Hb electrophoresis

Essentials and how to read it

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Why we test

- Microcytic anemia with normal iron studies
- Sickle cells or target cells on the film
- Premarital screening (national programme since 2004)
- A relative or a partner who is a carrier
- Newborn and antenatal testing
- Monitoring: HbS after exchange transfusion, HbF on hydroxyurea

Quantity and quality

Quantity: thalassemia

- Too little of a normal chain
- Hb low, MCV low
- No new peak
- The mix shifts: HbA2 rises in β-thalassemia

Quality: a variant

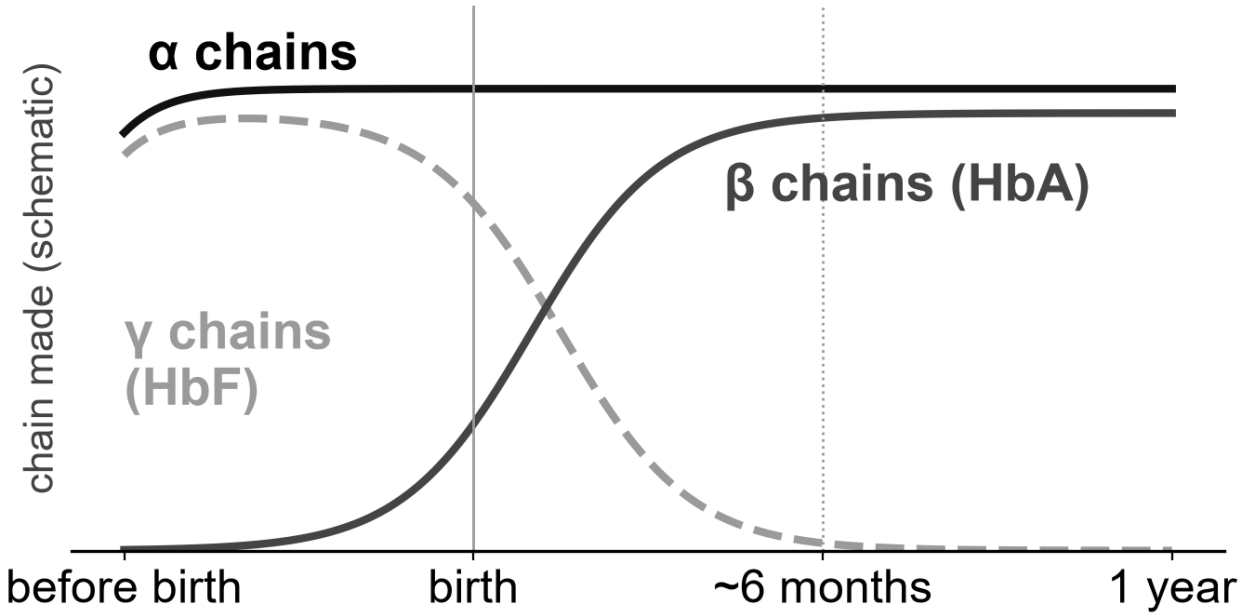
- An abnormal chain (S, C, D, E)
- A new peak at a new place
- A carrier can have a normal blood count
- Both can occur together: S/β-thalassemia

Normal hemoglobins

Hemoglobin	Chains	Adult %	Note
HbA	α2 β2	~97%	The main adult hemoglobin
HbA2	α2 δ2	2.5–3.5%	Rises in β-thalassemia trait
HbF	α2 γ2	<1%	The main hemoglobin before birth

- All three use α chains. Only HbA uses β chains.
- The 3.5% limit for HbA2 is a convention. Use the range on the report.

From HbF to HbA



- At birth most hemoglobin is HbF
- The switch is nearly complete by about 6 months
- β disorders stay hidden until then
- α disorders show at birth: Hb Bart's (γ4)
- Newborn Bart's 1–2%: suggests one α gene lost
- Newborn Bart's 5–10%: fits α0 trait, not diagnostic

Methods

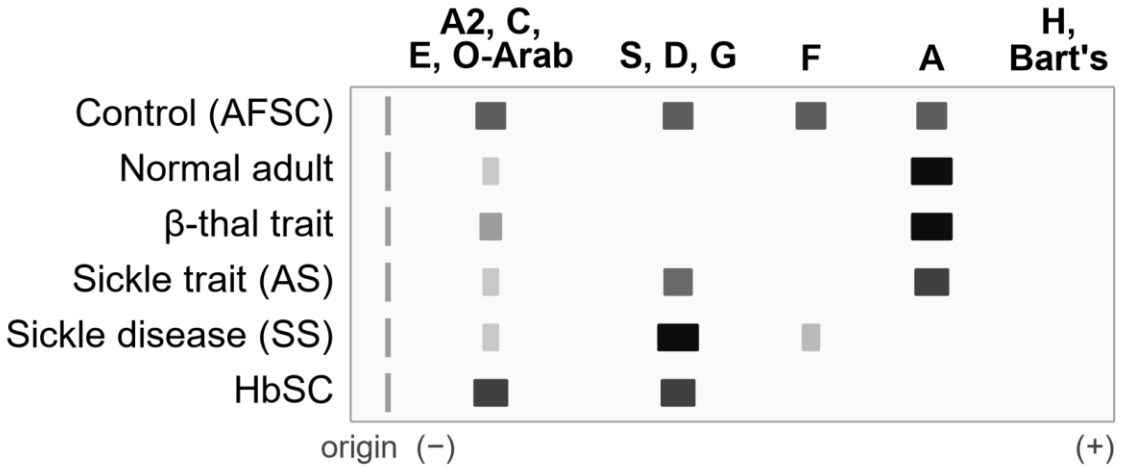
Method	Separates by	Result	Main limits
HPLC (in use)	Time to leave the column	Peaks with %	E falls in the A2 window on some instruments; A2 reads high with S
Capillary electrophoresis (in use)	Charge, in a thin capillary	Peaks with %	C not always separate from A2
Alkaline gel (historical)	Charge	Bands	No longer in routine use. A2/C/E/O-Arab and S/D/G ran together
Acid gel (historical)	Charge and binding to the gel	Bands	No longer in routine use. Was used to confirm alkaline gel bands

- In use: HPLC and capillary electrophoresis. Gels: historical only
- Suspected HbS: confirm with a sickle solubility test
- Any variant: confirm by a second method

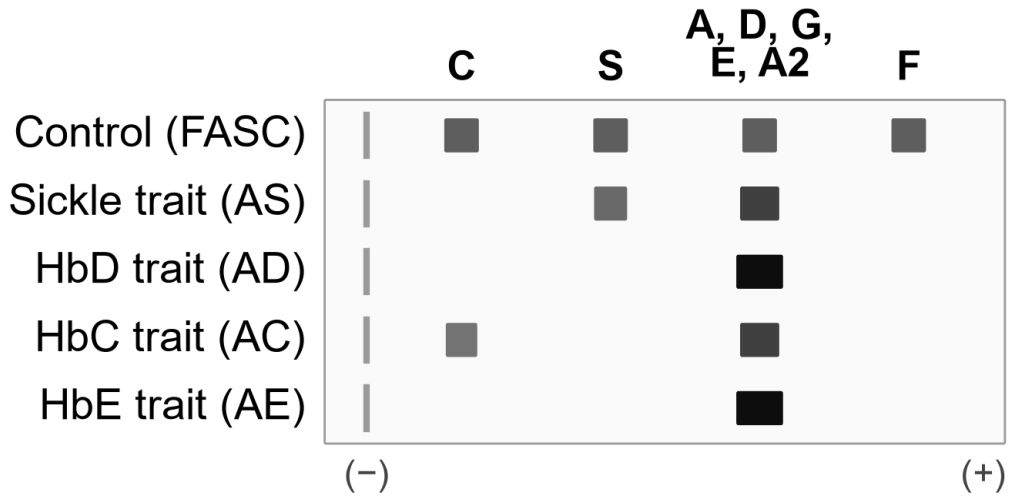
Gel electrophoresis (historical)

Historical: no longer in routine use. HPLC and capillary electrophoresis have replaced it.

