SHERLOCK HOLMES IN HET HEMATOLOGIE LABORATORIUM

Apr. Klin. Biol. Charlotte Matthys
Dr. Ariane Luyckx

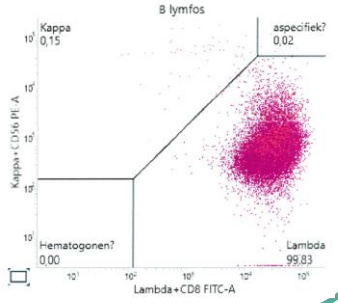
19/03/2024

MARIA MIDDELARES

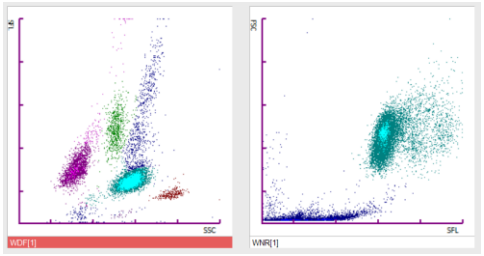
AZ Sint-Vincentius DEINZE

E17 ziekenhuis netwerk

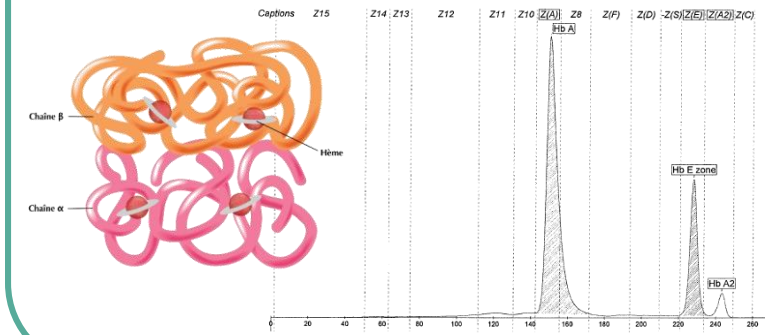
IMMUUNFENOTYPERING



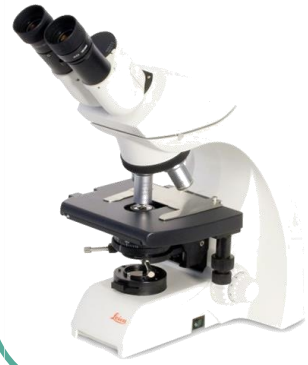
CELTELLING

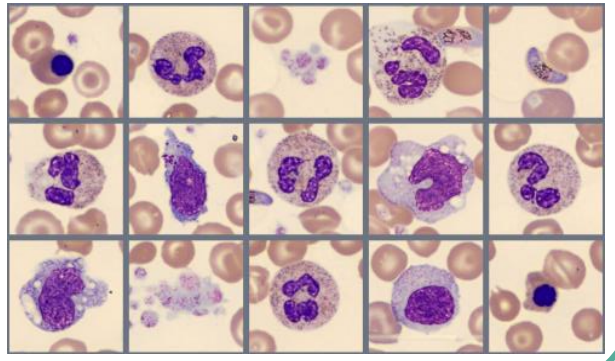


HB-ELEKTROFORESE

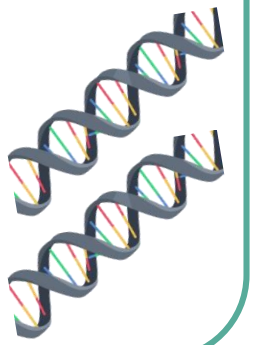


MICROSCOPIE





DNA SEQUENCING



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AZ Maria Middelares • AZ Sint-Vincentius Deinze

Congenitale

bloedcelafwijkingen

Hemolyse

…

Infecties

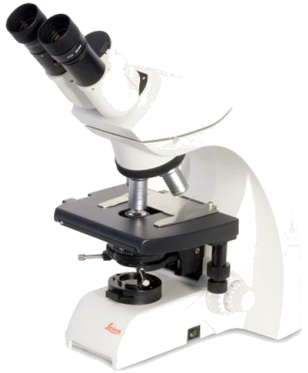
Nutritionele deficiënties

Lymfomen

Acute maligniteiten

Casus: vrouw, 35j

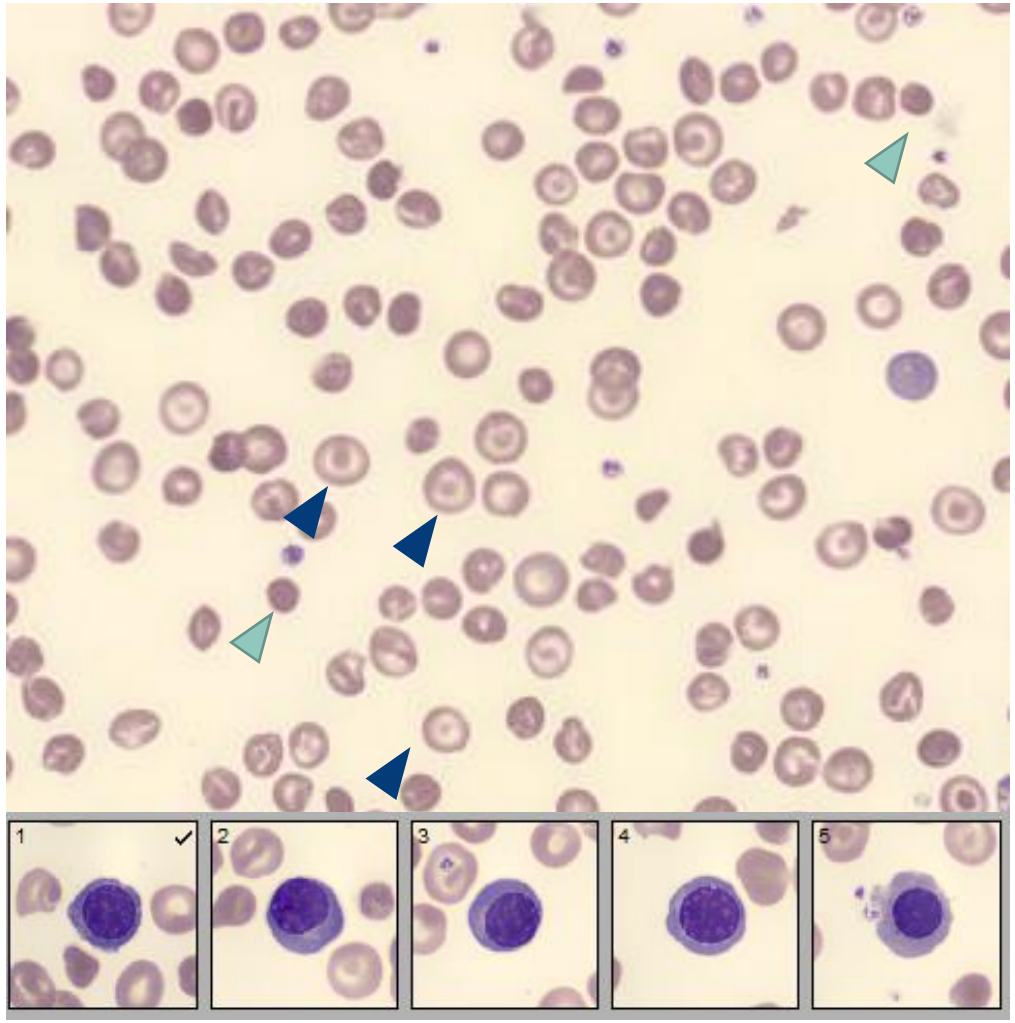
→ hevige buikpijn



Laboresultaten:

Analyse	Resultaat	Referentiewaarden
RBC (10^6/µL)	2,8	3,8-4,8
Hemoglobine (g/dL)	7,5	12,0-15,0
Hematocriet (%)	21	37-46
MCV (fL)	74	76-96
MCH (pg)	27	27-32
MCHC (g/dL)	36	31-37
LDH (U/L)	297	135-250
Ferritine (µg/L)	368	15-150

Microscopisch nazicht RBC:



microsferocyten

targetcellen +++

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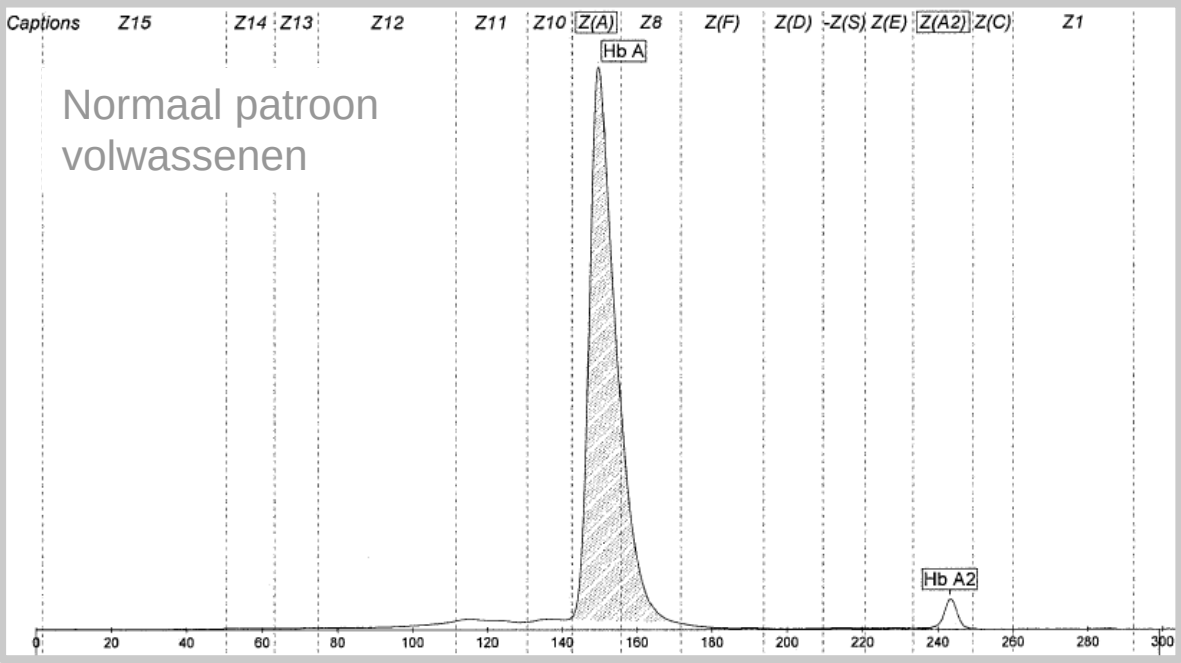
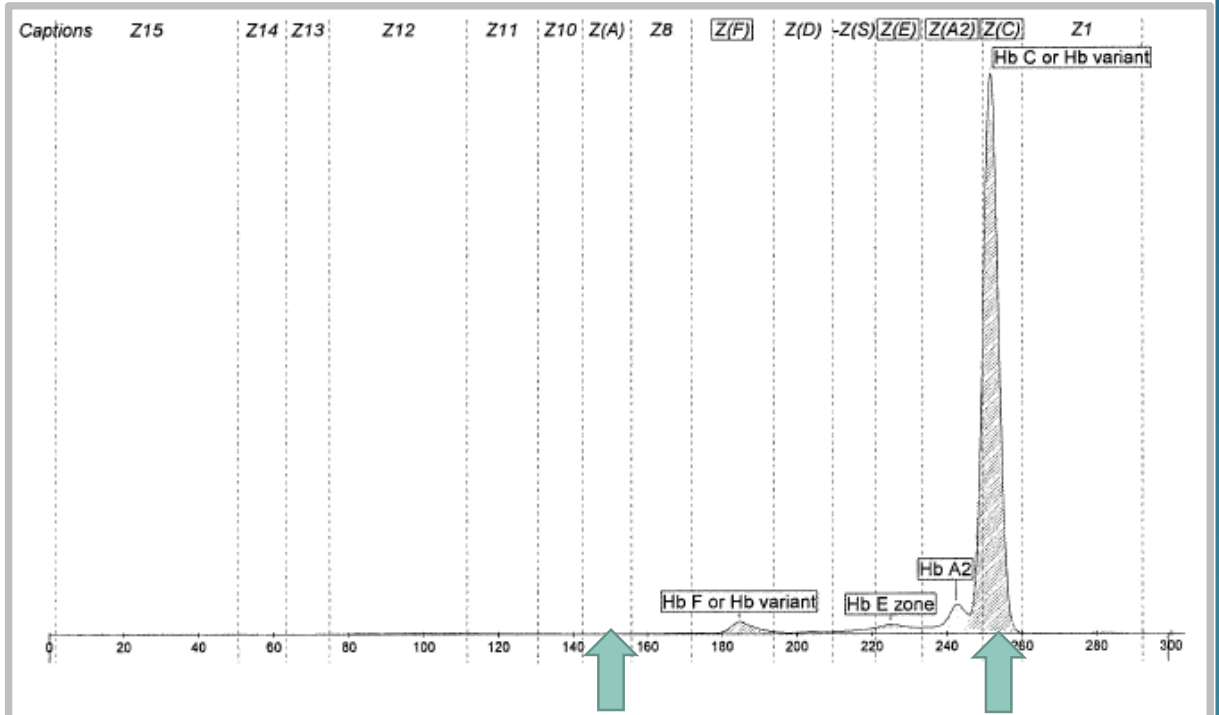
Hemoglobine-electroforese:



Normaal patroon volwassenen



Haemoglobin Electrophoresis

Name	%	Normal Values %
Hb F or Hb variant	2,6	
Hb E zone	1,1	
Hb A2	3,6	
Hb C or Hb variant	92,7	

CT abdomen: Opvallende splenomegalie met craniocaudale diameter tot 16 mm: hematologische problematiek?

