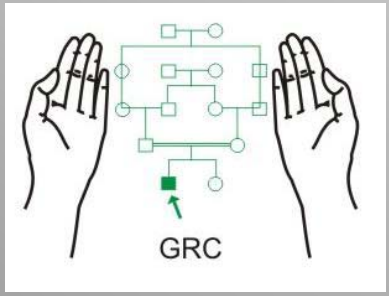
Red Cell Indices in Genetic Haemoglobin Disorders

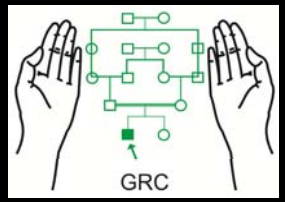
Maj Gen (R) Suhaib Ahmed, HI (M)
MBBS; MCPS; FCPS (Pak); PhD (London)

Genetics Resource Centre (GRC)



www.grcpk.com

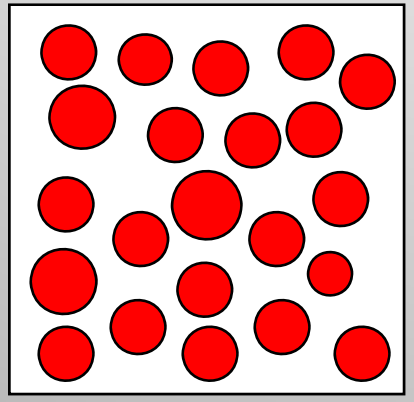
Red Cell Indices

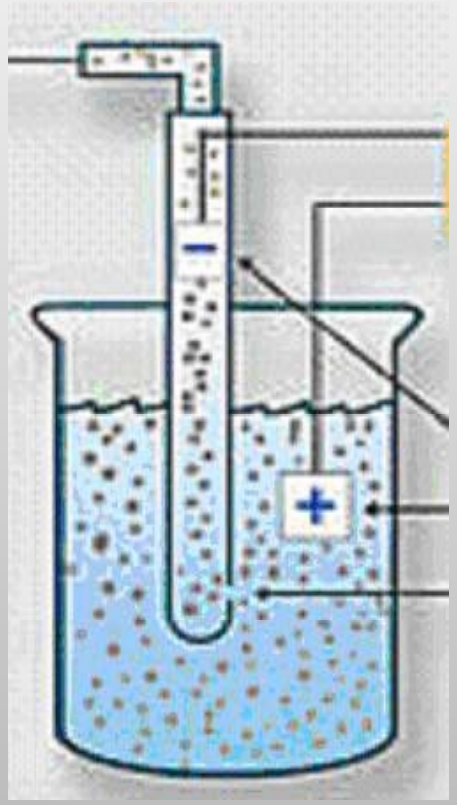


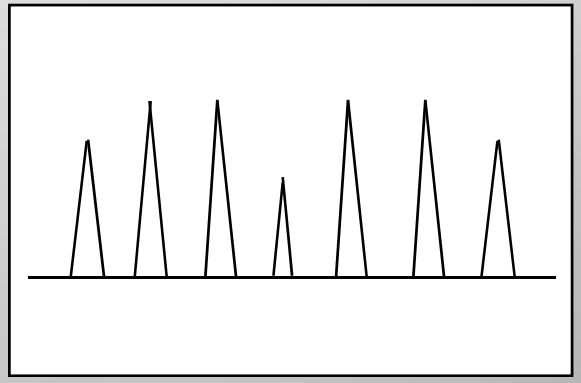
- TRBC
- MCV
- MCH
- MCHC
- RDW

No.		1613
Date		28/02/11 13:21
Mode		WB
WBC		9.0×10^9/L
RBC		5.00×10^{12}/L
HGB		14.5g/dL
HCT		0.427
MCV		85.4fL
MCH		29.0pg
MCHC		34.0g/dL
PLT		289×10^9/L
LYM%		33.4%
MXD%		8.4%
NEUT%		58.2%
LYM#		3.0×10^9/L
MXD#		0.8×10^9/L
NEUT#		5.2×10^9/L
RDW		51.4fL
PDW	-	8.8fL
MPV	-	7.8fL
P-LCR	-	10.3%

Blood Cell Counting
(Electrical Impedance Method)

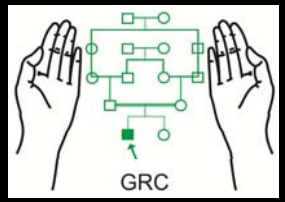






TRBC, MCV, RDW

Genetic Haemoglobin Disorders



Thalassaemia Syndromes

α-Thalassaemias:

- α^{o}-Thalassaemias
- α^{+}-Thalassaemias
  - Deletion
  - Non-deletion
- With α-chain Hb variants
- With β-chain Hb variants
- With β-Thalassaemia

β-Thalassaemias:

- β^{o}-Thalassaemias
- β^{+}-Thalassaemias
- With β-chain Hb variants
- With α-chain Hb variants
- With α-Thalassaemia

δβ-Thalassaemia:

- $(\delta\beta)^{o}$-Thalassaemia
- $({}^{A}\gamma\delta\beta)^{o}$-Thalassaemia
- $(\varepsilon\gamma\delta\beta)^{o}$-Thalassaemia
- δ-Thalassaemia
- γ-Thalassaemia

Hereditary Persistence of Fetal Haemoglobin (HPFH):

Structural Hb Variants

Sickling Disorders:

- With β-Thalassaemia
- With α-chain Hb variants
- With β-chain Hb variants

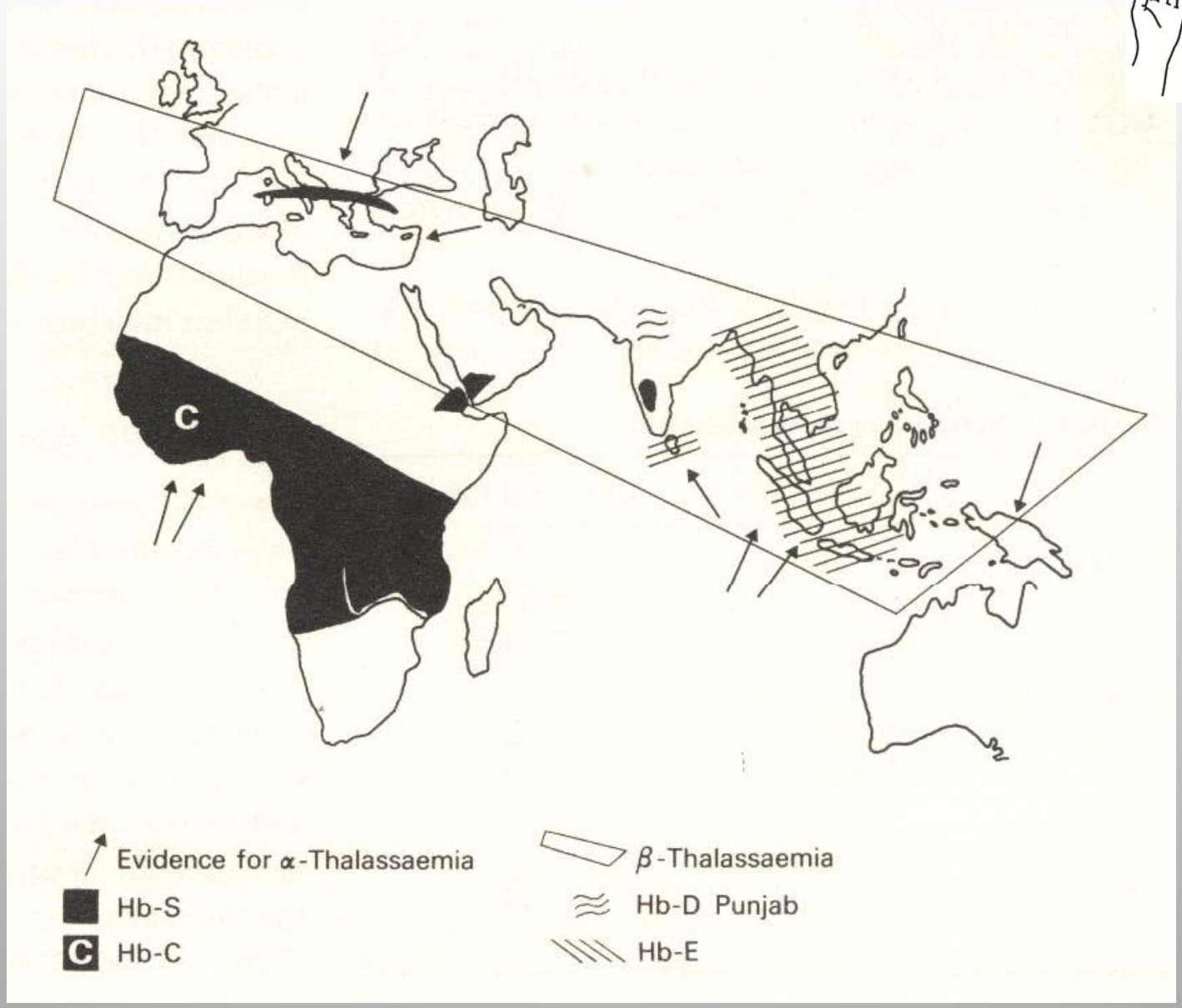
Other Structural Variants (Hb-E, C, D etc.):

