Moleculaire diagnostiek
Deel 2

Marjan vd Werken
Karlijn Stouten
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Programma

Opfrissen deel 1 van vorig jaar: Rob

Introductie verworven mutaties: Rob

Inleiding ‘integrale diagnostiek’ en myeloproliferatieve aandoeningen: Rob

Achtergrond complexere DNA-technieken: Marjan

Uitleg over nieuwe DNA-ruimten: Marjan

Uitleg en resultaten van onderzoek Karlijn: Karlijn

Casuïstiek: Marjan en Rob

Moleculaire diagnostiek

Met laboratoriumtesten de genetische code (DNA) onderzoeken.

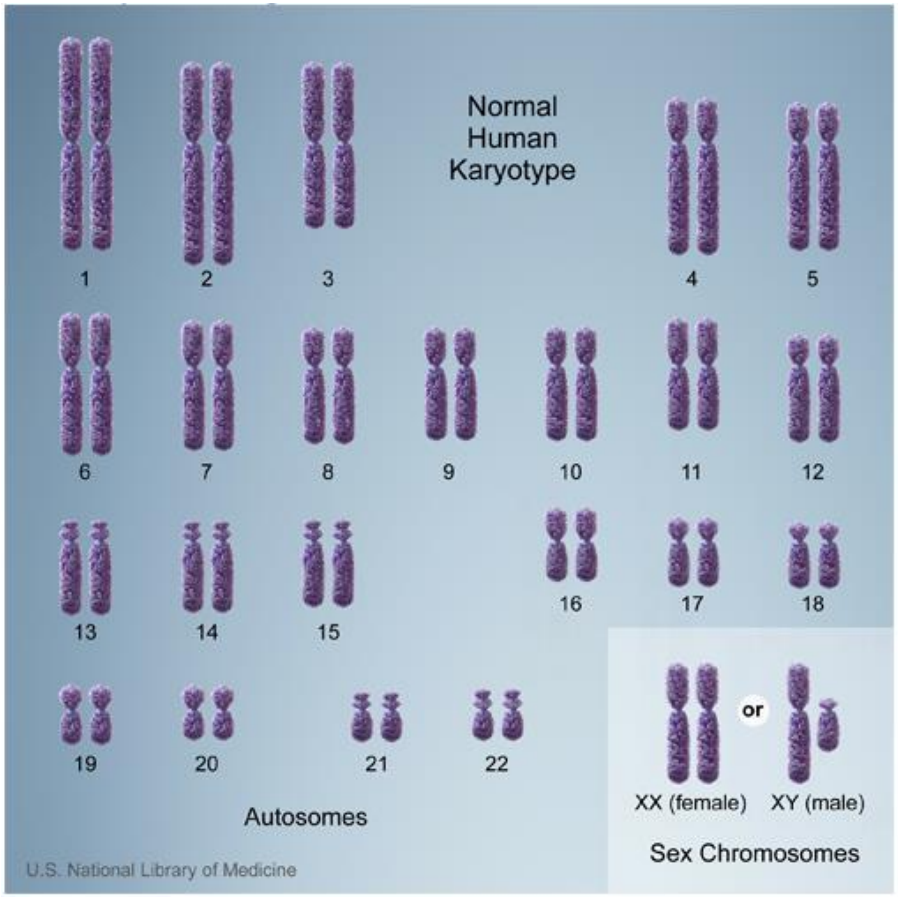
=

een van de speerpunten van Result Laboratorium

DNA: de genetische code
“een receptenboek”

- DNA-code bestaat uit (maar) vier verschillende letters:
  A, T, G en C
- Menselijke code is drie miljard (3.000.000.000) letters lang.
- Alle DNA van een cel uitgerold: snoer van 1 - 1,5 meter.
- Klein gedeelte (1%) van de DNA-code zijn genen.
- Aantal genen van een mens: circa 22.000.
- Een gen is het recept voor een eiwit.
- Ieder mens heeft zijn eigen, unieke, DNA-code.
- Tussen mensen verschilt DNA-code van genen 0,1%.

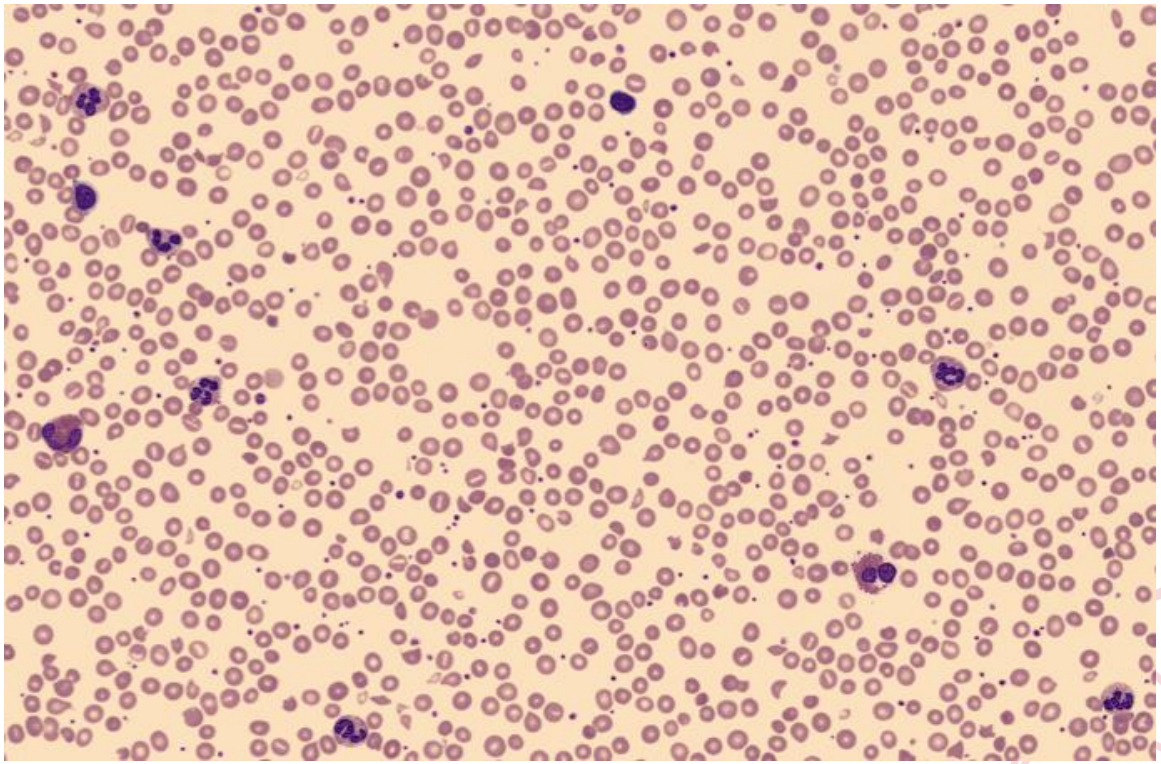
DNA: niet een aaneengesloten snoer, maar verdeeld over chromosomen



Van elk chromosoom twee exemplaren: een van je vader en een van je moeder.

http://ghr.nlm.nih.gov/handbook/illustrations/normalkaryotype

De 46 chromosomen zitten in de celkern.



