

The Quest for the Missing Diagnosis

A neonatal diagnostic challenge

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A 14-day-old term newborn

INITIAL PRESENTATION



Born at term, G1P1



Vaginal delivery,
vacuum-assisted



Appropriate
birth weight

Initial stay in the maternity ward

MATERNITY WARD

Neonatal jaundice requiring phototherapy
from day 2 to day 10



Initial stay in the maternity ward

MATERNITY WARD

Neonatal jaundice requiring phototherapy
from day 2 to day 10



Would this jaundice concern you?
What would you investigate?

Initial work-up

Investigations for neonatal jaundice



Direct Coombs test

Negative



Initial work-up

Investigations for neonatal jaundice



Direct Coombs test

Negative



G6PD activity

(normal range: 7–10 U/g Hb)

8.2 U/g Hb

Normal



Pyruvate kinase activity

(normal range: 10–15 U/g Hb)

12.5 U/g Hb

Normal



Direct Coombs test	Negative
G6PD activity	8.2 U/g Hb Normal
Pyruvate kinase activity	12.5 U/g Hb Normal
Red cell indices	
Haemoglobin electrophoresis	
Bilirubin	

Initial work-up

Investigations for neonatal jaundice



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Red cell indices

MCHC / MCV

Normal



Haemoglobin electrophoresis

HbA 95%
HbA₂ 2.5%
HbF 2.5%

Reported as
normal



The first **strange** clue

Unexpected low oxygen saturation



Day 10

SpO₂ 88–90% in room air

SpO₂
89 %



The first **strange** clue

Unexpected low oxygen saturation



Day 10

SpO₂ 88–90% in room air



What would you do
at this point?



The first **strange** clue

Unexpected low oxygen saturation



Day 10

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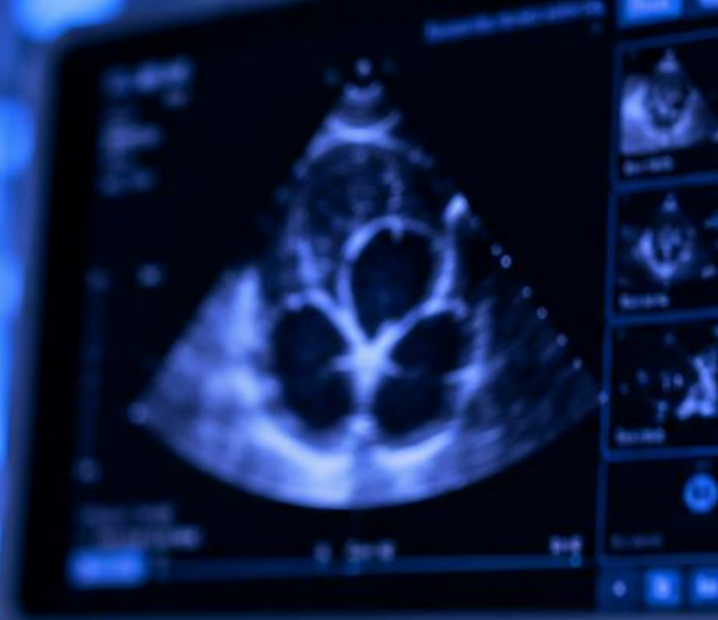
Clinical examination

Normal



Echocardiography

Normal



Finally, **all good?**

Repeat assessment before discharge



Repeat saturation measurement
Normal



Discharged home on day 11



Day 14 – Emergency Department

A clinical deterioration



Parents present at the ED
for episodes of **apnea**
reported by the parents

URGENCES



Day 14 – Emergency Department

A clinical deterioration



Parents present at the ED
for episodes of **apnea**
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What would you do
with this patient?

URGENCES



Day 14 – Emergency Department

A clinical deterioration



Vital parameters



Heart rate (FC) **122 bpm**



Respiratory rate (FR) **42 /min**



Temperature (T°) **37.0 °C**



Blood pressure (TA) **60/31 (42)**
mmHg (MAP)



SpO₂ **88–90%**
in room air



Clinical examination



No cyanosis



Mild axial hypotonia



Mildly jaundiced
(subicteric)



Systolic murmur 1/6

URGENCES

Day 14 – Emergency Department

A clinical deterioration



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At this point...

What is your differential diagnosis?

