GLU → GLN)

- Prevalent in Indian and Pakistani in every 100 persons about 1 trait (1% of the population).
- Trait are usually healthy.
- Homozygous D/D have mild to moderate anaemia.
- Combined double heterozygotes Hb S/D can give rise to moderate to a severe anaemia and symptoms of sickle cell disease.

Hb E:

- ($\alpha_2\beta_2$ 26 GLU → LYS) is one of the most common beta-chain variants.
- It is very prevalent in South East Asia (50%) of the population are heterozygotes.
- Patients who are homozygous generally have mild haemolytic anaemia, microcytic hypochromic red cells and mild enlargement of the spleen.
- Carriers are symptomless unless they have combined other mutations such as the one for alpha thalassemia, or beta-thalassemia trait.

Hb O Arab

($\alpha_2\beta_2$-121 GLU → LYS)

- Heterozygotes are not symptomatic.
- Double heterozygous with sickle S/O are clinically severe.
- Hb O- Arab enhance the polymerization of HbS.

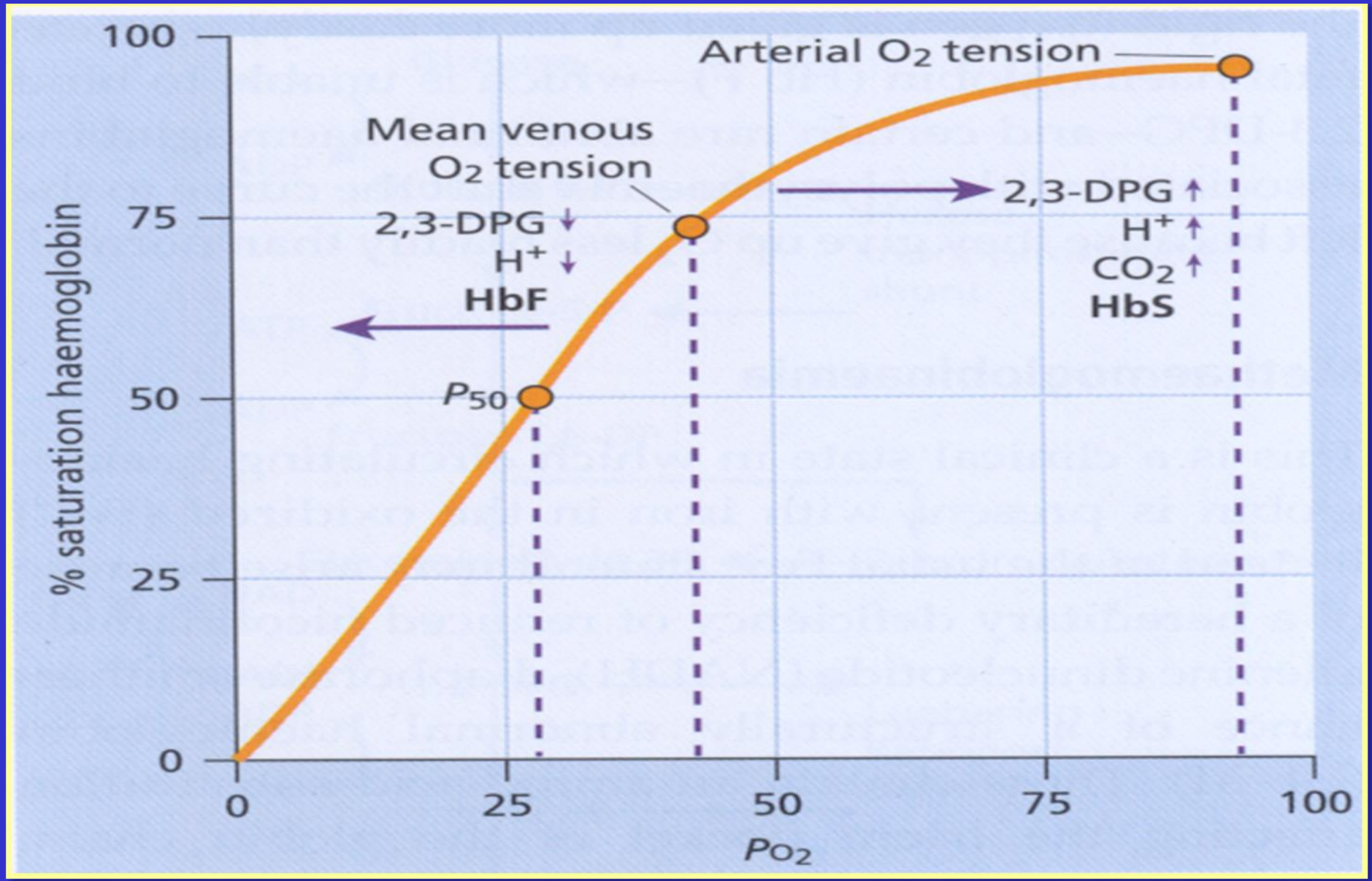
High Oxygen affinity haemoglobins

Hb Chesapeake:

(α_2-92 ARG → LEU β_2).

- Carriers are without clinical symptoms.
- Homozygous of erythrocytosis (polychemia) due to increased O_2 affinity.
- The patients have no splenomegaly. (except for patient's with concomitant β-thalassemia).
- They have normal WBC, and normal platelets.

* High Hb, High RBCs count and high haematocrit. (HCT).



The haemoglobin oxygen (O_2) dissociation cruve. 2,3-DPG, 2,3-diphosphoglycerate.

Unstable Haemoglobins

Hb koln ($\alpha_2\beta_2$-98 VAL → MET)

Hb Hammersmith ($\alpha_2\beta_2$ 42 PHE → SER)

Hb Hasharon (α_2-47 ASP → HIS β_2).

- **These abnormal haemoglobin cause haemolysis in the newborn (congenital non-spherocytic haemolytic anaemia).**
- **Heinz body hemolytic anaemia with sensitivity to oxidant drugs, such as sulfonamides.**
- **Reticulocytosis out of proportion to the level of Hb.**
- **Increased formation of methemoglobin.**
- **Spontaneous or drug induced haemolytic anaemia due to instability of the haemoglobin and consequent intracellular precipitation.**
- **Thalassaemia – like peripheral blood picture.**

  Clinically: The patient have anemia, jaundice, splenomegaly / hepatomegaly and gall stones.

Low oxygen affinity haemoglobins

- **More than 50 variants with reduced oxygen affinity have been identified.**

- **Hb kansas ($\alpha_2\beta_2$102 ASN → THR)**
- **Hb Aukland ($\alpha_2\beta_2$ 25 GLY → ASP)**
- **Rare as homozygotes.**
- **Patients have anaemia and congenital cynosis due to reduced oxygen affinity.**

Congenital Methaemoglobinaemia

- **Hb M Boston (α_2 58 HIS → TYR - β_2)**
- **Hb M Saskatoon (α_2-β_2-63 HIS → TYR)**
- **Hb M Hyde park ($\alpha_2\beta_2$ 92 HIS → TYR)**
- **Hb M IWATE ($\alpha_2$87 HIS → TYR-β_2)**

Cynosis in homozygotes due to congenital methaemoglobinaemia as a consequences of substitution of amonoacids near or in haem pocket.

Hb Indianapolis

- **(α_2-$\beta_2$112 CYS – ARG)**
- **Is a rare and slightly unstable beta-globin variant.**
- **Carriers are clinically normal with only mild reticulocytosis.**
- **Homozygons have haemolytic anaemia and renal failure in severe cases.**
- **Thalassaemia-like syndrome due to marked instability of the Hb.**

EFFECTS OF HAEMOGLOBIN VARIANTS

Variant	Clinical and haematological abnormalities
HbS	Recurrent painful crises (in adults) and chronic haemolytic anaemia; both related to sickling of red cells on deoxygenation*
HbC	Chronic haemolytic anaemia due to reduced red cell deformability on deoxygenation, * deoxygenated HbC is less soluble than deoxygenated HbA.
Hb Köln, Hb Hammersmith	Spontaneous or drug-induced haemolytic anaemia due to instability of the Hb and consequent intracellular precipitation.
HbM Boston, HbM Saskatoon	Cyanosis due to congenital methaemoglobinaemia as a consequence of a substitution near or in the haem pocket.
Hb Chesapeake	Hereditary polycythaemia due to increased O_2 affinity.
Hb Constant Spring, Hb Lepore, HbE	Thalassaemia-like syndrome due to decreased rate of synthesis of normal chains.
Hb Indianapolis	Thalassaemia-like syndrome due to marked instability of Hb

** Only in homozygotes*

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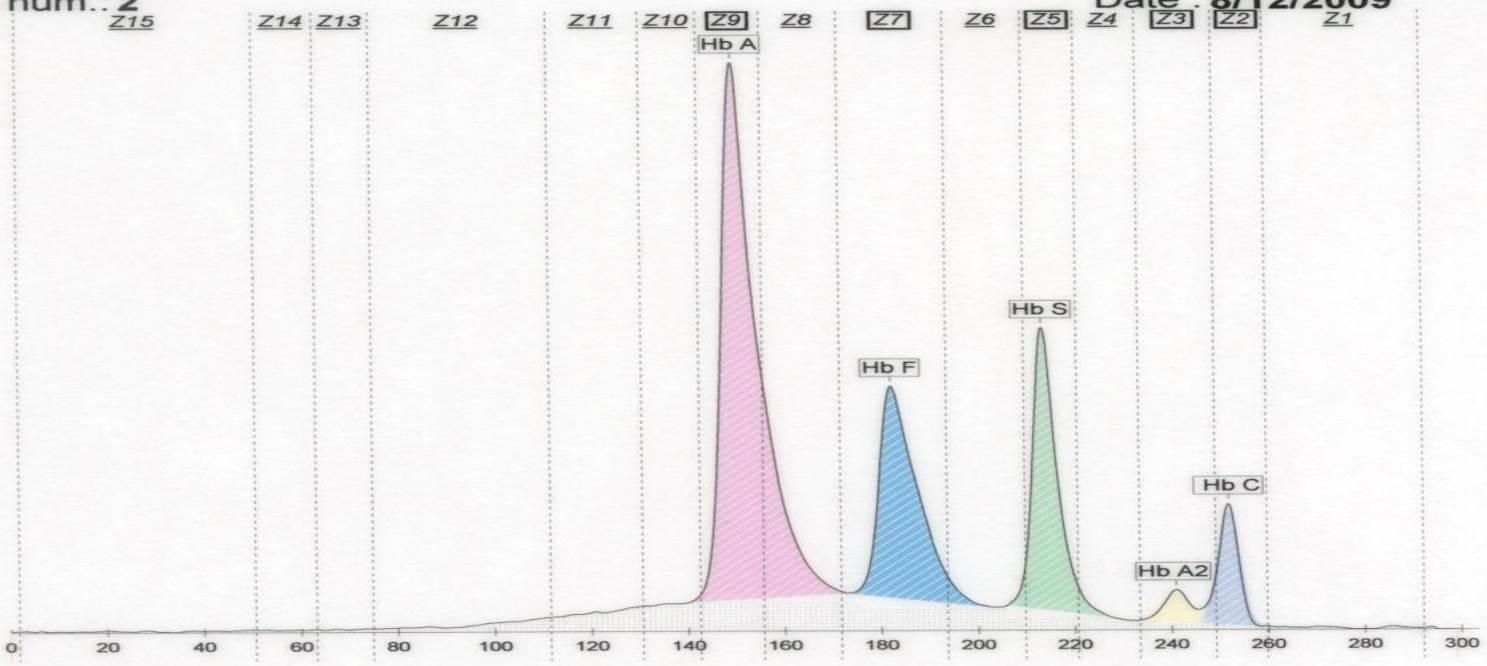
Hb Electrophoresis