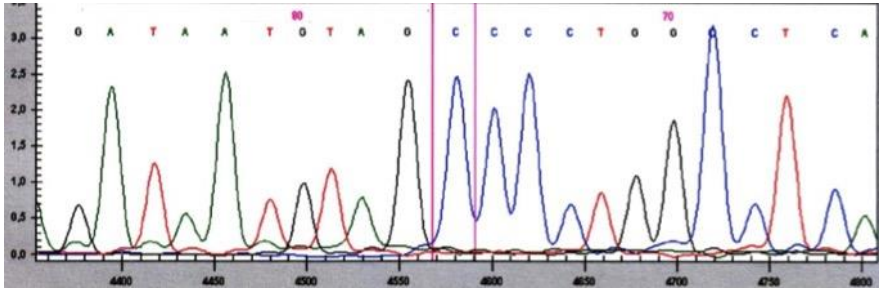
Blijft je genetisch code hetzelfde?

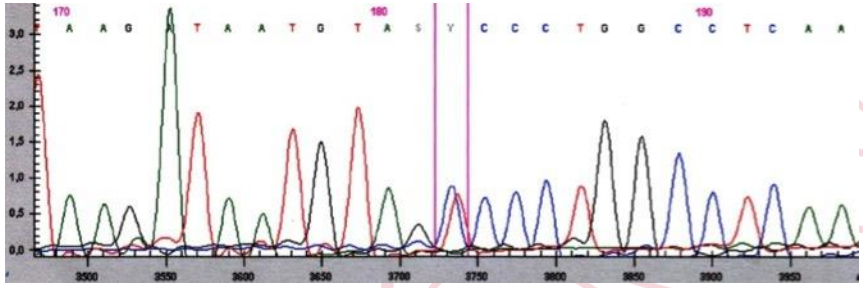
De DNA-code erf je van je ouders,

maar tijdens je leven ontstaan in sommige cellen veranderingen in de genetische code.

Uitdaging:

Hoe toon je die verworven mutaties aan?





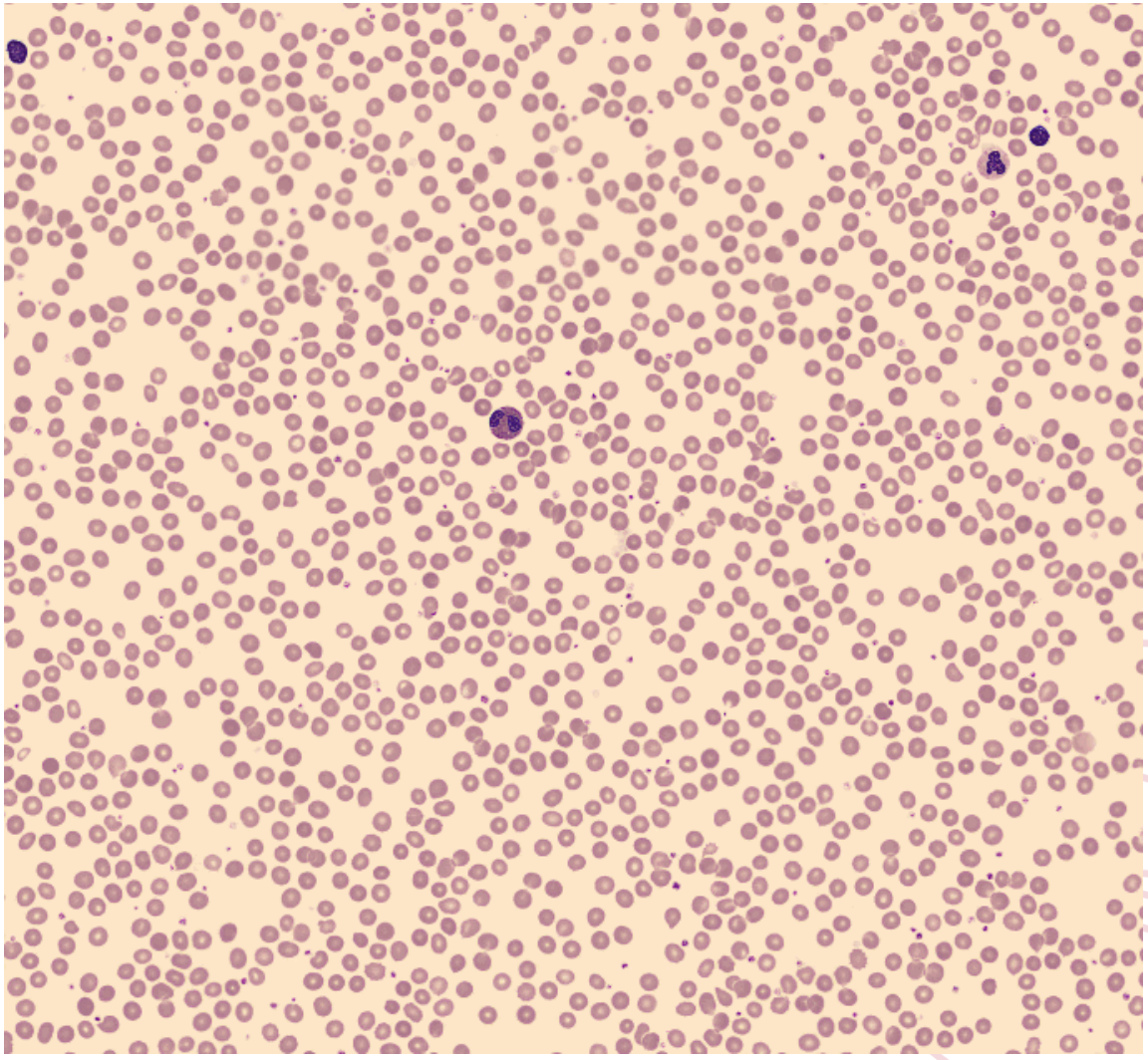
Moleculaire diagnostiek wordt een steeds belangrijker onderdeel van ‘integrale diagnostiek’.

Bijvoorbeeld: hemato-oncologie.