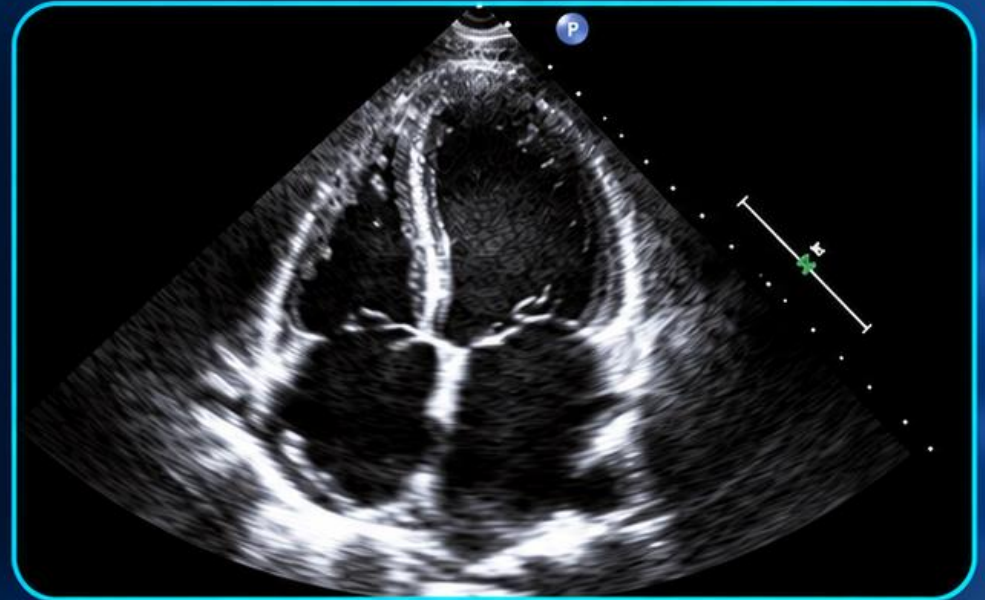
What we ruled out

Common causes first



Heart

Normal echocardiography,
normal function,
no intracardiac shunt
(except for a **tiny PFO**)



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Infection

Negative cultures, negative CRP,
normal WBC

What we ruled out

Common causes first



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Normal echocardiography, normal function,
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Infection

Negative cultures, negative CRP,
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Pulmonary

Normal chest X-ray,
no signs of respiratory infection,
no recurrent apneas in the ED



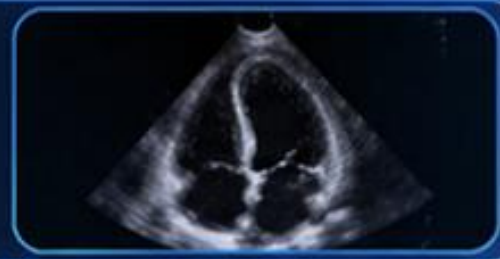
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Metabolic

Normal glucose, electrolytes,
acid-base status, no metabolic disorder suspected

Initial discussion

Family history at admission



**"No particular medical history
in the family."**



Mother
of Polish origin



Father
of Mexican origin



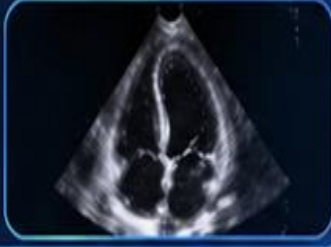
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Infection

Negative cultures, negative CRP, normal WBC



Pulmonary

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Metabolic

Normal glucose, electrolytes, acid-base status, no metabolic disorder suspected



This patient still has a **persistent saturation of 88%**

What treatment would you start?



We treated the number

Escalating respiratory support without effect

Oxygen

Nasal cannula



SpO₂ 88%



High flow

HFNC



SpO₂ 88%



CPAP

Nasal CPAP



SpO₂ 88%



Increasing FiO₂

FiO₂ up to 40–50%



SpO₂ 88%



Despite escalating respiratory support and increasing FiO₂,
the SpO₂ remains at 88%.

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Escalating respiratory support without effect

Oxygen

Nasal cannula



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Nasal CPAP



SpO₂ 88%

Increasing FiO₂

FiO₂ up to 40–50%



SpO₂ 88%



Despite escalating respiratory support and increasing FiO₂,
the SpO₂ remains at 88%.

What additional examination(s) would you perform now?

Venous blood gas

Venous blood gas analysis



Venous blood gas

Sample	Venous
pH	7.38
pCO ₂	40 mmHg
pO ₂	30 mmHg
Base excess	-1.1 mmol/L
Bicarbonate	23 mmol/L
Total hemoglobin	11.5 g/dL
Lactate	2.4 mmol/L



How do you
interpret
these results?



Further discussion with the father



Arrived in Belgium
2 years ago.



Underwent a **cholecystectomy**
due to **gallstones**.
The cause of the gallstones
remained unknown.



During this hospitalization,
he was found to have
a **chronic hemolytic anemia**.
He has since been followed
by a hematologist in Belgium.



His oxygen saturation
is usually around **85–88%.**



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**We contacted
his hematologist.**

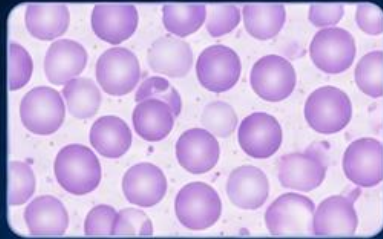
**What investigations
did he perform?**

Investigations performed by the father's hematologist

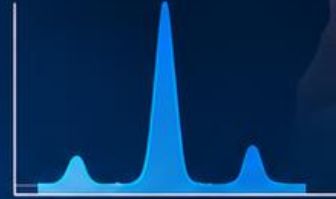
Complete blood count



Peripheral blood smear



Hemoglobin electrophoresis



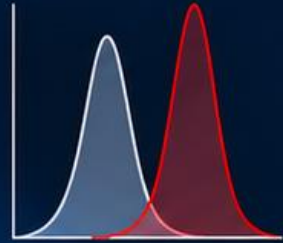
Direct Coombs test



Red cell indices (MCHC)

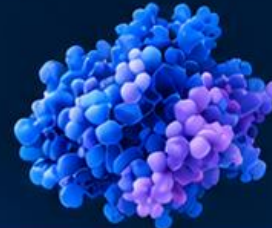


EMA test (for hereditary spherocytosis)