Hospital No.: **QC Hb AFSC CONTROL-**

ID : **Hb AFSC CONTROL-2**

Sample num.: **2**

Date : **8/12/2009**



Hb Electrophoresis

Fractions	%	Ref. %
Hb A	**51.3**	46.7 - 56.9
Hb F	**21.4**	17.4 - 22.4
Hb S	**18.3**	17.3 - 22.3
Hb A2	**2.3**	2.1 - 3.3
Hb C	**6.7**	4.6 - 7.0

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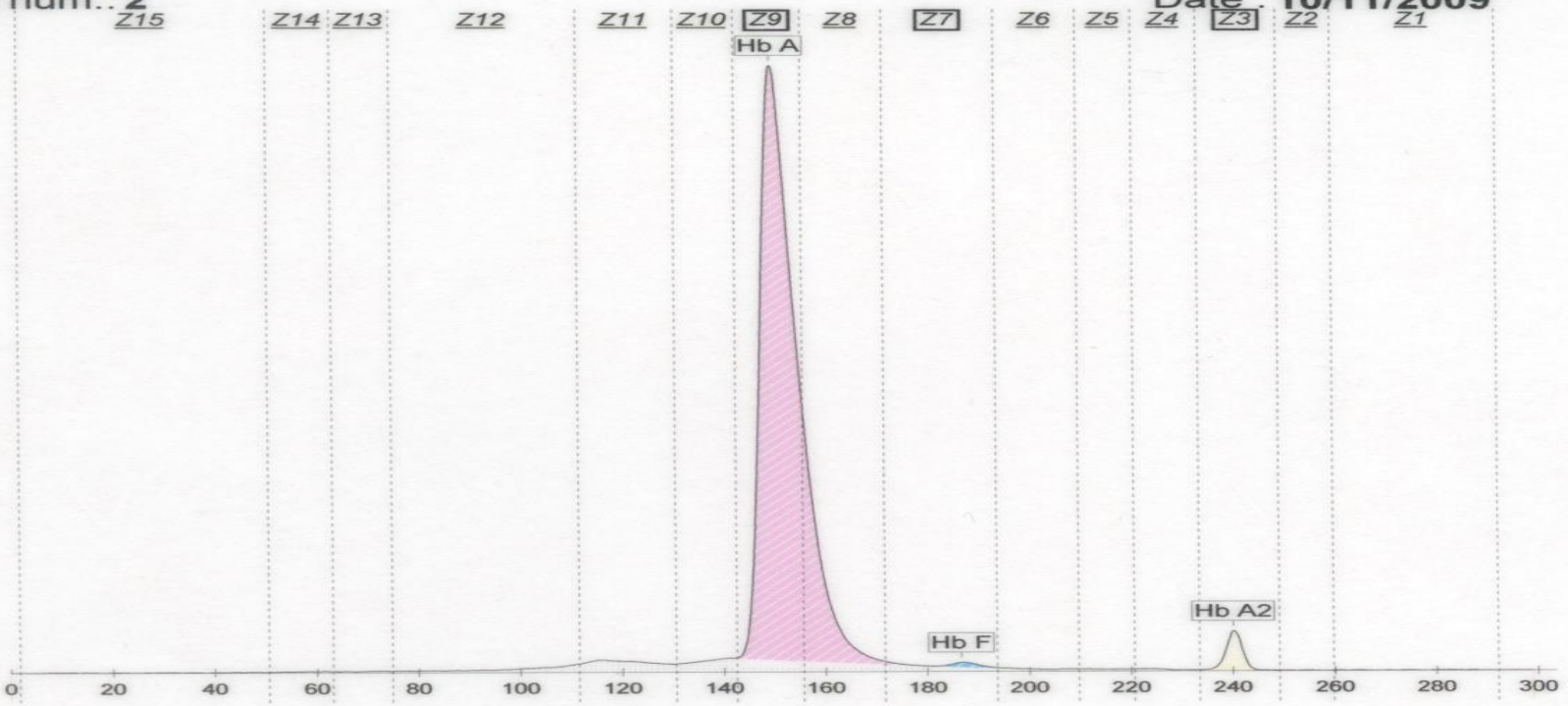
Hb Electrophoresis

Hospital No.: **933376**

ID : **061773**

Sample num.: **2**

Date : **10/11/2009**



Hb Electrophoresis

Fractions	%	Ref. %	
Hb A	**96.7**	96.8 - 97.8	
Hb F	**0.5**	=< 2.0	<
Hb A2	**2.8**	1.5 - 3.5	

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Hb Electrophoresis

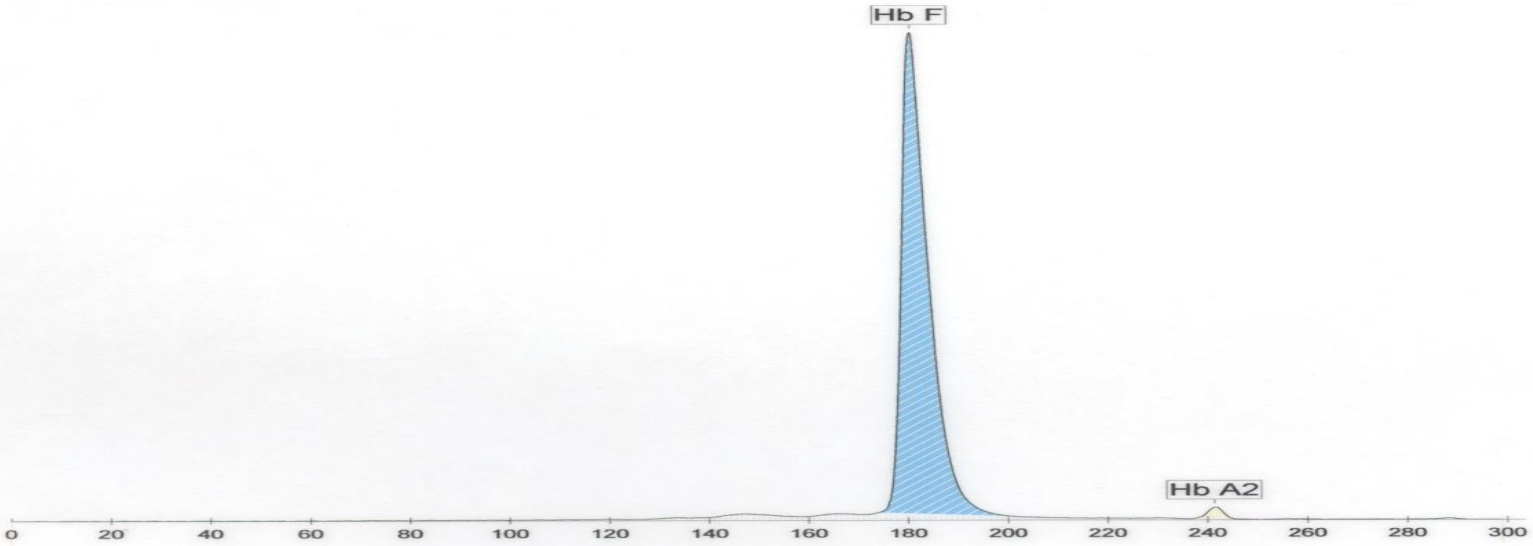
INSTRUMENT ID : KKUH : 24509

Hospital No.: **921107**

Sample No **54**

ID : **063761**

Date : **09/05/2010**



Fractions	%	Ref. %
Hb F	98.5	
Hb A2	1.5	

Comment :

