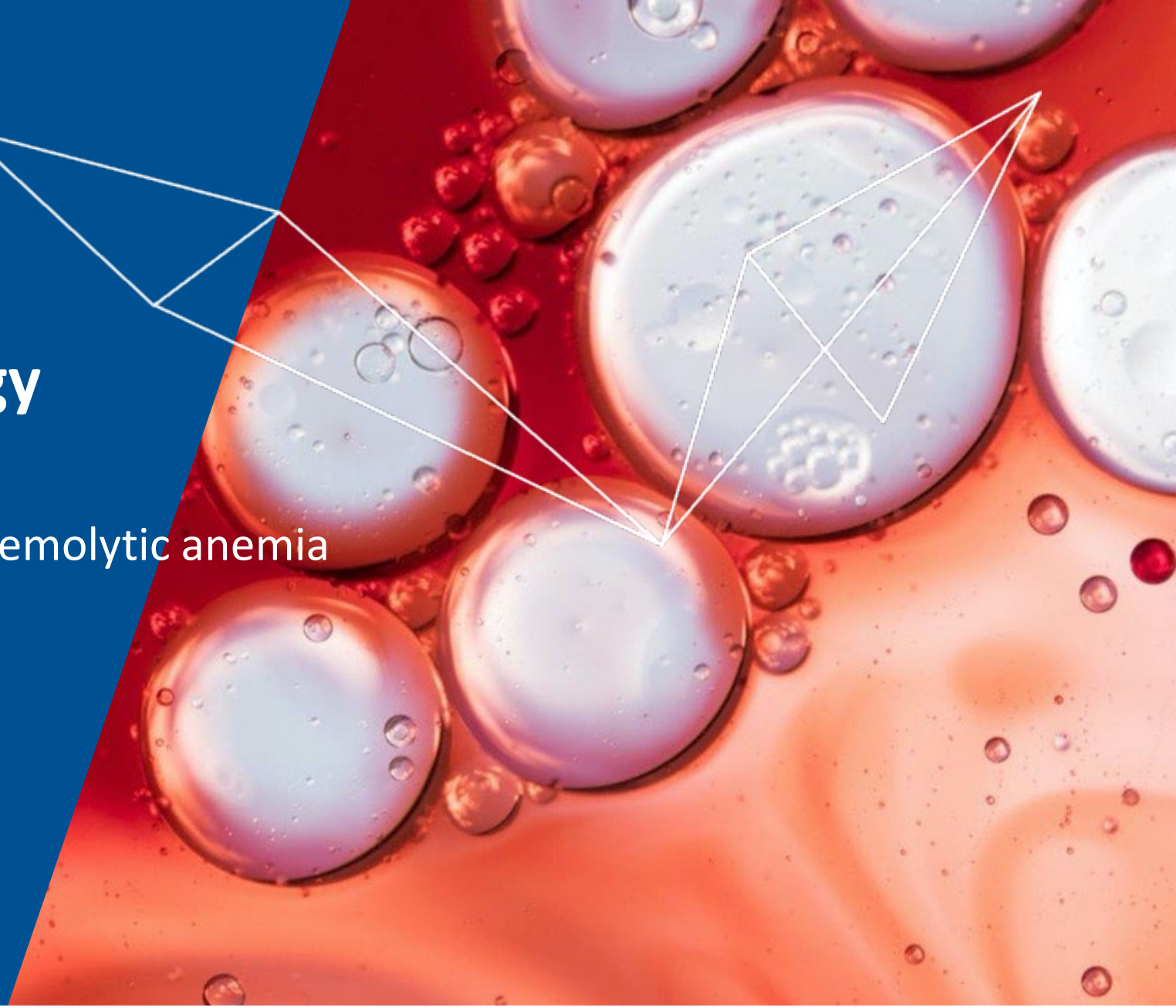
EUROPEAN HEMATOLOGY ASSOCIATION

EHA-ISHBT Hematology Tutorial

Self-assessment Case – Session Hemolytic anemia

Speaker: Pr Lydie Da Costa

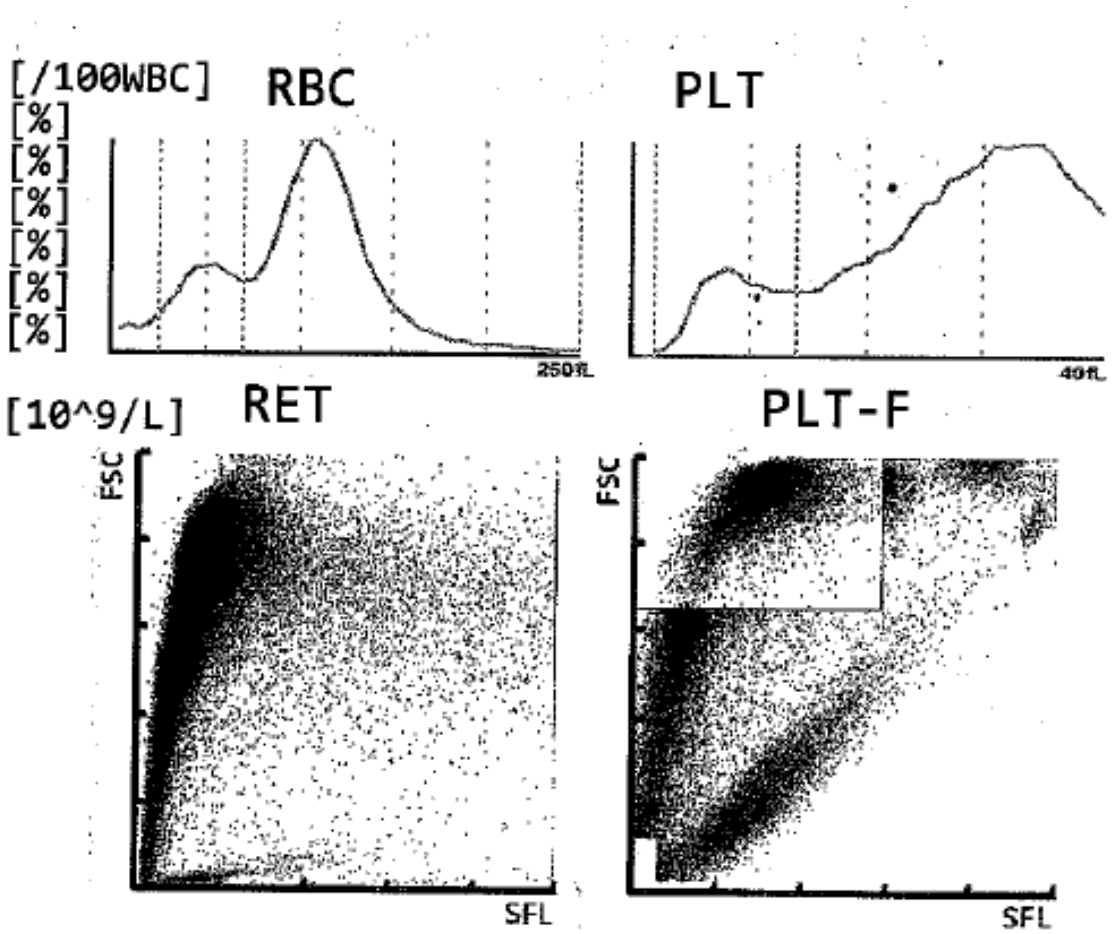
Hyderabad, India
March 1-3, 2024



Introduction

Case of K., referred to the Hospital at Day 3 of life for neonatal jaundice (second child of the family)

leukocytes	14.32 G/L	(6.2-17.1)
RBC	5.88 T/L	(3.78-6.17)
Hb	20.7 g/dL	(13.1-21.9)
Ht	55.7 %	(39.2-62.7)
MCV	94.7 fL	(92-112)
MHC	35.2 pg	(32-39)
MCHC	37.2 g/dL	(31.5-36.5)
PLA	96 G/L	(150-400)
RET	7.02 %	
	412.8G/L	(170-323)



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Questions can be answered by scanning the QR on your phone to access Slido.

For each question you have 15 seconds.

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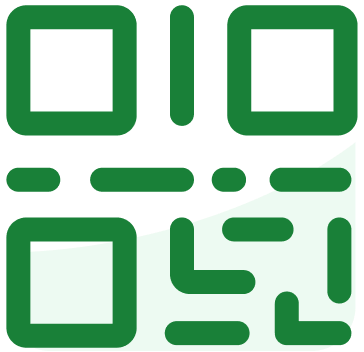

Join at slido.com
#3199010

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Q1) What do you see on the Complete Blood count? (only one response)

1. Anemia
2. Hyperleukocytosis
3. Thrombocytosis
4. Regeneration with a high reticulocyte count
5. Hypochromia

leukocytes	14.32 G/L	(6.2-17.1)
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2.21 What do you see on the Complete Blood count? (only one response)

ⓘ Start presenting to display the poll results on this slide.