Normaal voorkomen van de lever. Galblaas in contractie.

Geen dilatatie van de galwegen.

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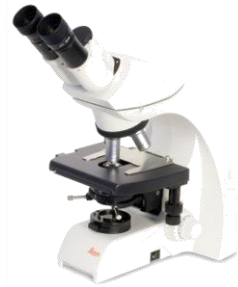
Homozygote Hemoglobine C variant

Klinische impact:

- Milde chronische hemolytische anemie
- Risico op splenomegalie, icterus en galstenen → monitoring
- Doorverwijzing hematoloog
- Kinderwens: partnerscreening

Casus: vrouw, 25j

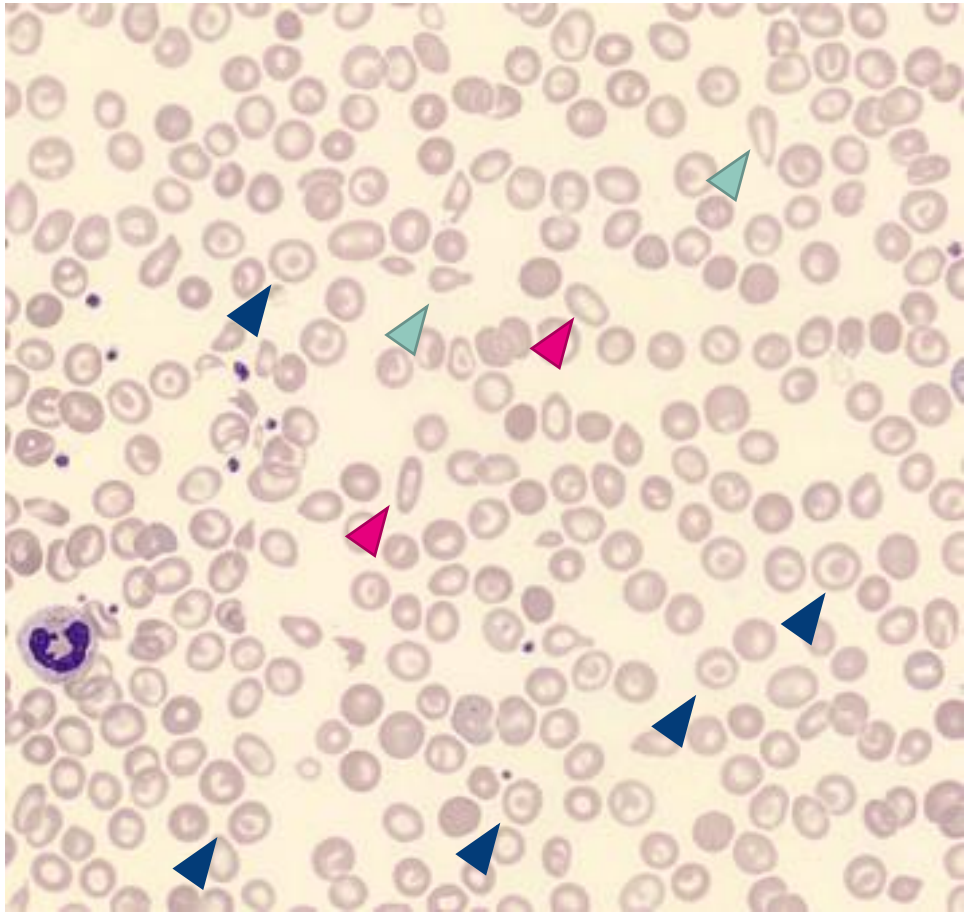
→ controle voor extractie wijsheidstanden



Laboresultaten:

Analyse	Resultaat	Referentiewaarden
RBC (10^6/µL)	6,1	3,8-4,8
Hemoglobine (g/dL)	10,8	12,0-15,0
Hematocriet (%)	36	37-46
MCV (fL)	58	76-96
MCH (pg)	18	27-32
MCHC (g/dL)	30	31-37
Reticulocyten abs.w. (10^3/µL)	155.1	50-100
Ferritine (µg/L)	377	15-150

Microscopisch nazicht RBC:



- Targetcellen ++
- Traandruppelcellen +
- Ovalocyten +

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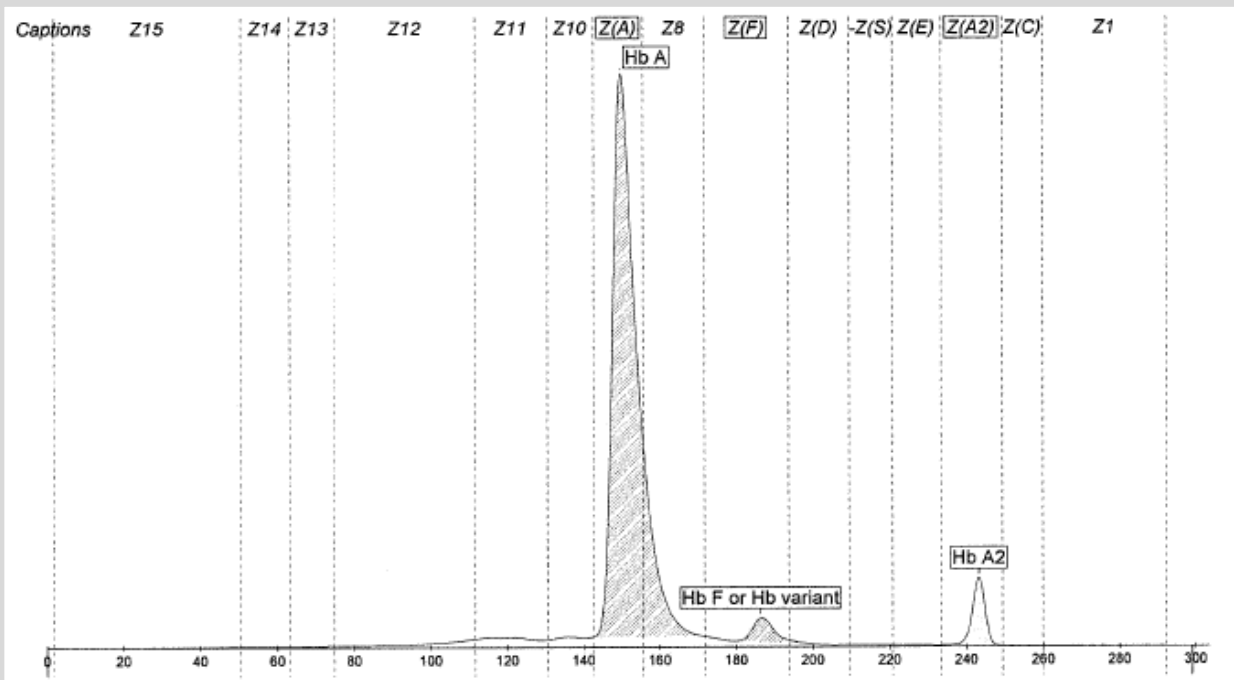
Hemoglobine-electroforese:



Haemoglobin Electrophoresis

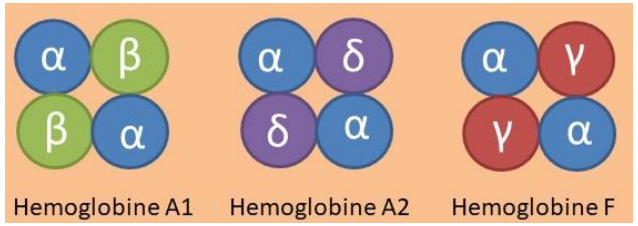
Name	%		Normal Values %
Hb A	**90,8**	<	96,8 - 97,8
Hb F or Hb variant	**3,5**	>	=< 0,5
Hb A2	**5,7**	>	2,2 - 3,2

Beta-thalassemie trait

Klinische impact:

- Meestal asymptomatisch
- Milde anemie
- Kinderwens: partnerscreening en doorverwijzing naar hematoloog