28/3/2010

CBC Hb 98

MCV 73

NRBC 34

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Hb Electrophoresis

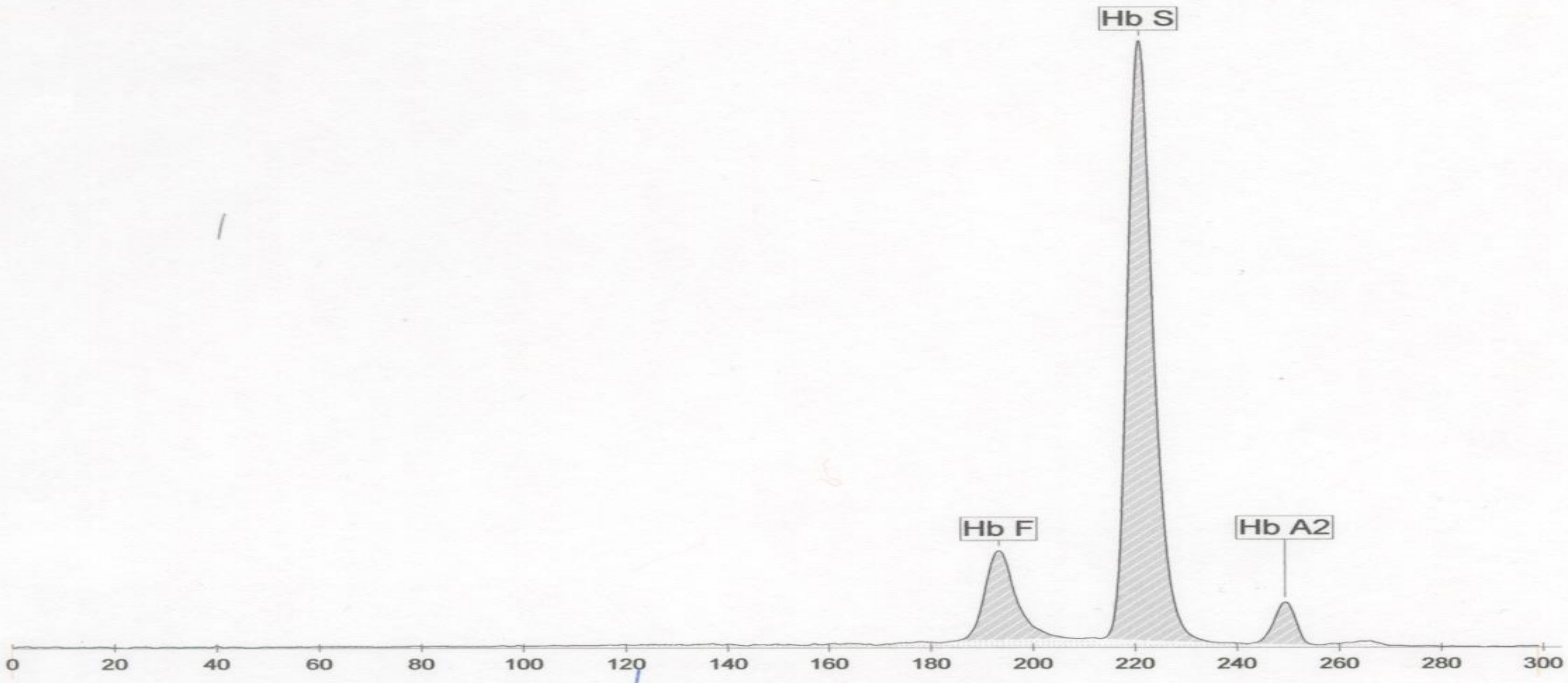
INSTRUMENT ID : KKUH : 24509

Hospital No.: 233095

Sample No 20

ID : **063478**

Date : **17/04/2010**



Fractions	%	Ref. %
Hb F	14.7	
Hb S	80.5	
Hb A2	4.8	

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Hb Electrophoresis

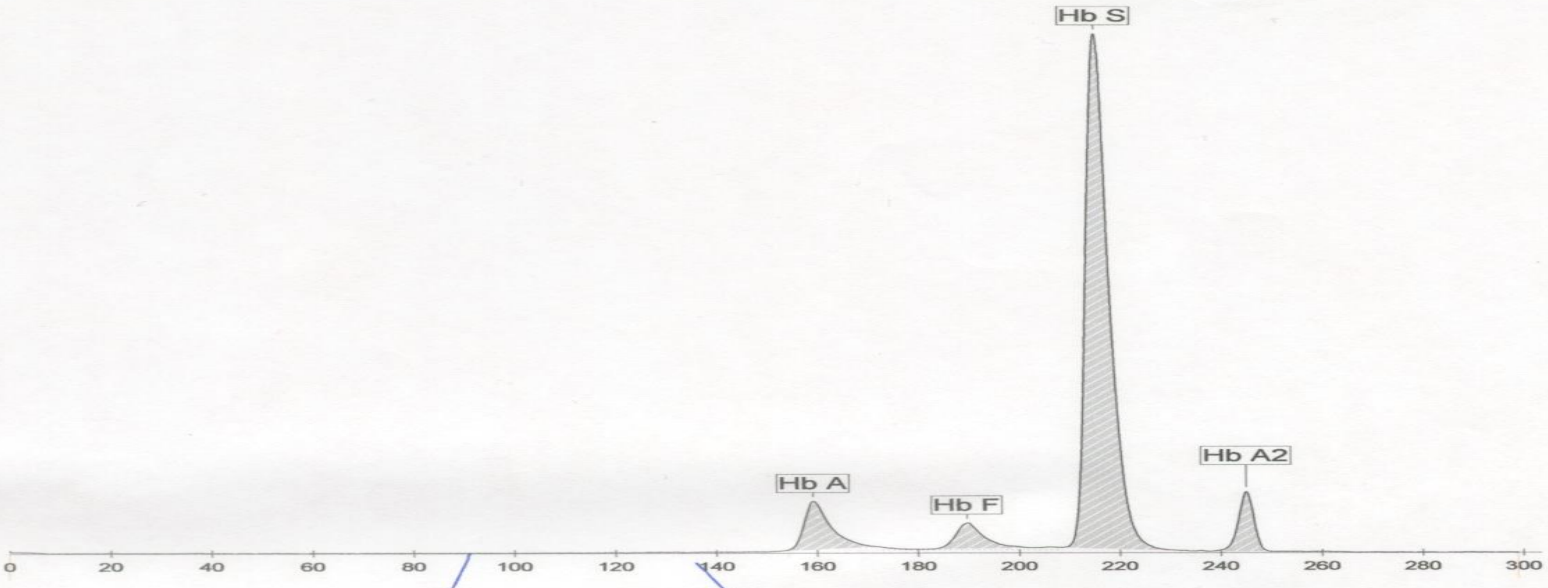
INSTRUMENT ID : KKUH : 24509

Hospital No.: **913628**

Sample No **34**

ID : **063511**

Date : **19/04/2010**



Fractions	%	Ref. %
Hb A	**8.7**	
Hb F	**4.9**	
Hb S	**80.1**	
Hb A2	**6.3**	

Comment :