Case of K., referred to the Hospital at Day 3 of life for neonatal jaundice

Leukocytes	14.32 G/L	(6.2-17.1)
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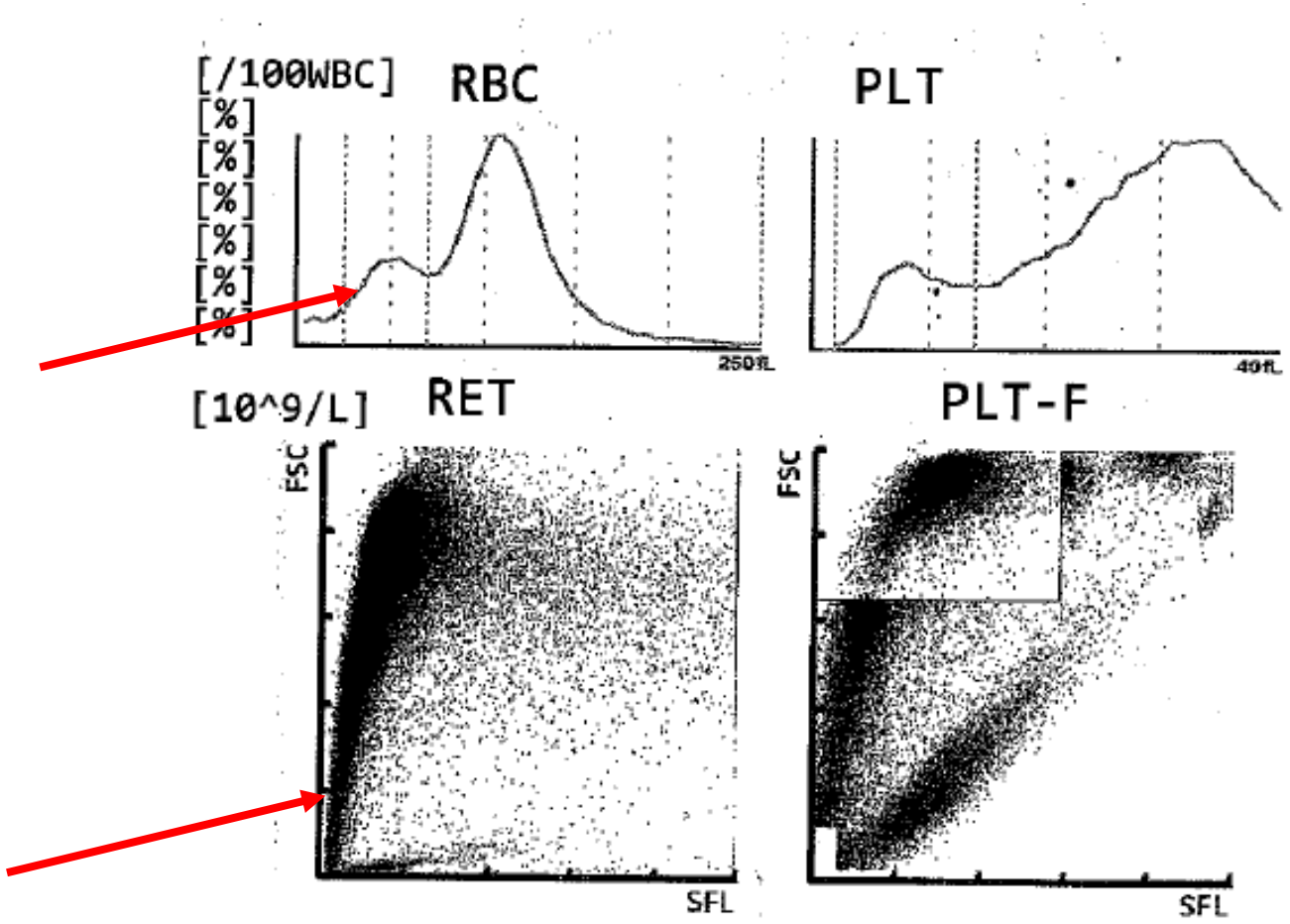
Hyperchromia

False thrombopenia = platelet aggregation

Reticulocytosis without anemia → Hemolysis compensated

Q2) Which cells are represented with the arrows? (only one response)

1. Normal red cell
2. Platelets
3. Erythrocyte fragments
4. Reticulocytes
5. Leukocytes



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2.22 Which cells are represented with the arrows? (only one response)

ⓘ Start presenting to display the poll results on this slide.

Case of K., referred to the Hospital at Day 3 of life for neonatal jaundice

IN RESUME FOR CBC analysis

Leukocytes	14.32 G/L	(6.2-17.1)
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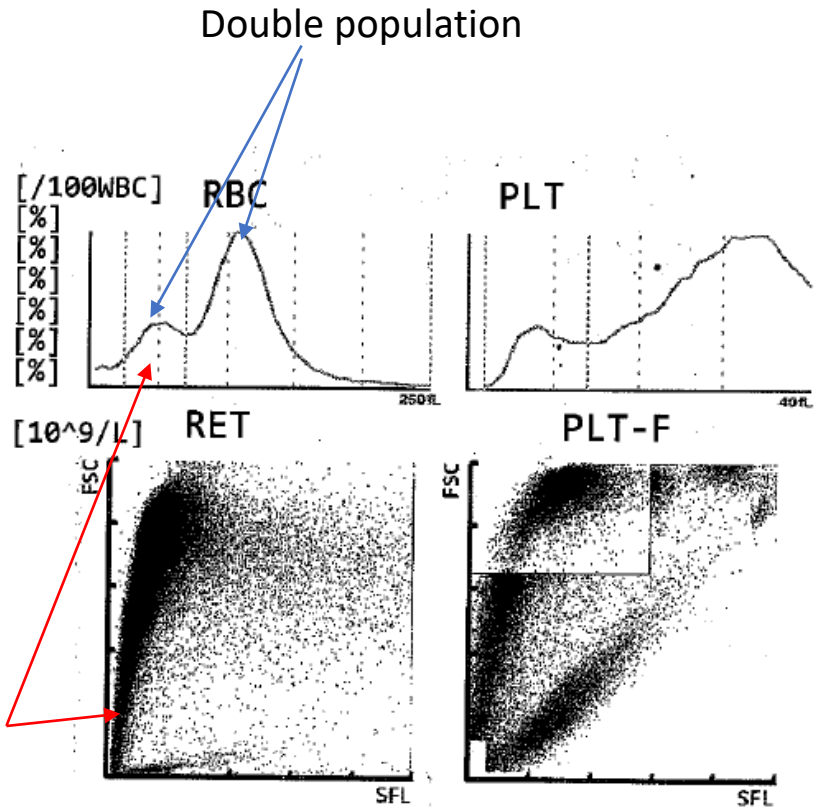