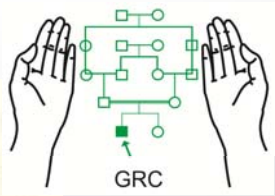
- With β-Thalassaemia
- With α-Thalassaemia

Unstable Haemoglobins:

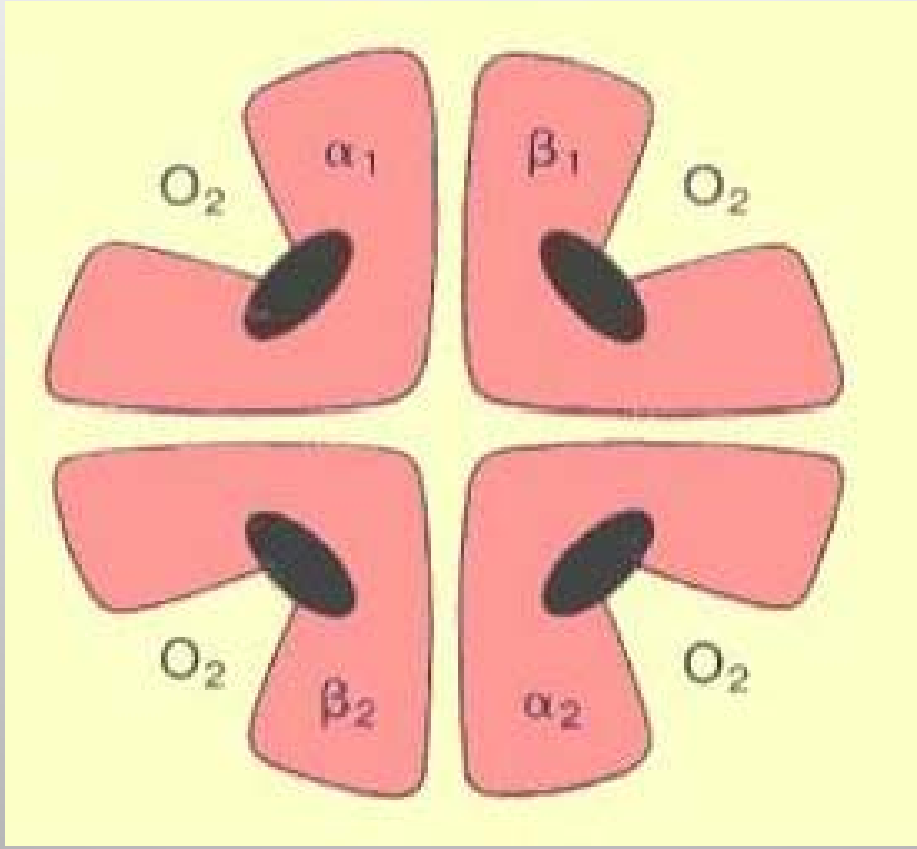
M Haemoglobins:

Altered Affinity Haemoglobins:

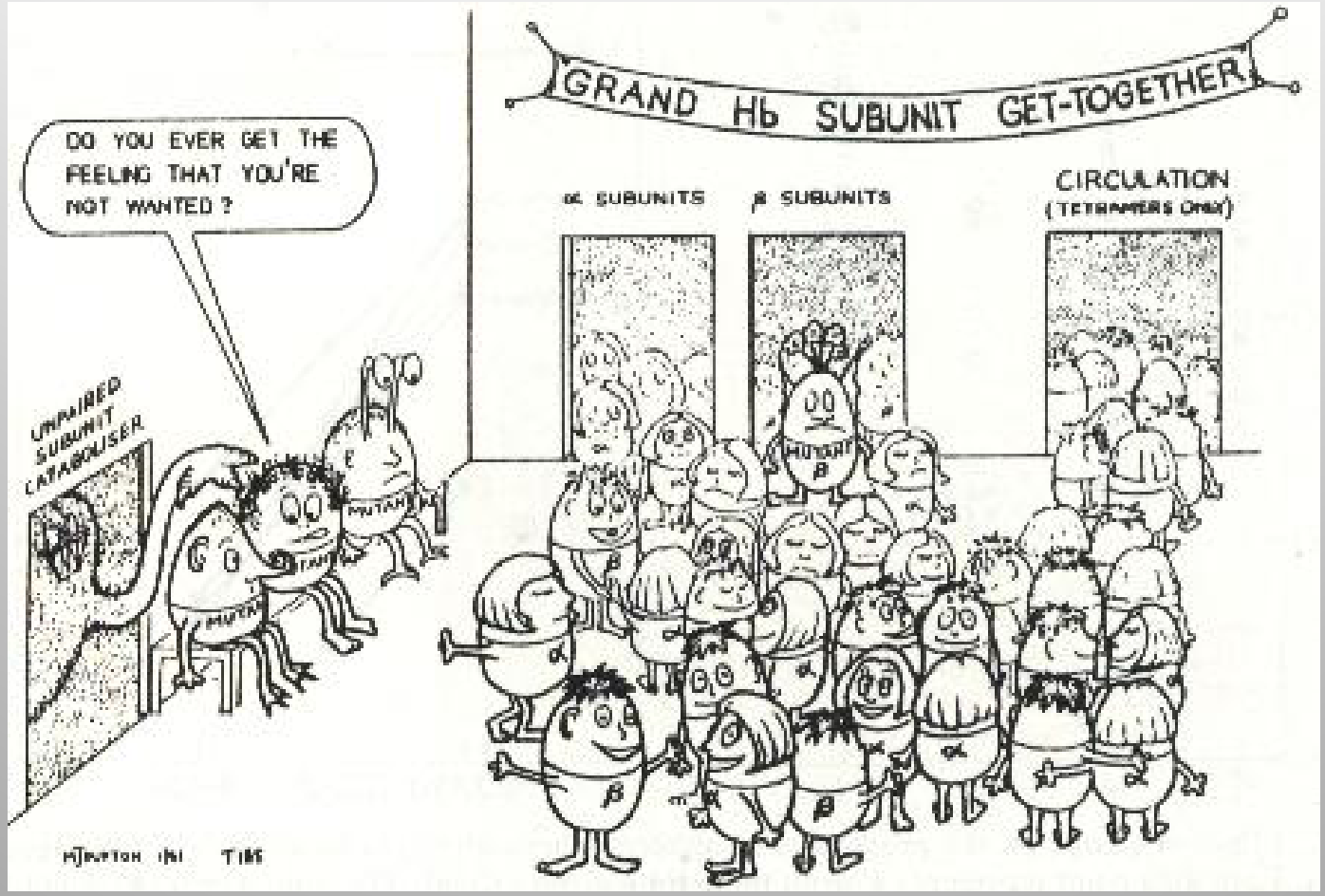

C
Evidence for α-Thalassaemia
Hb-S
C Hb-C
β-Thalassaemia
Hb-D Punjab
Hb-E


GRC

Haemoglobin

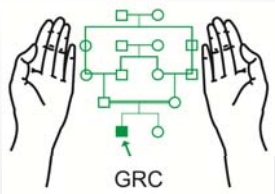


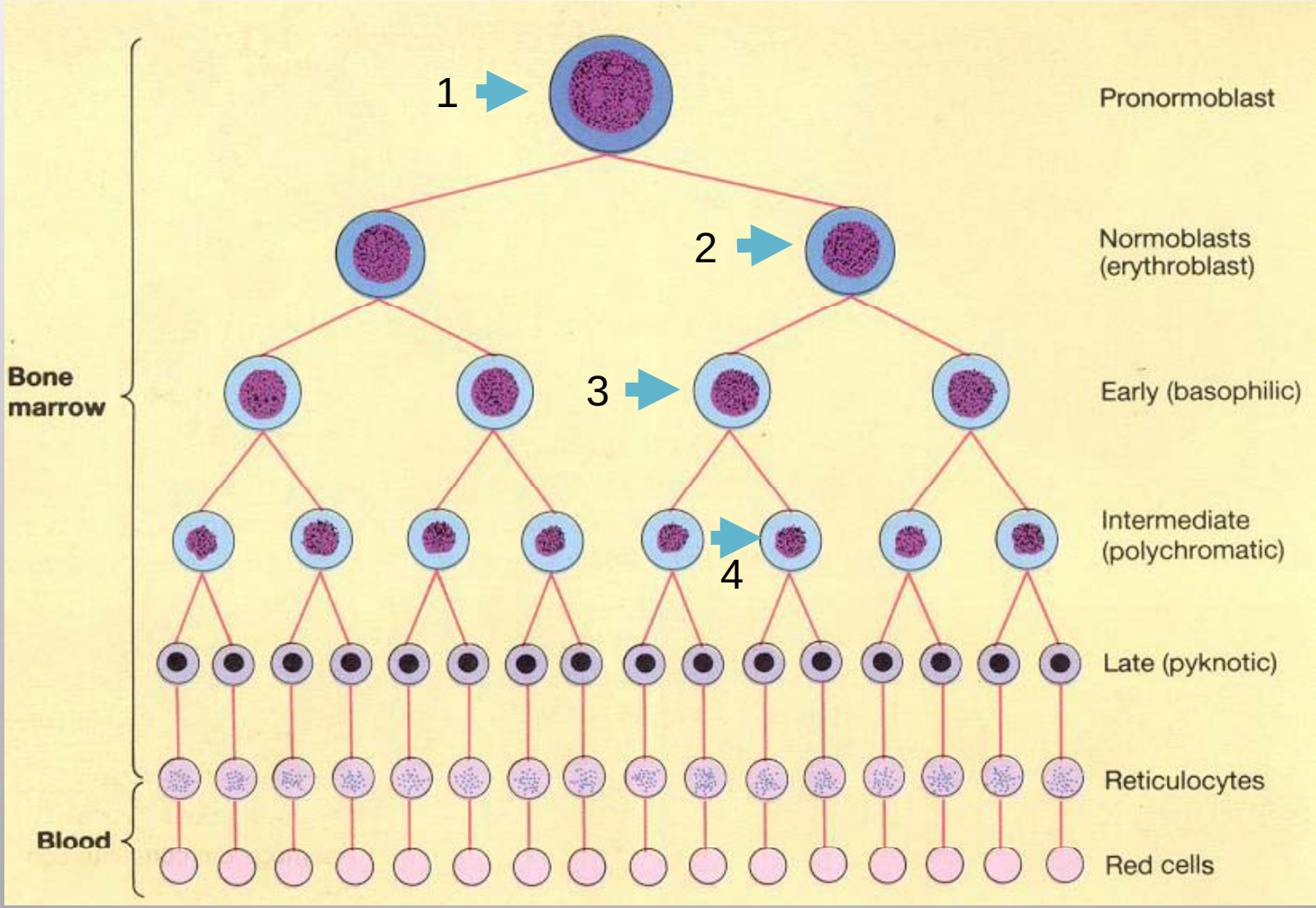
Haemoglobin Synthesis

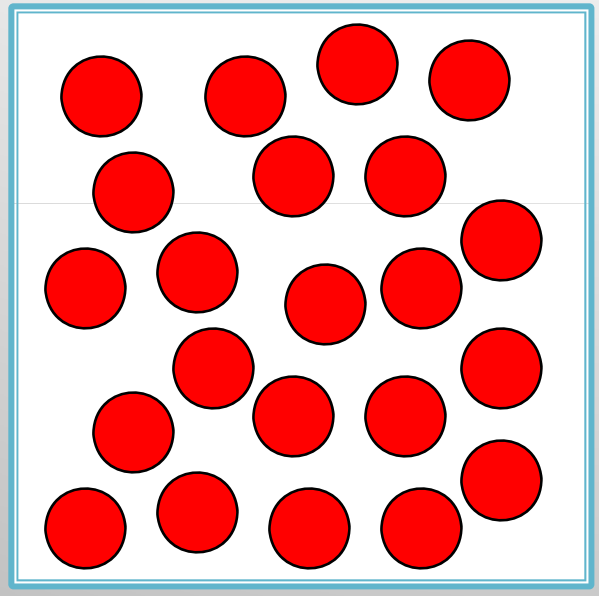


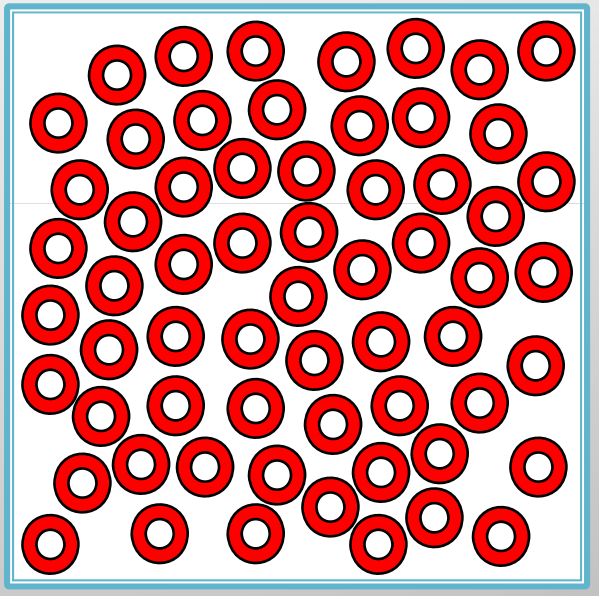
Thalassaemia

Poor Haemoglobinization of Red Cells


GRC


1
2
3
4
Pronormoblast
Normoblasts
(erythroblast)
Early (basophilic)
Intermediate
(polychromatic)
Late (pyknotic)
Reticulocytes
Red cells
Bone
marrow
Blood