NORMAAL BEELD volwassenen



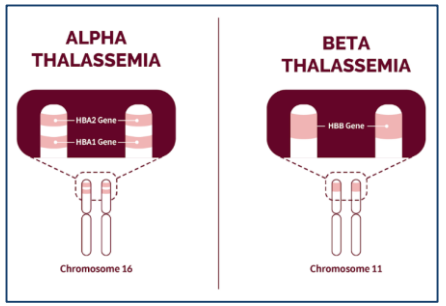
96.7-97.8% **2.2-3.2%** **< 0.5%**

HEMOGLOBINOPATHIE

Kwanitatieve afwijkingen

THALASSEMIE

→ Verminderde globine productie



Kwalitatieve afwijkingen

HEMOGLOBINE VARIANT

→ Productie van abnormaal globine

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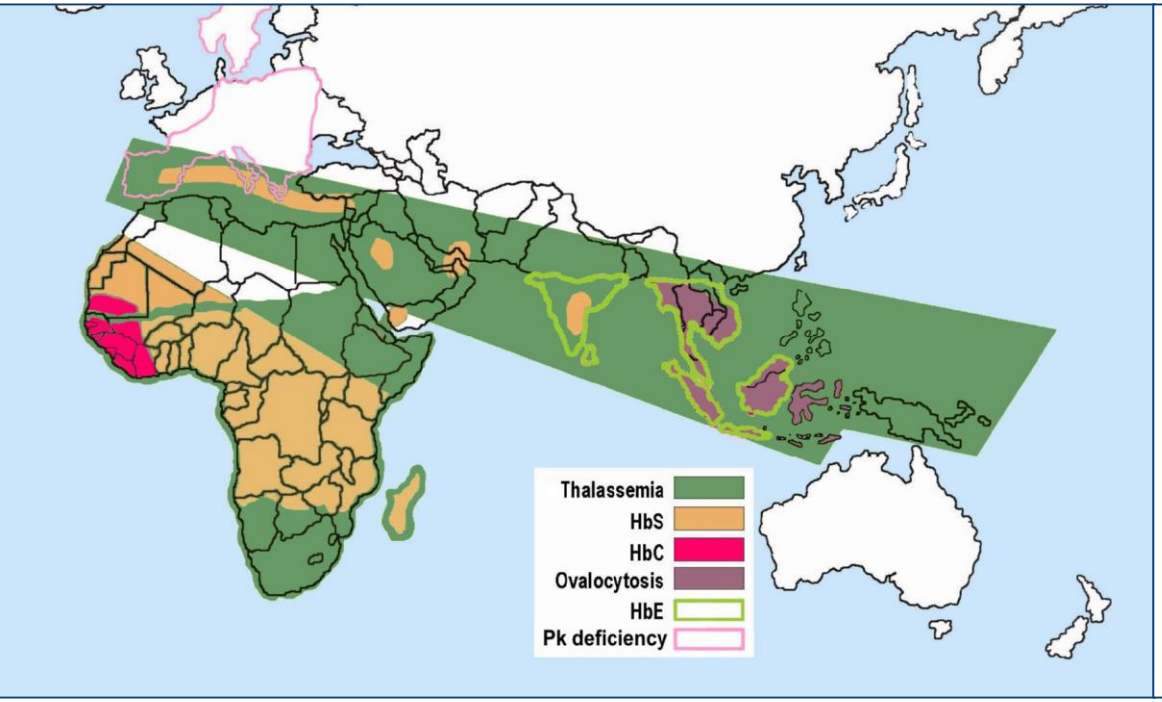
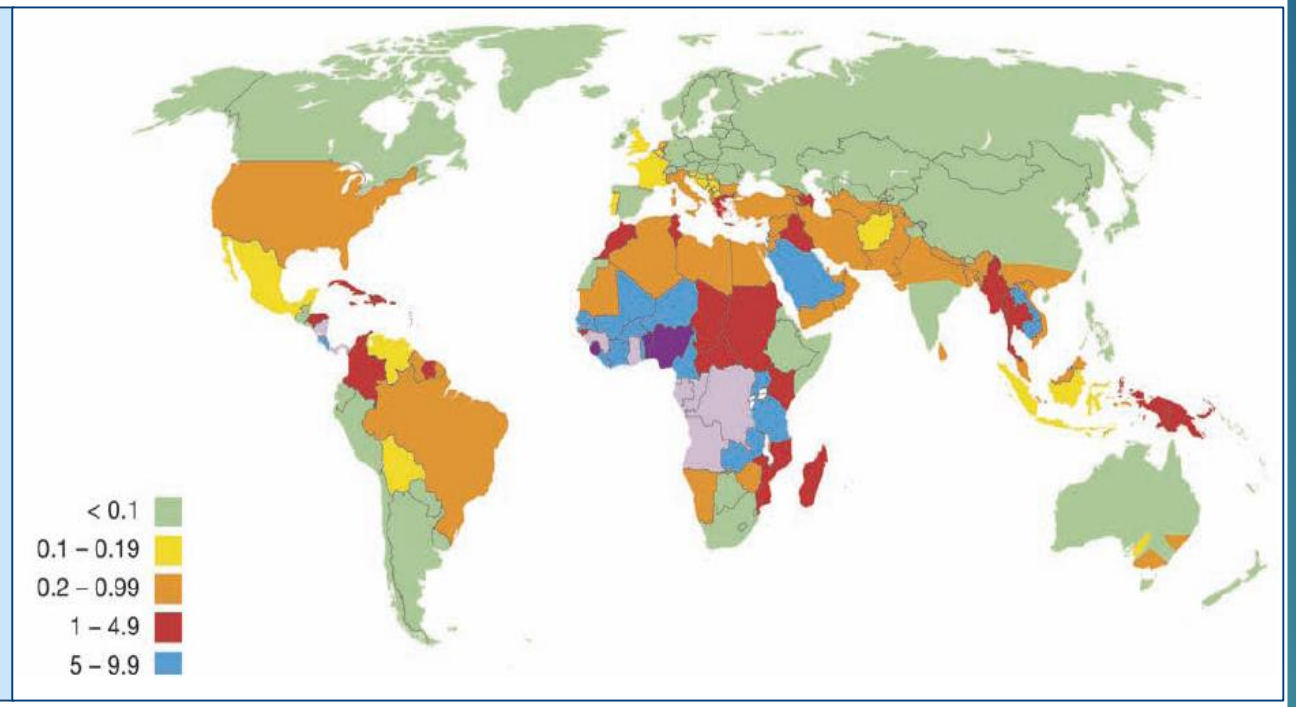
Hemoglobinopathie in de wereld



Lopez at al. Gene 2010; 467: 1-12



WHO 1996

Geboorten met een hemoglobinopathie per 1.000 levendgeborenen

Epidemiologie België

Table 2 | ESTIMATIONS OF THE NUMBER OF CARRIERS IN THE COUNTRIES STUDIED[14]

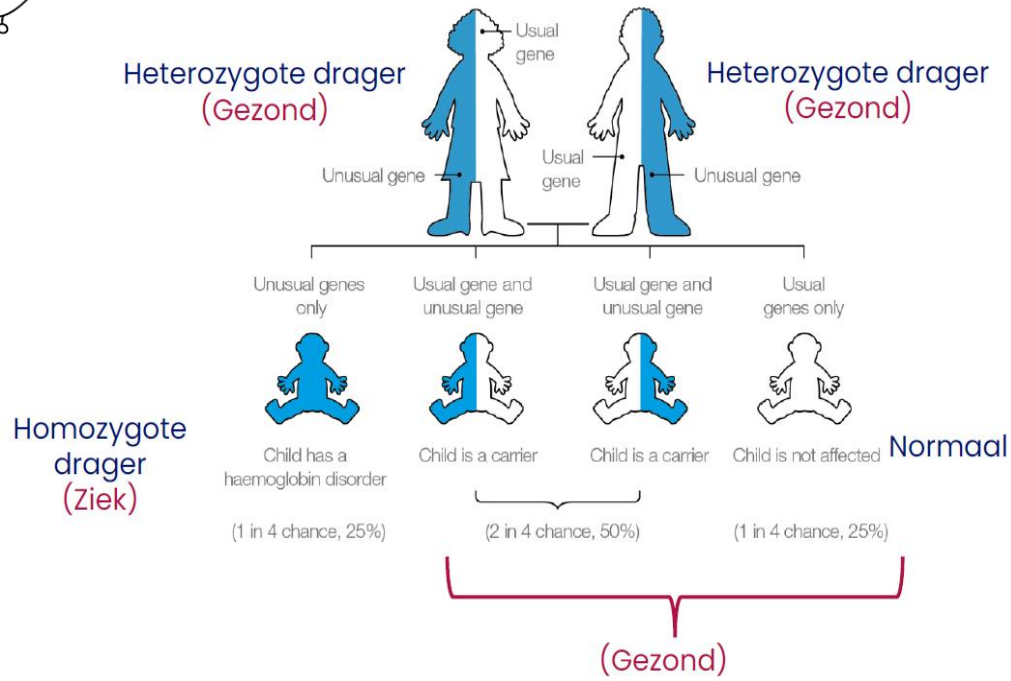
COUNTRY	TOTAL POPULATION	TOTAL NUMBER OF IMMIGRANT CARRIERS OF β THAL	TOTAL NUMBER OF CARRIERS OF β-THALASSAEMIA IN THE INDIGENOUS POPULATION	TOTAL NUMBER OF IMMIGRANT CARRIERS OF HbE	TOTAL NUMBER OF IMMIGRANT CARRIERS OF SICKLE CELL	TOTAL NUMBER OF IMMIGRANT CARRIERS OF HbC	CARRIER IMMIGRANTS AS A PROPORTION OF THE TOTAL POPULATION	CARRIERS OF Hb DISORDERS AS A PROPORTION OF THE TOTAL POPULATION
AUSTRIA	8,210,281	11,842	8,210	2,453	4,675	708	0.24%	0.34%
BELGIUM	10,438,353	19,403	10,438	4,073	39,250	5,169	0.65%	0.75%
CYPRUS	840,407	3,991	121,019	354	583	20	0.58%	15.00%
DENMARK	5,543,453	6,772	5,543	4,083	2,277	330	0.24%	0.34%
FRANCE	64,057,792	98,219	64,058	32,607	172,600	47,884	0.54%	0.65%
GERMANY	82,329,758	128,419	82,330	22,955	53,883	7,135	0.25%	0.36%
GREECE	10,737,429	29,289	837,519	536	7,626	183	0.35%	8.70%
ITALY	61,261,254	75,748	2,572,972	9,463	72,870	21,416	0.29%	6.50%
THE NETHERLANDS	16,715,999	27,656	16,716	13,751	30,329	7,703	0.47%	0.57%
SPAIN	47,042,984	57,257	715,053	2,434	92,601	27,796	0.38%	1.90%
SWEDEN	9,482,855	21,092	9,483	12,593	8,720	912	0.46%	0.56%
UK	63,047,162	107,694	63,047	27,124	145,038	25,290	0.48%	0.58%

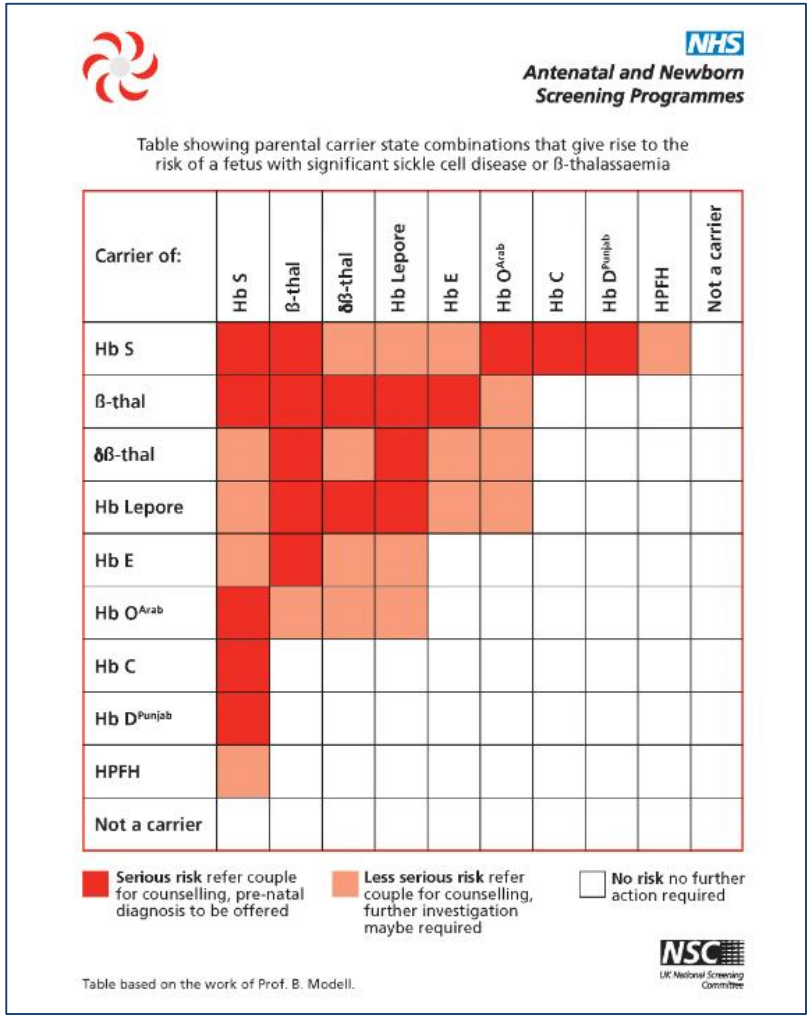
Haemoglobinopathies on the Move: Is Europe ready? Health and Migration Policy Perspectives. Augustus 2013

Indicaties hemoglobine-electroforese

- Onverklaarbare afwijkingen CBC e.g. anemie, afwijkende RBC-indices,…
- Screening: pre-conceptioneel, antenataal, neonataal, partner, pre-op
- Monitoring

Hemoglobinopathieën zijn erfelijke ziekten





NHS
Antenatal and Newborn Screening Programmes

Table showing parental carrier state combinations that give rise to the risk of a fetus with significant sickle cell disease or ß-thalassaemia

Carrier of:	Hb S	ß-thal	δß-thal	Hb Lepore	Hb E	Hb O^{Arab}	Hb C	Hb D^{Punjab}	HPFH	Not a carrier
Hb S										
ß-thal										
δß-thal										
Hb Lepore										
Hb E										
Hb O^{Arab}										
Hb C										
Hb D^{Punjab}										
HPFH										
Not a carrier										

Serious risk refer couple for counselling, pre-natal diagnosis to be offered

Less serious risk refer couple for counselling, further investigation maybe required

No risk no further action required

Table based on the work of Prof. B. Modell.