Homozygous sickle cell thal

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Hb Electrophoresis

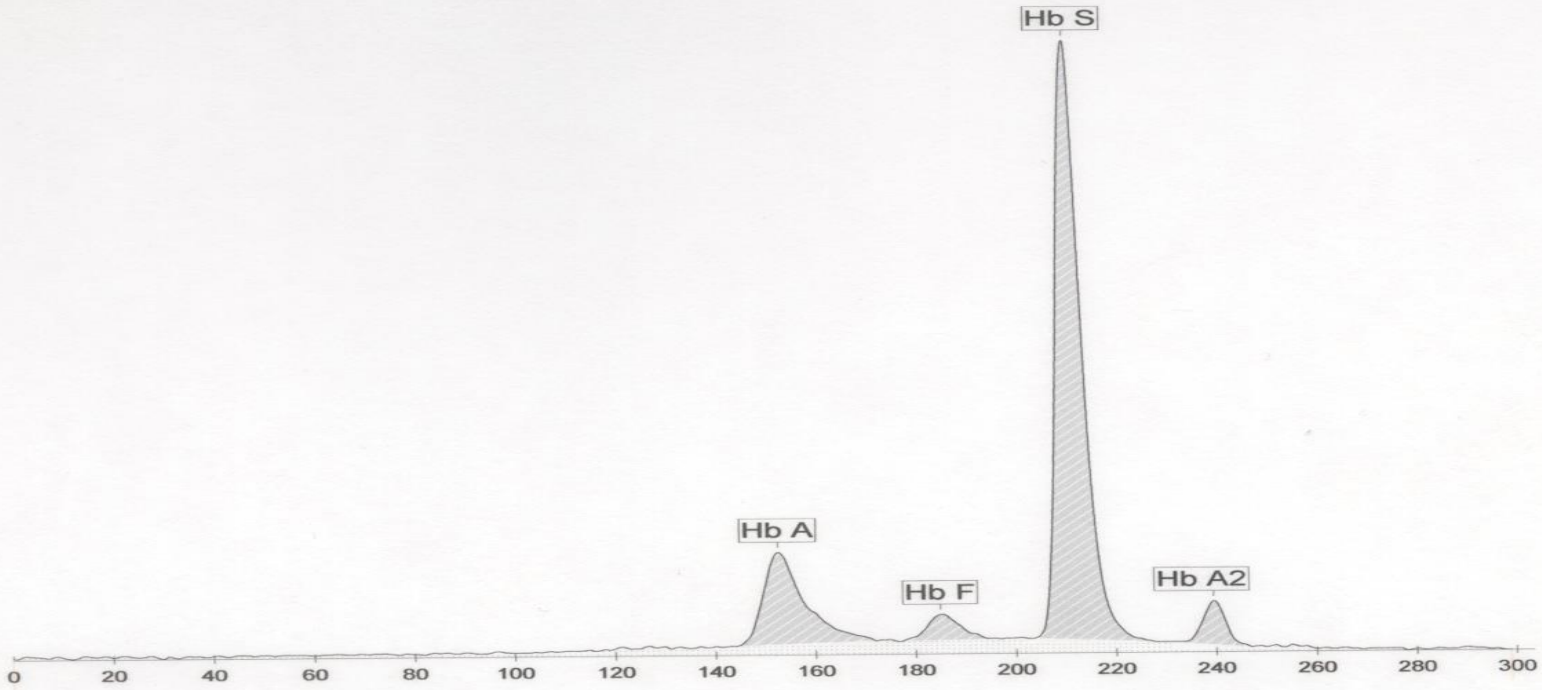
INSTRUMENT ID : KKUH : 24509

Hospital No.: **010755**

Sample No **19**

ID : **064209**

Date : **28/06/2010**



Fractions	%	Ref. %
Hb A	**18.0**	
Hb F	**4.0**	
Hb S	**73.3**	
Hb A2	**4.7**	

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Hb Electrophoresis

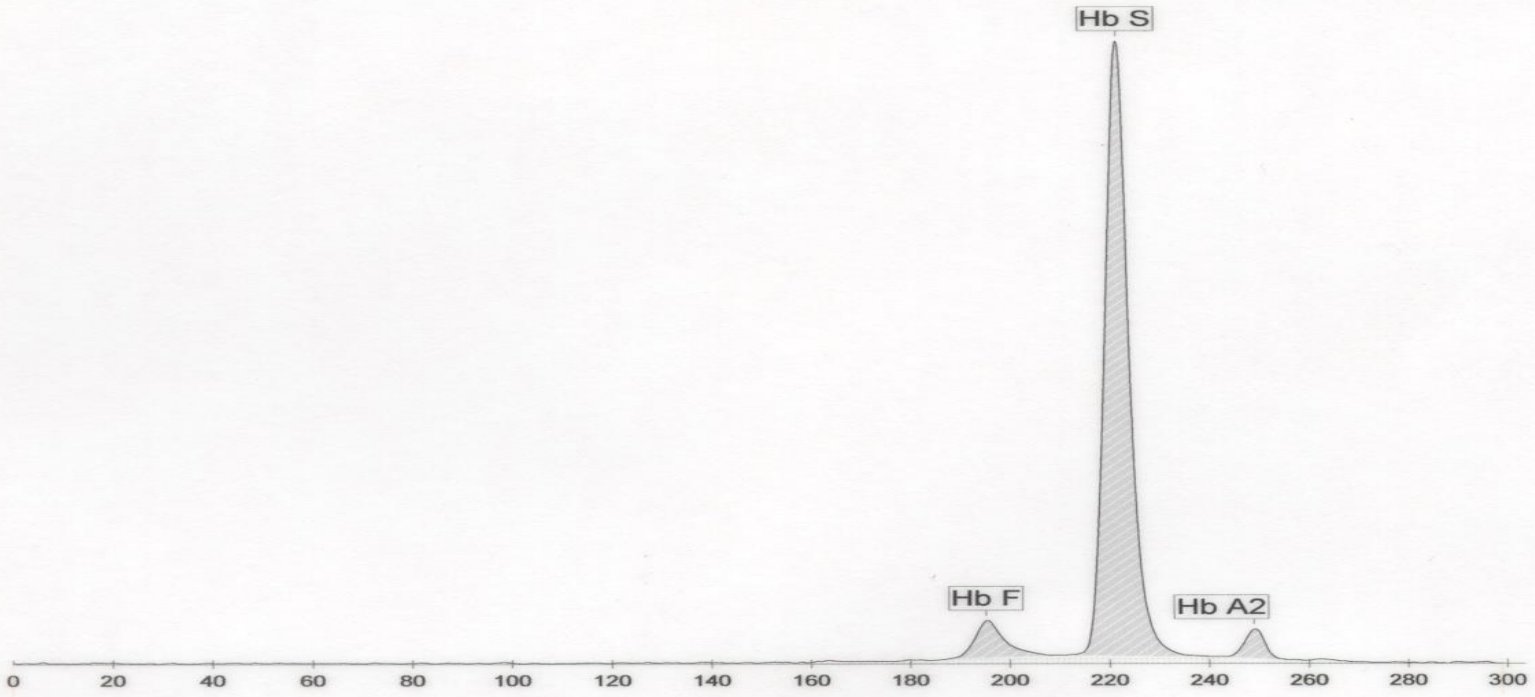
INSTRUMENT ID : KKUH : 24509

Hospital No.: 594729

Sample No 37

ID : **064199**

Date : **27/06/2010**



Fractions	%	Ref. %
Hb F	6.5	
Hb S	89.9	
Hb A2	3.6	

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Heamatology Unit

Hb Electrophoresis

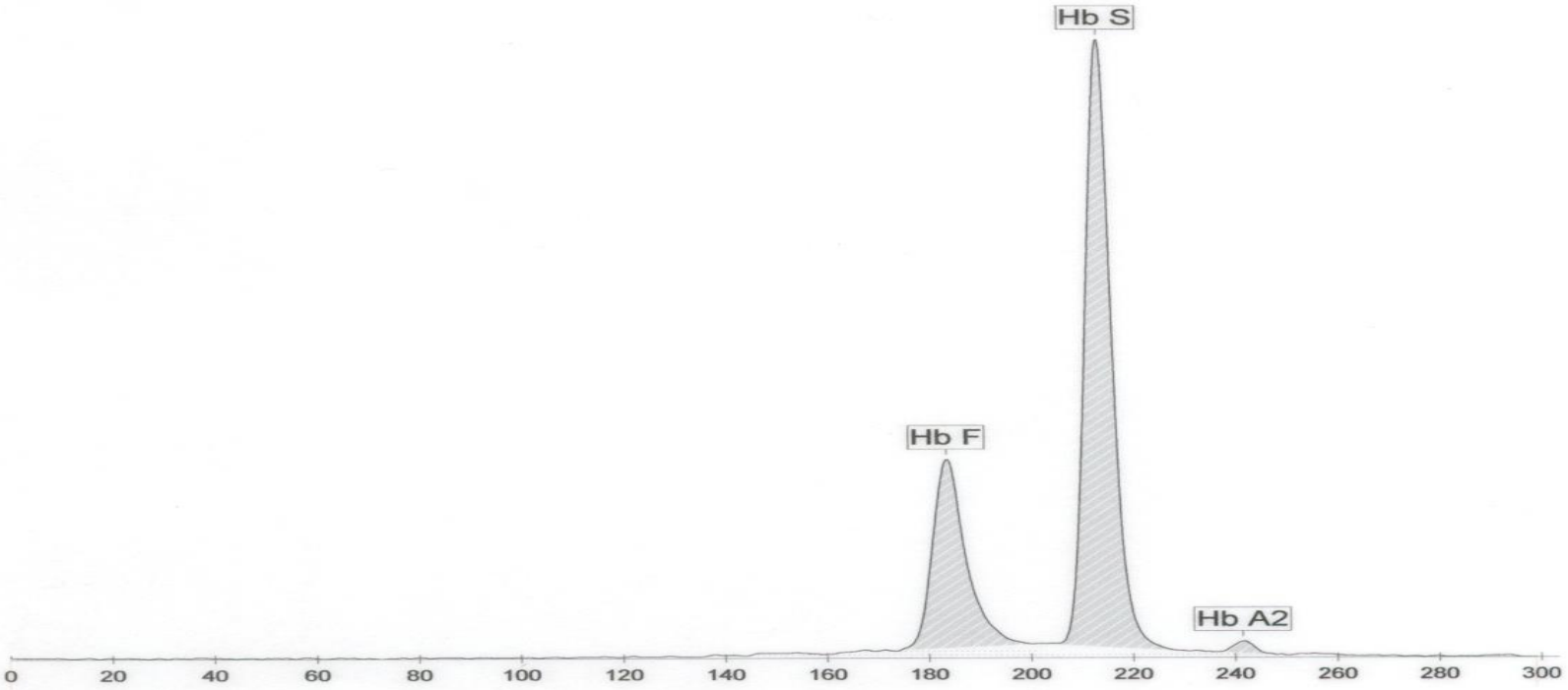
INSTRUMENT ID : KKUH : 24509

Hospital No.: 610043

Sample No 52

ID : 064229

Date : 29/06/2010



Fractions	%	Ref. %
Hb F	28.1	
Hb S	70.8	
Hb A2	1.1	

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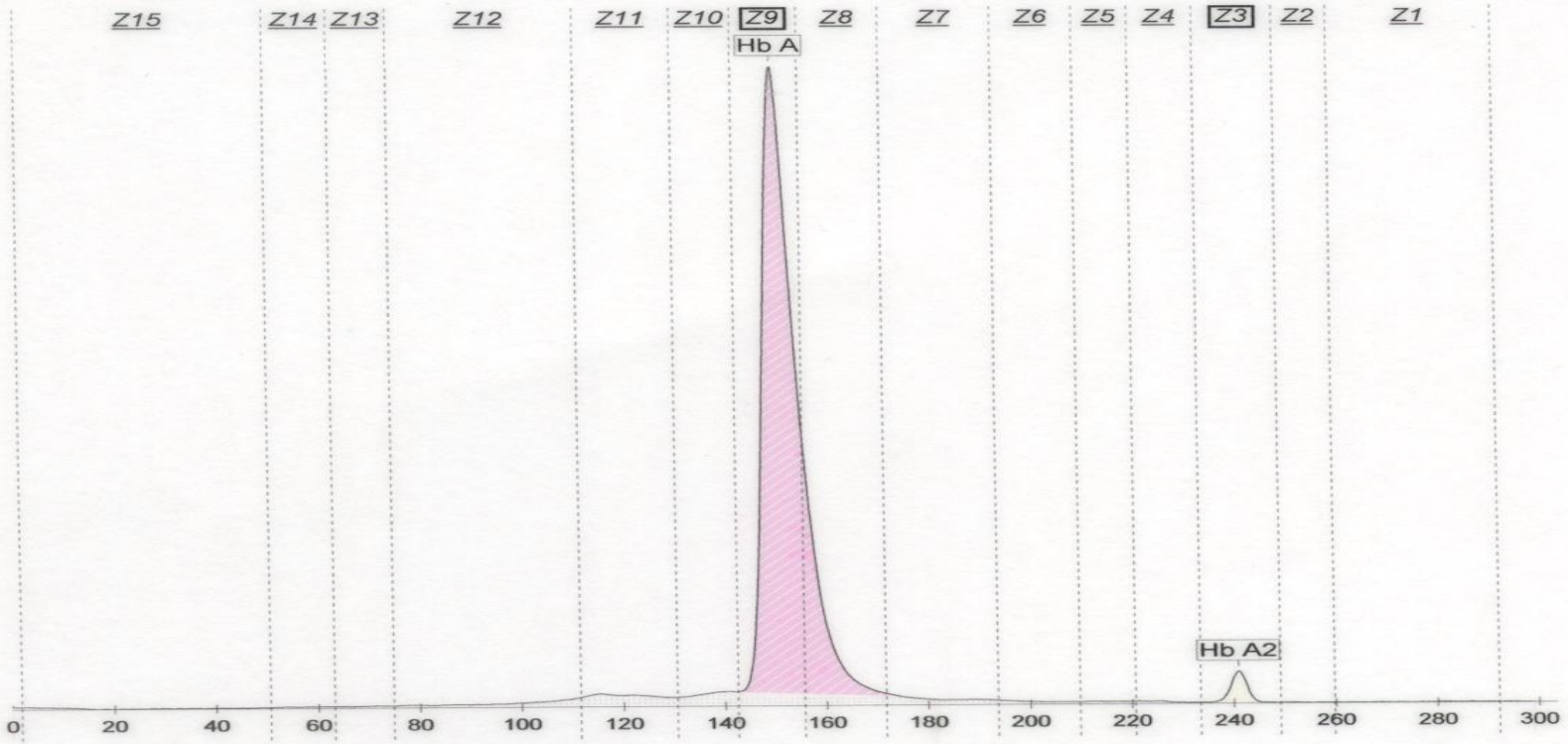
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: **Rack: SEBIA Pos.: 2** ID : **ABDULLAH**