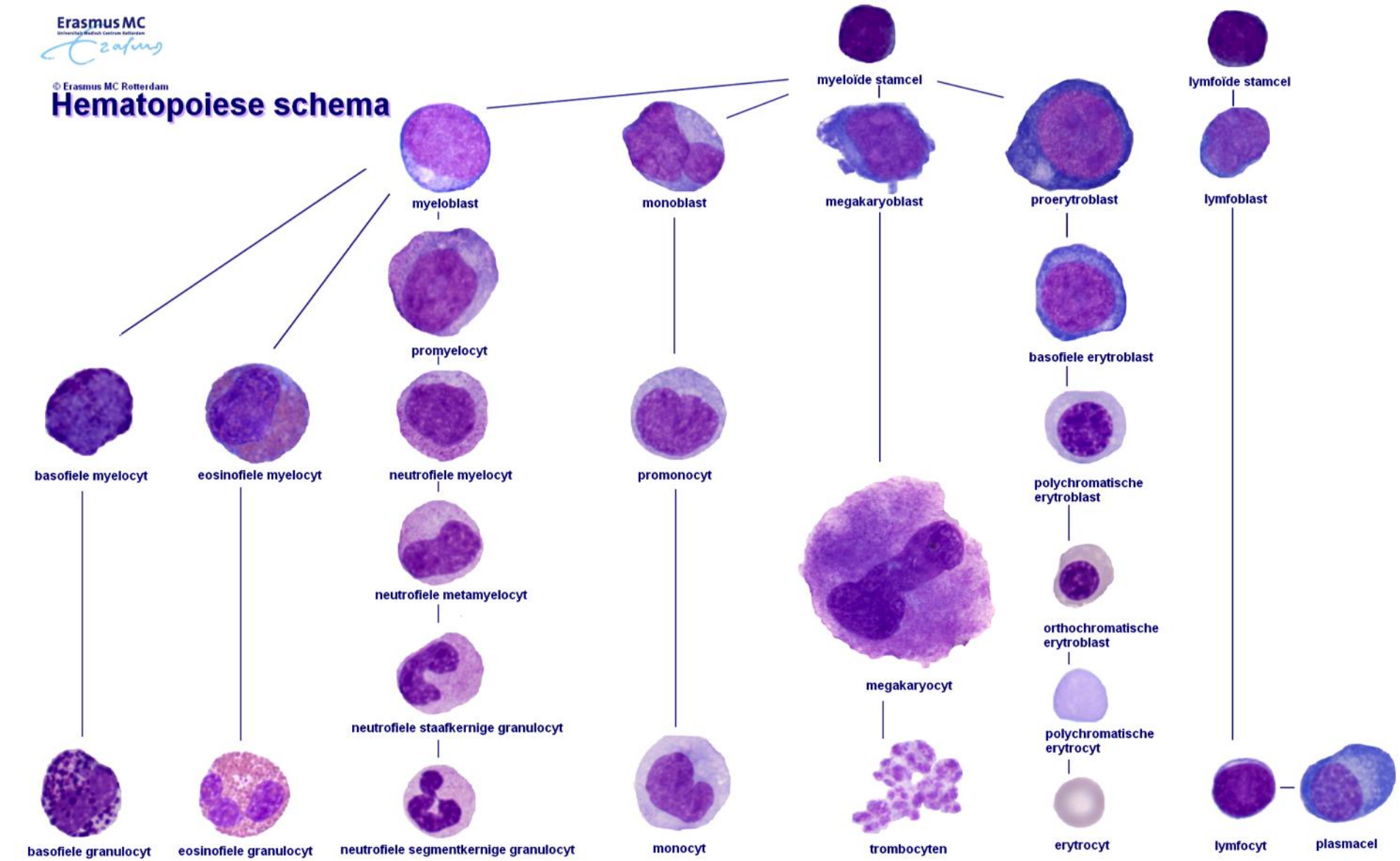
- lichamelijk onderzoek
- medische voorgeschiedenis
- routine laboratoriumtesten
- morfologie
- flowcytometrie
- moleculaire diagnostiek

Normale verhouding cellen bloed.

Rode bloedcellen ($4,2\text{-}6,2 \times 10^{12}$/L)
Witte bloedcellen ($4,3\text{-}10 \times 10^{9}$/L)
Bloedplaatjes ($150\text{-}400 \times 10^{9}$/L)



Hematopoiese

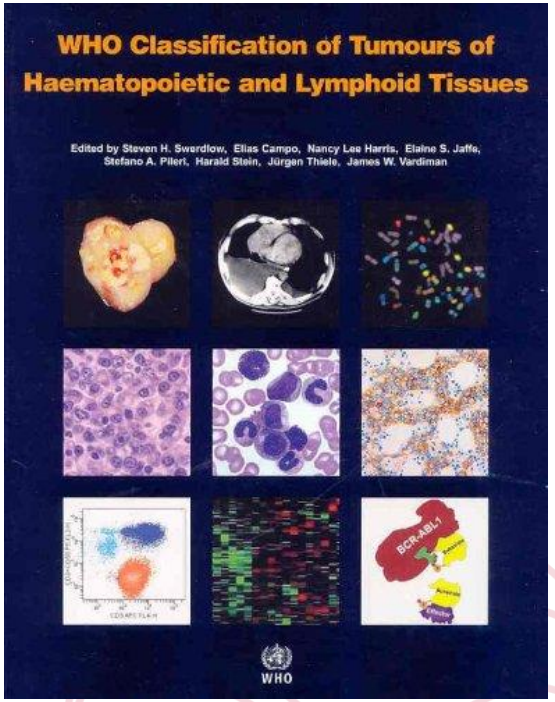


Myeloproliferatieve ziekten

MPN indeling volgens de 3de WHO editie (2008)

Tabel 1. Myeloproliferatieve neoplasmata (MPN).
chronische myeloïde leukemie, BCR-ABL1-positief (CML)
chronische neutrofiele leukemie
polycythemia vera (PV)
primaire myelofibrose (MF)
essentiële trombocytose (ET)
chronische eosinofiele leukemie, niet nader gespecificeerd (CEL NOS)
mastocytose
myeloproliferatief neoplasma, niet classificeerbaar

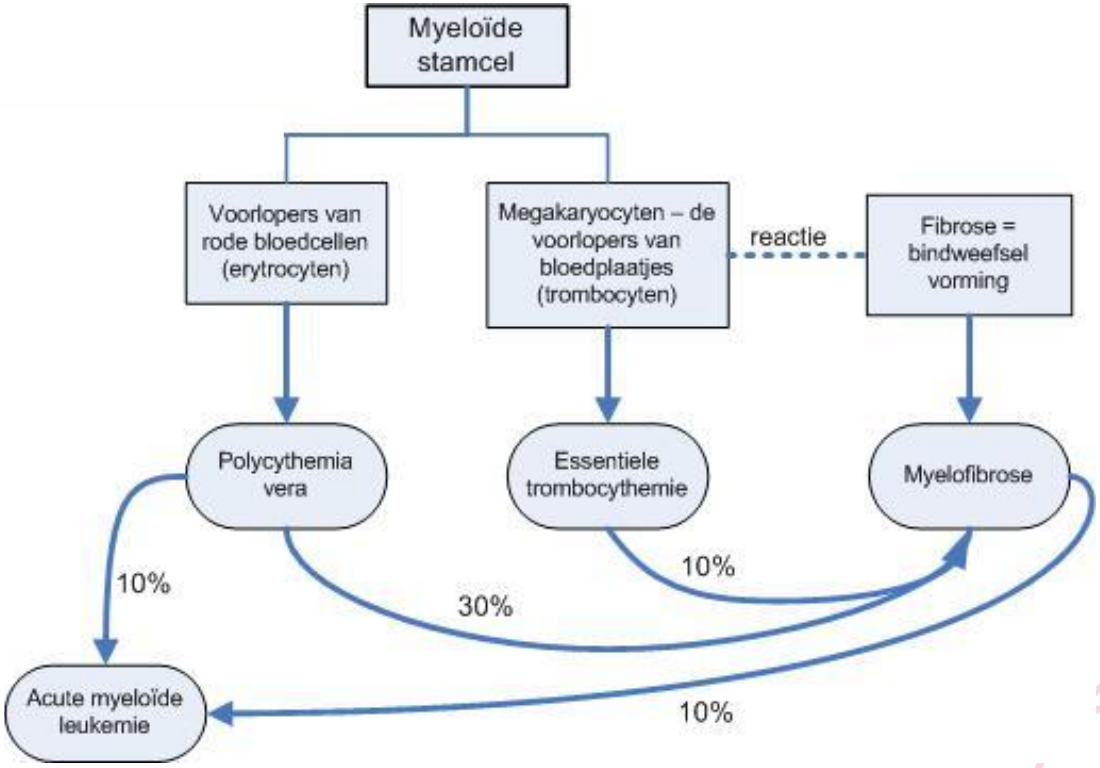
'Klassieke MPN'



Mastocytose: Aparte categorie in nieuwe 4de WHO editie 2016.

Ned Tijdschr Hematol 2009;6:247-253

Woekering van één (of meerdere) cellijnen.



Klinische problemen: verhoogd risico op veneuze en arteriële trombotische complicaties, en op bloedingen.