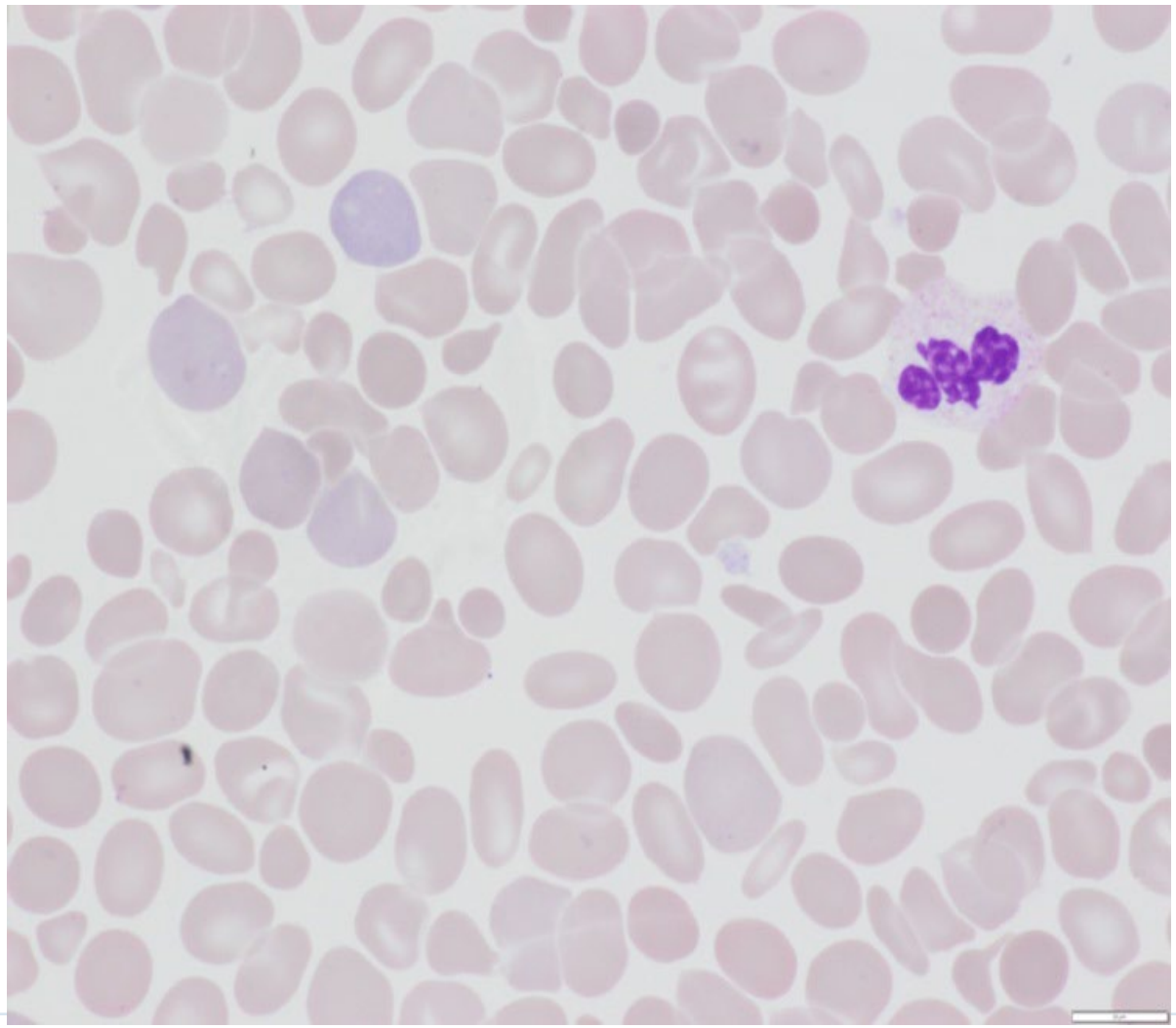
Hyperchromia

False thrombopenia = platelet aggregation

Reticulocytosis without anemia

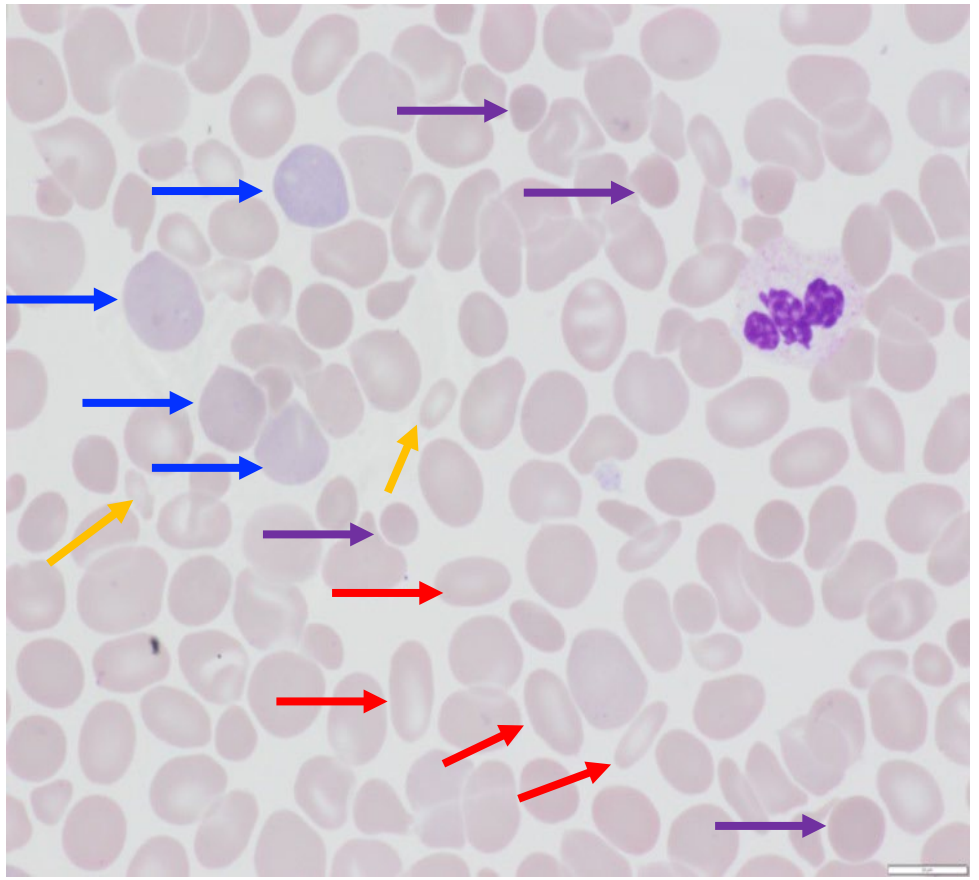
→ Hemolysis compensated

Blood smear



Q3) What do you see on the blood smear? All good answers except one, which one?

1. Sherocytes
2. Elliptocytes
3. Polychromatophilia
4. Stomatocytes
5. Erythrocyte fragments



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2.23 What do you see on the blood smear? All good answers except one, which one?

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Q4) What is the putative diagnosis?

1. Hereditary spherocytosis
2. Hereditary elliptocytosis
3. Hereditary stomatocytosis