Alkaline gel

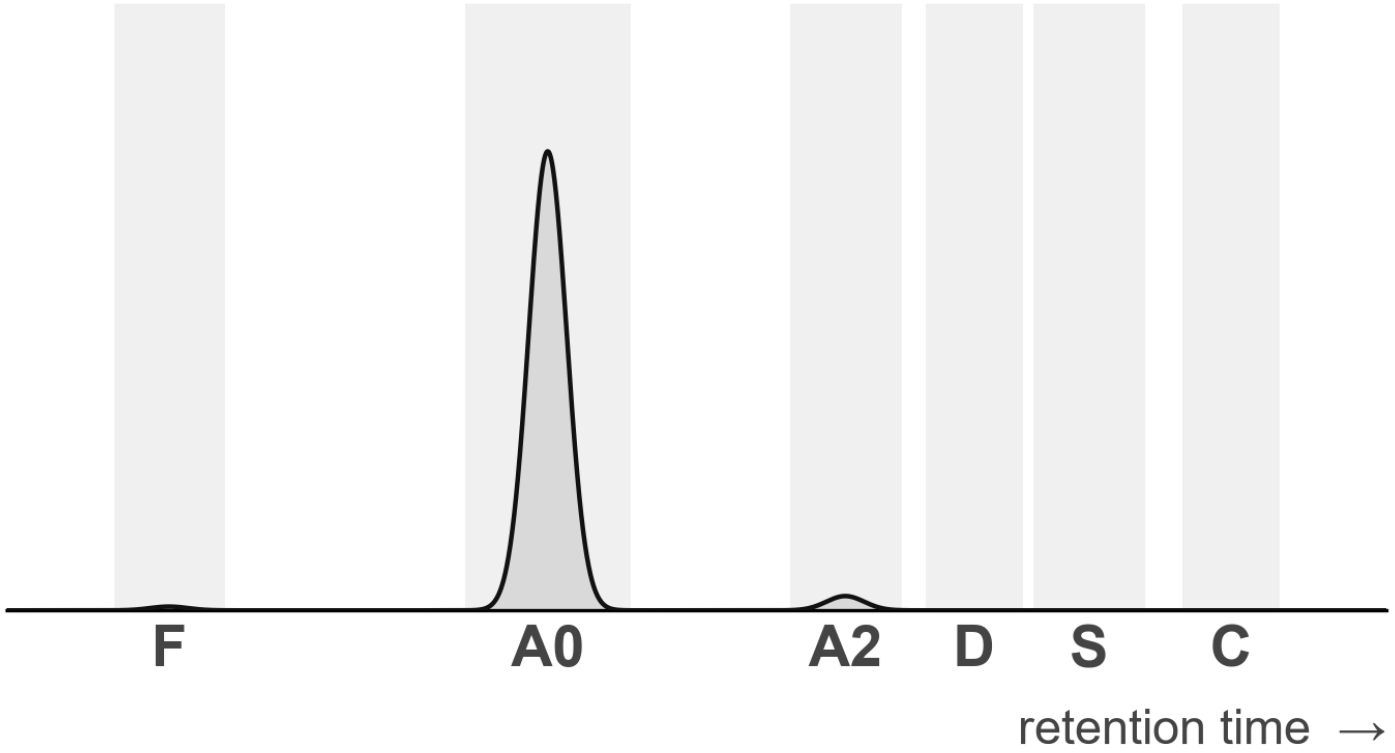


Acid gel



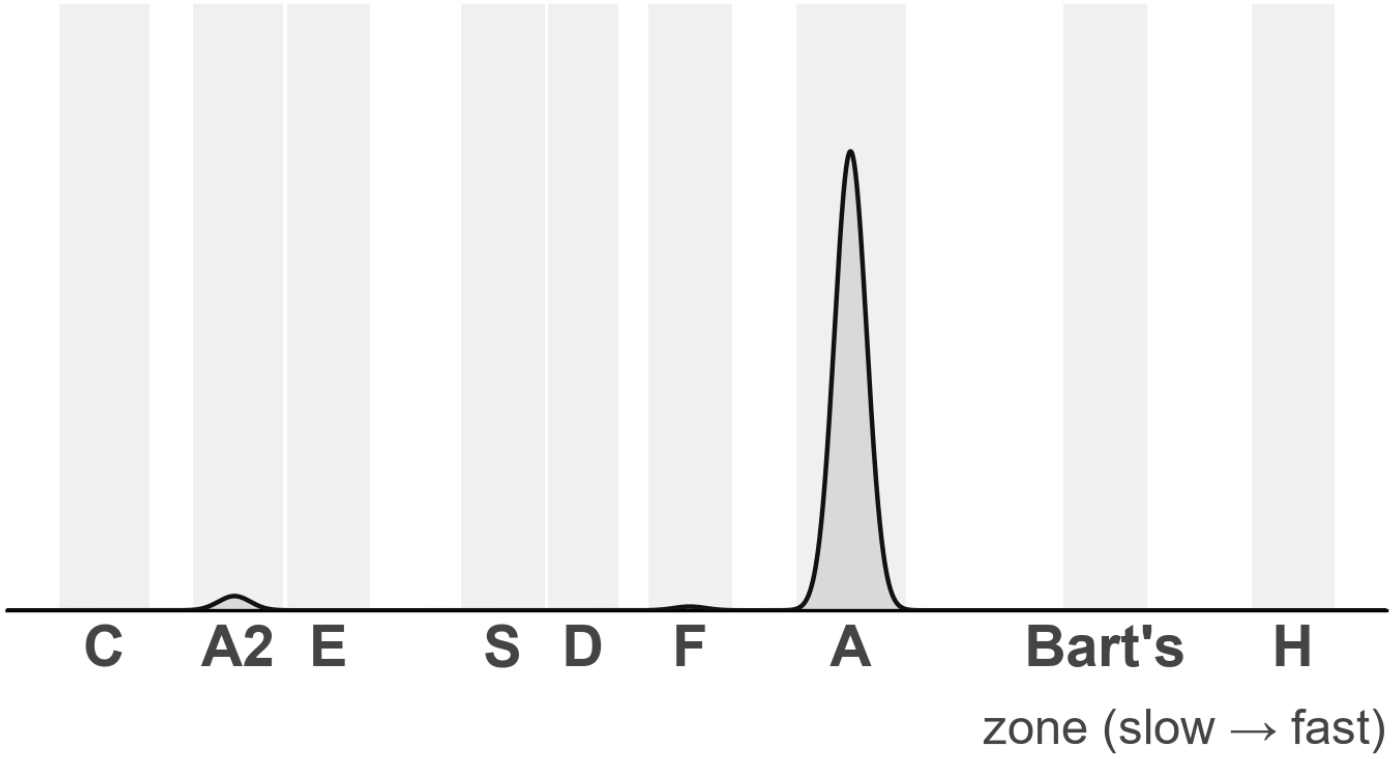
- Old teaching from the alkaline gel: A2, C, E and O-Arab ran together; so did S, D and G
- Today the overlaps to know are on HPLC and capillary (next two slides)

HPLC



- Each Hb leaves the column at its own time
- Peak area gives the percentage
- The standard way to measure A2 and F
- Separates D and G from S
- Traps: E in the A2 window on some instruments; A2 reads high with S

Capillary electrophoresis



- Charge separation inside a thin capillary
- Each peak falls in a zone relative to HbA
- Separates A2 from E; not always from C
- Small sample: suits newborn screening
- A2 with HbS: 3.1% here vs 4% by HPLC (means)

A reading routine

Before the peaks

1. Age: under 6 months? Pregnant? Family origin?
2. Transfusion in the last 3 months? Hydroxyurea?
3. CBC: Hb, MCV, MCH, red cell count
4. Iron status: ferritin

Then the peaks

5. Is HbA present?
6. A variant? Where, how much, confirmed?
7. HbA2: raised (>3.5%), normal or low?
8. HbF: raised?
9. Does the pattern fit the CBC?