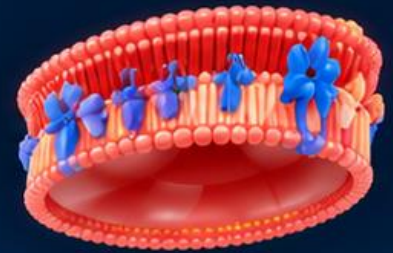


Enzymatic tests

- G6PD activity
- Pyruvate kinase activity



Membrane tests





Blood gas Baby

Venous sample

pH	7.38	
pCO ₂	40	mmHg
pO ₂	30	mmHg
HCO ₃ ⁻	23	mmol/L
Base excess	-1.1	mmol/L
Total CO ₂	23	mmol/L
O ₂ saturation (measured)	65.2	%
O ₂ saturation (calculated)	86	%
Total hemoglobin	11.5	g/dL
Oxyhemoglobin	62.1	%
Deoxyhemoglobin	33.2	%
Carboxyhemoglobin	0.5	%
Methemoglobin	4.2	%
Hematocrit	36	%
Lactate	2.4	mmol/L



Do you see
anything **abnormal**?





Blood gas Baby

Venous sample

pH	7.38	
pCO ₂	40	mmHg
pO ₂	30	mmHg
Base excess	-1.1	mmol/L
Bicarbonate	23	mmol/L
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Blood gas Father

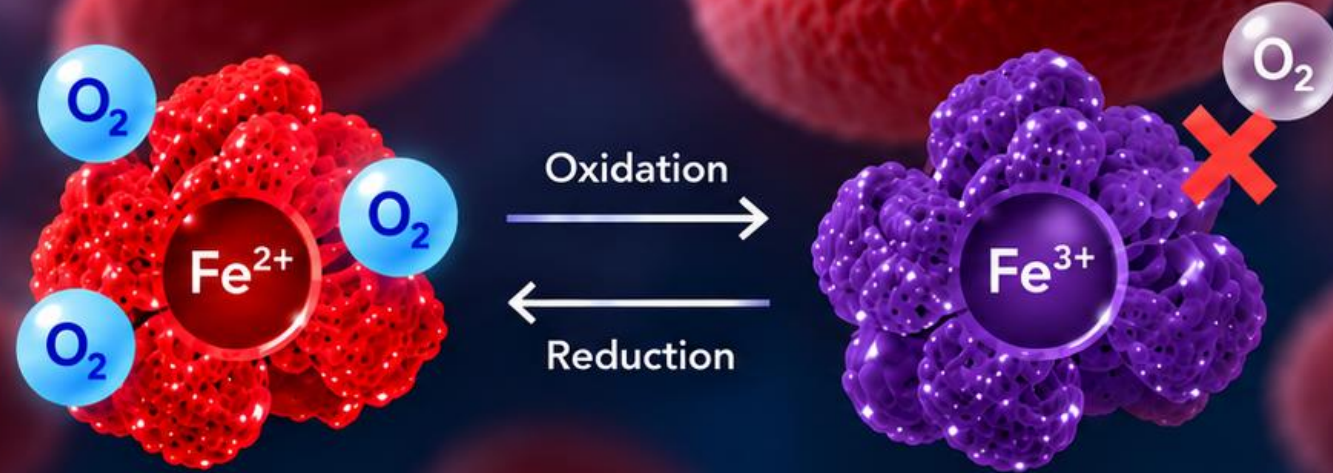
Venous sample

pH	7.38	
pCO ₂	40	mmHg
pO ₂	30	mmHg
Base excess	-1.0	mmol/L
Bicarbonate	24	mmol/L
O ₂ saturation (calculated)	64.1	%
Total hemoglobin	12.1	g/dL
Oxyhemoglobin	60.8	%
Deoxyhemoglobin	32.0	%
Carboxyhemoglobin	0.6	%
Methemoglobin	6.8	%
Hematocrit	37	%
Lactate	2.1	mmol/L



Do you notice the **same pattern** in both?

What is **methemoglobinemia**?



Ferrous iron (Fe^{2+})

Binds oxygen
Functional hemoglobin

Ferric iron (Fe^{3+})

Cannot bind oxygen
Methemoglobin



Definition

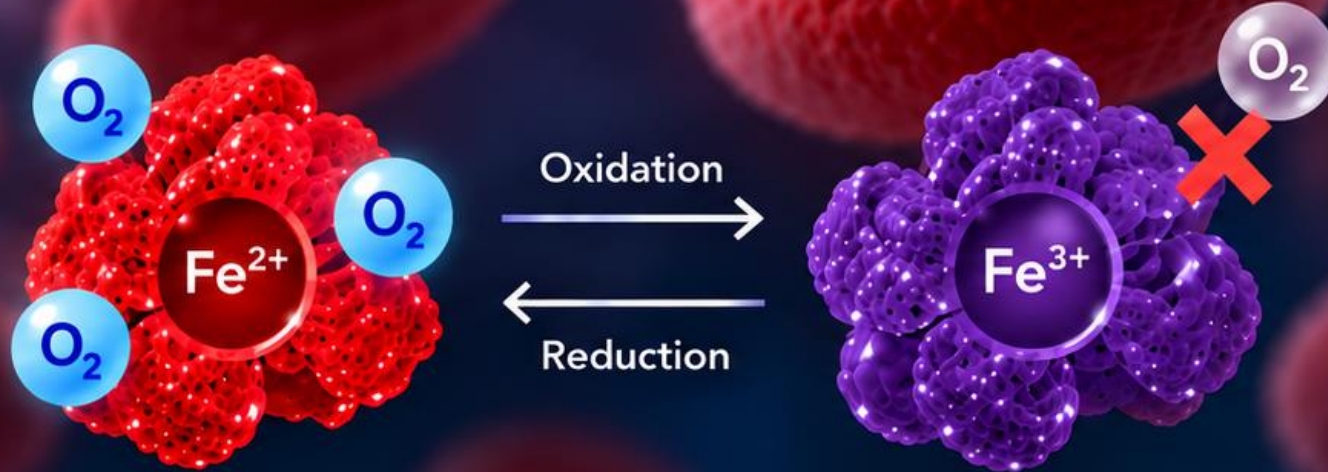
Methemoglobinemia is a condition in which the iron in hemoglobin is oxidized from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}), resulting in methemoglobin, which **cannot bind oxygen**.