4. G6PD deficiency

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2.24 What is the putative diagnosis?

ⓘ Start presenting to display the poll results on this slide.

Case of K., referred to the Hospital at Day 3 of life for neonatal jaundice

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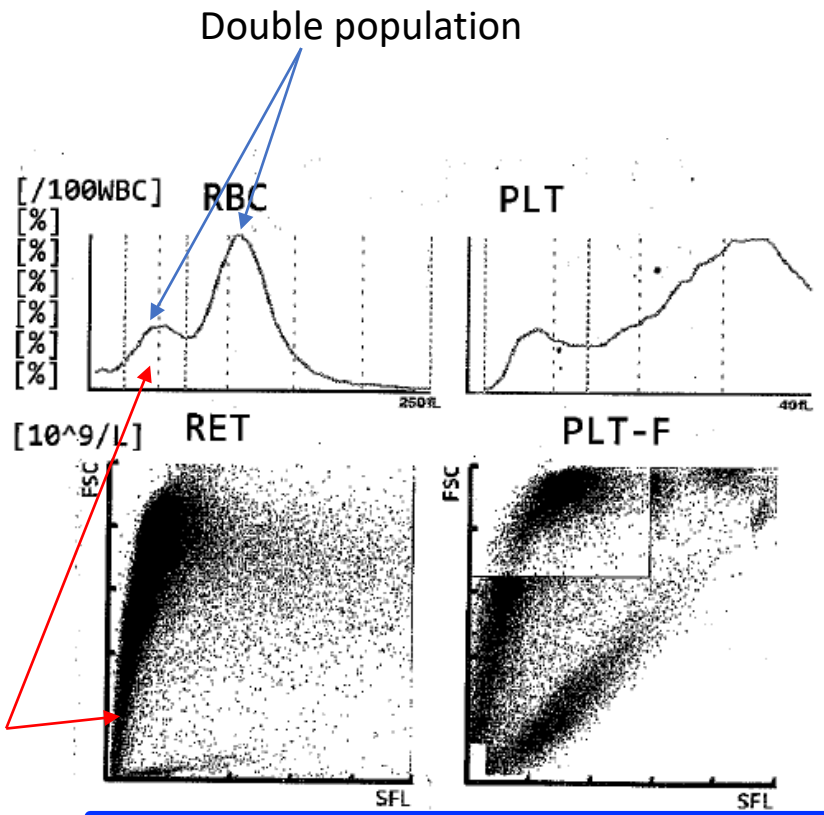

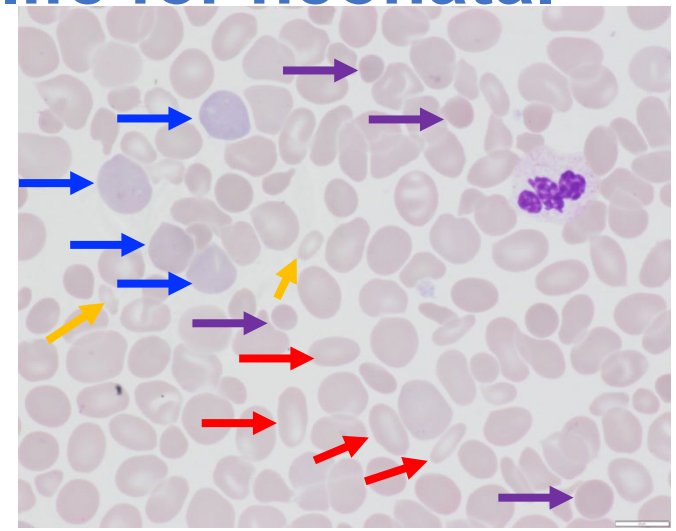

Poïkilocytosis
Polychromatophilia
Erythrocyte Fragments
Microspherocytes
Elliptocytes

Hyperchromia
False thrombopenia = platelet aggregation
Reticulocytosis without anemia
→ Hemolysis compensated

Fortuitous discovery of elliptocytosis
Is it a non severe HE with the poïkilocytosis of the neonates?
Is it a severe EH ?