Thalassemia patterns

Condition	HbA	HbA2	HbF	Other	CBC
Normal	~97%	2.5–3.5%	<1%	—	Normal
β-thal trait	90+%	>3.5%	Normal or ↑	—	Hb 10–12 g/dL, MCV low, RBC often high
β-thal major, before transfusion	Little or none	—	Main peak	—	Severe anemia
α-thal trait	Normal	Normal or low	Normal or low	No new peak	MCV low, iron normal
HbH disease	Present	Low	—	HbH 1–40% (usually 8–10%)	Hemolysis, golf-ball cells
Hb Bart's hydrops	None	None	None	Bart's 70–100%	Hydrops fetalis

Sickle patterns

Genotype	HbA	HbS	HbA2	MCV	The clue
AS (trait)	~60%	~40%	Normal	Normal	More A than S. A carrier.
SS	None	90+%	Normal	Normal	No A. Cells not small.
S/β0 thalassemia	None	90+%	Raised	Low	Like SS, but small cells
S/β+ thalassemia	10–30%	>60%	Raised	Low	Less A than S
SC	None	~Equal to C	Unreliable	—	S and C, no A

- Eastern Province adults (Arab-Indian haplotype): mean HbF 15.6%, so HbS can sit below 90% in true SS

High HbF: HPFH or δβ-thalassemia?

	HPFH (deletional carrier)	δβ-thalassemia trait
HbF	~15–30%	Raised, wide range (4–25%)
HbA2	Normal or low	Normal or low
MCV, MCH	Normal or slightly low	Low (thalassemic)
Anemia	None	Mild or none
HbF in red cells	Even (pancellular)	Uneven (heterocellular)

- Rough rule: HbF 5–10% favours δβ; 10% or more favours HPFH
- DNA analysis is needed to be sure

HbE and the rarer variants

- HbE carrier: 25–30% HbE, with small cells
- Old alkaline gel: E ran with A2 and C (historical)
- Some HPLC: E in the A2 window, so “A2” reads 25–30%
- Capillary electrophoresis separates E from A2

Variant	Saudi premarital 2011–2018 (n = 1,871,184)
HbC	0.04%
HbD	0.04%
HbO-Arab	0.007%
HbE	0.0007%

When the result misleads

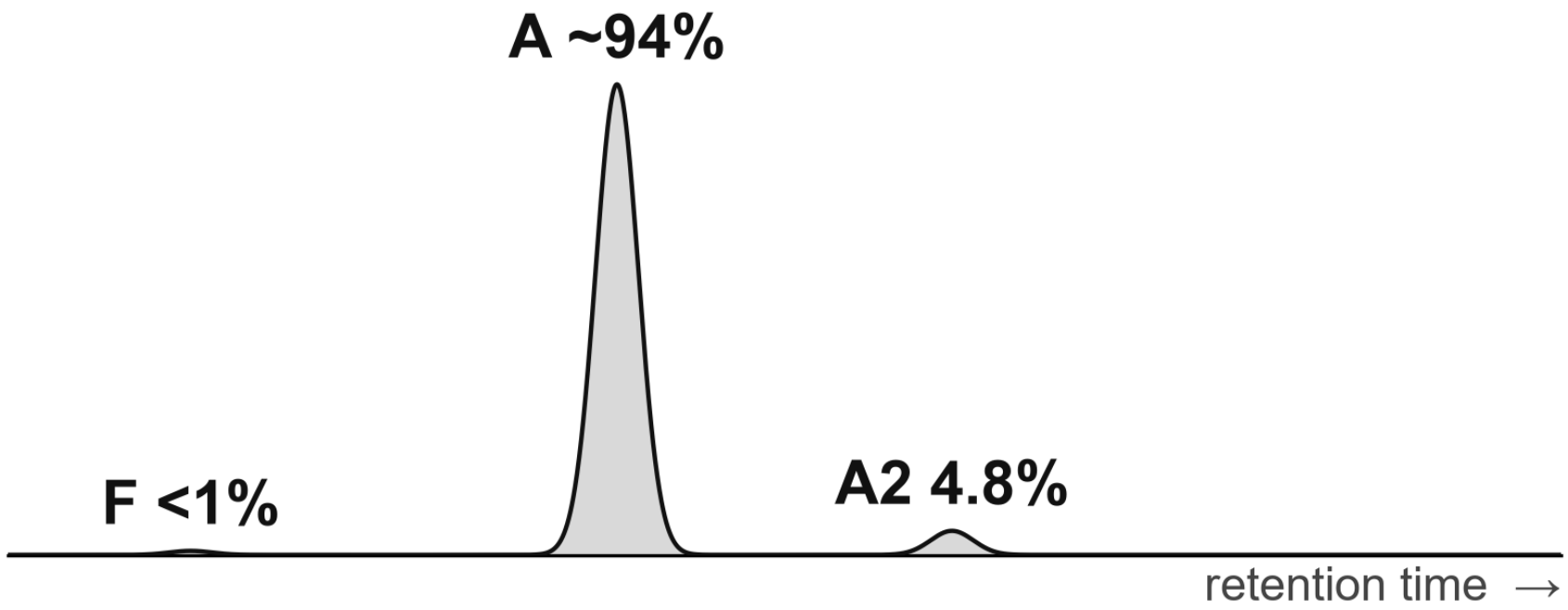
Situation	Effect	What to do
Iron deficiency	Lowers A2; can hide β trait	Correct iron, then repeat
Transfusion in last ~3 months	Donor HbA in the sample	Ask first; wait about 3 months
Hydroxyurea	Raises HbF	Ask about drugs
Age under 6 months	HbF high; β disorders hidden	Repeat later, or DNA
Peak in the S window	Usually S, not always	Solubility test, second method
HbS on HPLC	A2 reads high	Do not diagnose S/β0 on A2 alone
HbE on some HPLC	E in the A2 window	Confirm by another method
α-thal trait	Pattern looks normal	DNA test if it matters

Why it matters here

- Premarital screening mandatory since 2004
- First year (2004–05): 488,315 tested; sickle trait 4.20%, thalassemia trait 3.22%
- 2011–2018: 1,871,184 tested; HbS 4.7% (trait 4.4%, SS 0.3%); β-thalassemia 1.2%
- Sickle trait highest in Jazan (~136 per 1,000) and the Eastern Region (~114 per 1,000)
- β-thalassemia highest in Jazan, the Eastern Region and Aseer
- Eastern Province: Arab-Indian haplotype, high HbF. South-west: mostly African haplotypes

Case 1

A 25-year-old. Hb 11 g/dL, MCV 68 fL, red cell count 6.0 × 10^{12}/L. Iron studies are normal.

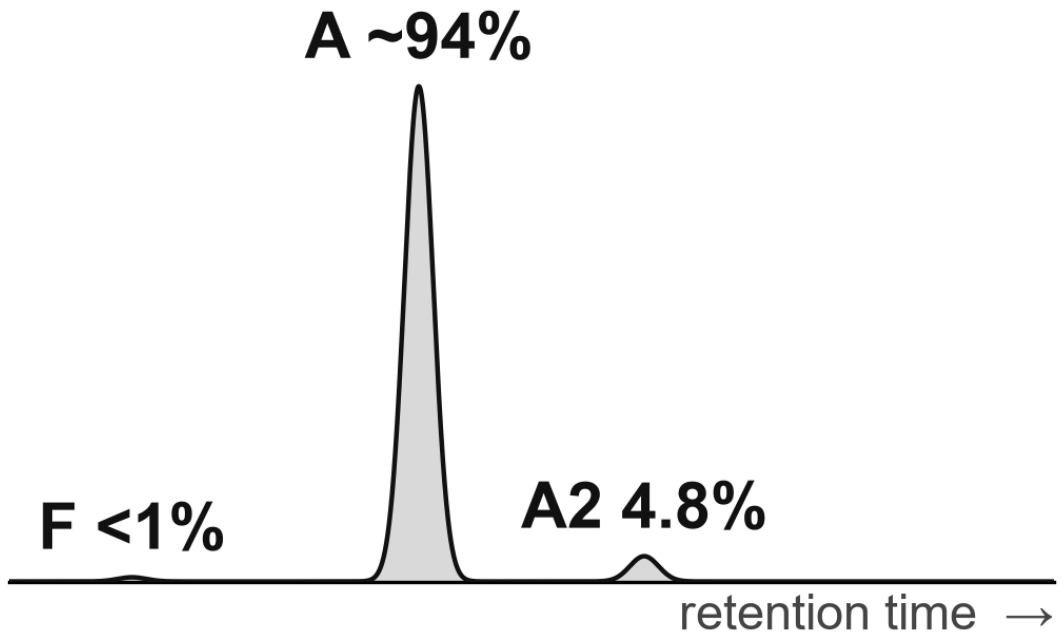


What is the diagnosis, and what do you do next?