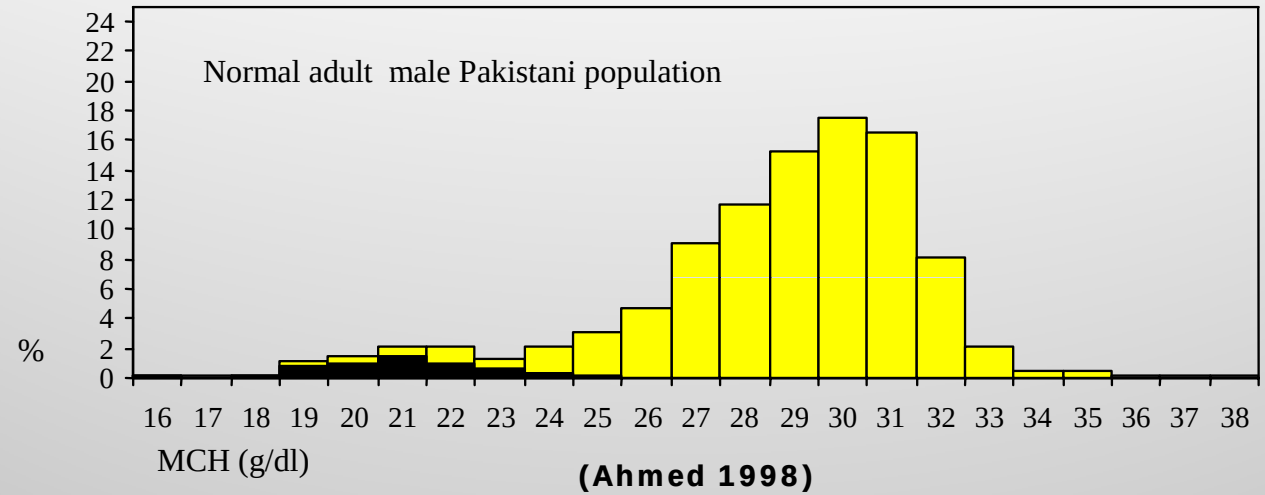




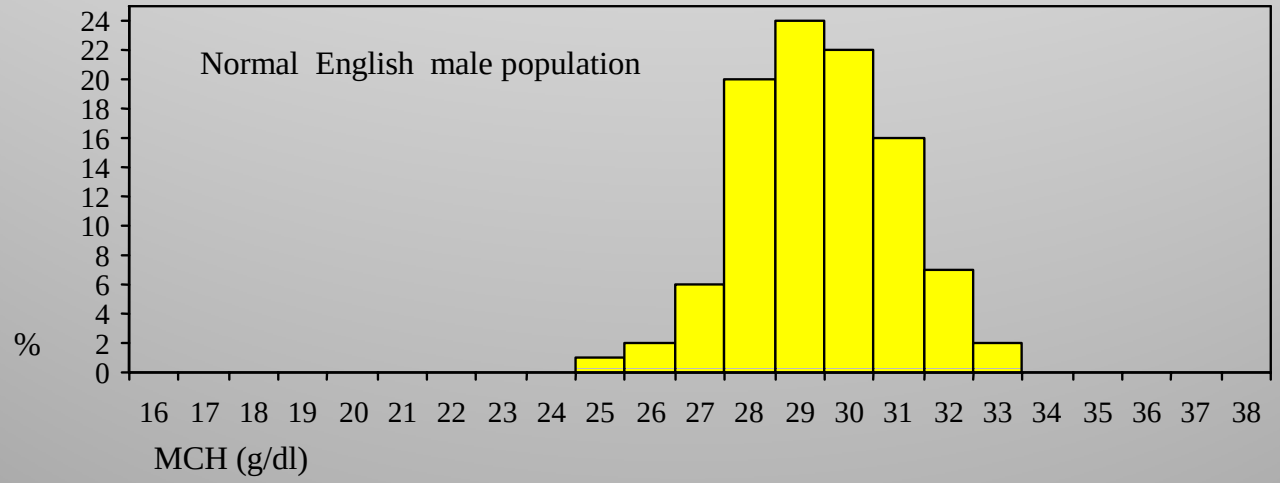
Is it possible to screen for thalassaemia by red cell indices?

Yes but….

MCV in the Population

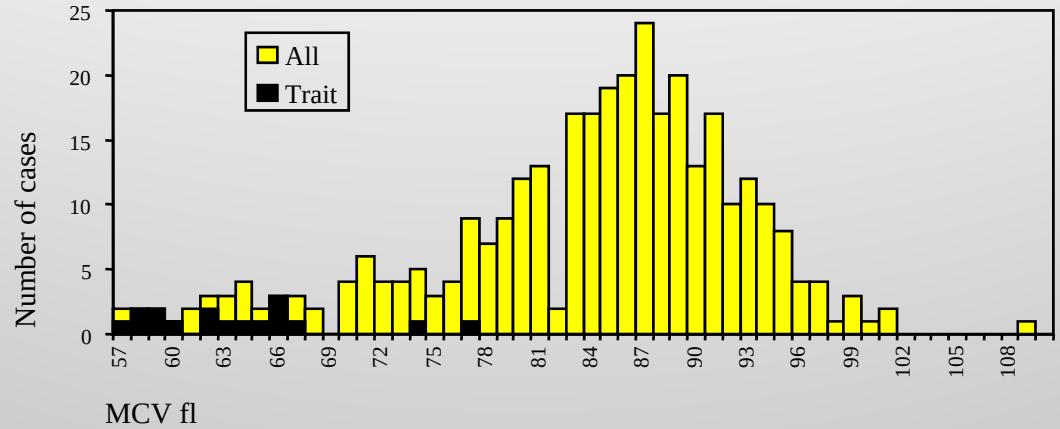


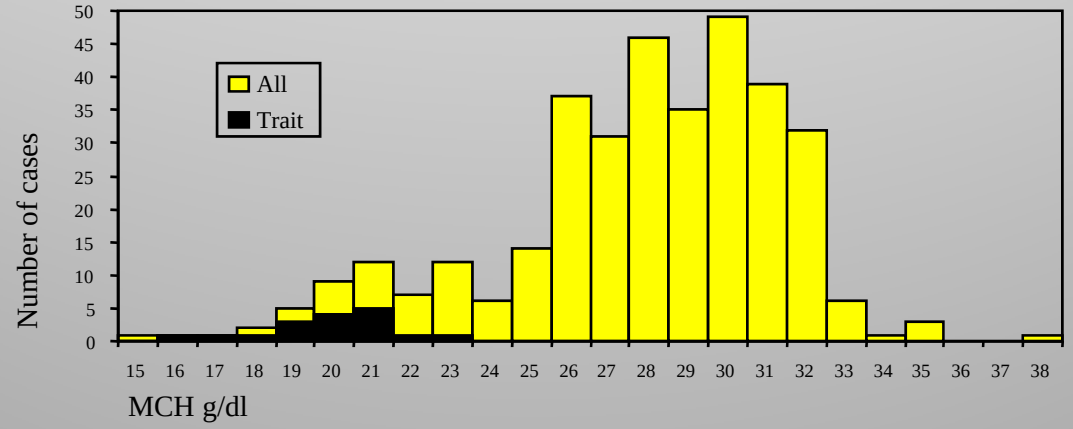
(Ahmed 1998)



(Modell & Berdukas 1984)

MCV & MCH in the population

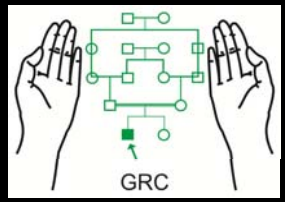




(S. Ahmed 1998)

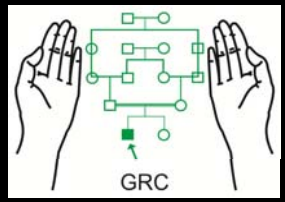
Thalassaemia & RBC Indices

Index	Formula	BTT	IDA
England & Fraser	MCV-(5xHb)-RBC	<0 (neg)	>0 (pos)
Mentzer	MCV/RBC count	<13.0	>13.0
Srivastava	MCH/RBC count	<3.8	>3.8
Shine & Lal	MCVxMCVxMCH/100	<1530	>1530
Green & King	MCVxMCVxRDW/(Hbx100)	<72	>72
Ricerca	RDW/RBC count	<3.3	>3.3
RDWI	MCVxRDW/RBC	<220	>220