Key consequences

- Leads to a **reduced oxygen-carrying capacity** of blood.
- Shifts the oxygen dissociation curve to the **left** for the remaining functional hemoglobin.
- Causes **tissue hypoxia** despite a **normal PaO_2** , because methemoglobin cannot carry O_2 and increases O_2 affinity of the remaining functional hemoglobin.

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Do you know some **causes** of methemoglobinemia?

Acquired causes of methemoglobinemia



Drugs and chemicals

- Nitrates and nitrites (e.g., nitroprusside, nitroglycerin)
- Local anesthetics (e.g., benzocaine, lidocaine, prilocaine)
→ **EMLA® cream** (lidocaine + prilocaine)
- Antimalarials (e.g., dapson, primaquine)
- Other oxidant drugs (e.g., sulfonamides, certain antibiotics)



Environmental exposures

- Nitrite-containing fertilizers, well water
- Industrial chemicals (e.g., aniline dyes)
- Smoke inhalation (e.g., from fires)



Dietary (infants)

- Nitrate-rich foods (e.g., poorly stored vegetables)
- Contaminated water used for formula preparation



Medical conditions

- Severe infections and sepsis
- Hemolytic states
- Metabolic acidosis, shock, hypoxia



In this patient, do you think the cause of methemoglobinemia is **acquired** or not?

Congenital causes of methemoglobinemia



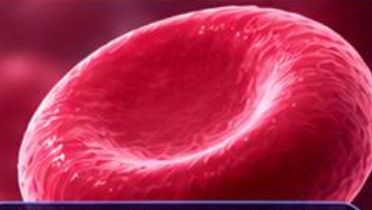
Hemoglobin variants (structural abnormalities)

- Point mutations in globin genes (most often HBA1, HBA2; less often HBB).
- Stabilize hemoglobin in the ferric state (Fe^{3+}) → reduced oxygen binding.
- Most common: **Hb M variants**.
- Other rare variants: Hb Denver, Hb Hammersmith, Hb Iwate, ...

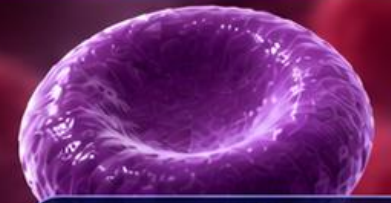


Congenital enzyme deficiencies

- Deficiency of enzymes that reduce methemoglobin back to ferrous iron (Fe^{2+}).
 - **NADH-cytochrome b_5 reductase deficiency**
 - Type I: isolated, usually benign
 - Type II: associated with neurologic abnormalities (developmental delay, seizures, microcephaly, hypotonia, ...).
 - **Cytochrome b_5 deficiency** (rare, similar mechanism).



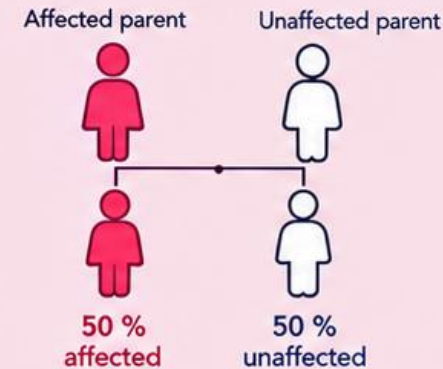
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cannot bind O_2

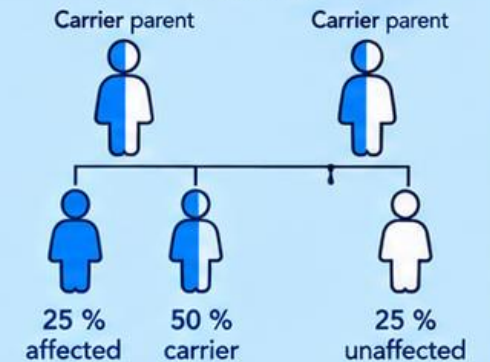
Inheritance patterns

Autosomal dominant (e.g. most Hb M variants)



- Often mild, sometimes incidental.
- Vertical transmission across generations.
- Variable penetrance and expressivity.

Autosomal recessive (e.g. NADH-cytochrome b_5 reductase deficiency)



- Usually more severe (especially type II).
- Consanguinity can be a risk factor.
- Both parents are asymptomatic carriers.