Sample No **20** Date : **19/05/2010**



Fractions	%	Ref. %
Hb A	**97.7**	95.0 - 99.0
Hb A2	**2.3**	1.5 - 3.5

KKUH

Heamatology Unit

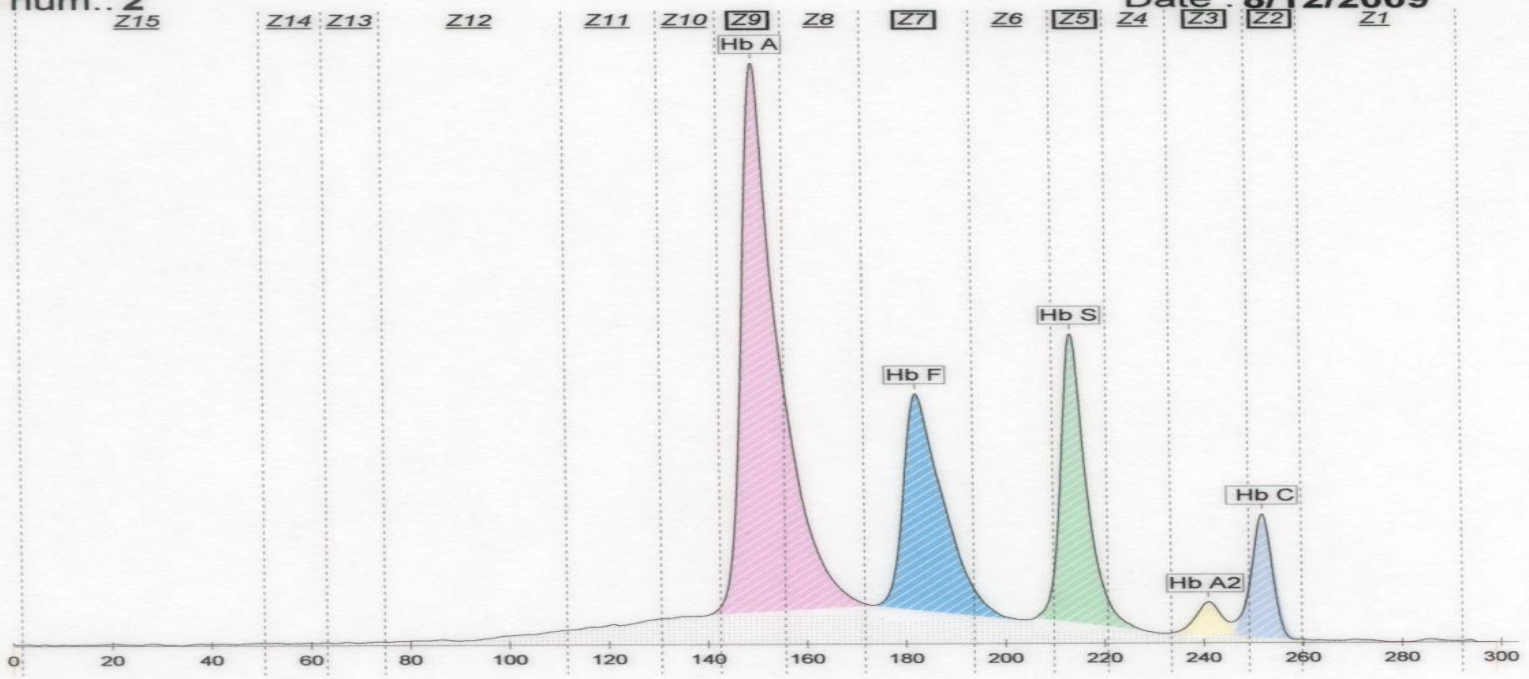
Hb Electrophoresis

Hospital No.: **QC Hb AFSC CONTROL-**

ID : **Hb AFSC CONTROL-2**

Sample num.: **2**

Date : **8/12/2009**



Hb Electrophoresis

Fractions	%	Ref. %
Hb A	**51.3**	46.7 - 56.9
Hb F	**21.4**	17.4 - 22.4
Hb S	**18.3**	17.3 - 22.3
Hb A2	**2.3**	2.1 - 3.3
Hb C	**6.7**	4.6 - 7.0

KKUH

Heamatology Unit

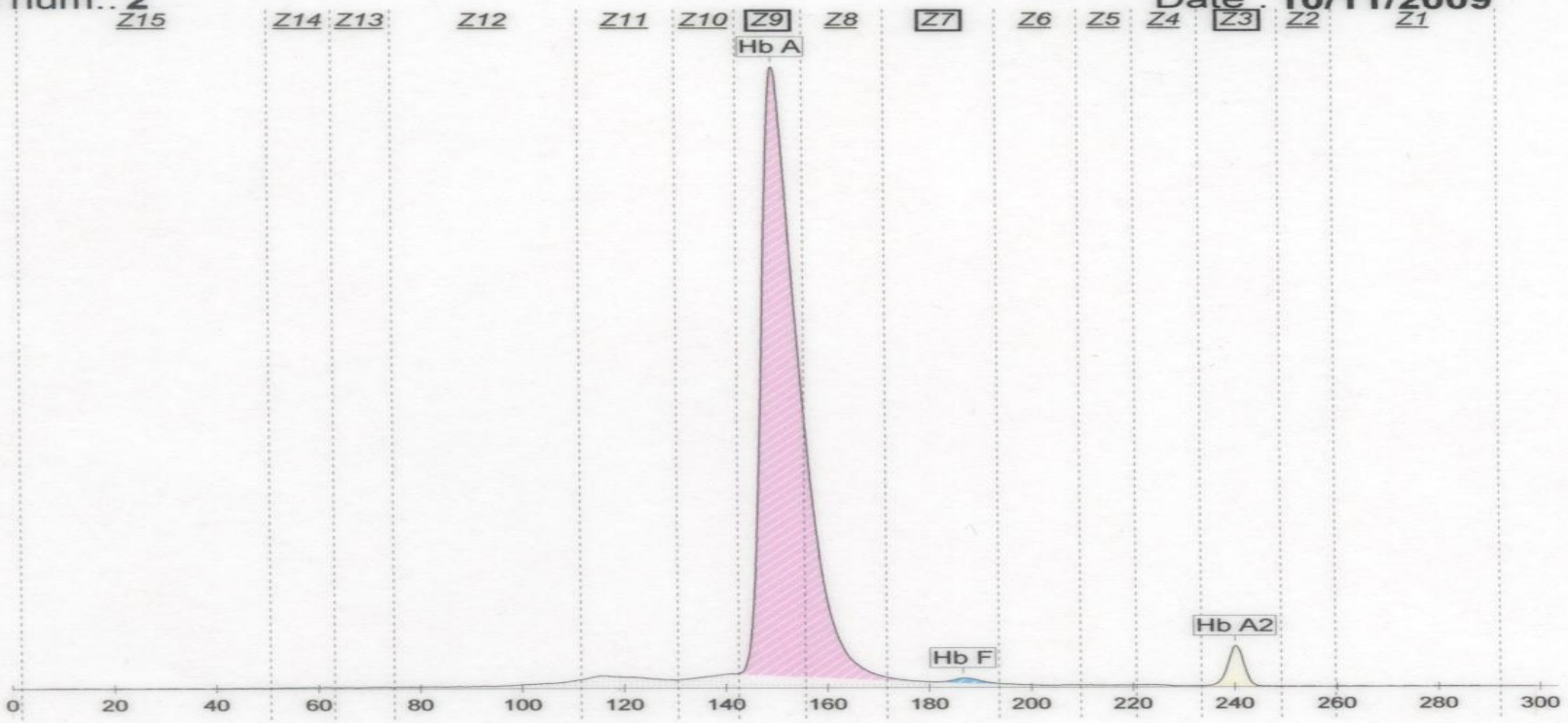
Hb Electrophoresis

Hospital No.: **933376**

ID : **061773**

Sample num.: **2**

Date : **10/11/2009**



Hb Electrophoresis

Fractions	%	Ref. %	
Hb A	**96.7**	96.8 - 97.8	<
Hb F	**0.5**	=< 2.0	
Hb A2	**2.8**	1.5 - 3.5	