Q5) What do you do, next (first test to do) to confirm the diagnosis ?

1. Ektacytometry
2. Red cell membrane protein electrophoresis
3. Hemoglobin electrophoresis
4. Molecular biology (targeted-NGS)
5. G6PD activity measurement

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2.25 What do you do, next (first test to do) to confirm the diagnosis ?

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Q5) What do you do, next (first test to do) to confirm the diagnosis ?

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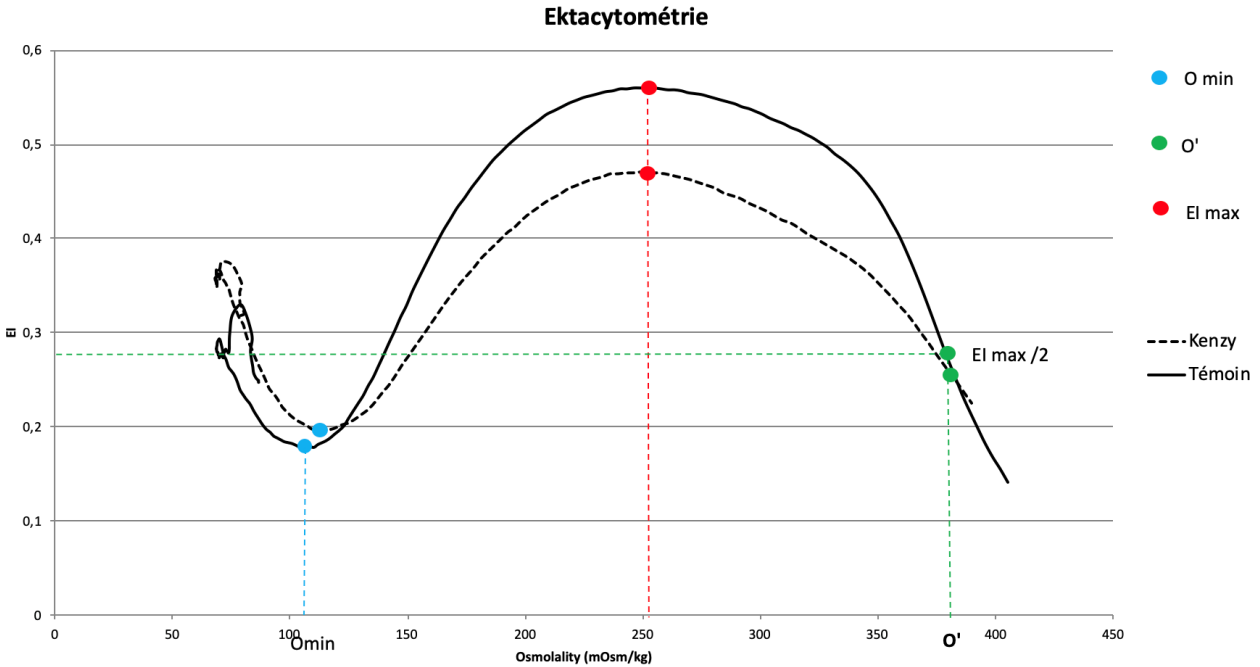
5. G6PD activity measurement

Ektacytometry



EI max : ↓
O'/ Point hyper: normal
Omin : normal

Trapézoïdal feature

Abnormal ektacytometry curve compatible with a hereditary elliptocytosis.

Q6) What do you do, next to make the differential diagnosis between a non severe HE with the important poïkilocytosis of the neonates or a severe EH (pyropoïkilocytosis or HPP)?

1. Ektacytometry
2. Red cell membrane protein electrophoresis
3. Hemoglobin electrophoresis
4. Molecular biology (t-NGS)
5. G6PD activity measurement

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2.26 What do you do, next to make the differential diagnosis between a non severe HE with the important poïkilocytosis of the neonates or a severe EH (pyropoïkilocytosis or HPP)?

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More test results

Molecular Biology

1. **Presence of a heterozygous duplication of 3 NT in exon 4 of the *SPTA1 gene:***

   SPTA1 gene (NM_003126.3) : rs757679761
   c.460_462dup
   p.(Leu155dup)

ClinVar (RCV000598724.1 and RCV000013700.19) : pathogenous variant. Identified in a lot of patients affected with hereditary elliptocytosis in our cohort and literature.

2. Heterozygous alpha-LELY* Polymorphism in exon 40 and intron 45 (Alpha V/41 polymorphism):

SPTA1 gene (NM_003126.3):
c.5572C>G
p.(Leu1858Val)
rs3737515, ClinVar RCV000247499.1
&
c.6531-12C>T
p. ?
rs28525570, ClinVar RCV000249337.1

** « Low-expression allele, Lyon ». Delaunay J, Dhermy D. Semin Hematol. 1993;30(1):21-33*

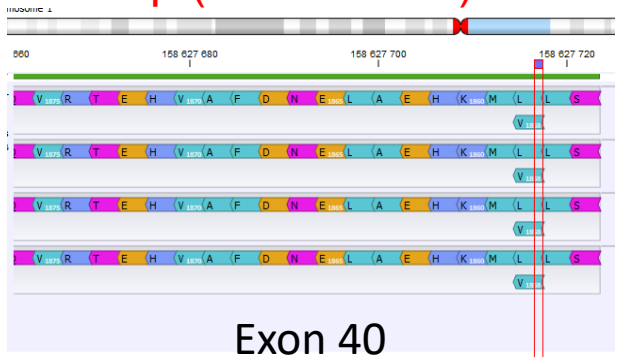
SPTA1 gene (NM_003126.3) :

rs757679761
c.460_462dup
p.(Leu155dup)