Congenital causes of methemoglobinemia



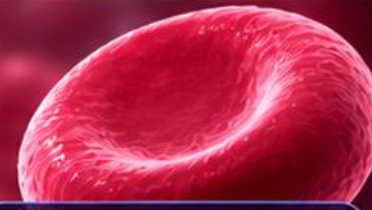
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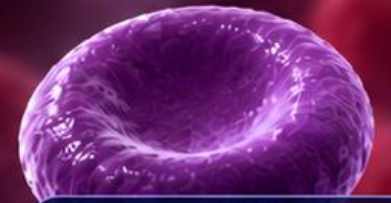


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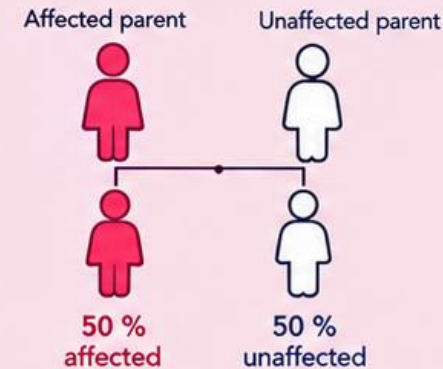
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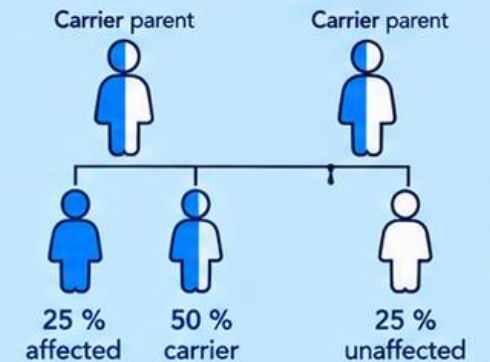
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- Usually more severe (especially type II).
- Consanguinity can be a risk factor.
- Both parents are asymptomatic carriers.



In this patient, do you think the congenital cause is **autosomal dominant** or **autosomal recessive** ?

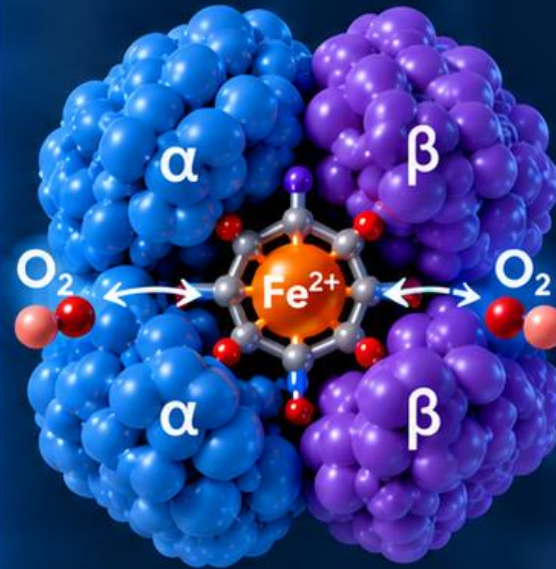


Congenital causes of methemoglobinemia

Hemoglobin M

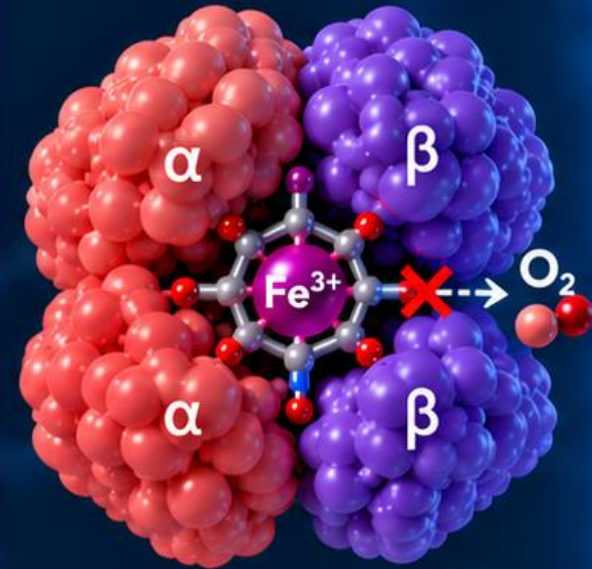
- **Point mutation in globin genes**
(most often HBA2, less often HBA1 or HBB).
- **Structural change** near the heme pocket
→ stabilizes hemoglobin in the **ferric state (Fe^{3+})** which cannot bind oxygen.
- Usually **autosomal dominant**.
- Results in a persistent, stable **mild to moderate** methemoglobinemia.

Normal hemoglobin



Fe^{2+} binds O_2

Hemoglobin M variant



Fe^{3+} cannot bind O_2

Hemoglobin M

Clinical presentation



**Persistent cyanosis /
low SpO₂**

Often around 85–90%



**Poor or absent
response to supplemental
oxygen**



**Chocolate-brown
blood**

Due to oxidized hemoglobin
(methemoglobin)



Usually clinically well

Especially in heterozygous
Hb M disease



The age at presentation
depends on which globin chain
is affected.



In this newborn, which globin chain
do you think is affected?

α-chain

β-chain

γ-chain

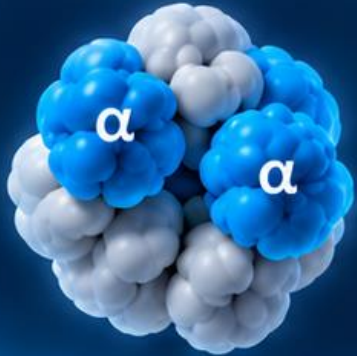
When does Hemoglobin M become apparent?

It depends on the affected globin chain

Main hemoglobin type during development

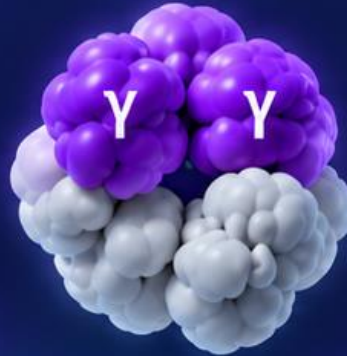


α -chain Hb M



Present
at birth.