KKUH

Heamatology Unit

Hb Electrophoresis

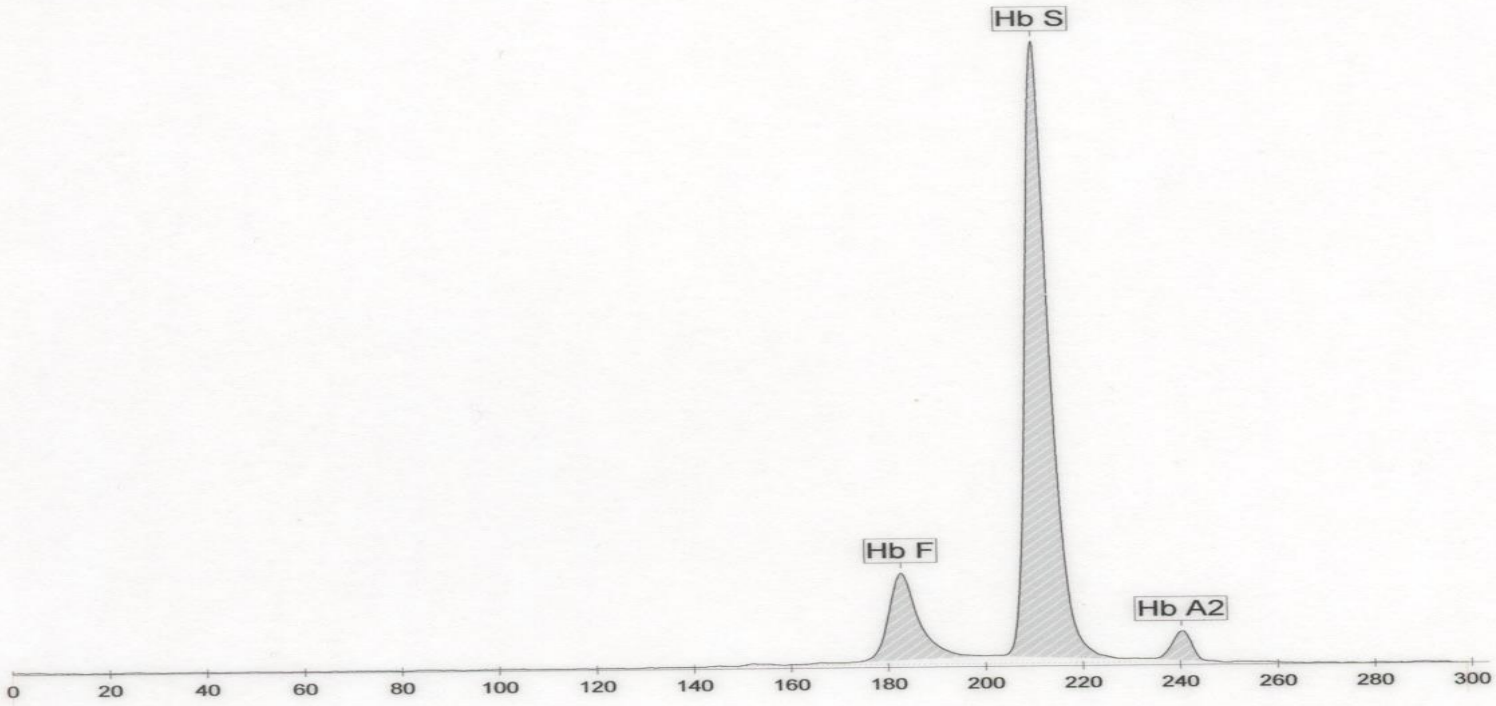
INSTRUMENT ID : KKUH : 24509

Hospital No.: **873506**

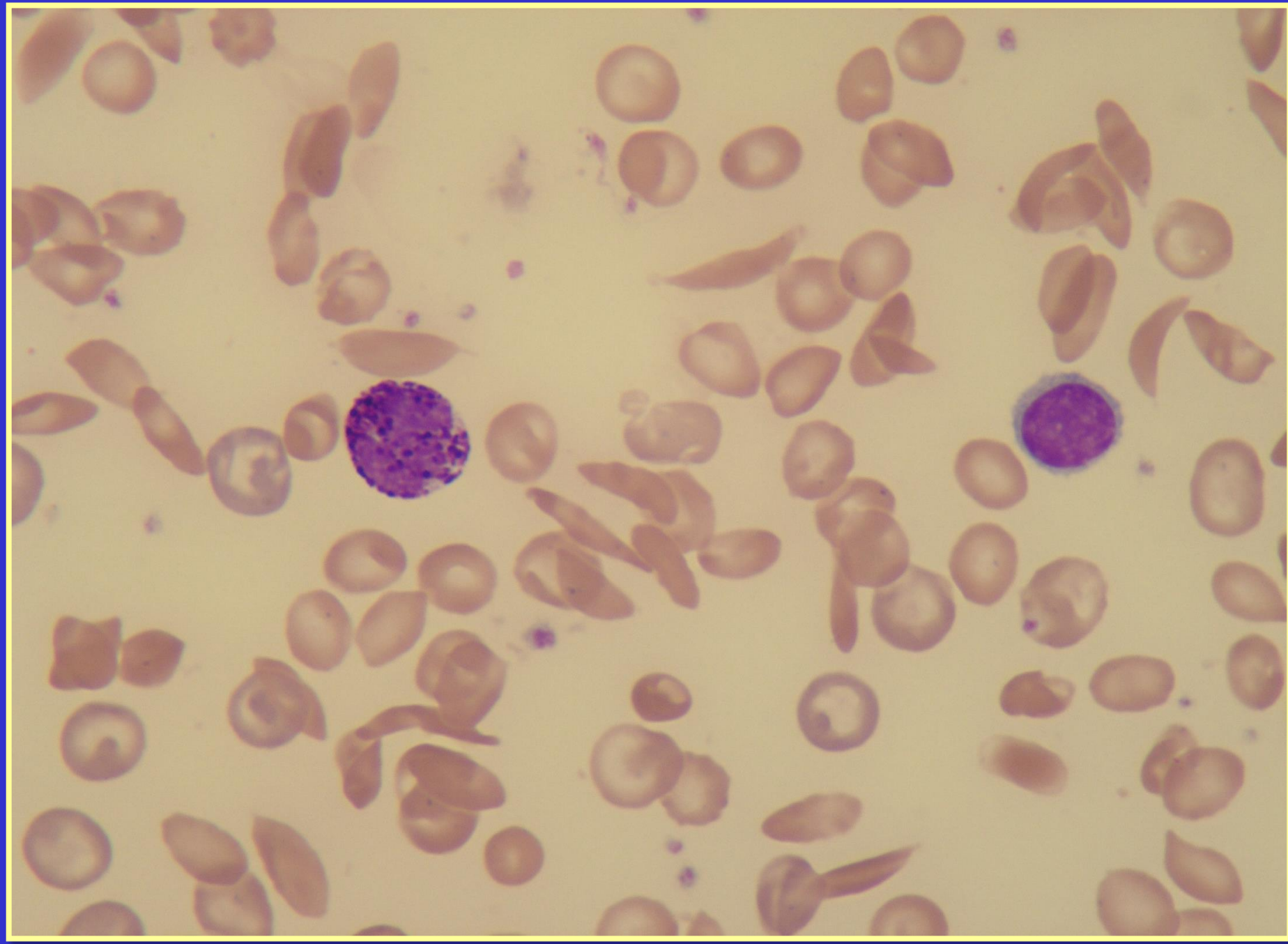
Sample No **53**

ID : **064230**

Date : **29/06/2010**



Fractions	%	Ref. %
Hb F	14.5	
Hb S	82.2	
Hb A2	3.3	



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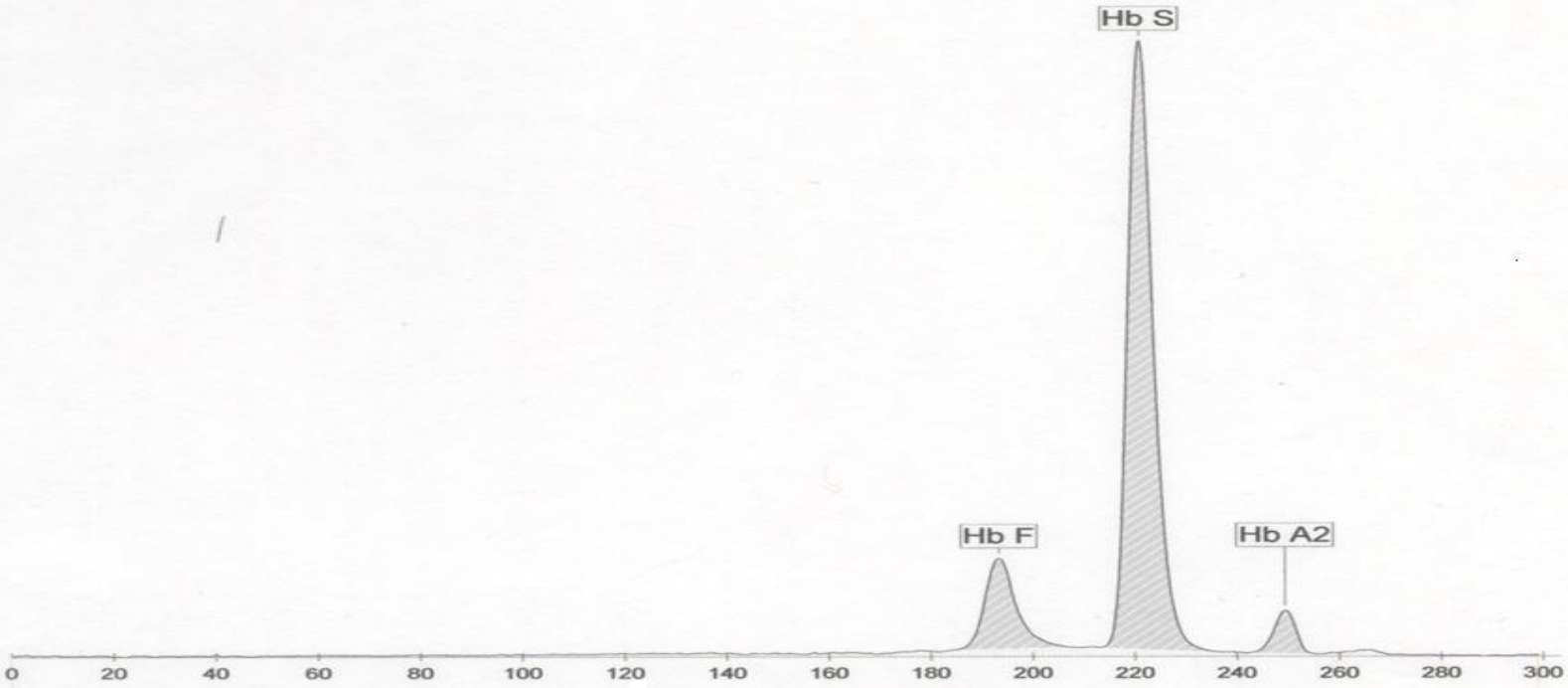
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: 233095 ID : 063478

Sample No 20 Date : 17/04/2010



Fractions	%	Ref. %
Hb F	14.7	
Hb S	80.5	
Hb A2	4.8	

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Heamatology Unit

Hb Electrophoresis

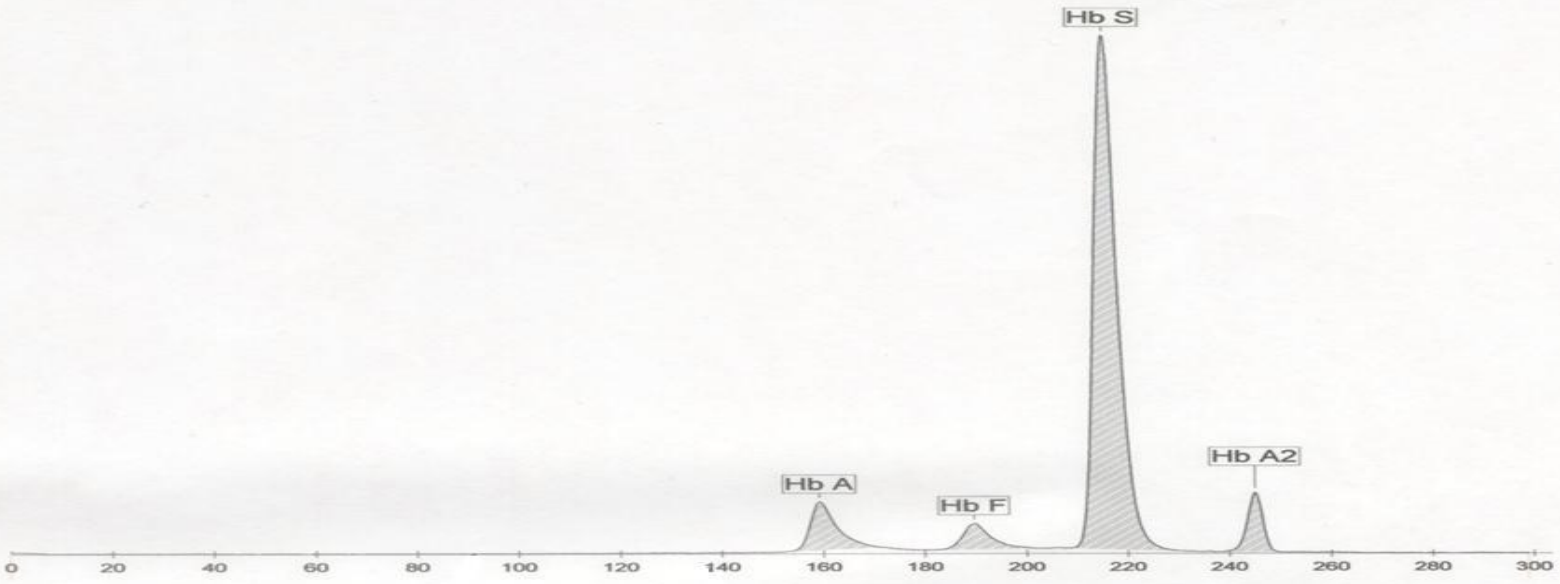
INSTRUMENT ID : KKUH : 24509

Hospital No.: 913628

Sample No 34

ID : 063511

Date : 19/04/2010



Fractions	%	Ref. %
Hb A	8.7	
Hb F	4.9	
Hb S	80.1	
Hb A2	6.3	

Comment :