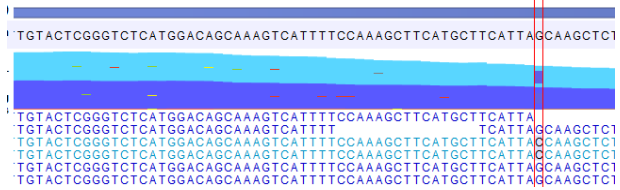
Exon 4

alpha Lely Polymorphism

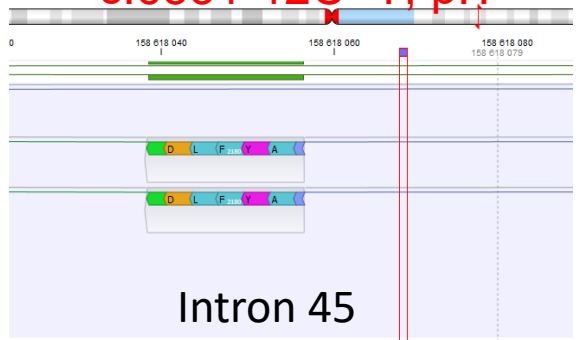
rs3737515
c.5572C>G
p.(Leu1858Val)



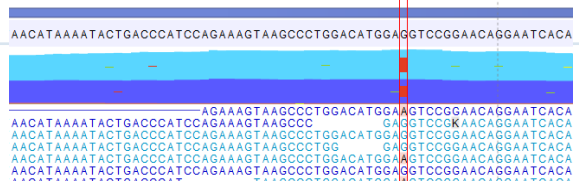
Exon 40



rs28525570,
c.6531-12C>T; p.?



Intron 45

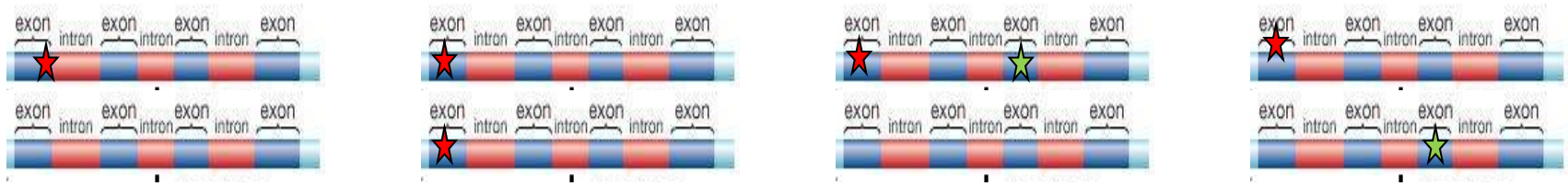


Alpha-Lely variant :

α LELY (Low Expression allele from Lyon)

Mutation de la spectrine

Polymorphisme aLely



α-LELY is a combination of two linked mutations (in exon 40 and intron 45) in the α-spectrin gene (*SPTA1*) :

- Mutation in exon 40 : substitution C > G leading to the amino acid change p.(Leu1857Val)
- Mutation in intron 45 : substitution C>T in minus 12 nucleotides from the splice site of the 46 exon.

46 Exon skipping in 50% of cases in the α spectrin mRNA which avoid the protein from this mutated allele to dimerize.

⇒ This polymorphism is responsible from the low allele expression,

⇒ The polymorphism is completely asymptomatic in the heterozygous patients,

⇒ But also in homozygous ones, because there is a large excess (X3 to 4 times) of the α - spectrin chains,

⇒ Anyway, when the α LELY polymorphism is associated *in trans* with a mutation into the α spectrin (*SPTA1*) gene, the number of the mutated α spectrin chains increased and are responsible for the pyropoïkilocytosis or HPP phenotype.

Q7) What do you do next, in order to confirm the involvement of the alpha Lely polymorphism in the case and its pathology?

1. Nothing: finding alpha Lely polymorphism in the case is enough
2. Screening of the Alpha Lely polymorphism in the first child of the family
3. Screening of each of the parents for Alpha Lely polymorphism
4. Screening of each of the parents for Alpha Lely polymorphism and the other *SPTA1* variant
5. Screening the whole genome in the case

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2.27 What do you do next, in order to confirm the involvement of the alpha Lely polymorphism in the case and its pathology?

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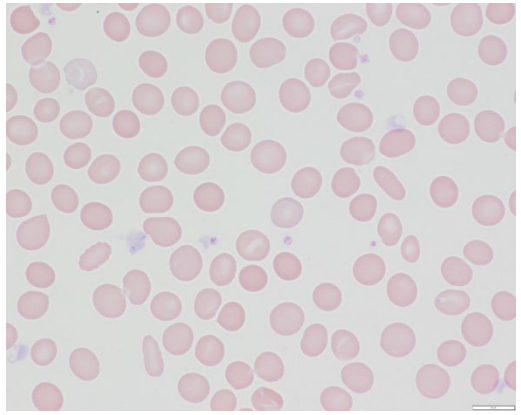
FAMILIAL SCREENING (FAMILIAL SEGREGATION) –