γ -chain Hb M



Present
in the
neonatal
period.

β -chain Hb M



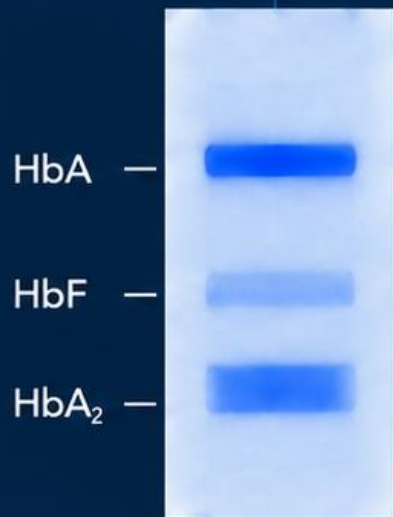
Appears
after the
first months
of life.



Our patient was symptomatic in the neonatal period → an α -chain variant.

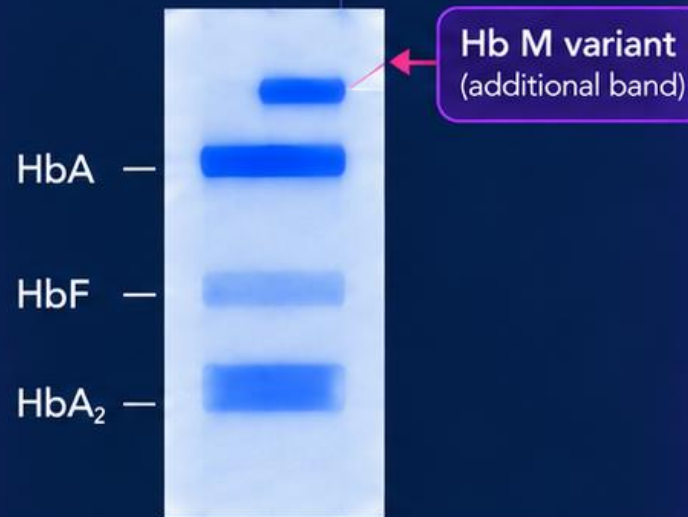
Hemoglobin electrophoresis in Hemoglobin M

Normal pattern



HbA, HbF and HbA₂
at their usual position.

Hemoglobin M pattern

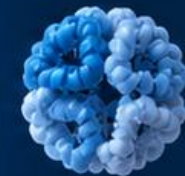


In many cases, an **additional band**
corresponding to **Hb M** is visible.

Why can electrophoresis be normal in Hemoglobin M ?



Hemoglobin M results from a **point mutation** in a globin gene (most often **α -chain**).



The mutation causes a subtle structural change with **little or no change in the electric charge or migration** of the molecule.



Therefore, the Hb M variant can **co-migrate** with normal hemoglobin (e.g. HbA), leading to a **normal electrophoresis pattern**.



A normal hemoglobin electrophoresis does not exclude Hemoglobin M.

Additional tests (e.g. co-oximetry, genetic analysis) are often required.

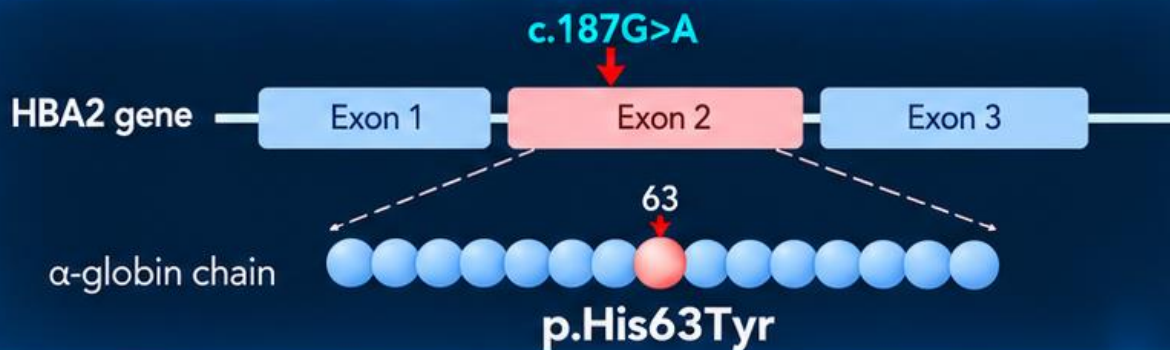
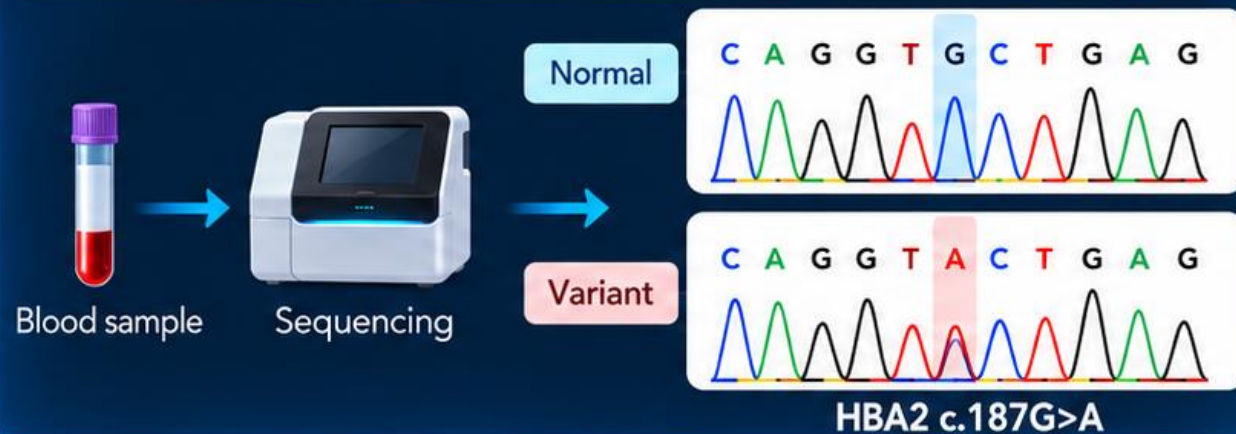