NSC UK National Screening Committee

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Praktisch

- Staaltype: EDTA-buis
- Voor een correcte interpretatie simultane bepaling van CBC en ijzerstatus.
- Graag klinische info (etniciteit, familiale context, zwangerschap, recente bloedtransfusie,…)
- Referentie-intervallen CBC, HbA, HbF en HbA2 zijn leeftijdsafhankelijk

* *Wong ECC[1], et al. Normal Pediatric Reference Intervals for Hemoglobins A, F, and A2 Using Capillary Zone Electrophoresis Methodology. Published abstract and poster presented at the 2009 meeting of the ISLH.*

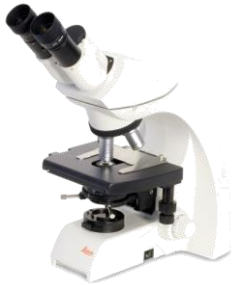
Results

Age Interval (n)	Hb A (%)[1]	Hb F (%)[1]	Hb A2 (%)[1]
0-6 days (47)	4.7-24.3	79.4-97.9	0.0-0.0
7-14 days (17)	6.6-38.3	66.0-98.4	0.0-0.4
15-45 days (28)	8.1-62.7	49.9-98.4	0.0-1.8
45 days to 3 months (32)	37.3-88.8	10.3-64.6	0.3-2.4
3-6 months (29)	67.0-96.7	5.2-34.5	1.7-2.7
6-9 months (21)	82.4-98.8	1.1-28.5	2.0-3.0
9-15 months (35)	91.9-98.7	0.2-9.9	2.2-3.2
15 months to 2 years (22)	94.9-97.8	0.0-5.5	2.4-3.1
2-6 years (92)	95.9-97.9	0.0-1.6	2.2-3.2
6-17 years (43)	96.4-98.2	0.0-0.0	1.9-3.3
≥18 years (78)	96.8-97.5	0.0-0.0	2.4-2.9

[1]Represents 2.5th to 97.5th percentiles

Casus: jongen, 9j

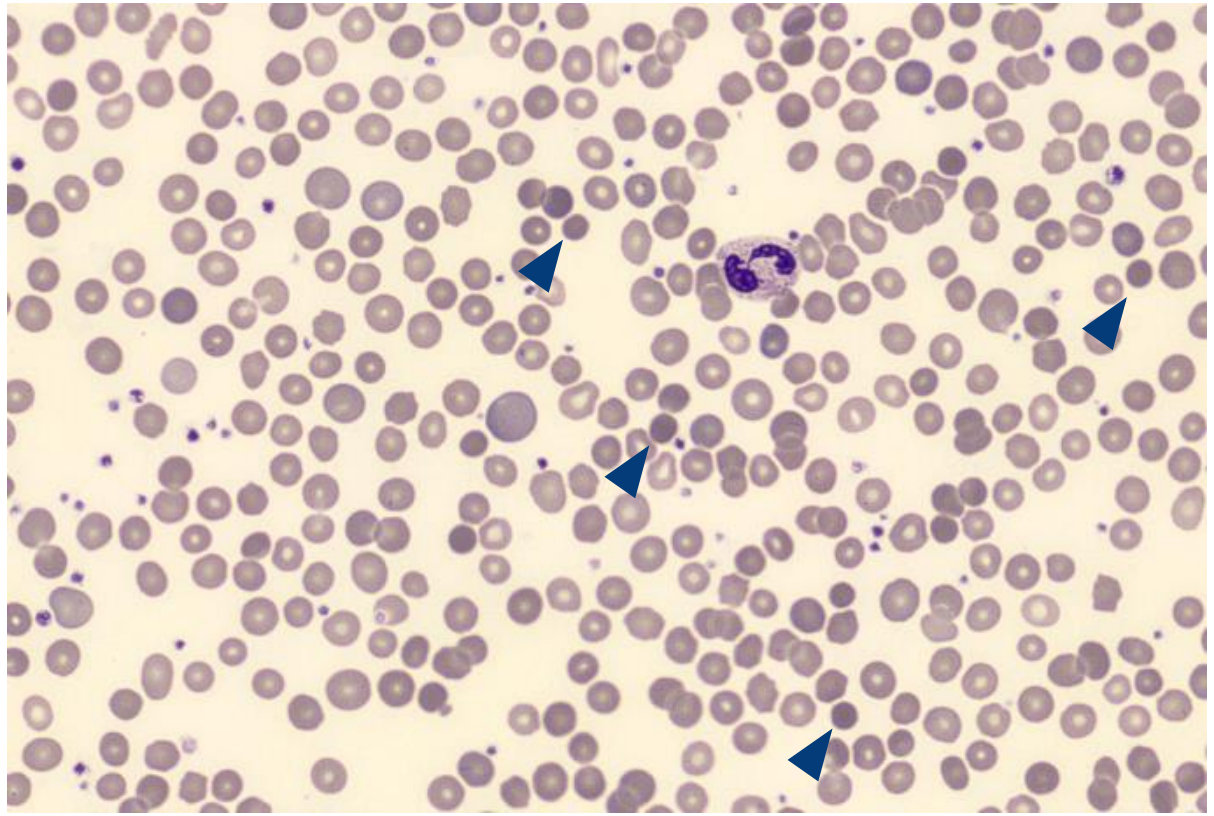
→ geen klinische info beschikbaar



Laboresultaten:

Analyse	Resultaat bij 37°C	Referentie-waarde
WBC (/µL)	10.040	3.750-13.090
Normoblasten (%)	0,3	<0,2
RBC (10^6/µL)	4,6	4,0-5,2
Hemoglobine (g/dL)	12,6	11,5-15,5
Hematocriet (%)	34	35-45
MCV (fL)	73	77-95
MCH (pg)	28	27-32
MCHC (g/dL)	37	31-37
Reticulocyten (/µL)	420.400	50.000-100.000

Microscopisch nazicht RBC:



Hyperdense sferocyten +++

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Casus: jongen, 9j

Bijkomende testen:

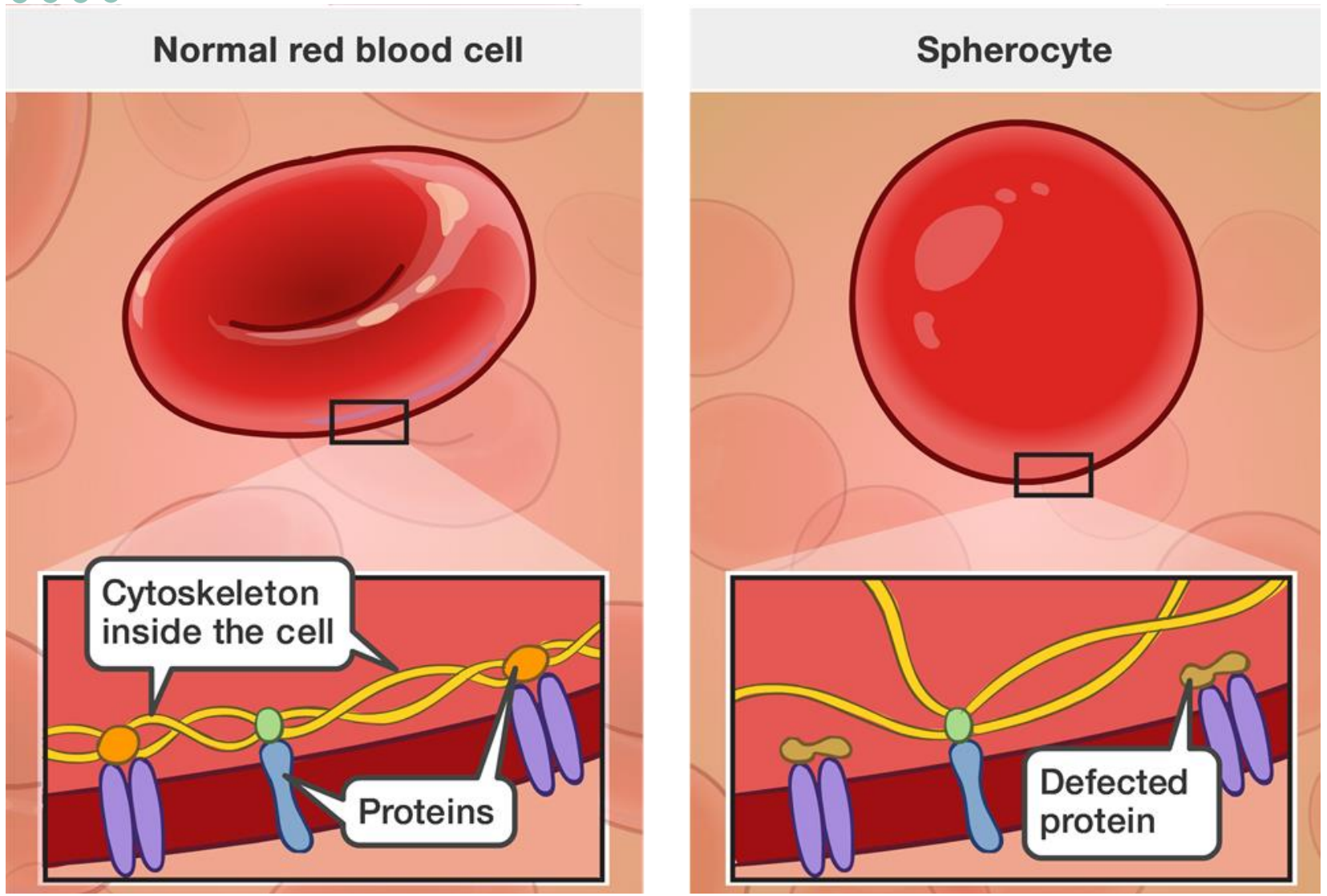
Analyse	Resultaat	Referentie-waarden
Haptoglobine (g/L)	<0,10	0,30-2,00
Kalium (mmol/L)	4,1	3,5-5,1
LDH (U/L)	2522	135-250
Directe Coombs	Negatief	
Hb elektroforese	Normaal beeld	
EMA test	0,67 Sterk suggestief voor hereditaire sferocytose	0,90-1,10 <0,80: significant

Hereditaire sferocytose

- Erfelijke vorm van hemolytische anemie
- RBC membraandefect
- 1/1000 geboortes

Klinische impact:

- Milde tot matige hemolyse (+/- anemie)
- Kan zich presenteren op elke leeftijd van mild tot zeer ernstig
- Splenomegalie


Normal red blood cell
Cytoskeleton inside the cell
Proteins
Spherocyte
Defected protein

Hemolyse

Intrinsieke vorm (erfelijk)

- Hemoglobinopathie
- RBC membraandefecten (vb. heriditaire sferocytose)
- RBC enzymdeficiënties (vb. glucose-6-phosphate dehydrogenase, pyruvate kinase)

Extrinsieke vorm (verworven)

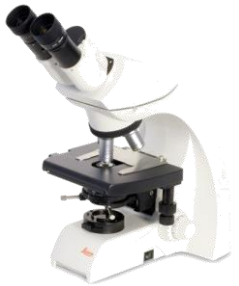
- Auto (vb. cold hemaglutinin disease) / allo immuun
- Hypersplenisme
- Trombotische microangiopathie
- Medicatie (vb dapsone)

Chronische vorm (gecompenseerd / asymptomatisch)

Acute vorm (hemolytische crisis)

Casus: man, 67j

→ longcarcinoom, respiratoire insufficiëntie

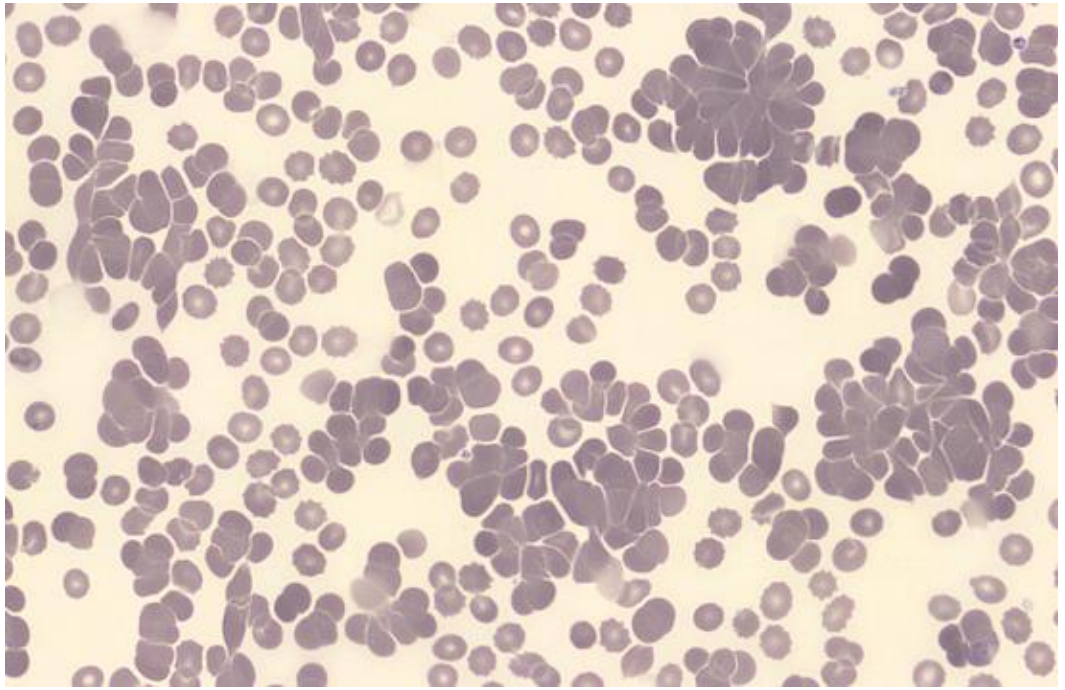


Laboresultaten:

Analyse	Resultaat bij KT	Resultaat bij 41°C	Referentie-waarde
WBC (/µL)	32.000	31.900	3.710-12.660
Normoblasten (%)	9,4	9,4	<0,2
RBC (10^6/µL)	2,9	3,1	3,8-4,8
Hemoglobine (g/dL)	10,8	10,8	12,0-15,0
Hematocriet (%)	27,8	31	37-46
MCV (fL)	98,5	95	76-96
MCH (pg)	41,8	30	27-32
MCHC (g/dL)	42,5	35	31-37

> 36,5 g/dL:
Meting wordt herhaald bij 41°C

Microscopisch nazicht RBC:



RBC agglutinatie met anemie en normoblastose

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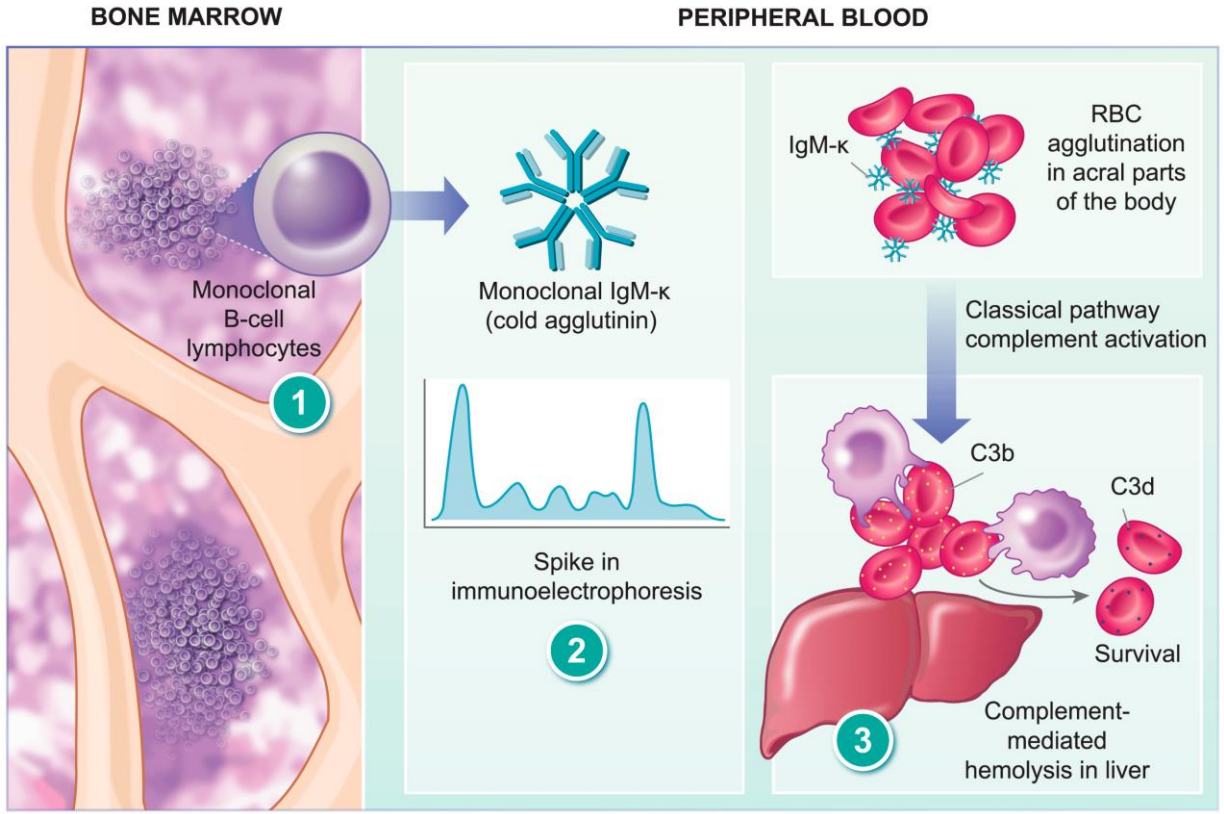
Casus: man, 67j

Bijkomende testen:

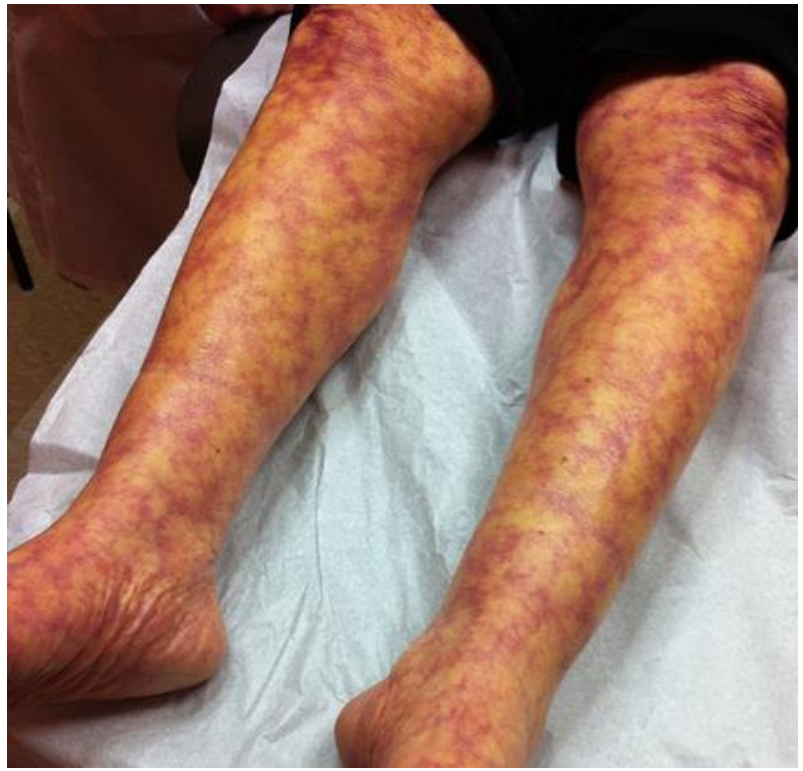
Analyse	Resultaat	Referentie-waarden
Haptoglobine (g/L)	<0,10	0,30-2,00
Bilirubine totaal (mg/dL)	4,0	<1,2
Kalium (mmol/L)	6,5	3,5-5,1
LDH (U/L)	2522	135-250
Directe Coombs	Positief	
Identificatie directe Coombs	Complement C3c + Complement C3d +	
Eluaat (IgG)	Negatief	
Indirecte Coombs	Positief bij KT, negatief bij 37°C	
Identificatie	Koude auto-antistoffen	



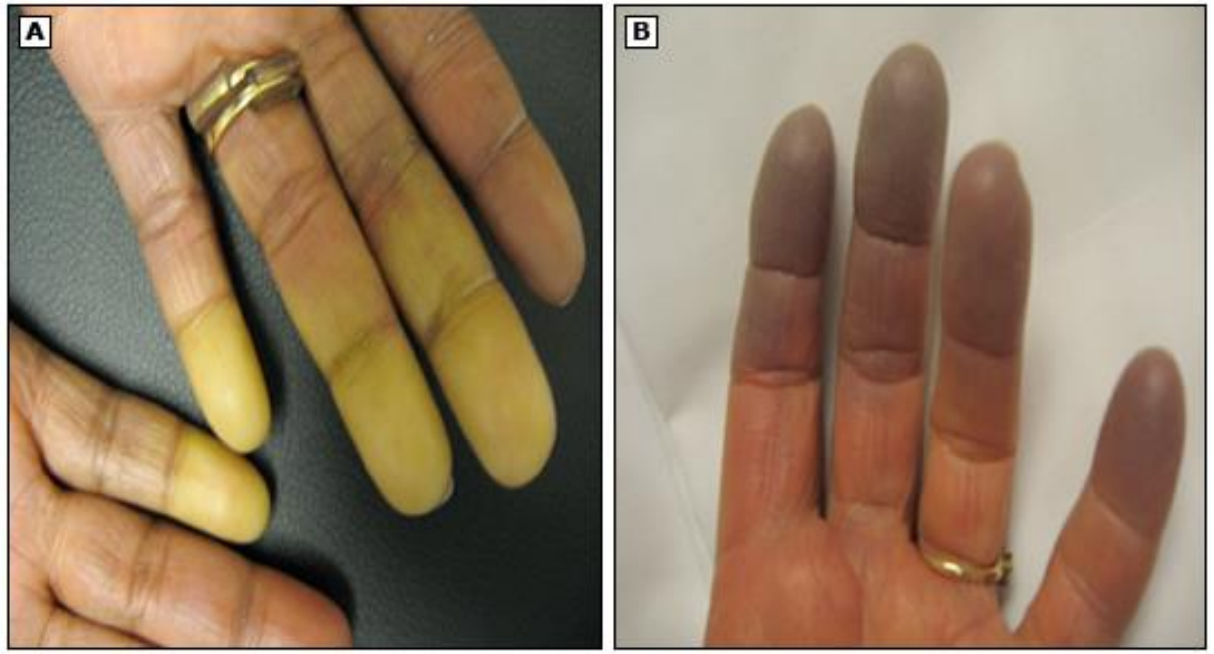
Sureda et al, Hemato 2022

In vivo hemolyse
→ CHAD = cold hemaglutinin disease

CHAD



Livido reticularis



Raynaud fenomeen

Casus: vrouw, 39j

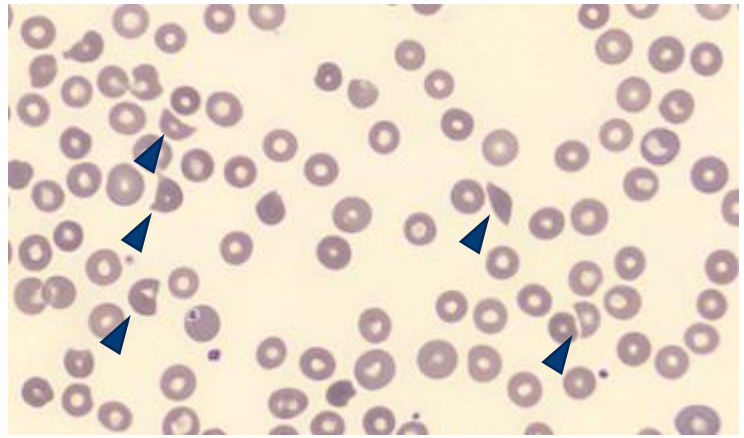
→ braken, neurologische symptomen

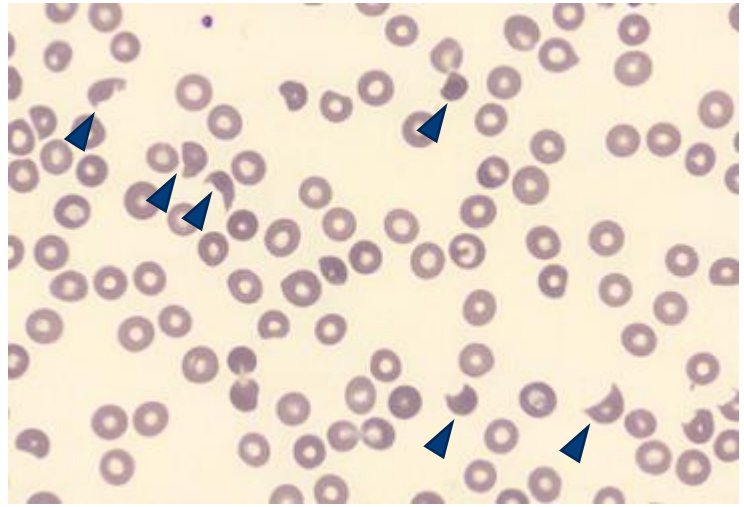
Laboresultaten:

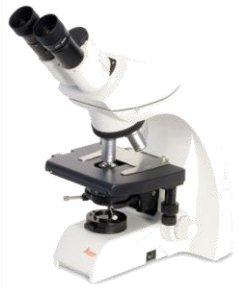
Analyse	Resultaat	Referentiewaarde
RBC (10^6/µL)	2,7	3,8-4,8
Hemoglobine (g/dL)	8,6	12,0-15,0
MCV (fL)	88	76-96
Reticulocyten (/µL)	136.600	50.000-100.000
WBC (/µL)	13.500	3.710-12.660
Neutrofielen (%)	74,8	37,0-74,7
Thrombocyten (/µL)	40.000	140.000-440.000

Anemie, trombopenie en schistocyten ++

Microscopisch nazicht RBC:







AZ Sint-Vincentius DEINZE

Casus: vrouw, 39j

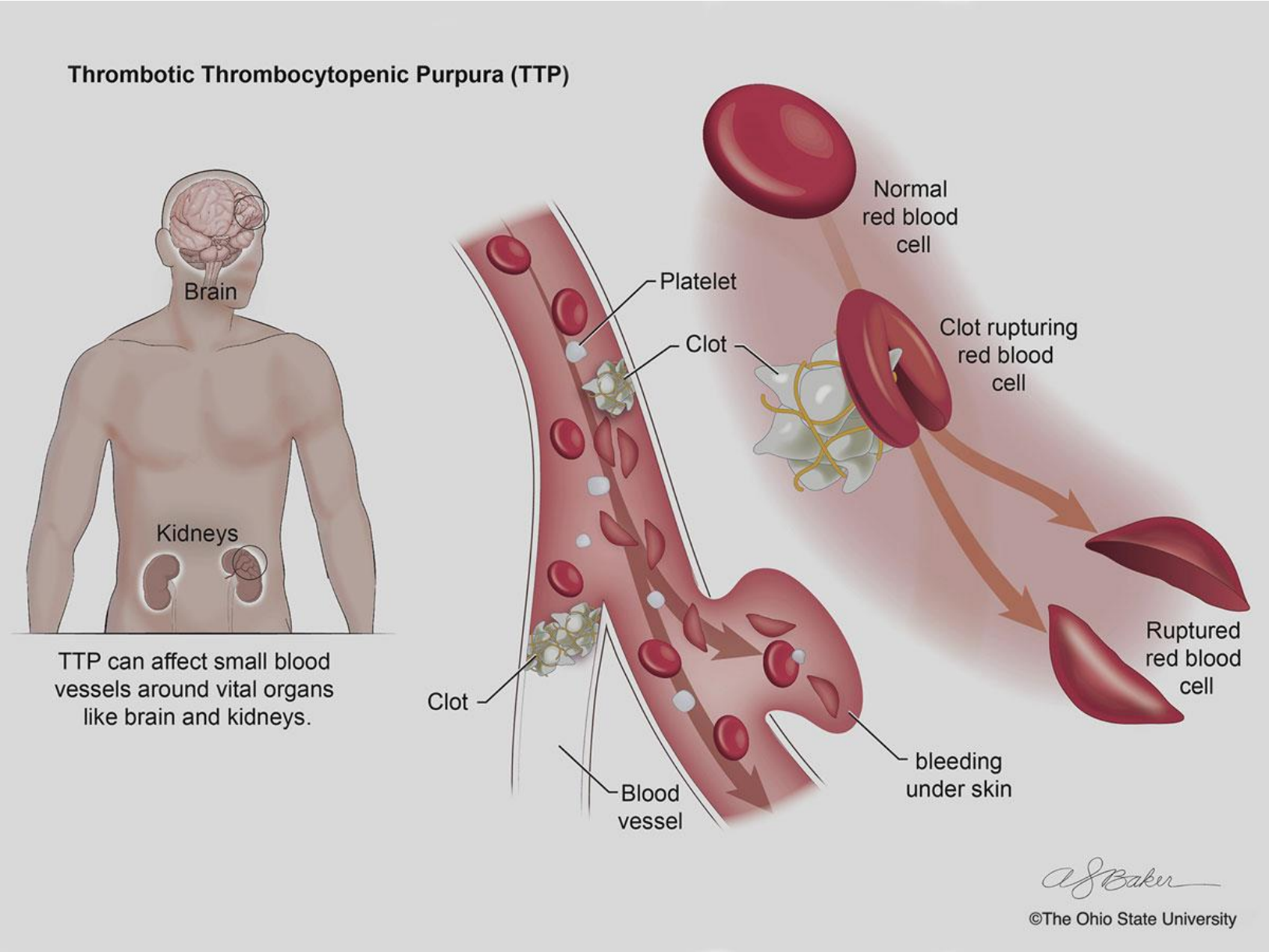
Bijkomende testen:

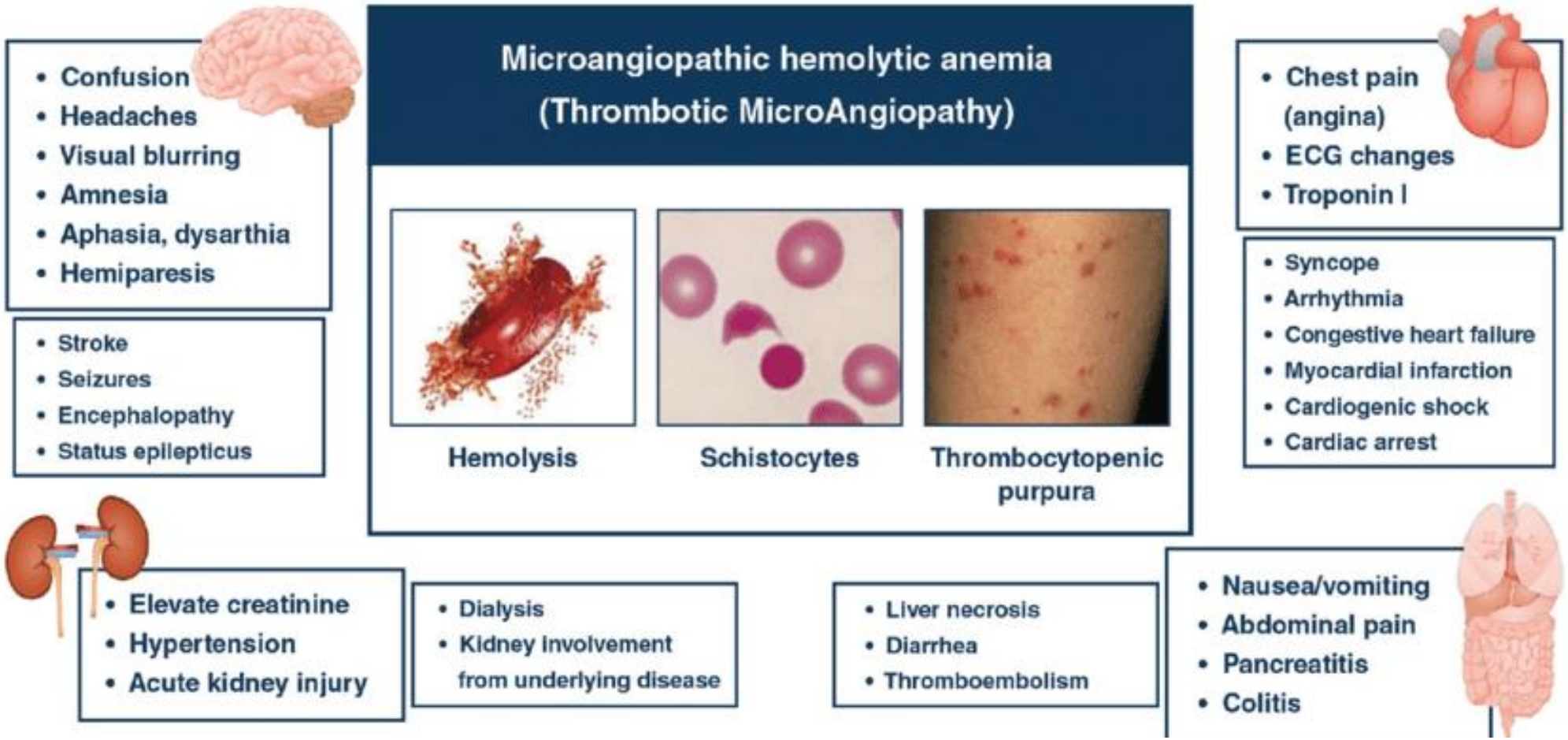
Analyse	Resultaat	Referentie-waarden
Haptoglobine (g/L)	<0,10	0,30-2,00
Bilirubine totaal (mg/dL)	0,5	<1,2
Kalium (mmol/L)	3,4	3,5-5,1
LDH (U/L)	422	135-250
Directe Coombs	Negatief	
PT (%)	>100	70-120
INR	0,9	0,8-1,2
APTT (s)	27,3	26,0-40,0
Fibrinogeen (mg/dL)	298	200-400
D-dimeren (ng/mL)	657	< 500
VWF cleaving protease ADAMTS13 (IU/dL)	0 ↓	95-100

Thrombotische thrombocytopenische purpura (TTP)

Klinische impact:

- Medische urgentie!
- Dringende behandeling


Thrombotic Thrombocytopenic Purpura (TTP)
Brain
Kidneys
TTP can affect small blood vessels around vital organs like brain and kidneys.
Normal red blood cell
Platelet
Clot
Clot rupturing red blood cell
Ruptured red blood cell
Clot
bleeding under skin
Blood vessel
©The Ohio State University



Zafrani et al, J. Clin. Med. **2022**

Congenitale

bloedcelafwijkingen

Hemolyse

…

Infecties

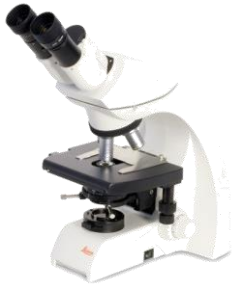
Nutritionele deficiënties

Lymfomen

Acute maligniteiten

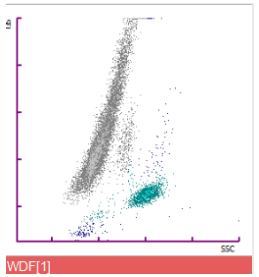
Casus: man, 23j

→ geen klinische info

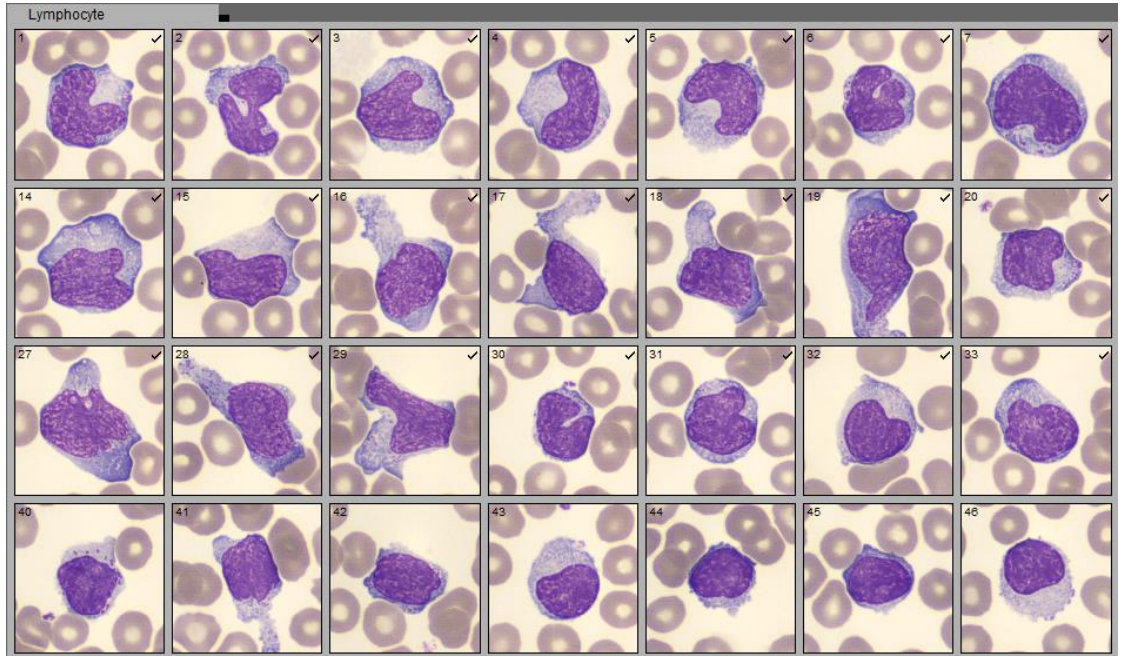


Laboresultaten:

Analyse	Resultaat	Referentiewaarde
Hemoglobine (g/dL)	13,3	13,0-17,0
WBC (/µL)	11.050	3.710-12.660
Neutrofielen (/µL)	4.080	1.640-8.800
Eosinofielen (/µL)	0	20-540
Basofielen (/µL)	0	10-80
Lymfocyten (/µL)	6.280	1.120-3.820
Monocyten (/µL)	600	270-970
Thrombocyten (/µL)	107.000	140.000-440.000



Microscopisch nazicht lymfocyten:



Reactieve lymfocyten +++

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AZ Sint-Vincentius DEINZE

Casus: man, 23j

Bijkomende testen:

Analyse	Resultaat	Referentie-waarden
Alkalisch fosfatase (U/L)	304	40-129
SGPT (U/L)	224	10-50
SGOT (U/L)	268	10-50
Gamma GT (U/L)	280	8-61
LDH (U/L)	779	135-250
EBV EBNA IgG	Negatief	
EBV VCA IgM	Positief (index 9,95)	
EBV VCA IgG	Positief (index 1,16)	

Acute EBV infectie

Klinische impact:

- Symptomatische behandeling

Congenitale

bloedcelafwijkingen

Hemolyse

…

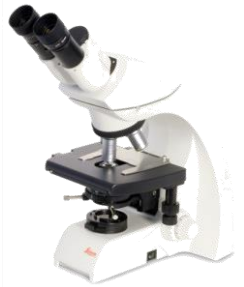
Infecties

Nutritionele deficiënties

Lymfomen

Acute maligniteiten

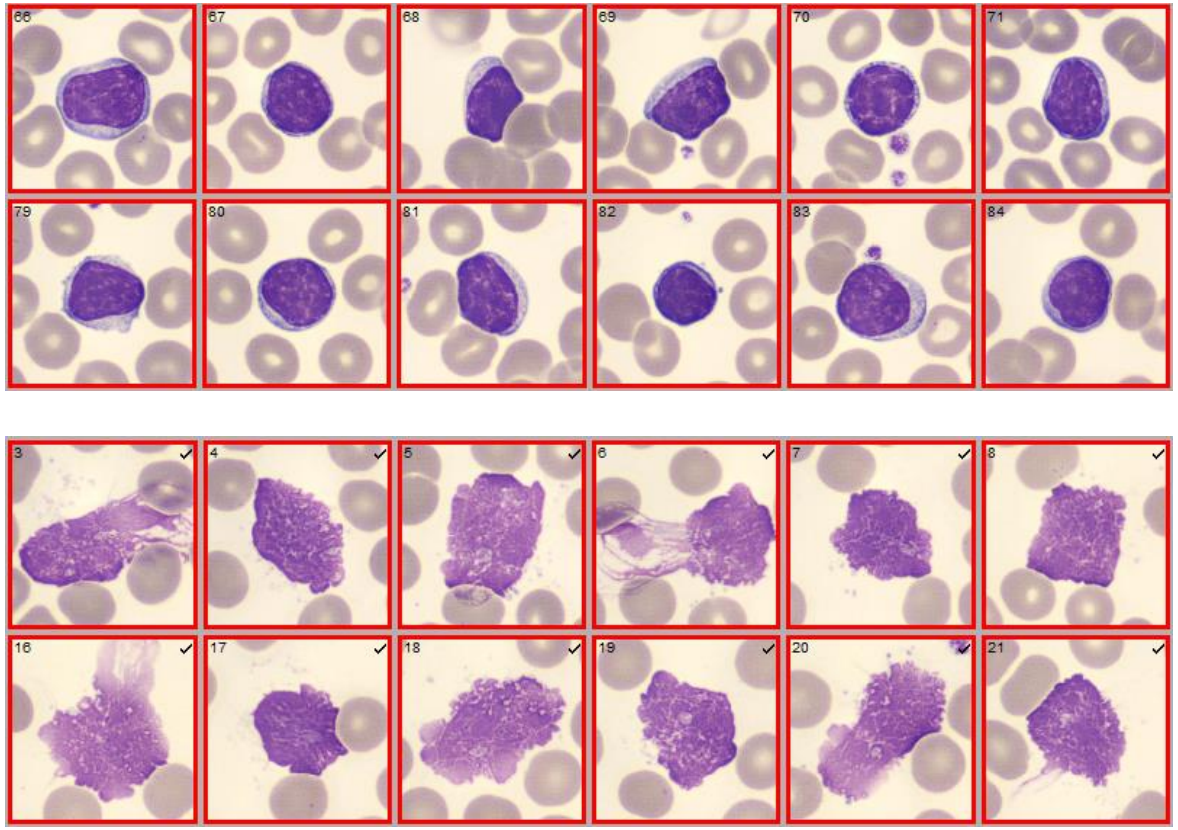
Casus: man, 64j → jaarlijkse controle, geen kliniek



Laboresultaten:

Analyse	Resultaat	Referentiewaarden
RBC (10^6/µL)	4,28	4,4-5,5
Hemoglobine (g/dL)	14,7	13,0-17,0
WBC (/µL)	11.020	3.600-11.820
Neutrofielen (/µL)	4250	1.500-8.400
Eosinofielen (/µL)	70	20-570
Basofielen (/µL)	60	10-80
Lymfocyten (/µL)	6.140	1.020-3.550
Monocyten (/µL)	480	260-1.070
Thrombocyten (/µL)	221.000	140.000-440.000

Microscopisch nazicht lymfocyten:



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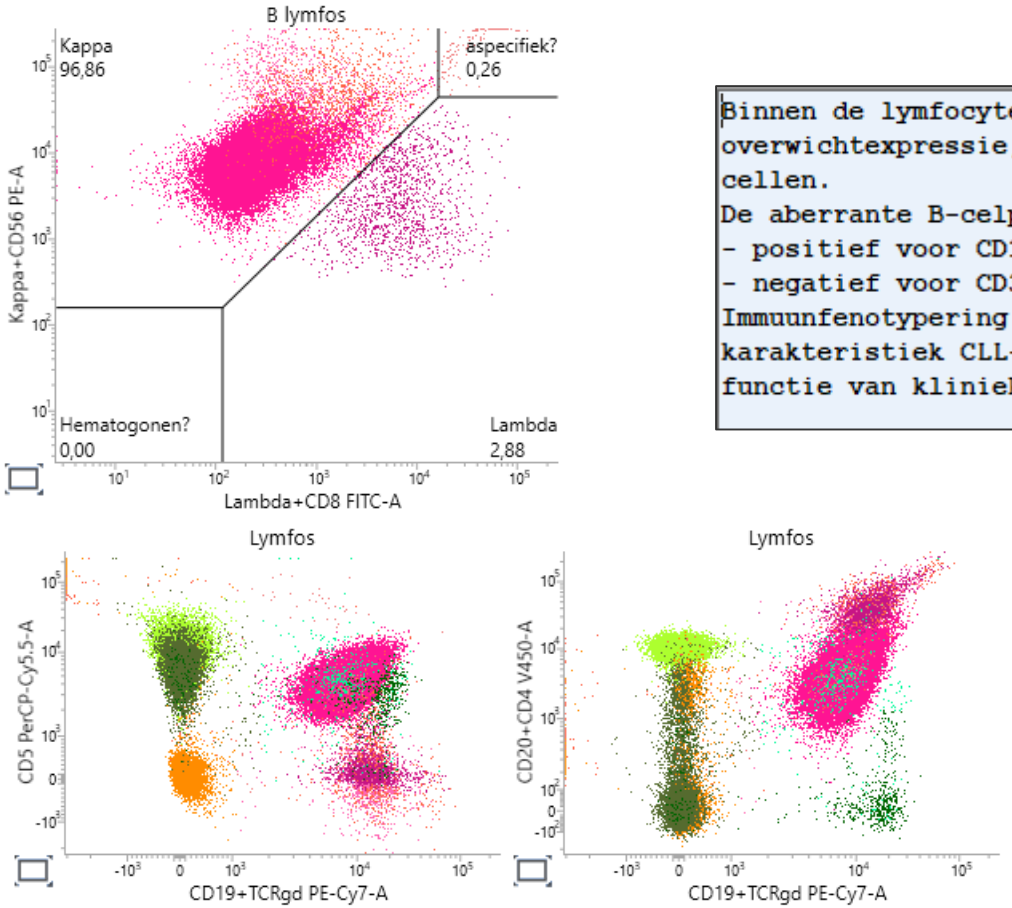
Immuunfenotypering:



Binnen de lymfocytengate zijn er 62 % B-lymfocyten (CD19+) met kappa lichte keten overwichtexpressie, 24 % T-lymfocyten (CD3+) met een normale CD4/CD8 verhouding en 13 % NK-cellen.
De aberrante B-celpopulatie vertoont volgend expressiepatroon:
- positief voor CD19, CD20 (zwak), CD5, CD23, CD43 en CD200
- negatief voor CD38, CD79b en CD10
Immuunfenotypering toont de aanwezigheid van een monoklonale B-celpopulatie met karakteristiek CLL-fenotype (+/- 57 % van lymfocyten, absoluut aantal: 3500 cellen/µL), in functie van kliniek passend bij een B-CLL/MBL.

High count MBL met CLL fenotype

- Klonale B-celpopulatie in PBO:

  500-5000 /µL
- Asymptomatisch
- 1-2% kans op progressie per jaar

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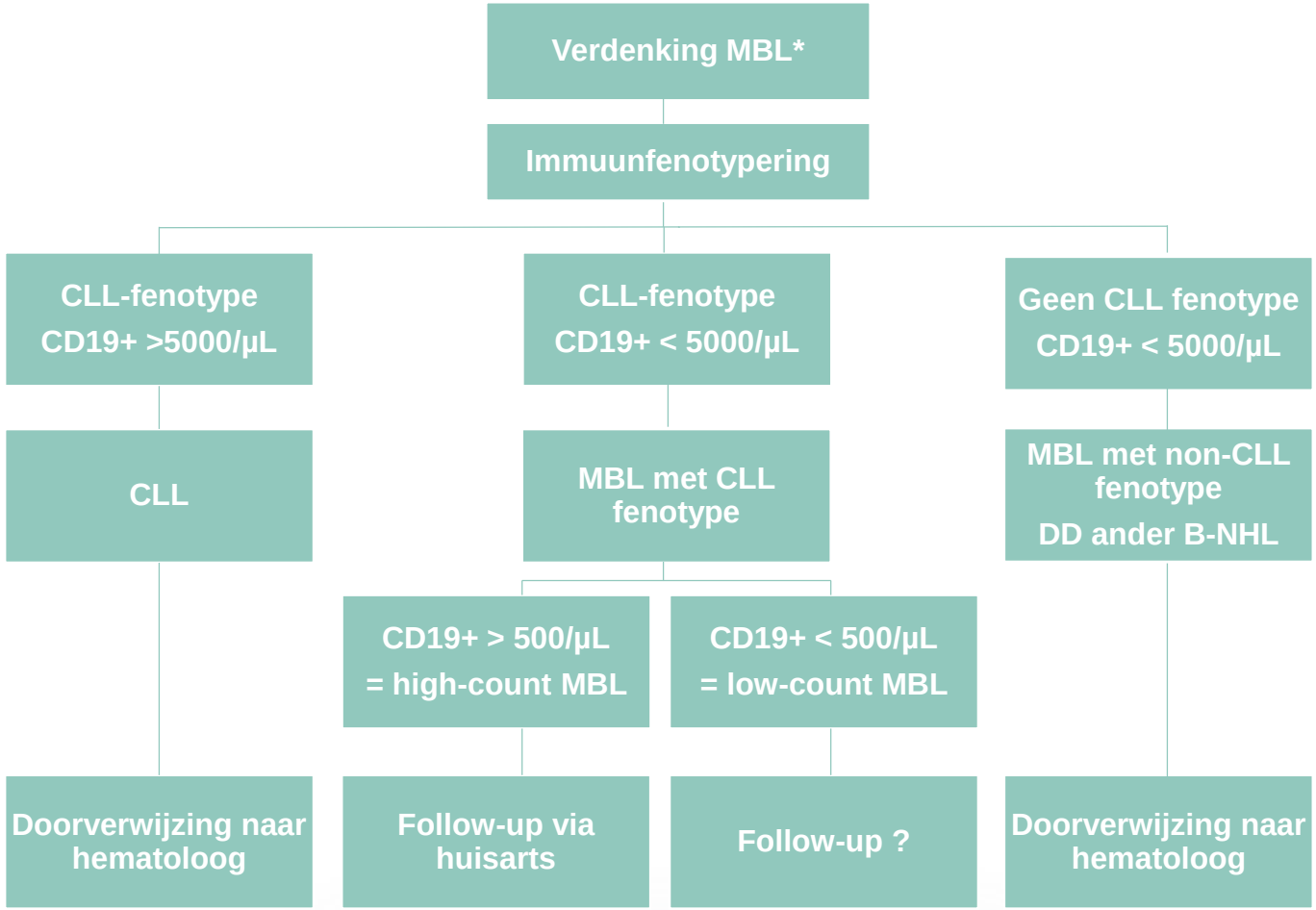

- **jaarlijkse bloedafname**
- **Alarmsignalen: B-symptomen, cytopenieën, (verdubbeling lymfocytose binnen 12 maanden)**