Case 1: answer

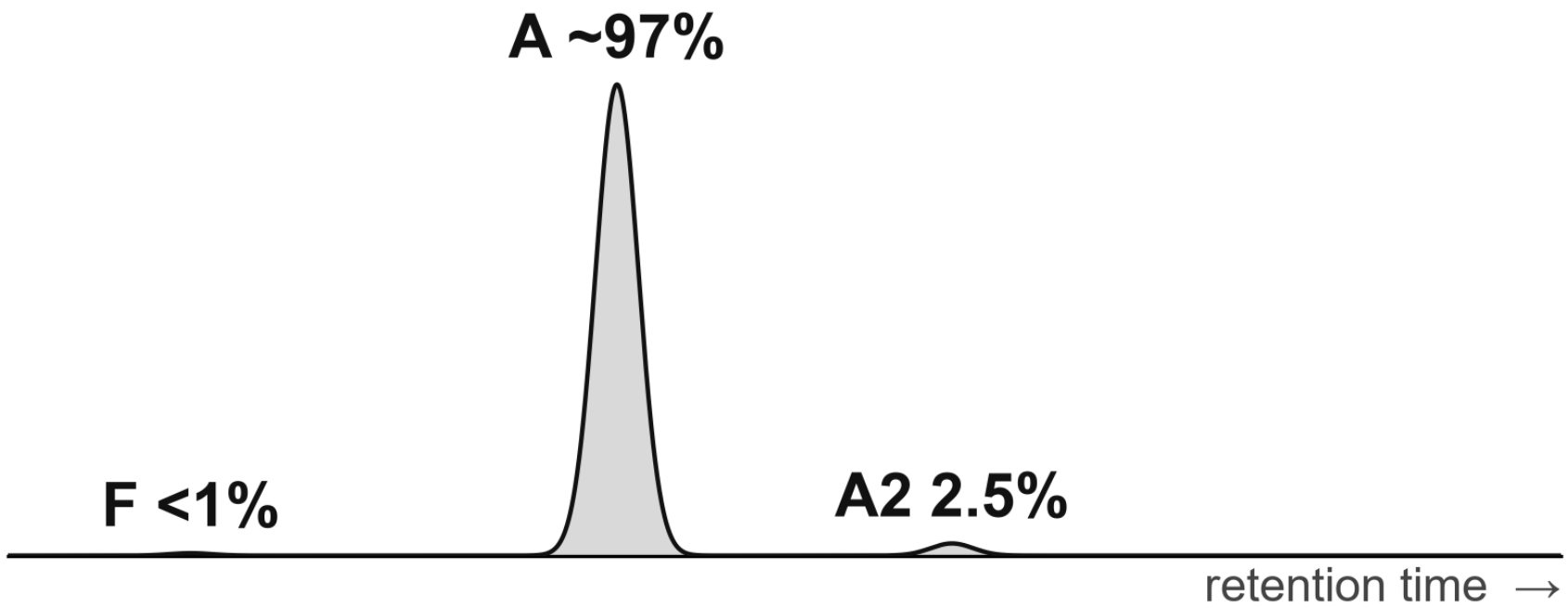
β-thalassemia trait

- Small cells, high red cell count, normal iron
- HbA present, no variant
- HbA2 4.8%: above 3.5%
- No iron. Explain the result
- Test the partner before marriage



Case 2

A 30-year-old woman with a mild microcytic anemia. Her red cell count is high-normal. Ferritin is normal.

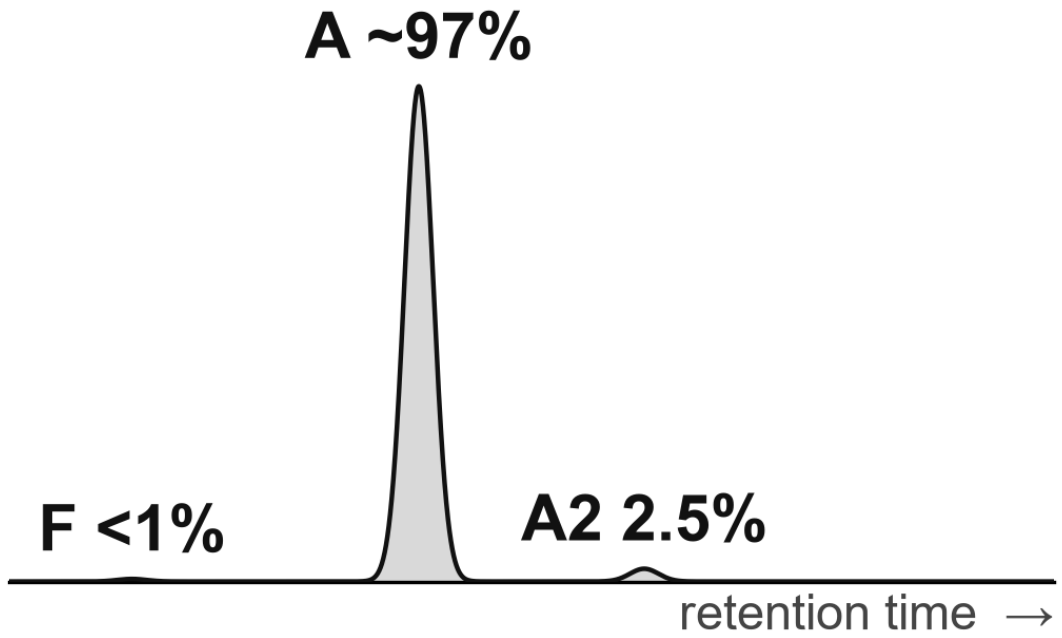


Is this result normal? What is the likely diagnosis?