Ned Tijdschr Hematol 2011;8:64-79

http://www.hematologienederland.nl/myeloproliferatieve-aandoeningen

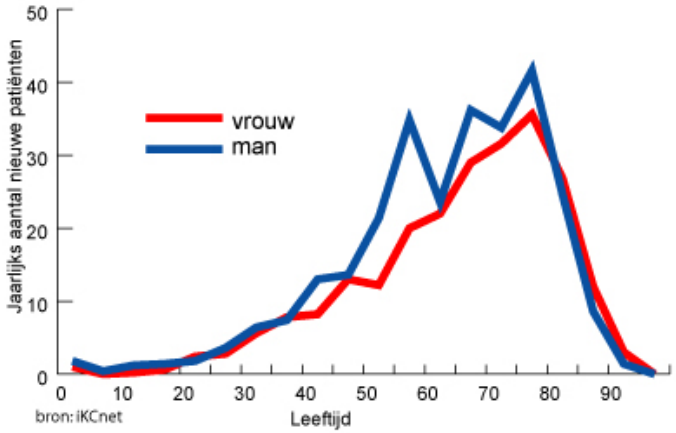
Prognose

PV
In het algemeen een gemiddelde overleving na diagnose van 10 - 20 jaar.

ET
Bij een goede behandeling, zal de levensverwachting van patiënten met ET niet sterk afwijken van die van mensen zonder ET.

MF
Patiënten kunnen de ziekte 10 jaar of langer overleven, vooral als de diagnose vroeg gesteld wordt. Zeer ernstige gevallen kunnen binnen 3 tot 6 jaar fataal zijn.

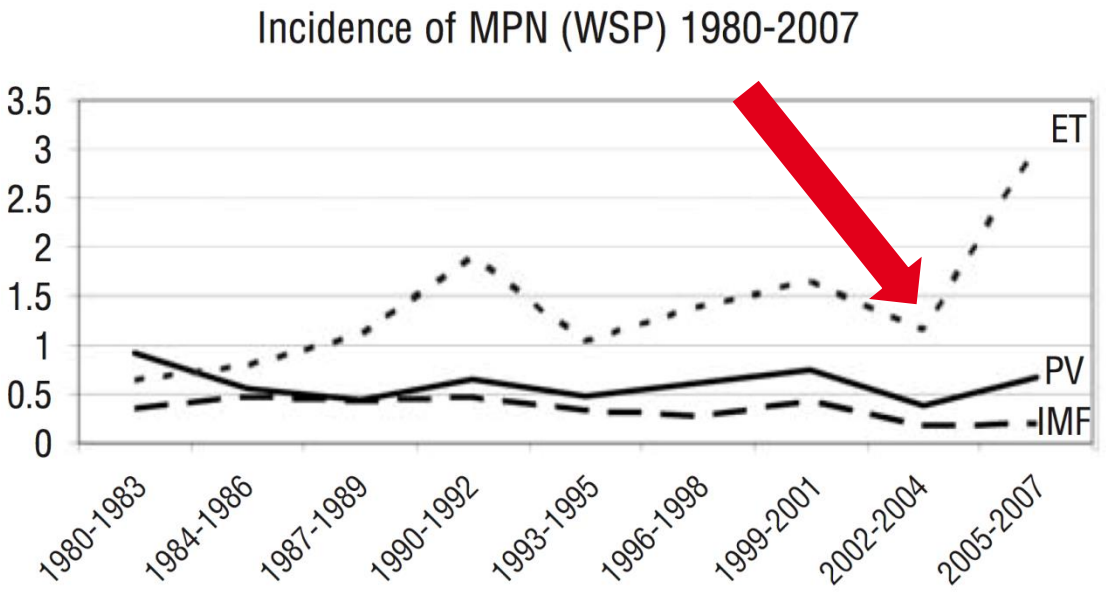
Zeldzaam?



- Voornamelijk >50 jaar, maar van alle leeftijden
- Incidentie: 6-10/100.000/jaar
- Jaarlijks 1.000 nieuwe patiënten in Nederland

Maar…

Incidentie neemt toe



F. Girodon et al. Haematologica 2009; 94(6)

2005

betere laboratoriumdiagnostiek:
moleculaire diagnostiek.

Onderscheid primair/secundair kan lastig zijn…

Tabel 8.22 Oorzaken van trombocytose.

primair:
- essentiële trombocytose*
- andere myeloproliferatieve ziekten*

secundair:
- postoperatief
- na grote bloedingen
- (chronische) infecties
- Fe-gebrek
- niet-hematologische maligniteiten

Tabel 3. Oorzaken van congenitale of verworven secundaire erytrocytose.

oorzaken congenitale secundaire erytrocytose
- erytropoëtinereceptordefect
- hemoglobinevariant met verhoogde zuurstofaffiniteit
- bisfosfoglyceraatmutasedeficiëntie
- verstoorde regulatie van de zuurstofhomeostase door 'hypoxia inducible factor' (HIF); mutaties in von hippel-lindau-tumorsuppressor (chuvash-erytrocytose), prolylhydroxylase-2 en HIF2-α

oorzaken van verworven secundaire erytrocytose
- centrale hypoxie (bijvoorbeeld door chronische longziekte, roken, slaapapneusyndroom, koolmonoxidevergiftiging, verblijf op grote hoogte)
- lokale, renale hypoxie (bijvoorbeeld nierarteriestenose, cyste nieren, postniertransplantatie)
- pathologische erytropoëtineproductie (bijvoorbeeld door cerebellair hemangioblastoom, meningioom, niercelcarcinoom, hepatocellulair carcinoom)
- exogene toediening van erytropoëtine

Moleculaire diagnostiek

2005: JAK2 V617F mutatie (95% PV, 60% ET, 50% MF)

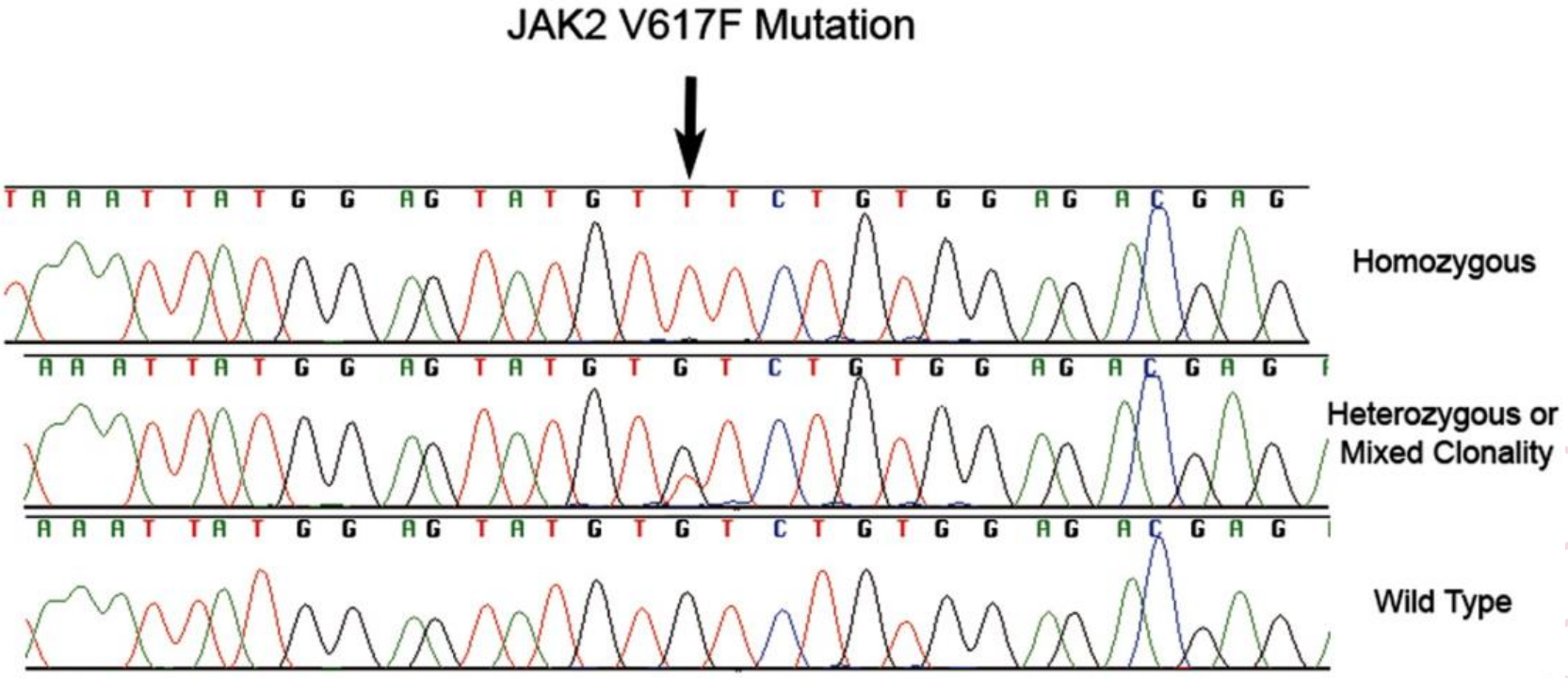
2006: MPL W515L/K (5-10% ET en MF)