→ doorverwijzing hematoloog

* Patiënt met persisterende lymfocytose zonder lymfadenopathie, organomegalie, cytopenie(ën) of B-symptomen

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Congenitale

bloedcelafwijkingen

Hemolyse

…

Infecties

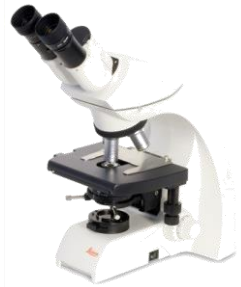
Nutritionele deficiënties

Lymfomen

Acute maligniteiten

Casus: vrouw, 24j

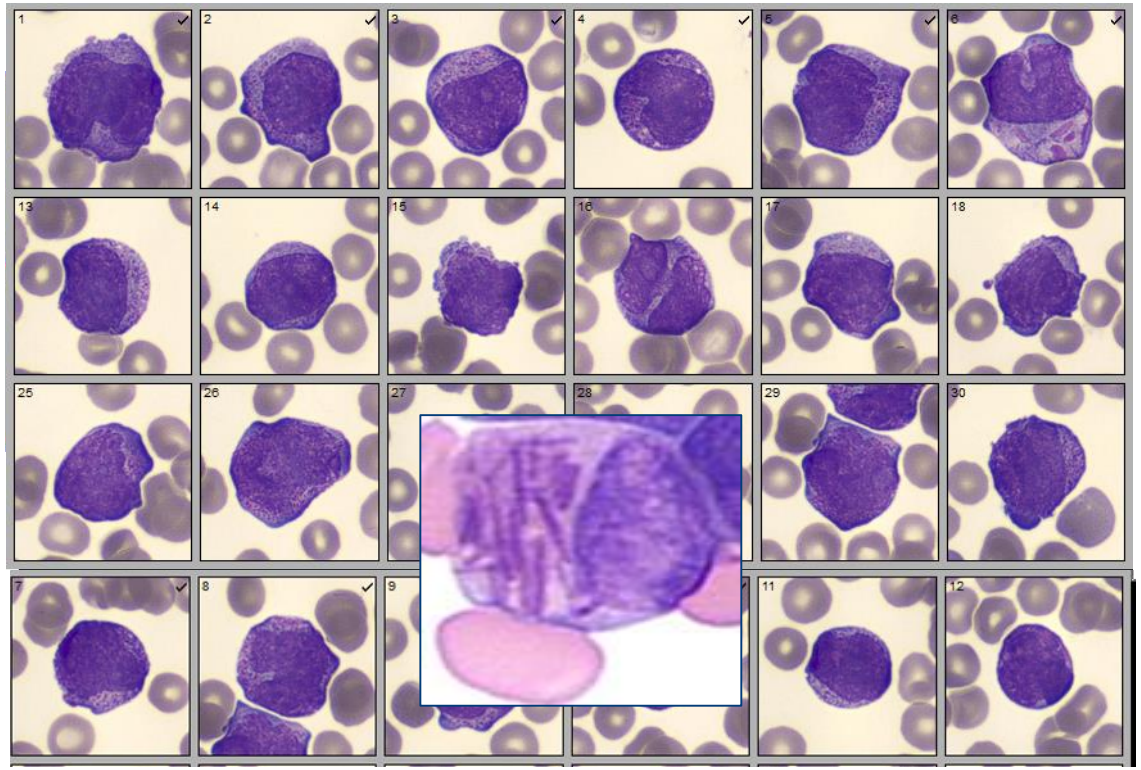
→ zwanger, neusbloedingen

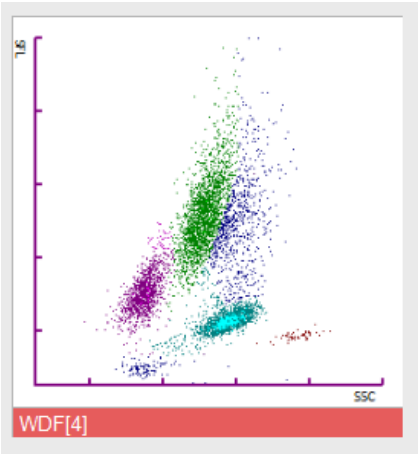


Laboresultaten:

Analyse	Resultaat	Referentiewaarden
RBC (10^6/µL)	3,0	3,8-4,8
Hemoglobine (g/dL)	10,2	12,0-15,0
Hematocriet (%)	27	37-46
WBC (/µL)	7.030	3.710-12.660
Thrombocyten (/µL)	13.000	140.000-440.000

Microscopisch nazicht:

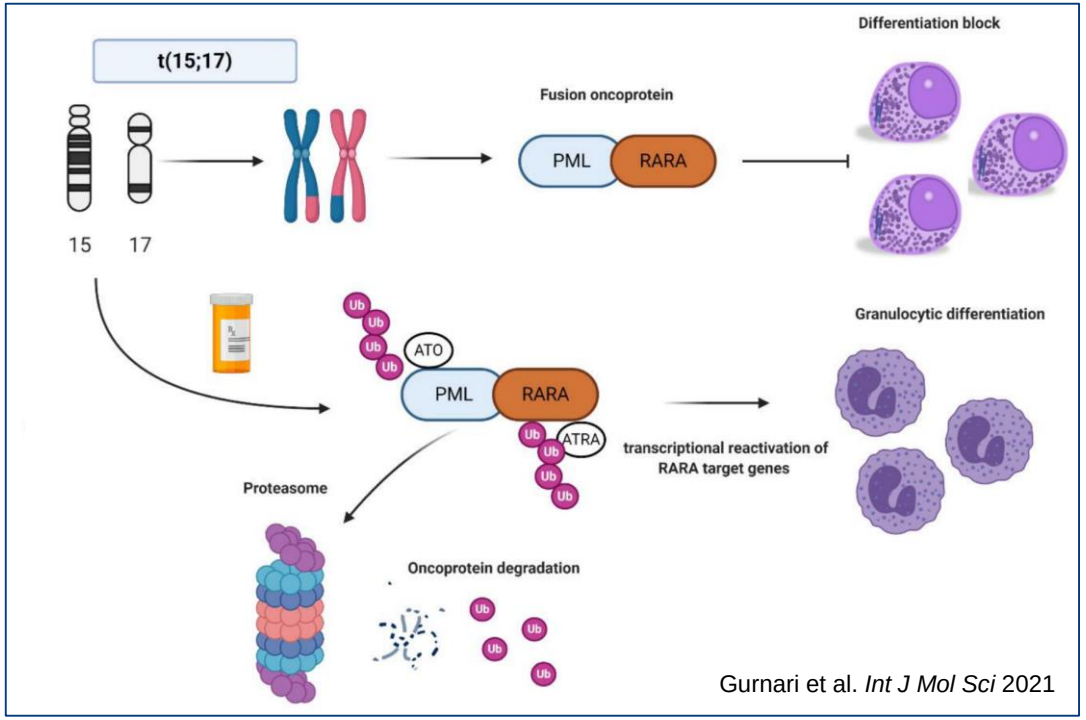




+/-30% blasten/promyelocyten met hypergranulatie en Auerstaven

Bijkomende testen:

Analyse	Resultaat	Referentie-waarden
PT (%)	49	70-120
APTT (sec)	35,6	26,0-40,0
Fibrinogeen (mg/dL)	62	200-400
D-dimeren (ng/mL)	>2000	<500
Immuunfenotypering	45% myeloblasten	
Fusietranscript PML-RARA	Aanwezig	



Gurnari et al. *Int J Mol Sci* 2021

- Maturatiestop ter hoogte van promyelocyten
- Promyelocytaire granules: pro-coagulerend
- DIC en hyperfibrinolyse

Acute promyelocytaire leukemie (APL)

Klinische impact:

- Medische urgentie!
- Hoge mortaliteit tgv DIC en hyperfibrinolyse
- Doorverwijzing UZ
- Onmiddellijk opstart R/ ATRA + ATO

Congenitale

bloedcelafwijkingen

Hemolyse

…

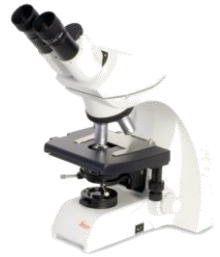
Infecties

Nutritionele deficiënties

Lymfomen

Acute maligniteiten

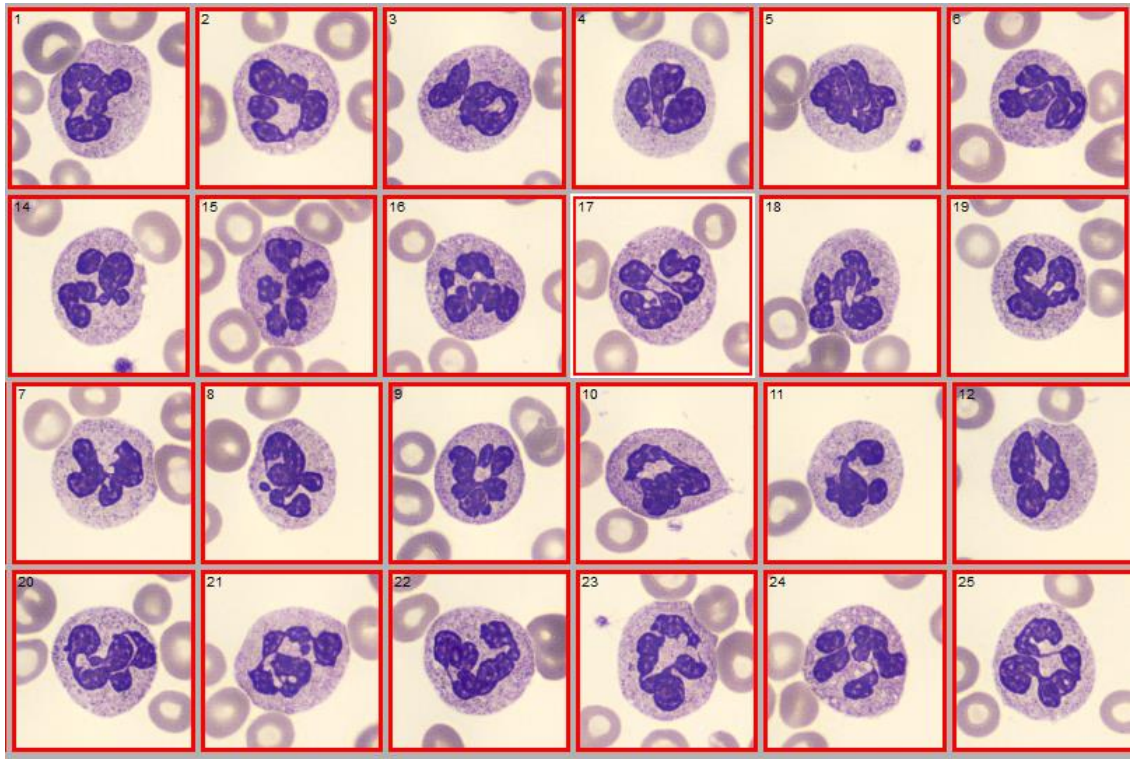
Casus: vrouw, 72j → gemetastaseerd melanoom



Laboresultaten:

Analyse	Resultaat	Referentiewaarde
RBC (10^6/µL)	2,1	3,8-4,8
Hemoglobine (g/dL)	6,7	12,0-15,0
MCV (fL)	101	76-96
WBC (/µL)	2.020	3.710-12.660
Neutrofielen (/µL)	990	1.640-8.800
Eosinofielen (/µL)	40	20-540
Basofielen (/µL)	0	10-80
Lymfocyten (/µL)	930	1.120-3.820
Monocyten (/µL)	50	270-970
Thrombocyten (/µL)	52.000	140.000-440.000
Reticulocyten abs. w. (10^3/µL)	21.2	50.0-100.0

Microscopisch nazicht neutrofielen:



Pancytopenie, macrocytaire anemie, hypersegmentatie van de neutrofielen

Bijkomende testen:

Analyse	Resultaat	Referentiewaarden
Serumijzer (µg/dL)	259	33-193
Transferrine (g/L)	2,11	2,00-3,60
Ferritine (µg/L)	150	15-150
Transferrineverzadiging (%)	97	10-45
Foliumzuur (µg/L)	1,1	>3,9
Vitamine B12 (ng/L)	216	197-771

Diep foliumzuurtekort

Klinische impact:

- Transfusienood
- Foliumzuursuppletie

MARIA MIDDELARES

AZ Sint-Vincentius DEINZE

Hartelijk dank voor jullie aandacht !