Case 2: answer

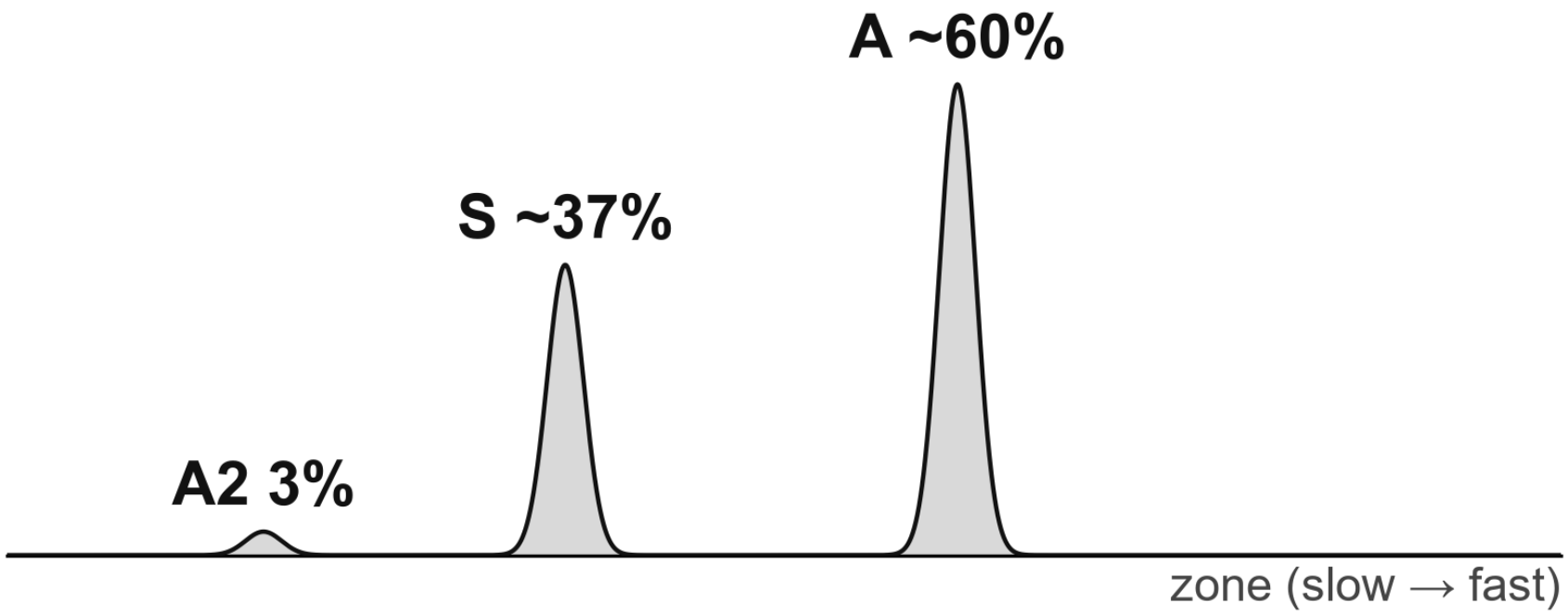
Probable α-thalassemia trait

- Small cells, normal iron, normal pattern
- α chains are in A, A2 and F, so all fall together
- A2 is often low-normal
- A diagnosis of exclusion
- Confirm by DNA when it matters, such as before marriage



Case 3

Premarital testing. A 24-year-old man. Hb and MCV are normal. Capillary electrophoresis:

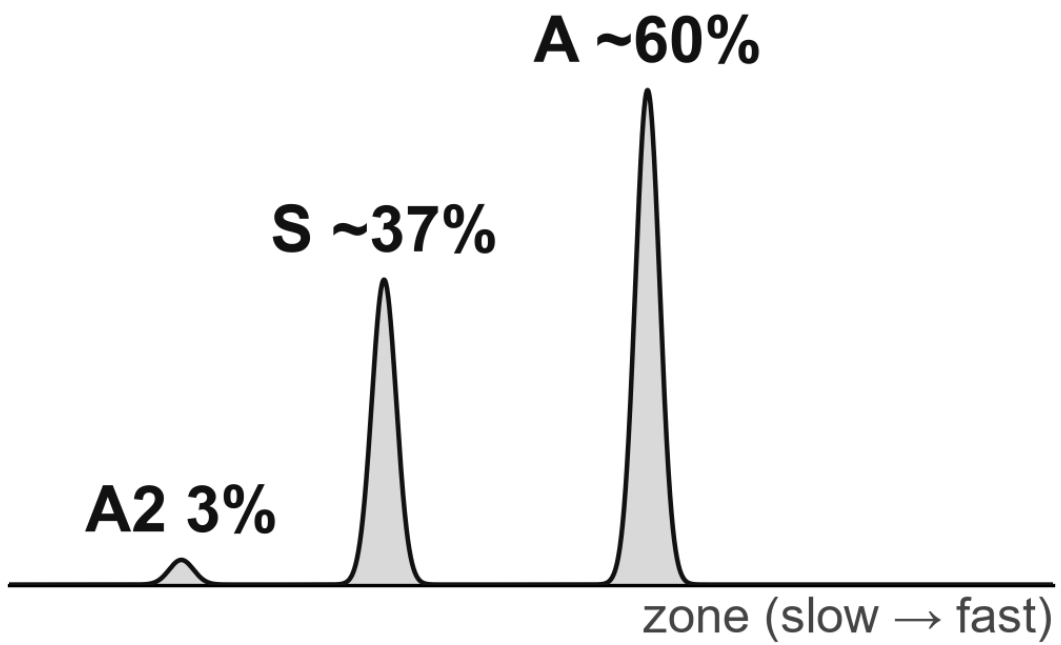


What is his result? What do you tell the couple?

Case 3: answer

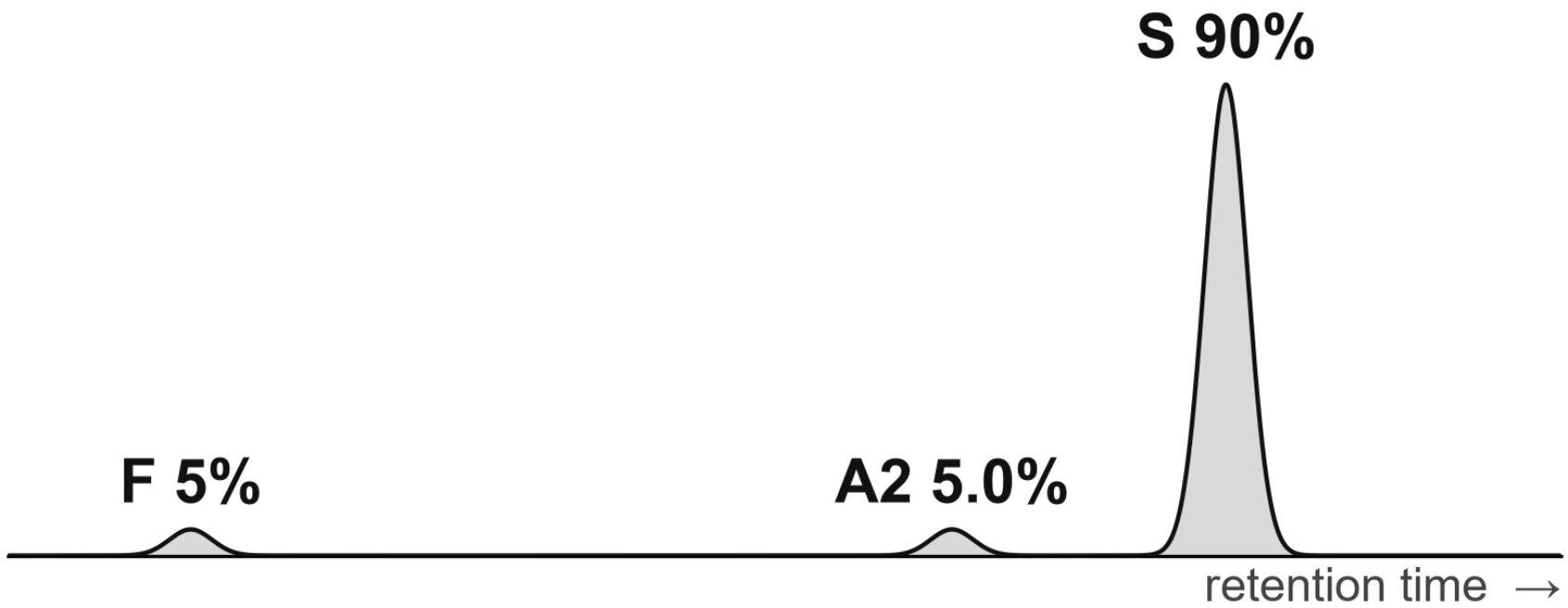
Sickle cell trait (AS)

- HbA present, and more A than S
- Normal blood count: a carrier, not a patient
- Confirm HbS with a solubility test
- Partner AS: 1 in 4 chance of SS in each pregnancy
- Partner β0 carrier: 1 in 4 chance of S/β0



Case 4

A 19-year-old with repeated bone pain crises. Hb 8.5 g/dL, MCV 66 fL. Ferritin is normal.