No.		1613
Date		28/02/11 13:21
Mode		WB
WBC		9.0×10⁹/L
RBC		5.00×10¹²/L
HGB		14.5g/dL
HCT		0.427
MCV		85.4fL
MCH		29.0pg
MCHC		34.0g/dL
PLT		289×10⁹/L
LYM%		33.4%
MXD%		8.4%
NEUT%		58.2%
LYM#		3.0×10⁹/L
MXD#		0.8×10⁹/L
NEUT#		5.2×10⁹/L
RDW		51.4fL
PDW	-	8.8fL
MPV	-	7.8fL
P-LCR	-	10.3%

No.		1452
Date		28/02/11 13:06
Mode		WB
WBC		12.9×10⁹/L
RBC		4.87×10¹²/L
HGB		10.1g/dL
HCT		0.322
MCV	-	66.1fL
MCH	-	20.7pg
MCHC		31.4g/dL
PLT	+	645×10⁹/L
LYM%		7.6%
MXD%		7.2%
NEUT%		85.2%
LYM#		1.0×10⁹/L
MXD#		0.9×10⁹/L
NEUT#		11.0×10⁹/L
RDW		53.6fL
PDW		10.3fL
MPV	-	8.6fL
P-LCR		17.3%



No.	8849
Date	28/02/11 13:05
Mode	WB
WBC	6.6×10⁹/L
RBC	+ 7.00×10¹²/L
HGB	13.5g/dL
HCT	0.449
MCV	- 64.1fL
MCH	- 19.3pg
MCHC	- 30.1g/dL
PLT	222×10⁹/L
LYM%	28.7%
MXD%	2.9%
NEUT%	68.4%
LYM#	1.9×10⁹/L
MXD#	0.2×10⁹/L
NEUT#	4.5×10⁹/L
RDW	38.5fL
PDW	14.8fL
MPV	10.5fL
P-LCR	32.3%

No.	8850
Date	28/02/11 13:07
Mode	WB
WBC	10.8×10⁹/L
RBC	5.07×10¹²/L
HGB	9.8g/dL
HCT	0.325
MCV	- 64.1fL
MCH	- 19.3pg
MCHC	- 30.2g/dL
PLT	237×10⁹/L
LYM%	24.5%
MXD%	5.4%
NEUT%	70.1%
LYM#	2.6×10⁹/L
MXD#	0.6×10⁹/L
NEUT#	7.6×10⁹/L
RDW	38.3fL
PDW	16.5fL
MPV	10.9fL
P-LCR	34.4%

No.	1452
Date	28/02/11 13:06
Mode	WB
WBC	12.9×10⁹/L
RBC	4.87×10¹²/L
HGB	10.1g/dL
HCT	0.322
MCV	- 66.1fL
MCH	- 20.7pg
MCHC	31.4g/dL
PLT	+ 645×10⁹/L
LYM%	7.6%
MXD%	7.2%
NEUT%	85.2%
LYM#	1.0×10⁹/L
MXD#	0.9×10⁹/L
NEUT#	11.0×10⁹/L
RDW	53.6fL
PDW	10.3fL
MPV	- 8.6fL
P-LCR	17.3%

Thalassaemia & RBC Indices

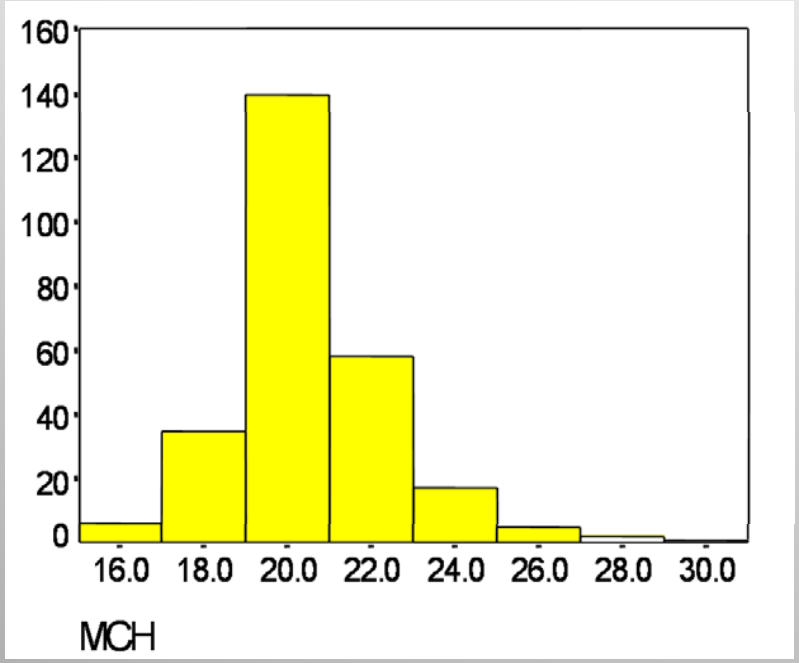
The cutoff limits ?

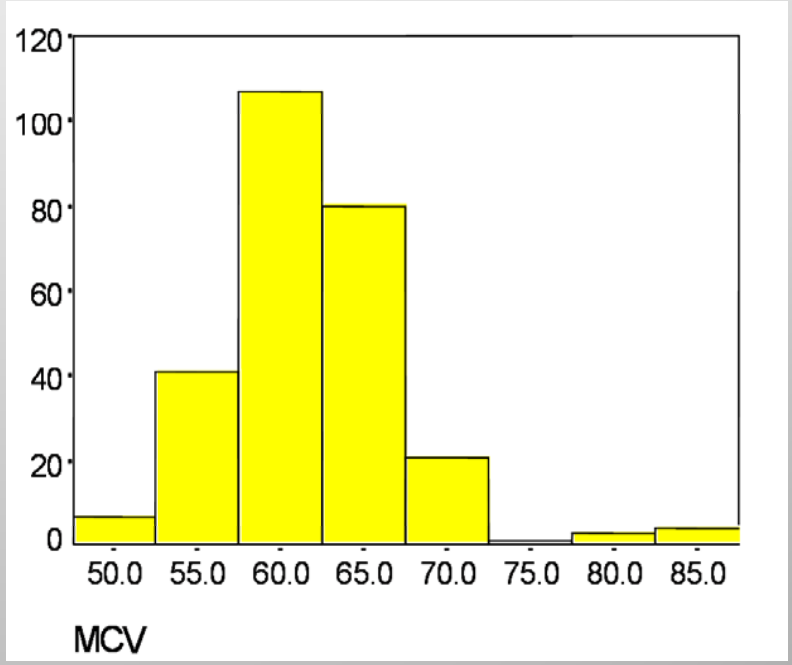
RBC Indices: Reference Ranges

Ethnic group:	Hb (g/dl):		TRBC ($X10^9$/L):		MCV (fl):		MCH (pg):	
	Range:	Mean ± SD:	Range:	Mean ± SD:	Range:	Mean ± SD:	Range:	Mean ± SD:
Punjabi: (n=290)	7.0-16.8	13.9 ± 1.9	3.0-6.2	4.95 ± 0.54	54-101	85.2 ± 7.7	16-34	28.1 ± 3.3
Pathan: (n=307)	7.6-19.8	14.2 ± 1.7	4.0-6.9	5.10 ± 0.51	61-103	87.5 ± 7.1	17-35	29.1 ± 3.1
Sindhi: (n=223)	9.4-18.4	13.7 ± 1.6	3.4-7.2	4.83 ± 0.53	64-110	87.4 ± 7.6	18-39	28.6 ± 3.2
Baluch: (n=300)	8.9-19.4	15.0 ± 1.5	3.9-7.2	5.41 ± 0.55	59-108	83.1 ± 8.0	19-36	28.1 ± 3.1
Urdu speaking: (n=225)	9.6-17.3	14.6 ± 1.6	3.8-7.0	4.91 ± 0.53	60-102	85.1 ± 7.2	18-33	29.2 ± 3.0
All groups: (n=1345)	7.0-19.8	14.3 ± 1.7	3.0-7.2	5.04 ± 0.53	54-110	85.7 ± 7.5	16-39	28.6 ± 3.1