2007: JAK2 exon 12 (5% PV)

2013: CALR exon 9 (25% ET en MF)

Klampfl T et al. N Engl J Med 2013; 369(25):2379-90.

JAK2 V617F



Blood 2005 106:1207-1209; doi:10.1182/blood-2005-03-1183

JAK2 exon 12 mutaties

SUBSTITUTIONS

wildtype	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIF
F533IK539L	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMIQMVFHLIRNEDLIF
F537IK539L	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVIHLIRNEDLIF
H538QK539L	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFQLIRNEDLIF
H538DK539LI540S	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFDLSRNEDLIF
K539L	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHLIRNEDLIF
K539LL545V	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHLIRNEDVIF
I540T	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKTRNEDLIF
D544G	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEGLIF
L545S	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDSIF
F547L	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIL
F547V	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIV

DELETIONS

wildtype	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIF
F537-K539delinsK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVKIRNEDLIF
F537-K539del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVIRNEDLIF
F537-K539delinsL	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVLIRNEDLIF
H538del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFKIRNEDLIF
H538-K539del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFIRNEDLIF
H538-K539delinsF	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFFIRNEDLIF
H538-K539delinsI	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFIIRNEDLIF
H538-K539delinsL	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFLIRNEDLIF
I540-N542delinsS	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKSEDLIF
I540-N542delinsK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKKEDLIF
I540-E543delinsKK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKKKDLIF
I540-E543delinsMK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKMKDLIF
I540-D544delinsMK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKMKLIF
I540S,R541-E543delinsK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKSKDLIF
R541-E543delinsK	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIKDLIF
R541-D544del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKILIF
N542-E543del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRDLIF
N542-D544delinsN	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNLIF
E543-D544del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNLIF
D544-L545del	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEIF

DUPLICATIONS

wildtype	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIF
V536-I546dup11	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQM**VFHKIRNEDLI**VFHKIRNEDLIF
V536-F547dup12	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQM**VFHKIRNEDLIF**VFHKIRNEDLIF
V536F, F37-I546dup10	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQM**FFHKIRNEDLI**FHKIRNEDLIF
F537-F547dup11	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMV**FHKIRNEDLIF**FHKIRNEDLIF
F537-I546dup10,F547L	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMV**FHKIRNEDLIL**FHKIRNEDLIF
F547L, I540-F547dup8	(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHK**IRNEDLIFL**IRNEDLIF

Am. J. Hematol. 86:668–676, 2011.

MPL mutaties

W515K
W515L
V501A
S505C
A506T
V507I
G509C
L510P
R514K
W515R
R519T
S204P
Y591N
S505N

The Journal of Molecular Diagnostics, Vol. 15, No. 6, November 2013

CALR exon 9 mutaties

Type 1	TRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 2	NCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 3	QRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 4	RRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 5	GQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 6	RRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 7	RRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 8	RRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 9	RQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 10	MCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 11	DQRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 12	RRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 13	QRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 14	RRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 15	RRRERTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 16	QRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 17	RRQWTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 18	RMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 19	RQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 20	GRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 21	AFKRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 22	NAKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 23	CVRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 24	RRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 25	RQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 26	NAKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 27	CFAKRRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 28	RRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 29	PPLCLRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 30	DHPCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 31	GNCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 32	CRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 33	CRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 34	TCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 35	ICRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-
Type 36	CRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA-

N Engl J Med 2013;369:2379-90.

WHO 4de editie

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Table 4. WHO criteria for PV

WHO PV criteria
Major criteria
1. Hemoglobin >16.5 g/dL in men 10,2 mmol/L
Hemoglobin >16.0 g/dL in women 9,9 mmol/L
or,
Hematocrit >49% in men
Hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†

*More than 25% above mean normal predicted value.

†Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

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Table 5. WHO criteria for ET

WHO ET criteria
Major criteria
1. Platelet count ≥450 × 10^9/L
2. BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR*, or *MPL* mutation
Minor criterion
Presence of a clonal marker or absence of evidence for reactive thrombocytosis
Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion

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Table 6. WHO criteria for prePMF

WHO prePMF criteria
Major criteria
1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1*, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for *BCR-ABL1*$^+$ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of minor reactive BM reticulin fibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

‡Minor (grade 1) reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Table 7. WHO criteria for overt PMF

WHO overt PMF criteria
Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*$^+$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

‡BM fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

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Table 7. WHO criteria for overt PMF

WHO overt PMF criteria
Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*$^{+}$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq 11 \times 10^9$/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

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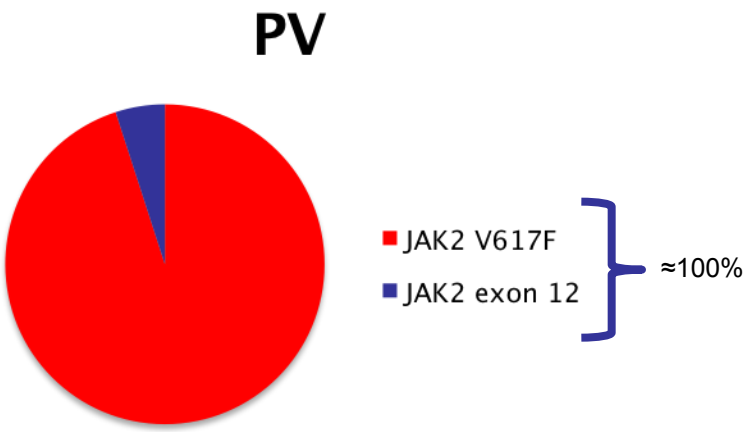
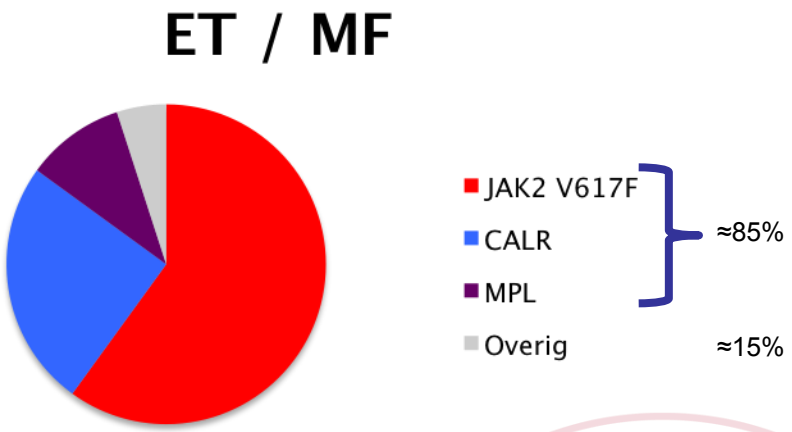
Moleculaire diagnostiek