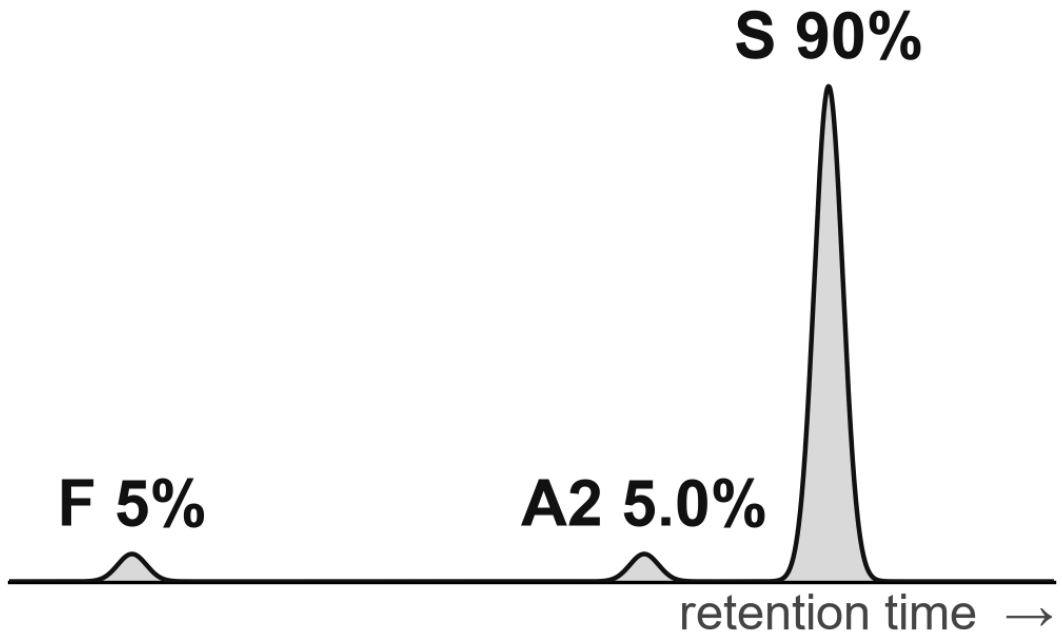


SS or something else? What is the clue?

Case 4: answer

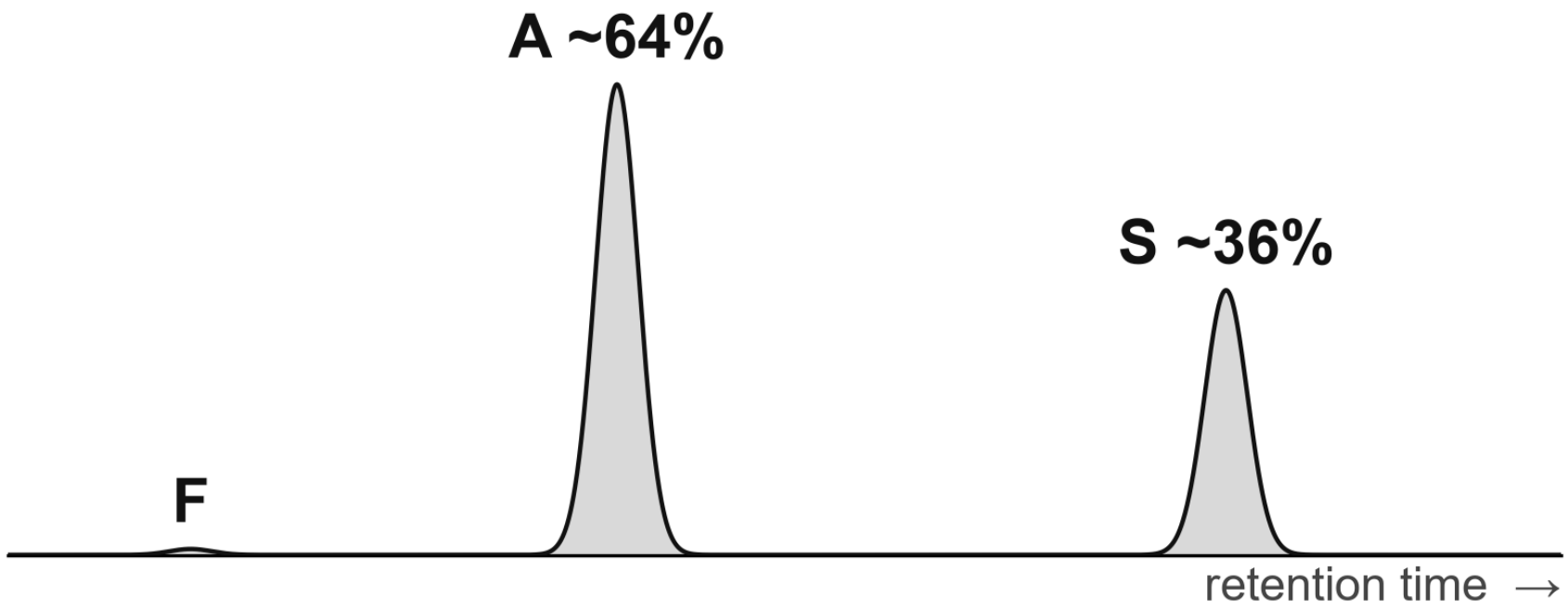
Sickle/β0 thalassemia

- No HbA: sickle cell disease, not trait
- Small cells with normal iron: think S/β0
- A2 raised, but do not rely on A2 alone
- Behaves clinically like SS
- Family studies or DNA settle it



Case 5

A 22-year-old with known SS. Three weeks ago he had an exchange transfusion for acute chest syndrome. The report says “pattern consistent with sickle trait”.

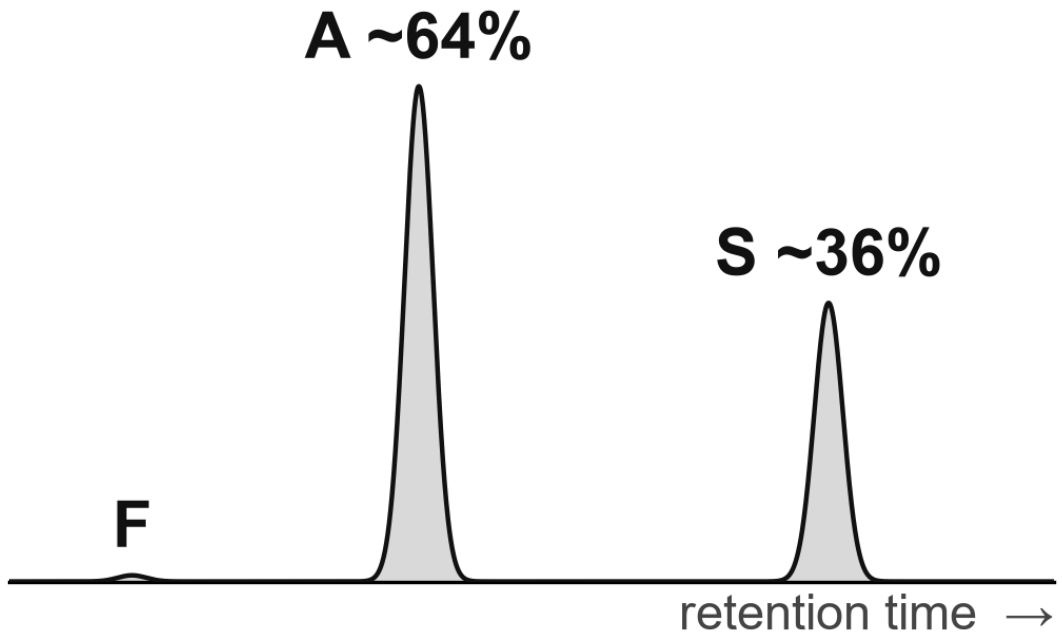


Do you accept the report?