Genetic analysis in Hemoglobin M

Genetic analysis confirms the diagnosis

- ✓ Detects the **exact mutation**
- ✓ Confirms **Hemoglobin M**
- ✓ Our patient:
HBA2 c.187G>A (p.His63Tyr)

Mutation identified



Diagnosis confirmed: Hb Evans

Why does the pulse oximeter remain ~85% in methemoglobinemia / Hemoglobin M?

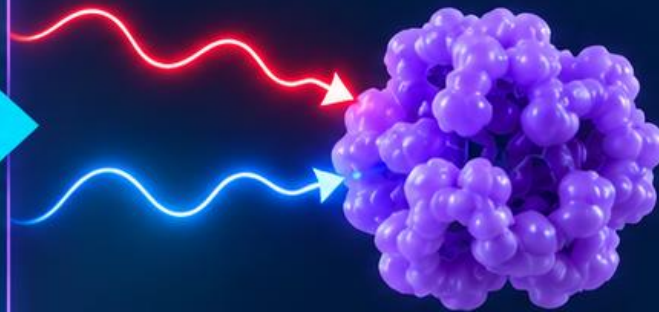
1 Normal pulse oximetry

Uses **two wavelengths** of light to distinguish **oxyhemoglobin** from **deoxyhemoglobin**.



2 Hemoglobin M

Absorbs red and infrared light in a **similar way**.



The two wavelengths are absorbed similarly → the oximeter measures a **ratio ≈ 1** .

3 Oximeter calculation

A ratio of ≈ 1 is interpreted by the pulse oximeter algorithm as a **saturation of ~85%** (depending on the device).

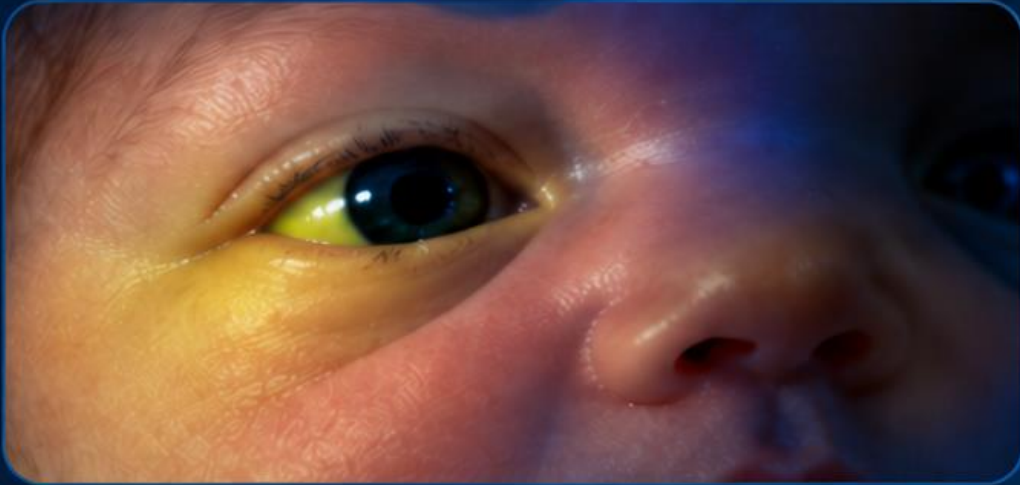


And suddenly, the whole story makes sense

All the clues fit together

1

**Persistent jaundice
in the newborn**



Prolonged neonatal jaundice
despite normal initial work-up.



And suddenly, the whole story makes sense

All the clues fit together

1 Persistent jaundice in the newborn



Prolonged neonatal jaundice
despite normal initial work-up.



2 Low oxygen saturation not improving with oxygen



Persistent SpO₂ 85–88%
with no significant increase
despite supplemental oxygen.

And suddenly, the whole story makes sense

All the clues fit together

1

Persistent jaundice
in the newborn



Prolonged neonatal jaundice
despite normal initial work-up.

2

Low oxygen saturation
not improving with oxygen



SpO₂ 85%
100
90
80
72

Persistent SpO₂ 85–88%
with no significant increase
despite supplemental oxygen.