KKUH

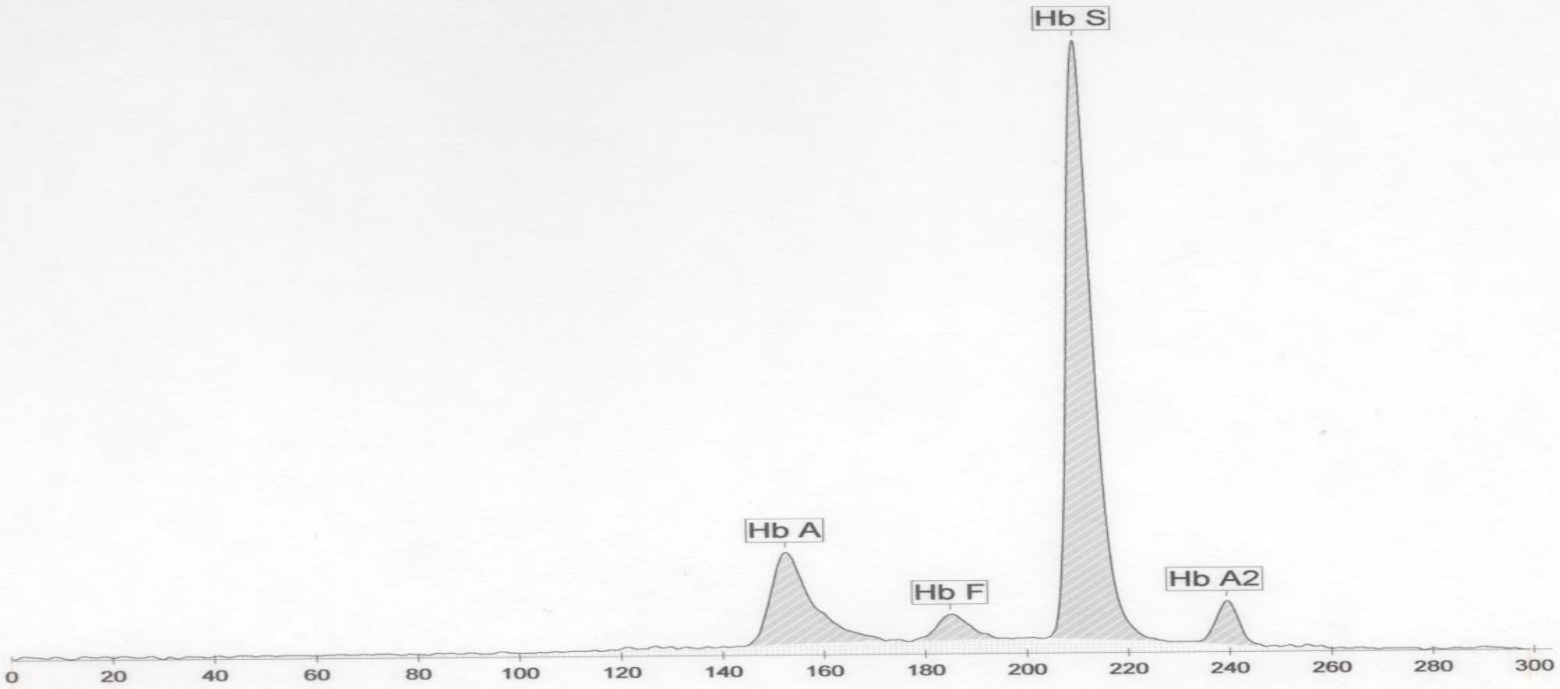
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: 010755 ID : 064209