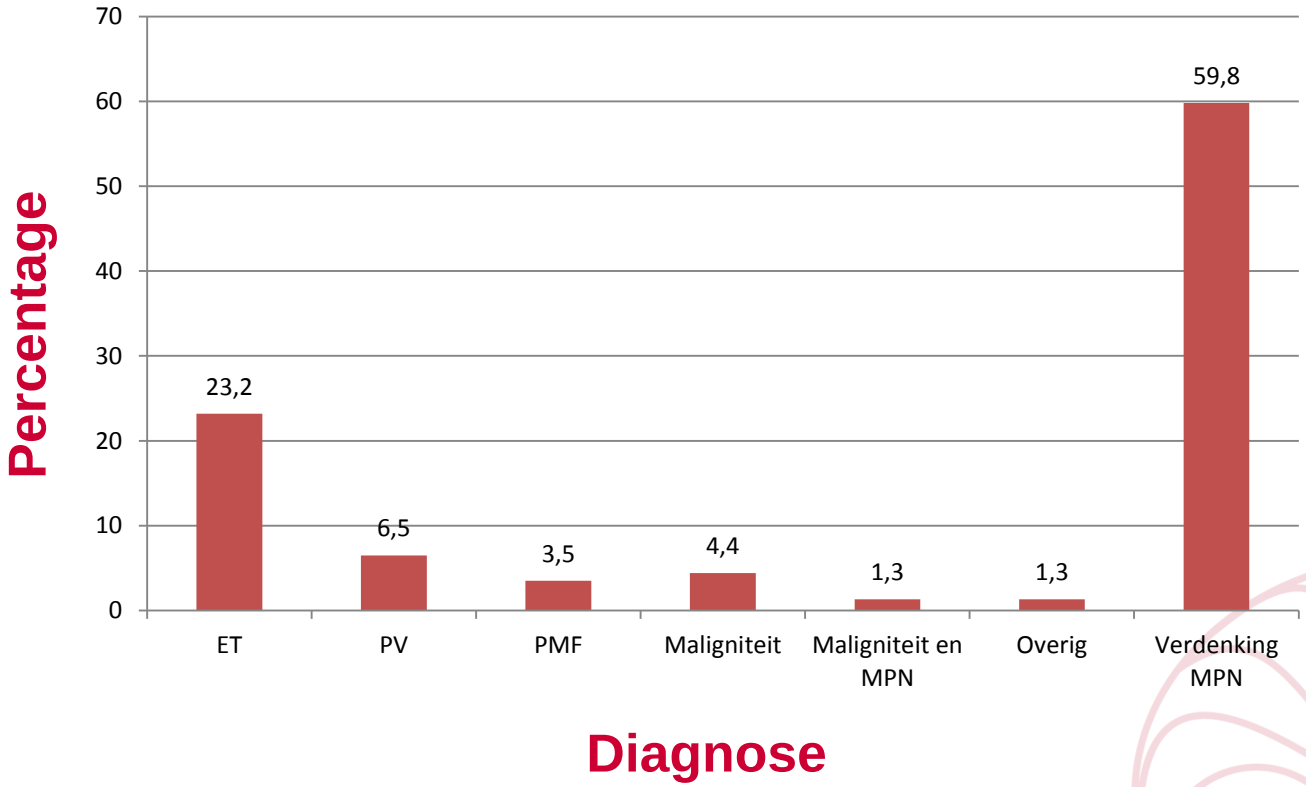


Klampfl T et al. N Engl J Med 2013; 369(25):2379-90.

Database Result

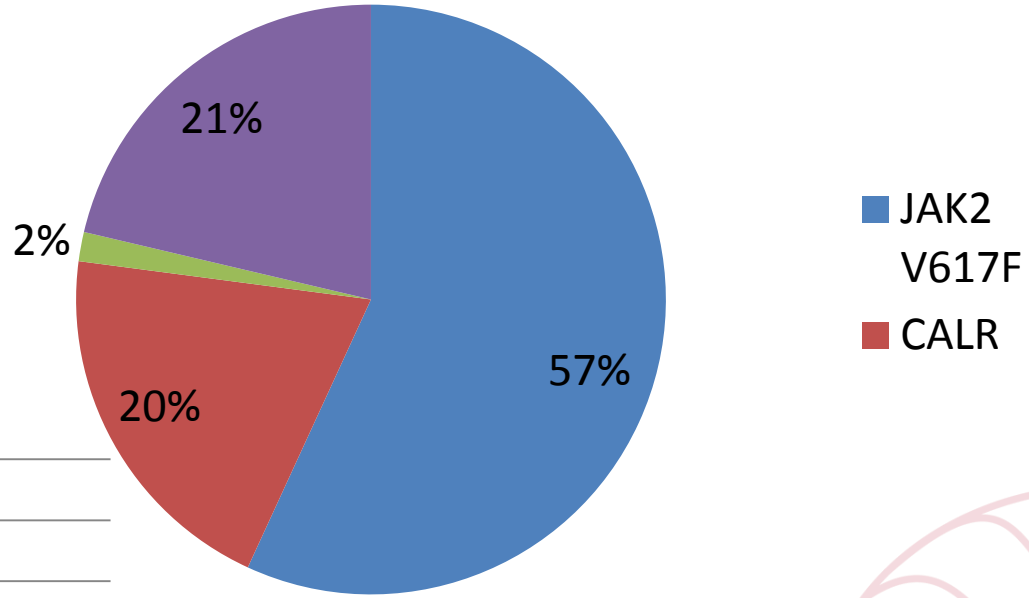
- DNA van alle patiënten waarvoor een JAK2 V617F bepaling is aangevraagd
- 981 patiënten sinds maart 2012
- Gevonden mutatie en gebruikte techniek worden geregistreerd
- 850 patiënten (86.6%) hebben bekende diagnose

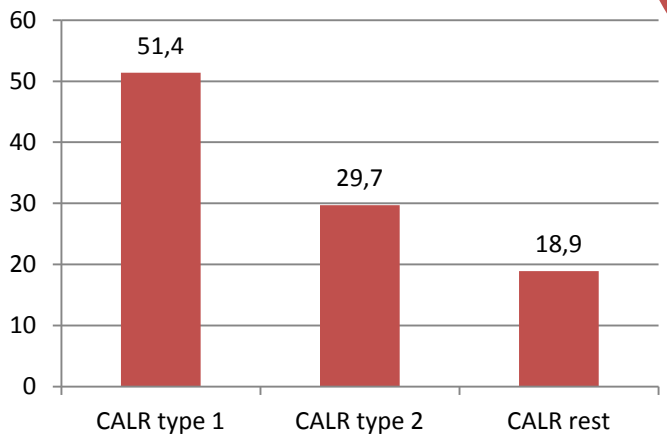
Diagnoses


Percentage
70
60
50
40
30
20
10
0
23,2
6,5
3,5
4,4
1,3
1,3
59,8
ET
PV
PMF
Maligniteit
Maligniteit en MPN
Overig
Verdenking MPN
Diagnose