3

The father is also affected
with similarly low SpO₂

Newborn
SpO₂ 85–88%

Father
SpO₂ 85–88%

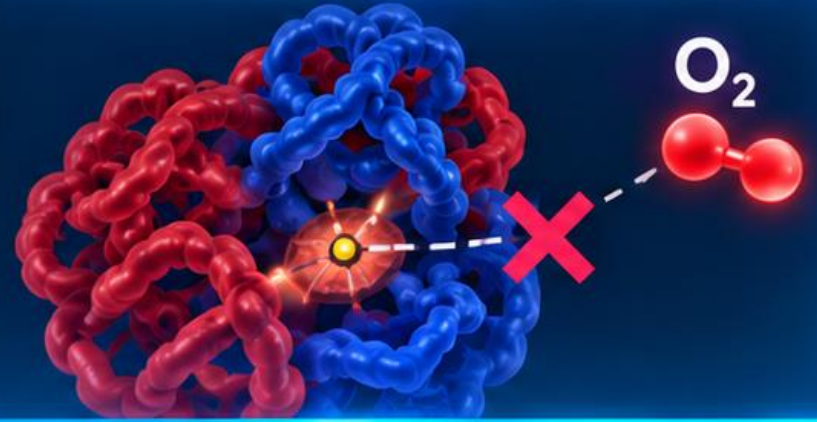


Low oxygen saturation in both the newborn
and his father suggests a **congenital cause**
such as **Hemoglobin M**.

Take home **messages**

1

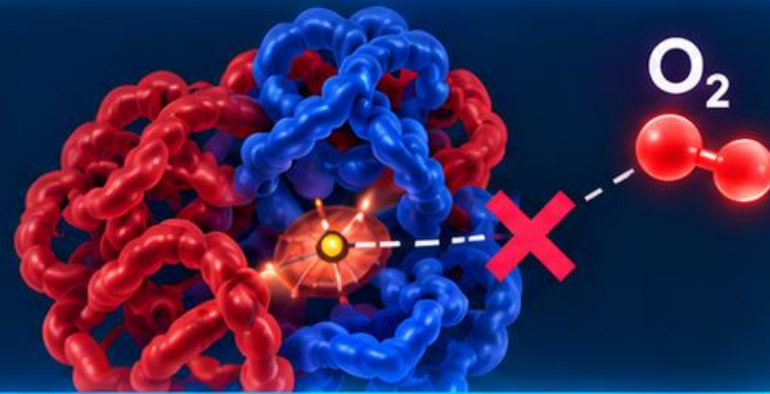
Hemoglobin M is a **rare congenital cause** of **persistent low oxygen saturation**.



Take home **messages**

1

Hemoglobin M is a **rare congenital cause** of **persistent low oxygen saturation**.



2

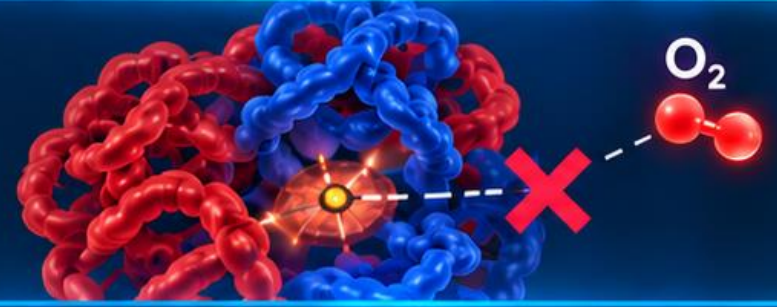
Think of Hb M when there is **unexplained desaturation** with normal respiratory and cardiac evaluation.



Take home **messages**

1

Hemoglobin M is a **rare congenital cause** of **persistent low oxygen saturation**.



2

Think of Hb M when there is **unexplained desaturation** with normal respiratory and cardiac evaluation.



3

It can be **familial**.
Parents and other relatives may also have low SpO₂.



Newborn
SpO₂ 85–88%

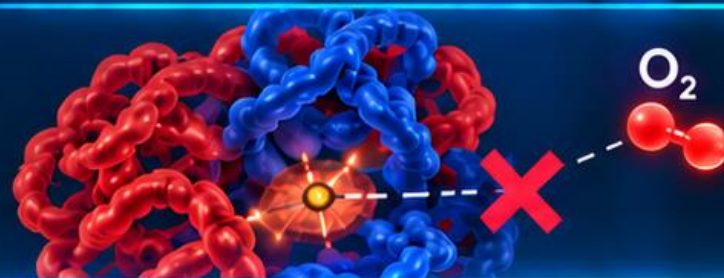


Father
SpO₂ 85–88%

Take home **messages**

1

Hemoglobin M is a **rare congenital cause** of **persistent low oxygen saturation**.



2

Think of Hb M when there is **unexplained desaturation** with normal respiratory and cardiac evaluation.



SpO₂ 100
90
85%
80
72

3

It can be **familial**.
Parents and other relatives may also have low SpO₂.



Newborn
SpO₂ 85–88%



Father
SpO₂ 85–88%

4

Confirm the diagnosis with **hemoglobin electrophoresis** and **genetic analysis**.



HBA2
c.187G>A

Take home messages

1

Hemoglobin M is a **rare congenital cause of persistent low oxygen saturation**.



2

Think of Hb M when there is **unexplained desaturation** with normal respiratory and cardiac evaluation.



3

It can be **familial**.
Parents and other relatives may also have low SpO₂.



4

Confirm the diagnosis with **hemoglobin electrophoresis** and **genetic analysis**.



5

No specific treatment is required.
Recognition avoids unnecessary investigations and reassures families.



A newborn baby is sleeping peacefully in a hospital bed, covered with a patterned blanket. In the background, medical monitors are visible, displaying vital signs such as a heart rate of 98 and a respiratory rate of 120. The scene is dimly lit with a blue tint, creating a calm and professional atmosphere.

Thank you

Questions?

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