(S. Ahmed 1998)

MCV & MCH in Thalassaemia





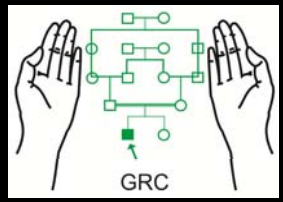
(S. Ahmed 1998)

Thalassaemia & RBC Indices

Ethnic group:	MCV≤75fl or MCH ≤25pg:	β-thalassaemia carriers:			95% Confidence interval:
		Number:	% of low MCV or MCH	% of total	
Punjabi: (n=290)	33 (11.3%)	13	39.4%	4.5%	2.1-6.9%
Pathan: (n=307)	30 (9.8%)	16	53.3%	5.2%	2.7-7.7%
Sindhi: (n=223)	21 (9.4%)	11	52.4%	5.2%	4.2-8.2%
Baluchi: (n=300)	57 (19.0%)	27	47.4%	9.0%	5.7-12.3%
Mohajir: (n=225)	23 (10.2%)	12	52.2%	5.2%	2.3-8.1%
All ethnic groups: (n=1345)	164 (12.2%)	71	43.3%	5.3%	4.1-6.5%

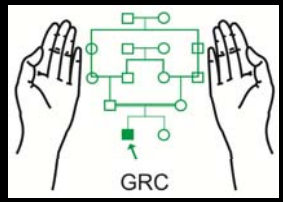
(S. Ahmed 1998)

RBC Indices in Unusual Thalassaemias



- Promoter mutations
- Splice junction mutations
- Co-existing α-thalassaemia
- Co-existing Iron Deficiency
- δβ-thalassaemia

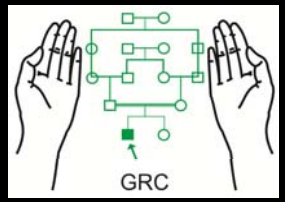
Promoter region Mutations



- Parent of a child with Transfusion dependent anaemia
- Hb-A_2: 2.6%
- PCR: Cap+1 (A-C)

No.		1
Date		03/02/11 08:40
Mode		WB
WBC		8.3×10⁹/L
RBC	+	5.54×10¹²/L
HGB		14.4g/dL
HCT		0.446
MCV		80.5fL
MCH		26.0pg
MCHC		32.3g/dL
PLT	AG	274×10⁹/L
LYM%		30.8%
MXD%		18.9%
NEUT%		50.3%
LYM#		2.6×10⁹/L
MXD#		1.6×10⁹/L
NEUT#		4.1×10⁹/L
RDW		41.9fL
PDW		12.0fL
MPV		9.9fL
P-LCR		24.0%

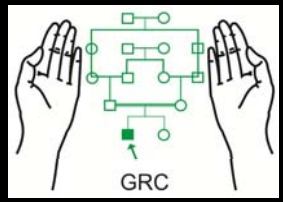
Cap+1 Mutation



Sex	TRBC	Hb	MCV	MCH	Mutation	Sex	TRBC	Hb	MCV	MCH	Mutation
F	4.77	13.3	83.0	27.9	Cap+1	M	6.57	12.9	71.8	19.6	IVSI-5
F	4.47	11.5	81.3	25.2	Cap+1	M	7.50	13.6	54.5	18.1	Fr 41-42
F	4.18	10.9	82.8	26.1	Cap+1	M	6.78	13.1	62.1	19.3	IVSI-5
M	4.96	14.9	85.9	30.1	Cap+1	M	6.93	14.2	62.8	20.5	Del 619
F	4.06	12.1	85.2	29.8	Cap+1	M	6.21	12.0	59.7	19.3	Del 619
F	5.41	13.4	76.0	24.8	Cap+1	F	5.35	10.3	58.5	19.3	Cd 15
F	4.05	11.2	86.4	27.7	Cap+1	M	6.73	13.3	61.1	19.8	Cd 5
F	4.17	11.3	80.6	27.1	Cap+1	F	5.16	9.9	55.7	19.2	IVSI-1
M	4.83	14.2	87.6	28.8	Cap+1	M	6.76	15.8	65.0	23.4	IVSI-5
F	4.19	11.2	80.7	26.7	Cap+1	F	5.15	11.3	66.0	21.9	IVSI-5
F	3.80	9.9	77.9	26.1	Cap+1	M	6.80	12.6	60.7	18.5	IVSI-5
F	3.37	9.9	87.5	29.4	Cap+1	F	5.50	10.7	53.0	19.5	IVSI-5
M	6.05	15.4	82.6	25.5	Cap+1	M	7.79	14.5	61.0	18.6	Cd 30
F	4.15	10.0	76.9	24.1	Cap+1	F	4.92	9.8	68.3	19.9	Cd 30

(S. Ahmed unpublished data)

Splice Junction Mutations



- Parent of a child with transfusion dependent anaemia
- Hb-Electrophoresis
  - Hb-E Trait

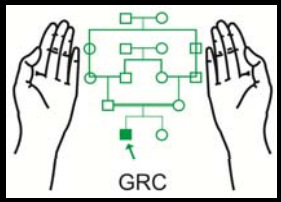
WBC	8.0×10⁹/L
RBC	5.05×10¹²/L
HGB	11.1g/dL
HCT	0.365
MCV	- 72.3fL
MCH	- 22.0pg
MCHC	- 30.4g/dL
PLT	319×10⁹/L
LYM%	30.3%
MXD%	8.6%
NEUT%	61.1%
LYM#	2.4×10⁹/L
MXD#	0.7×10⁹/L
NEUT#	4.9×10⁹/L
RDW	46.6fL
PDW	12.3fL
MPV	10.0fL
P-LCR	26.1%

RBC Indices in Thalassaemia

Mutation	Hb (g/dl)	TRBC (X 109/L)	MCV (fl)	MCH (pg)
β^+ Trait (Cap+1)	12.1 9.9-15.4	4.46 3.37-6.05	82.5 76.0-87.6	27.1 24.1-30.1
Hb-E Trait	10.5 8.8-13.5	4.40 3.47-5.55	73.7 65.0-81.0	23.9 21.0-26.0
β^o-Thal Trait	12.4 9.8-15.8	6.30 4.92-7.79	61.4 53.0-71.8	19.8 18.1-24.3

(S. Ahmed unpublished data)