Case 5: answer

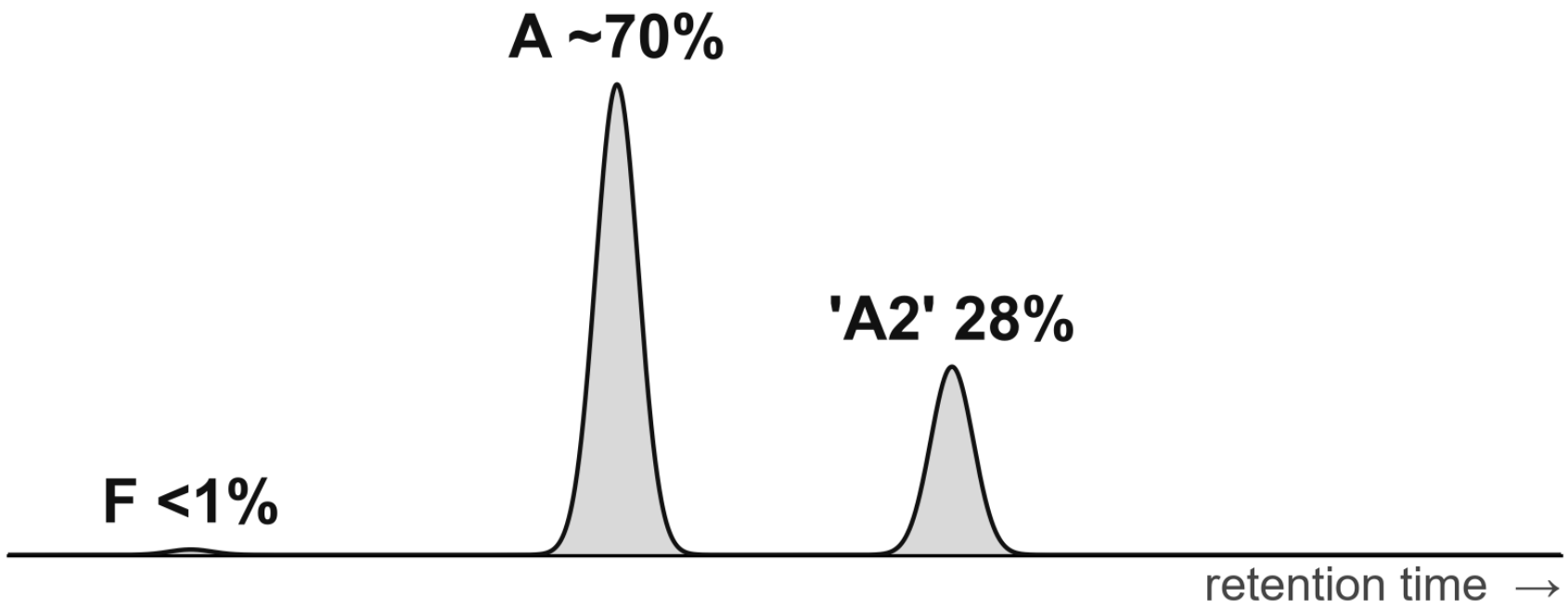
No. This is donor HbA

- He cannot make HbA: the A came from donors
- Always ask about transfusion in the last ~3 months
- Here the result is useful: it shows how much HbS is left
- For diagnosis, wait about 3 months



Case 6

A 28-year-old woman. MCV slightly low. Ferritin normal. The HPLC report reads “HbA2 28%”.

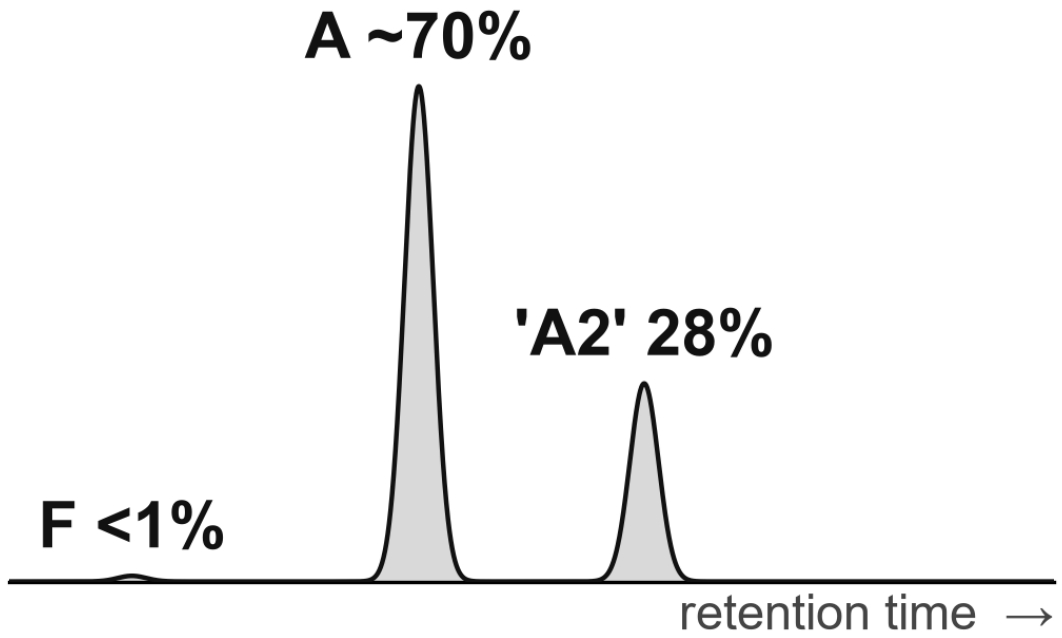


Is this β-thalassemia trait?

Case 6: answer

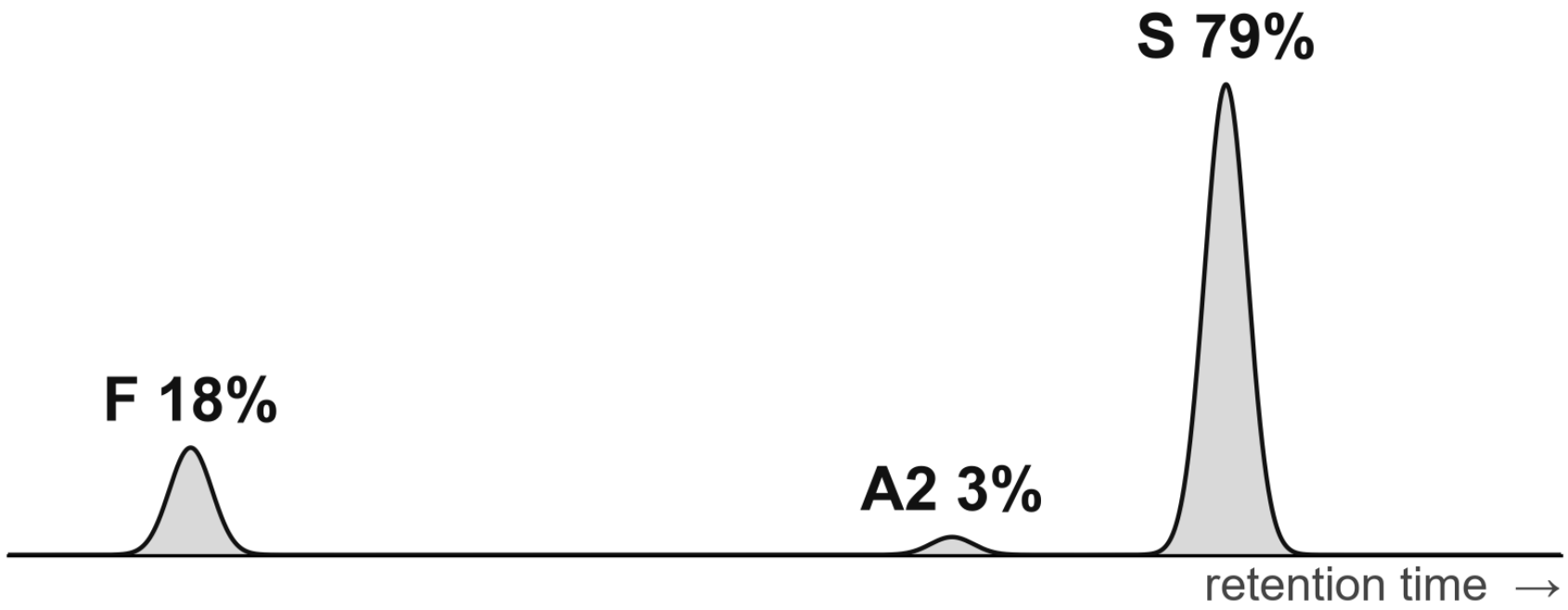
No. A variant in the A2 window

- No β-thal trait has an A2 of 28%
- HbE comes off in the A2 window on some HPLC
- HbE carriers: 25–30% HbE, small cells
- Confirm by capillary electrophoresis
- Rare in Saudis screened: ask about family origin