Sample No 19 Date : 28/06/2010



Fractions	%	Ref. %
Hb A	18.0	
Hb F	4.0	
Hb S	73.3	
Hb A2	4.7	

KKUH

Heamatology Unit

Hb Electrophoresis

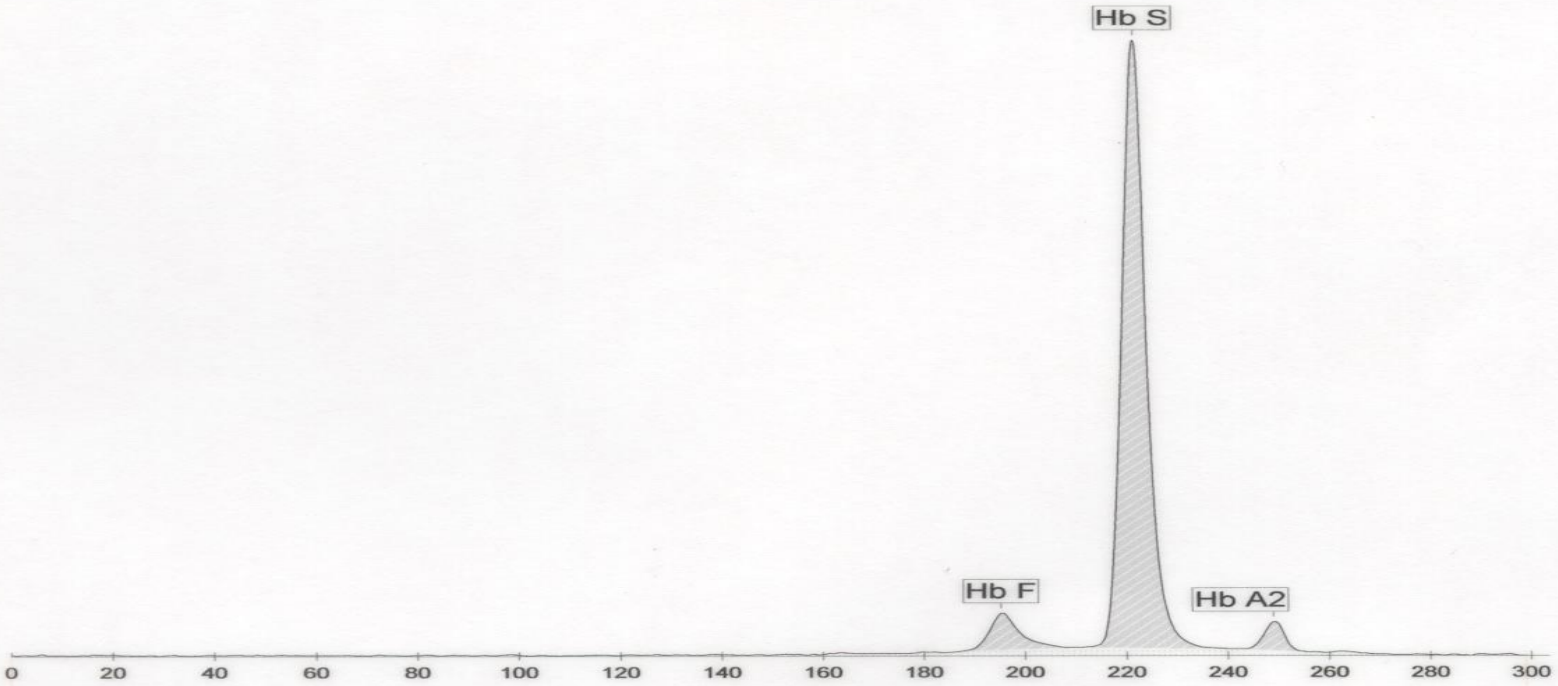
INSTRUMENT ID : KKUH : 24509

Hospital No.: 594729

Sample No 37

ID : **064199**

Date : **27/06/2010**



Fractions	%	Ref. %
Hb F	**6.5**	
Hb S	**89.9**	
Hb A2	**3.6**	

KKUH

Heamatology Unit

Hb Electrophoresis

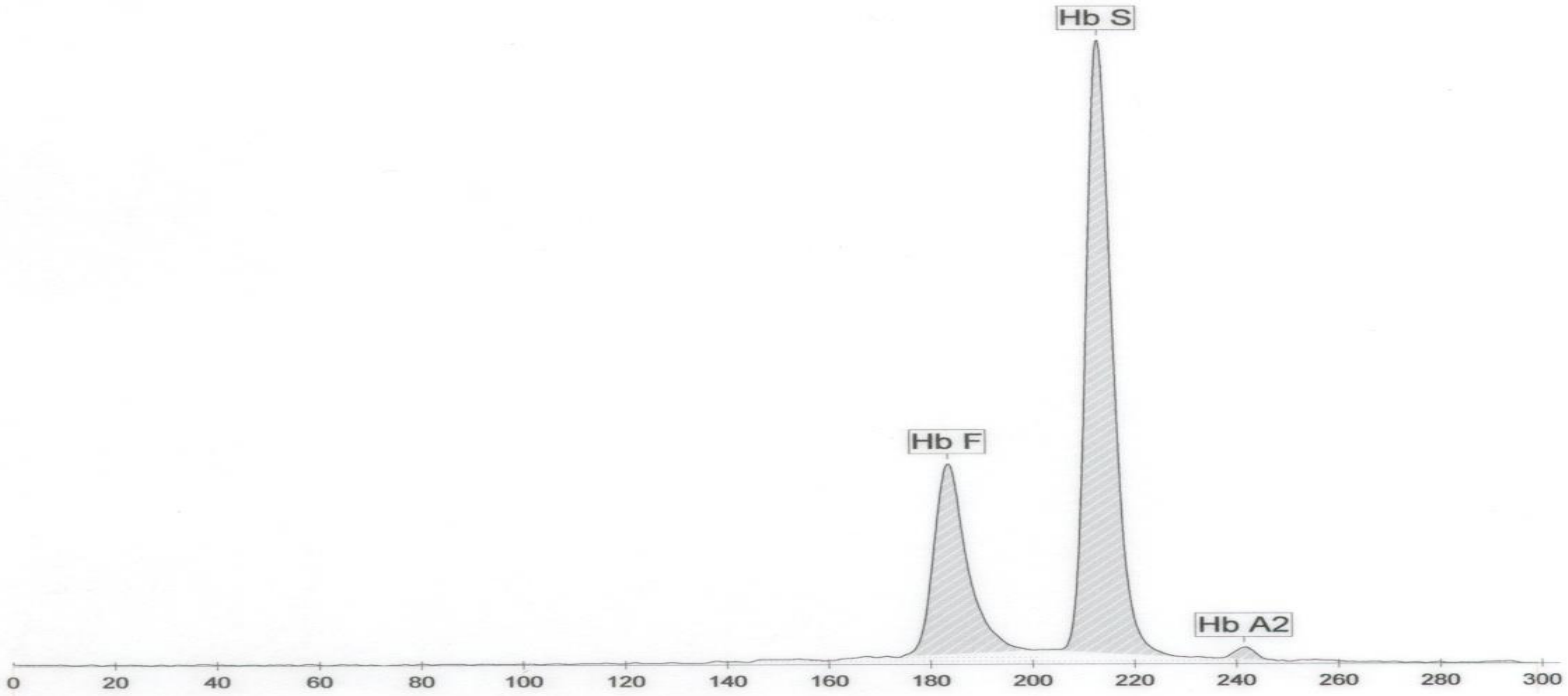
INSTRUMENT ID : KKUH : 24509

Hospital No.: 610043

Sample No 52

ID : 064229

Date : 29/06/2010



Fractions	%	Ref. %
Hb F	28.1	
Hb S	70.8	
Hb A2	1.1	

KKUH

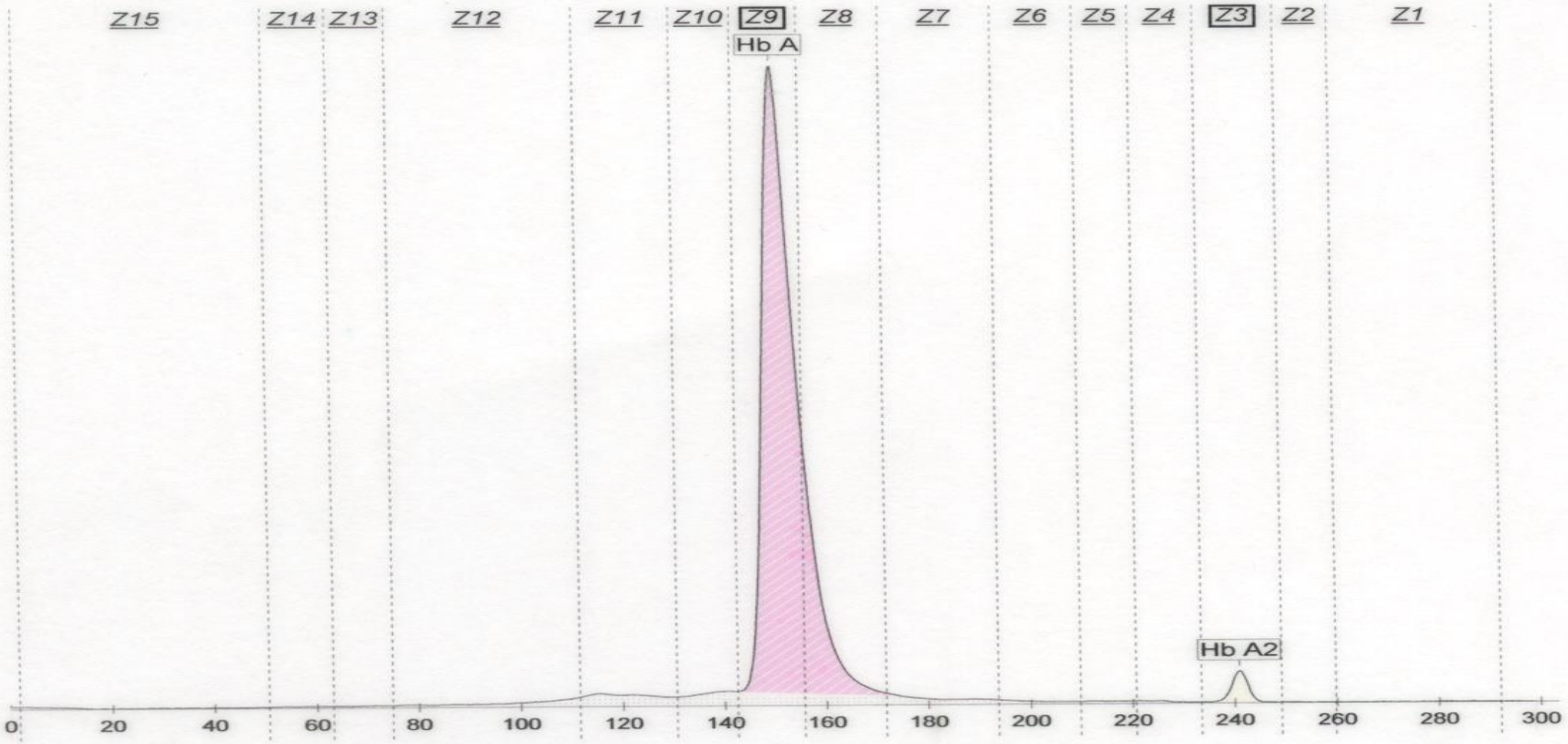
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: **Rack: SEBIA Pos.: 2** ID : **ABDULLAH**

Sample No **20** Date : **19/05/2010**



Fractions	%	Ref. %
Hb A	**97.7**	95.0 - 99.0
Hb A2	**2.3**	1.5 - 3.5

KKUH

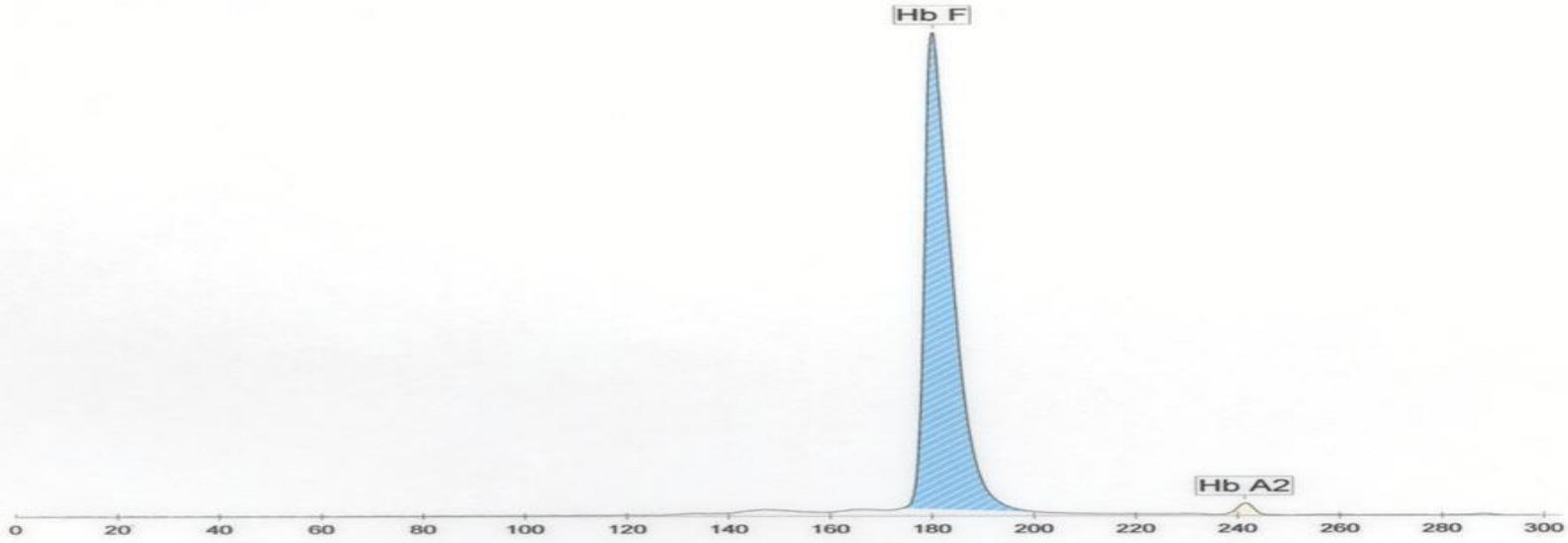
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: 921107 ID : 063761

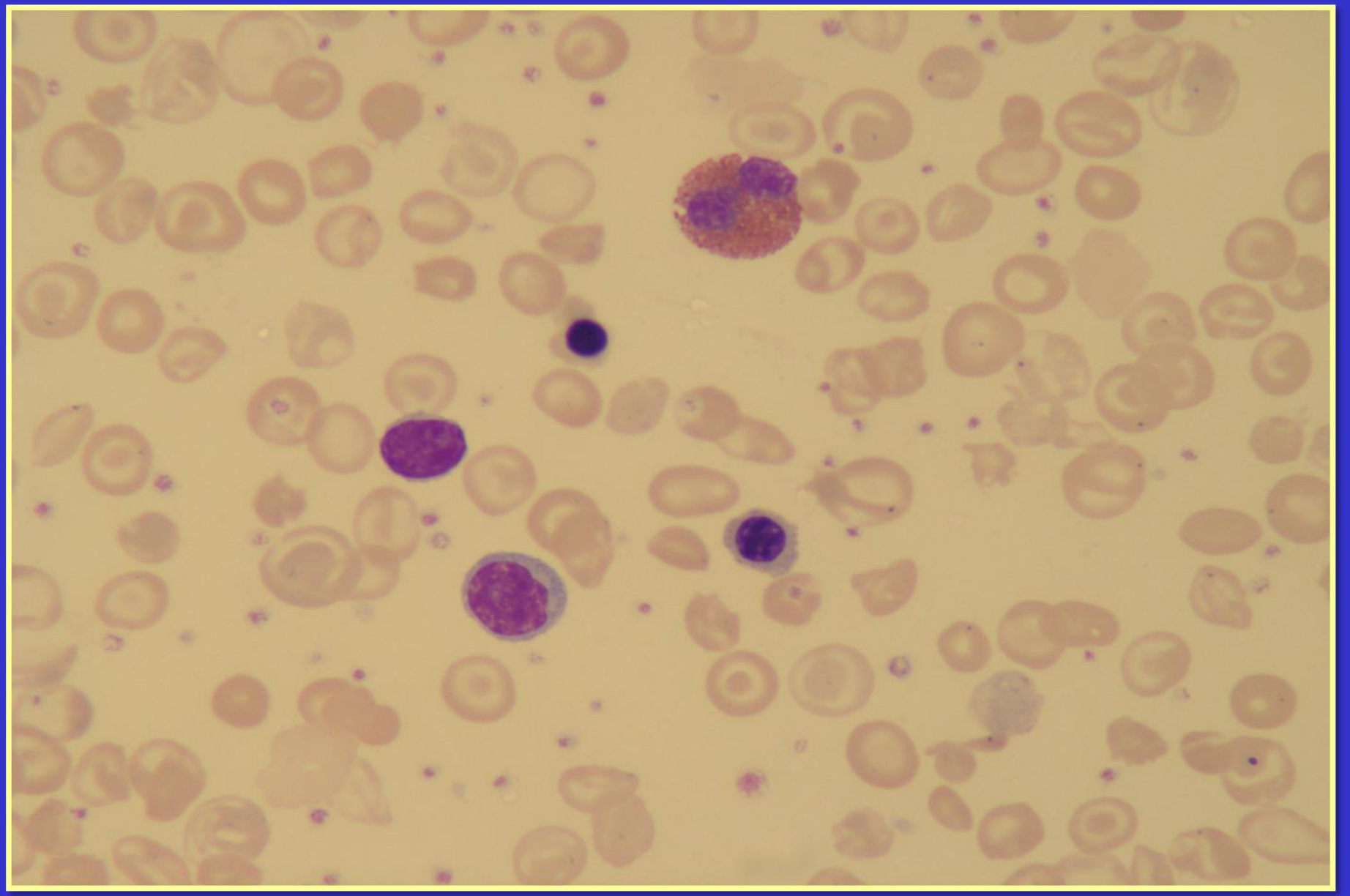
Sample No 54 Date : 09/05/2010



Fractions	%	Ref. %
Hb F	98.5	
Hb A2	1.5	

Comment :

Beta Thalassaemia Major




Thank you!
TESCHIO 99