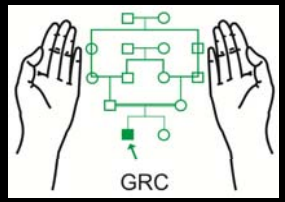
δβ-Thalassaemia in Pakistan

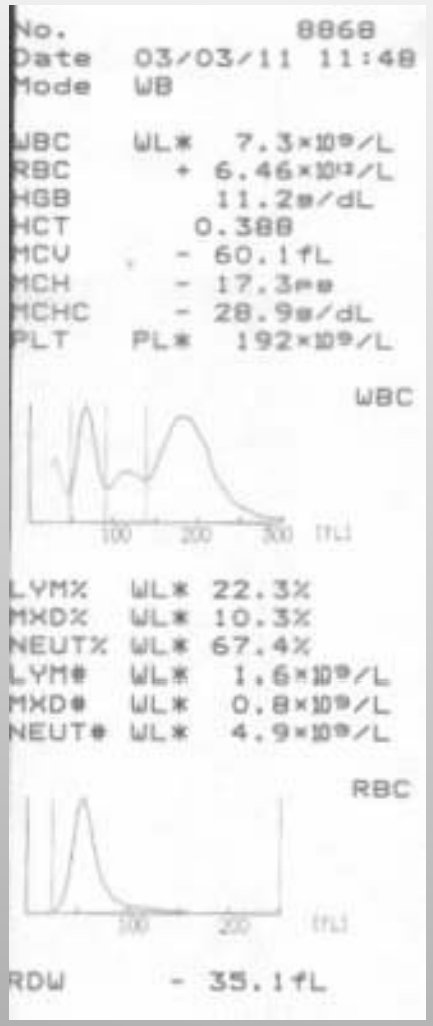


Sr.	Fam	Ethnic	Sex	Age	Trans	Spl	Hb	TRBC	MCV	MCH	HbF	HbA_2	Mutation	Xmn-I
Heterozygous ($^{A}\gamma\delta\beta)^{o}$/Nor)														
1.	1.	Pathan	F	30	No	-	11.5	5.15	73.2	22.3	15%	2.4%	$(^{A}\gamma\delta\beta)^{o}$/Nor	-/+
2.	2.	Punjabi	M	33	No	-	13.4	5.61	78.3	23.9	17%	2.9%	$(^{A}\gamma\delta\beta)^{o}$/Nor	-/+
3.	2.	Punjabi	F	28	No	-	10.4	4.48	72.1	23.2	15%	2.6%	$(^{A}\gamma\delta\beta)^{o}$/Nor	+/+
4.	3.	Punjabi	F	27	No	-	12.5	5.67	71.6	22.0	12%	2.8%	$(^{A}\gamma\delta\beta)^{o}$/Nor	-/+
5.	3.	Punjabi	M	6	No	-	11.3	5.91	64.0	19.1	12%	2.4%	$(^{A}\gamma\delta\beta)^{o}$/Nor	-/+
6.	3.	Punjabi	F	4	No	-	10.5	5.41	66.2	19.4	14%	2.3%	$(^{A}\gamma\delta\beta)^{o}$/Nor	-/+
Mean							11.6	5.37	70.9	21.7	13%	2.6%		
Homozygous $(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$														
7.	2.	Punjabi	M	8	No	+	10.5	5.50	63.3	19.1	100%	Nil	$(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$	+/+
8.	3.	Punjabi	M	33	No	+	10.5	6.30	65.1	20.0	100%	Nil	$(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$	+/+
9.	3.	Punjabi	F	2	Yes	++	5.0	2.27	71.8	22.0	70%*	1.3%	$(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$	+/+
10.	4.	Punjabi	F	34	No	++	11.2	4.40	76.9	25.4	100%	Nil	$(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$	+/+
11.	5.	Bohra	F	10	No	++	10.3	5.32	70.1	19.0	100%	Nil	$(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$	+/+
12.	6.	Bohra	M	29	No	++	10.7	5.17	70.6	20.7	100%	Nil	$(^{A}\gamma\delta\beta)^{o}/(^{A}\gamma\delta\beta)^{o}$	+/+
Mean							9.7	4.82	69.6	21.0	100%	Nil		
Compound heterozygous $(^{A}\gamma\delta\beta)^{o}$/IVSI-5 (G-C)														
13.	6.	Bohra	F	3	Yes	++	8.0	3.8	72.3	21.3	20%*	1.3%	$(^{A}\gamma\delta\beta)^{o}/\beta^{o}$	-/+

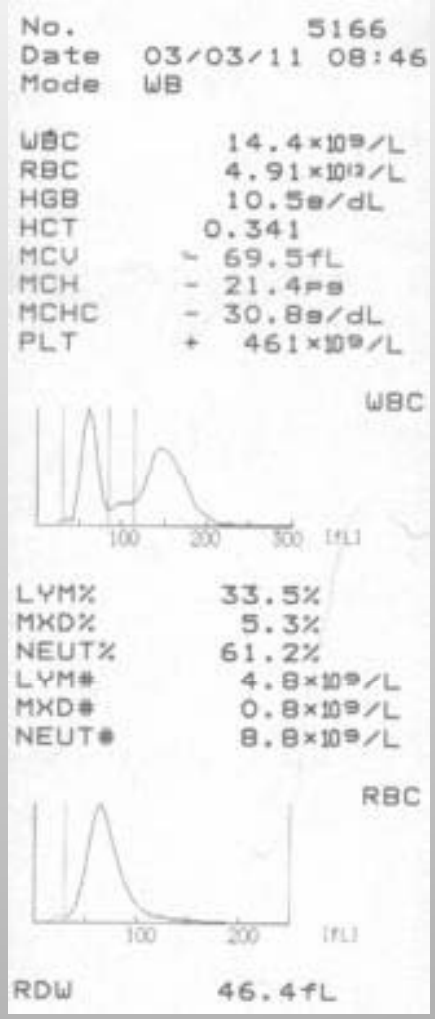
(Ahmed & Anwar 2006)

RDW: Thalassaemia Trait vs Iron Deficiency Anaemia (IDA)





No.		8868
Date		03/03/11 11:48
Mode		WB
WBC	WL*	7.3×10⁹/L
RBC	+	6.46×10¹²/L
HGB		11.2g/dL
HCT		0.388
MCV	-	60.1fL
MCH	-	17.3pg
MCHC	-	28.9g/dL
PLT	PL*	192×10⁹/L
LYM%	WL*	22.3%
MXD%	WL*	10.3%
NEUT%	WL*	67.4%
LYM#	WL*	1.6×10⁹/L
MXD#	WL*	0.8×10⁹/L
NEUT#	WL*	4.9×10⁹/L
RDW	-	35.1fL



No.		5166
Date		03/03/11 08:46
Mode		WB
WBC		14.4×10⁹/L
RBC		4.91×10¹²/L
HGB		10.5g/dL
HCT		0.341
MCV	-	69.5fL
MCH	-	21.4pg
MCHC	-	30.8g/dL
PLT	+	461×10⁹/L
LYM%		33.5%
MXD%		5.3%
NEUT%		61.2%
LYM#		4.8×10⁹/L
MXD#		0.8×10⁹/L
NEUT#		8.8×10⁹/L
RDW		46.4fL

RDW: Thalassaemia Trait vs Iron Deficiency Anaemia (IDA)

RDW (fl)	≤32	32-36	36-40	40-44	44-48	48-52	52-56	>56
Thal Trait	10	35	44	10	1	0	0	0
IDA	0	0	2	18	22	12	4	5

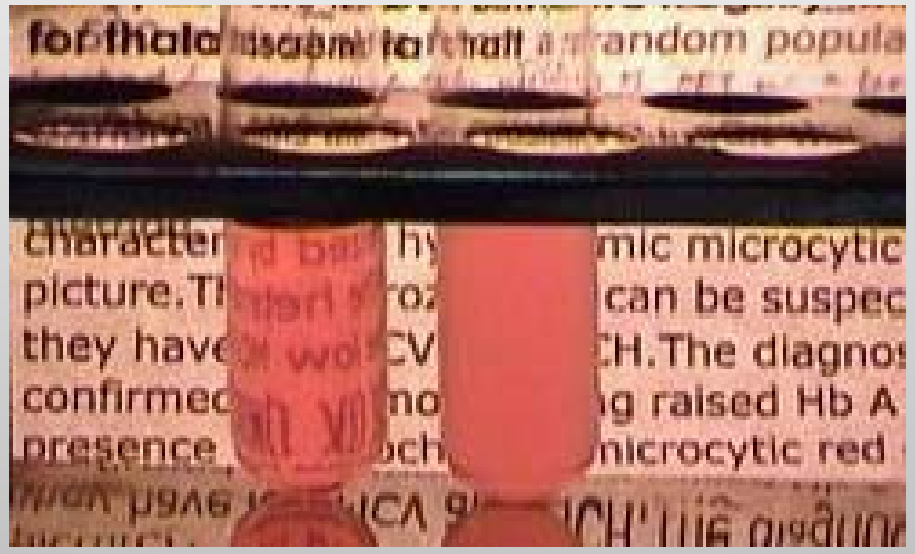
(Fatima & Ahmed 2008)

β-Thalassaemia Major in Previously Transfused Patients

	Untransfused (n=171)		Transfused (n=109)		*p* value
	Mean	Range	Mean	Range	
Hb (g/dl)	6.3	1.9 - 9.0	6.2	2.3 – 11.2	0.20
MCV (fl)	70	57 - 83	74	58 - 98	0.014
MCH (pg)	21	15 - 29	24	16 - 31	0.015
Hb-F (%)	95	35 - 97	31	0.5 - 97	< 0.001

(S. Ahmed et al, 2003)

An low-cost alternate to MCV in Thalassaemia Screening ?