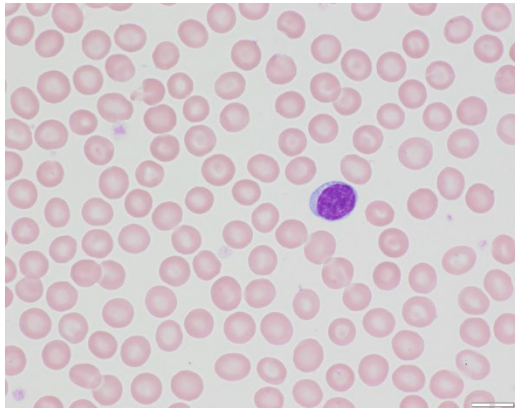
– Ektacytometry (including CBC+retic count+cytology/ektacytometry/EMA test)

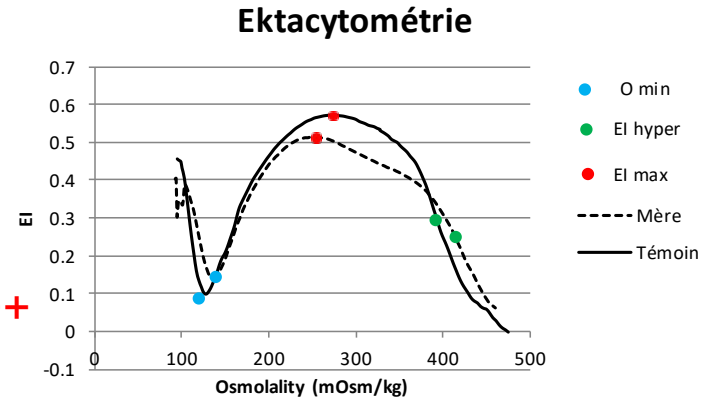
– Molecular Biology (both variant screening : Alpha Lely polymorphism and the other c.460_462dup *SPTA1* variant

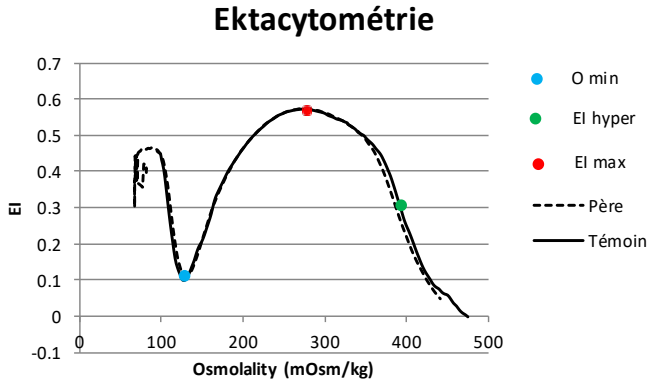
Mother



Father







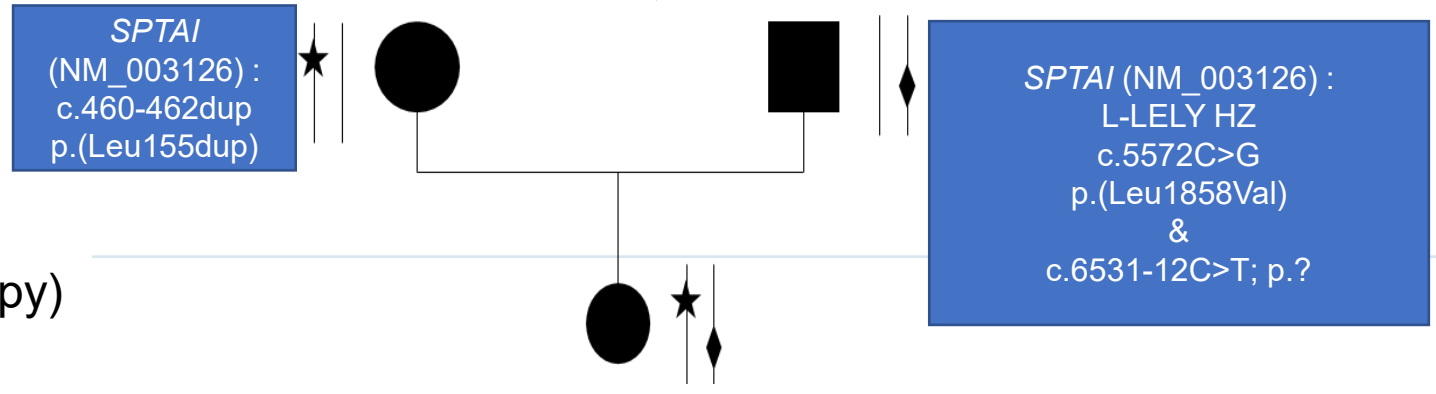
Genetic Counselling +++

Risk of severe ellitocytosis HPP: 25%

→fœtal death *in utero*

→ *Hydrops fetalis*

→neonatal jaundice (phototherapy)



Discussion

- Importance of the accurate interpretation of the CBC and graphs from the automated haematology analyzer
- Importance of the blood smear analysis (cytology of the RBC and reticulocytes and other cell lineage)
- First clue of the diagnosis : both CBC+retic+graphs+cytology in order to prescribe more specialized tests (ektacytometry, molecular biology)
- Gravity to establish (HPP versus non severe HE)
- Importance of the familial segregation study for the diagnosis of HPP and the genetic counselling
- Prenatal diagnosis possible in the very severe form of HPP

References

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