Mutaties ET

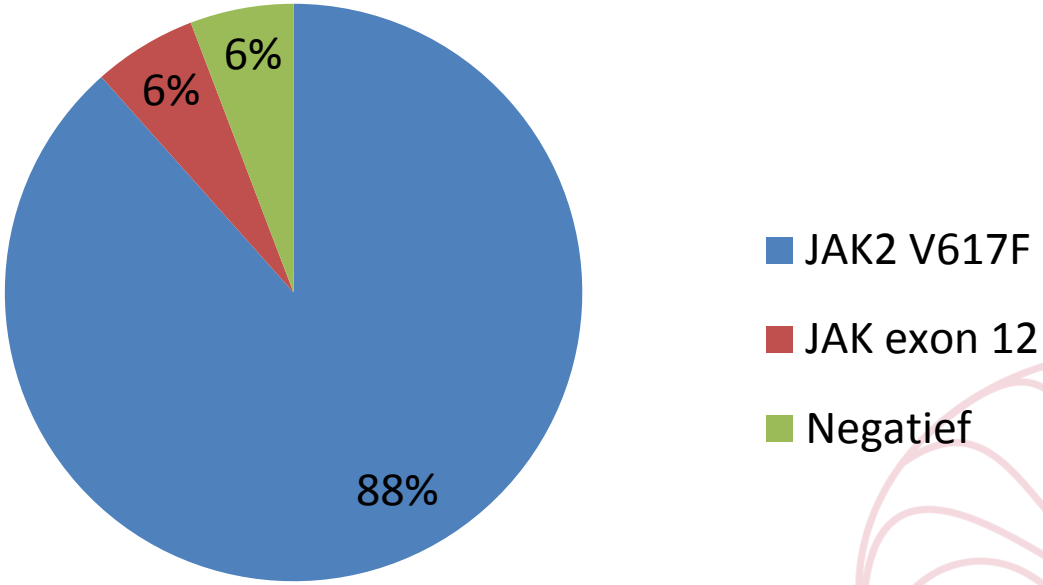
- **197 patiënten met ET → 182 volledig getest**





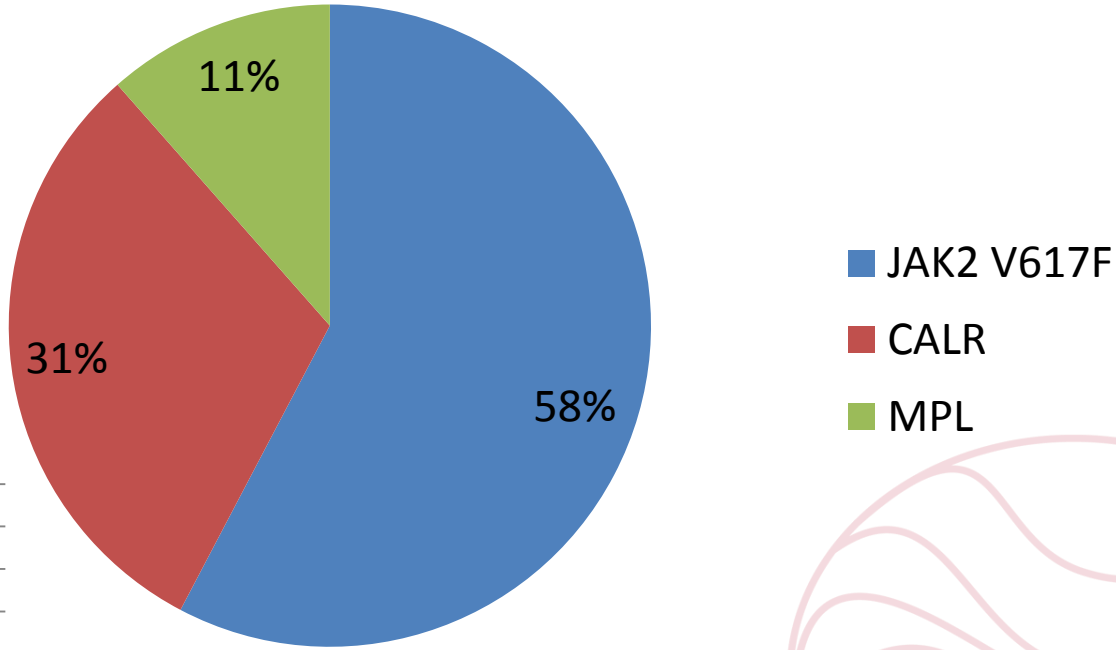
Mutaties PV

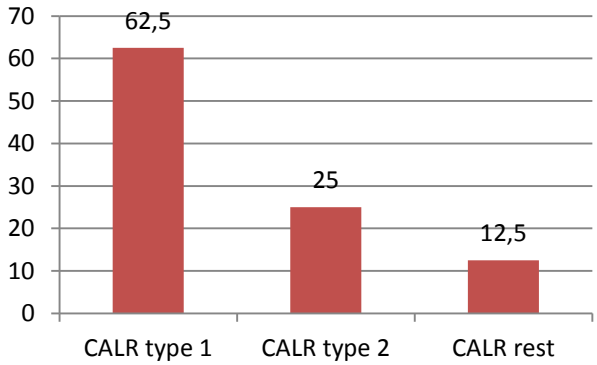
- **55 patiënten met PV → 52 volledig getest**