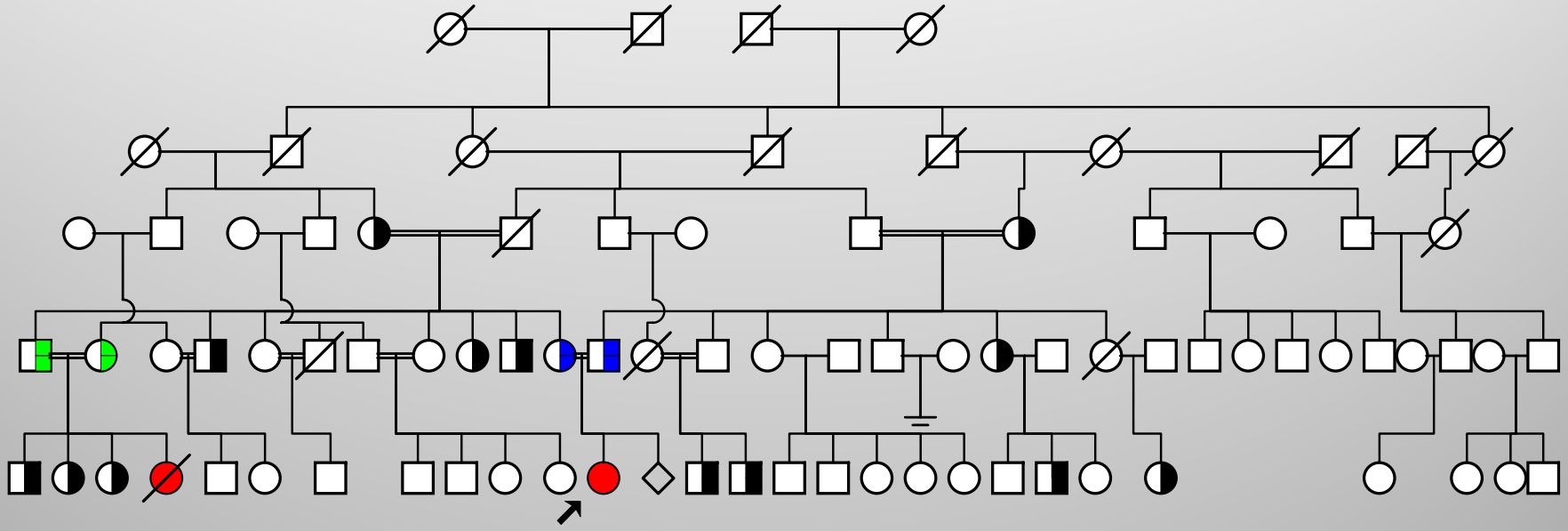


0.36% Saline

(Yazdani & Ahmed 2007)

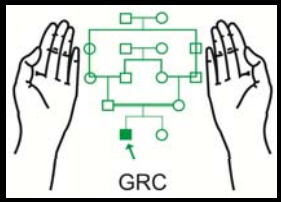
One Tube Osmotic Fragility Test

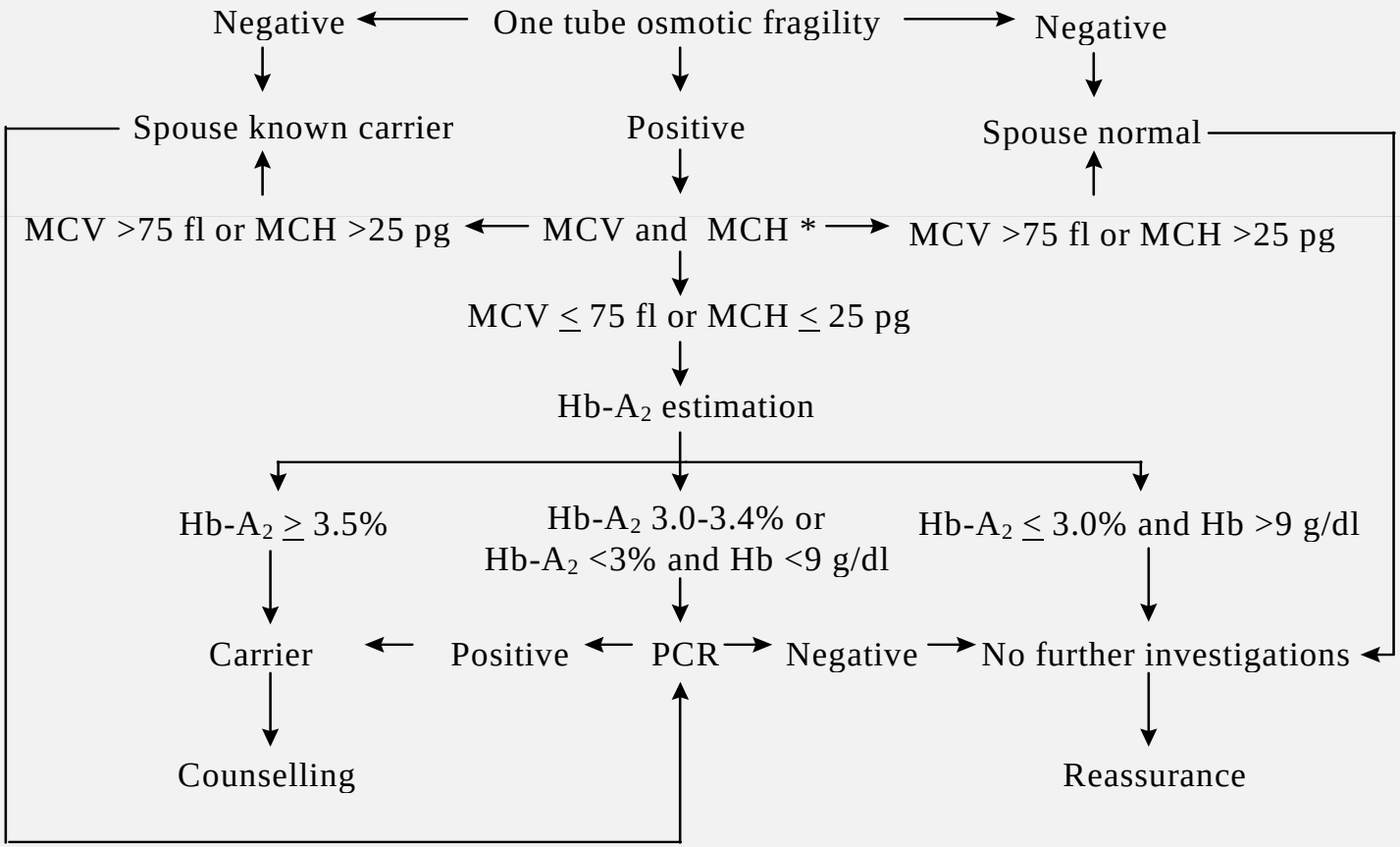
Family Screening for Thalassaemia



(S. Ahmed et al, NEJM 2002)

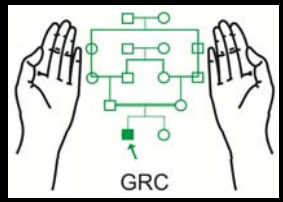
A Strategy for Thalassaemia Screening





(S. Ahmed 1998)

Summary



- RBC Indices can be extremely useful in screening for thalassaemia
- Prior knowledge of the local population data is an essential requirement
- Simpler is better
- Good calibration of the instrument is important
- One tube osmotic fragility may be very useful for working in the field area