Case 7

A 26-year-old from Al-Ahsa with sickle cell disease. Not on hydroxyurea. No recent transfusion. Hb 9 g/dL, MCV normal.

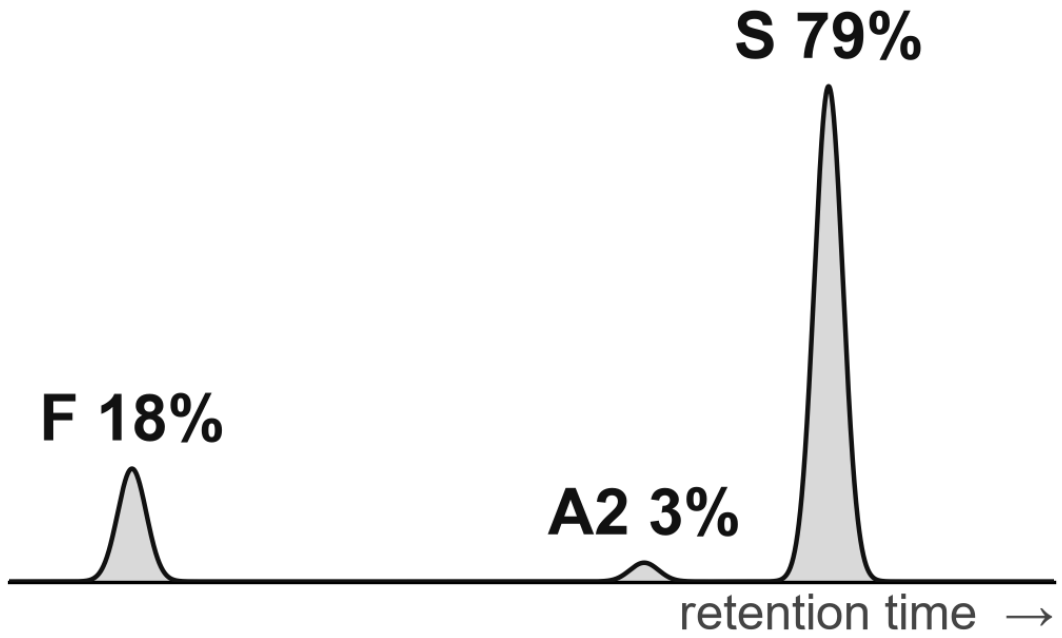


HbS is only 79%. Can this be SS?

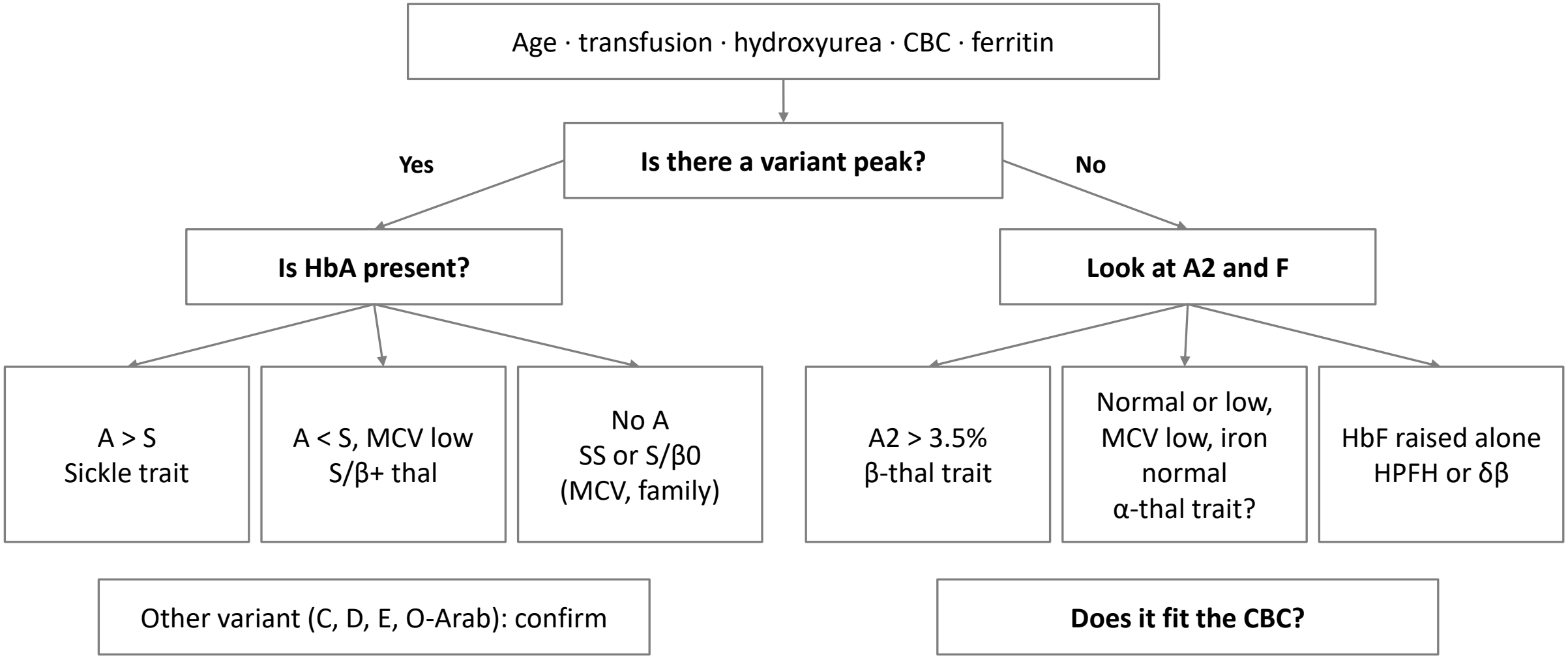
Case 7: answer

Yes. SS with high HbF

- No HbA: sickle cell disease
- HbF takes a share, so HbS is below 90%
- Arab-Indian haplotype: mean HbF 15.6% in Eastern Province adults
- Rule out hydroxyurea and transfusion first
- S/HPFH is milder: Hb above 12 g/dL, HbF 20–30%



Putting it together



Reading checklist

Steps

1. Age under 6 months? Pregnant? Family origin?
2. Transfusion in the last 3 months?
3. Hydroxyurea?
4. Hb, MCV, MCH, red cell count
5. Ferritin: is iron deficiency present?
6. Is HbA present?
7. A variant? Where, how much, confirmed?
8. HbA2: >3.5%, normal or low?
9. HbF: raised?
10. Does the pattern fit the CBC?

Traps

- Low iron hides β-thal trait
- Donor HbA after transfusion
- A peak at S: confirm with a solubility test
- A2 reads high with HbS on HPLC
- “A2” of 25–30% is a variant (often E)
- α-thal trait looks normal
- High HbF can push HbS below 90% in SS