Mutaties PMF

- 30 patiënten met PMF → 26 volledig getest





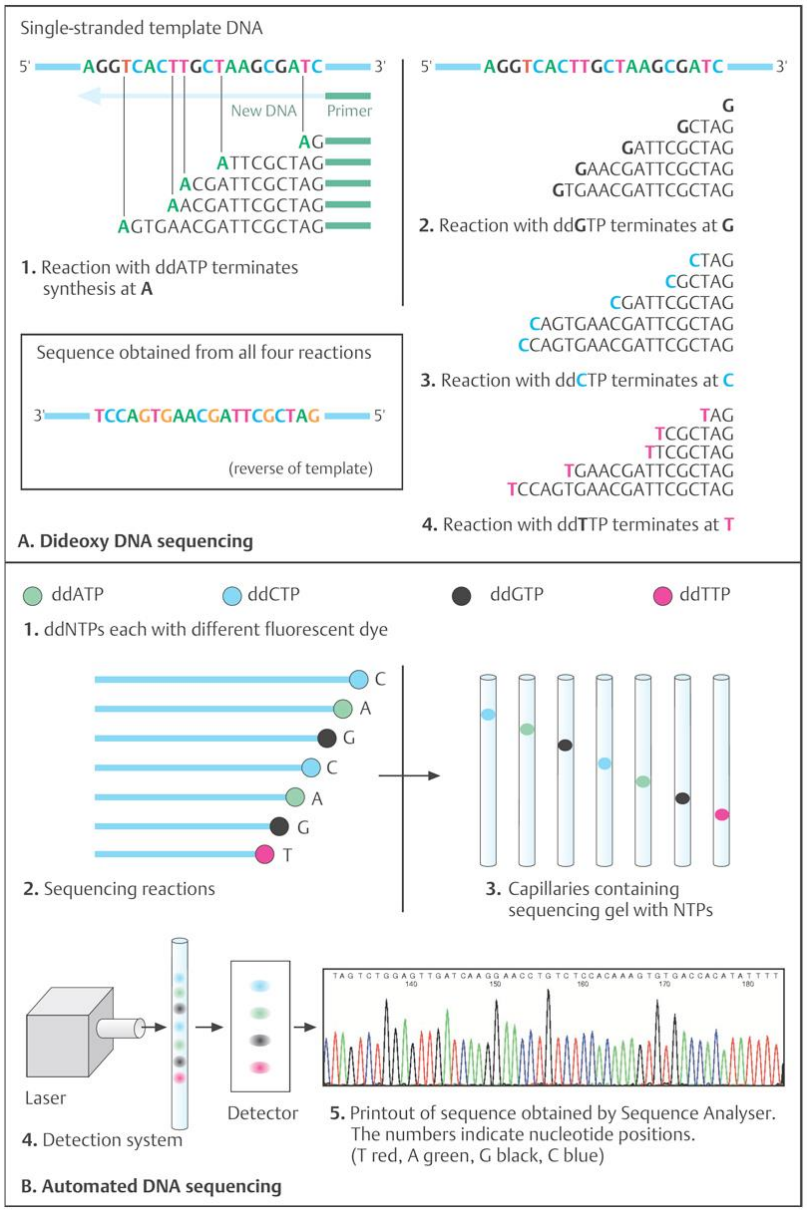
MPN onderzoeken

- JAK2 V617F
- JAK2 exon 12
- Calreticuline (CALR)
- MPL mutaties

Technieken voor MPN onderzoek

- Sequentie analyse
- MLPA
- Real Time
- PCR

Sequentie analyse


Single-stranded template DNA
5' AGGTCACTTGCTAAGCGATC 3'
New DNA
Primer
AG
ATTCGCTAG
ACGATTCGCTAG
AACGATTCGCTAG
AGTGAACGATTCGCTAG
1. Reaction with ddATP terminates synthesis at A
Sequence obtained from all four reactions
3' TCCAGTGAACGATTCGCTAG 5'
(reverse of template)
5' AGGTCACTTGCTAAGCGATC 3'
G
GCTAG
GATTCGCTAG
GAACGATTCGCTAG
GTGAACGATTCGCTAG
2. Reaction with ddGTP terminates at G
CTAG
CGCTAG
CGATTCGCTAG
CAGTGAACGATTCGCTAG
CCAGTGAACGATTCGCTAG
3. Reaction with ddCTP terminates at C
TAG
TCGCTAG
TTCGCTAG
TGAACGATTCGCTAG
TCCAGTGAACGATTCGCTAG
4. Reaction with ddTTP terminates at T
A. Dideoxy DNA sequencing
ddATP
ddCTP
ddGTP
ddTTP
1. ddNTPs each with different fluorescent dye
C
A
G
C
A
G
T
2. Sequencing reactions
3. Capillaries containing sequencing gel with NTPs
Laser
4. Detection system
Detector
5. Printout of sequence obtained by Sequence Analyser. The numbers indicate nucleotide positions. (T red, A green, G black, C blue)
B. Automated DNA sequencing

Voordelen

Toont puntmutaties en kleine deleties of inserties goed aan

Nadelen

Toont geen grote deleties of inserties aan

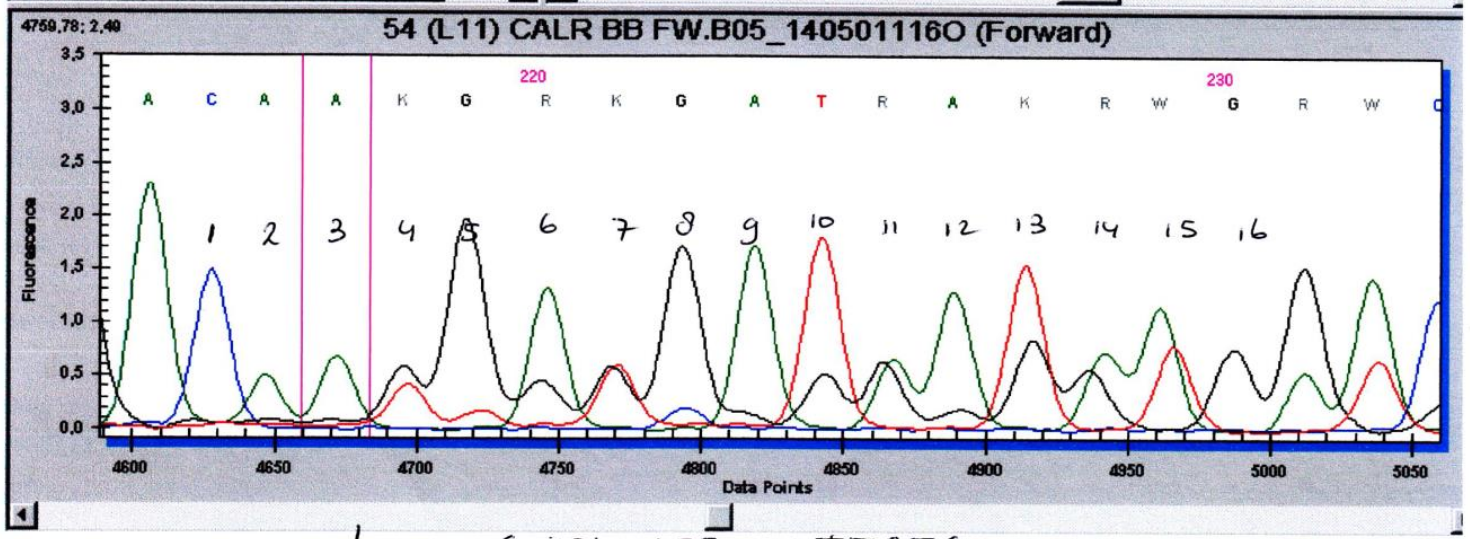
Lage gevoeligheid (20%)

Result
Laboratorium

CALR mutaties

Type 1	c.1092_1143del	→ 52 bp deletie
Type 2	**c.1154_1155insTTGTC**	**→ 5bp insertie TTGTC**
Type 3	c.1095_1140del	→ 46 bp deletie
Type 4	c.1102_1135del	→ 34 bp deletie
Type 5	c.1091_1142del	→ 52 bp deletie
Type 6	c.1094_1139del	→ 46 bp deletie
Type 7	c.1102_1153del	→ 52 bp deletie
Type 8	c.1104_1137del	→ 34 bp deletie
Type 9	c.1140del	→ 1 bp deletie
Type 10	c.1154delinsTGTGTC	→ 1 bp deletie en insertie van TGTGTC
Type 11	[c.1092G>C;1095_1140del]	→ 1092 G wordt C en deletie van 46 bp
Type 12	c.1098_1131del	→ 34 bp deletie
Type 13	c.1100_1134delinsA	→ 35 bp deletie en insertie A
Type 14	c.1101_1134del	→ 34 bp deletie
Type 15	[c.1102_1135del;1145C>G]	→ 34 bp deletie en C wordt G
Type 16	c.1102_1137delinsCA	→ 36 bp deletie en insertie CA
Type 17	[c.1104_1137del;1148A>T]	→ 34 bp deletie en A wordt T

Base Number	310			320				330				340	
Codon Number	102	103	104	105	106	107	108	109	110	111	112	113	114
Reference AA Translation		E	D	K	E	D	D	E	D	K	D	E	D
CALR BB FW+ CALR AA Rev.txt	CA	GAG	GAC	AAG	GAG	GAT	GAT	GAG	GAC	AAA	G-AT	GAG	GAT
54 (L11) CALR BB FW.B05_140501	CA	GAG	GAC	AAK	GRK	GAT	RAK	RWG	RWC	AAR	GAMT	RAG	RWT
Differences				?	??		?	?	??	??	?	<+?	?	??	?
Consensus	CA	GAG	GAC	AAK	GRK	GAT	RAK	RWG	RWC	AAR	GAMT	RAG	RWT
Consensus AA Translation		E	D	KN	*	D	*	*	*	K	DE X	*	X



L11 = C.1154_1155 ins TTgTC

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

C A A g g A g g A T g A T g A g

C A A T T g T C g g A g g A T g

insertie.

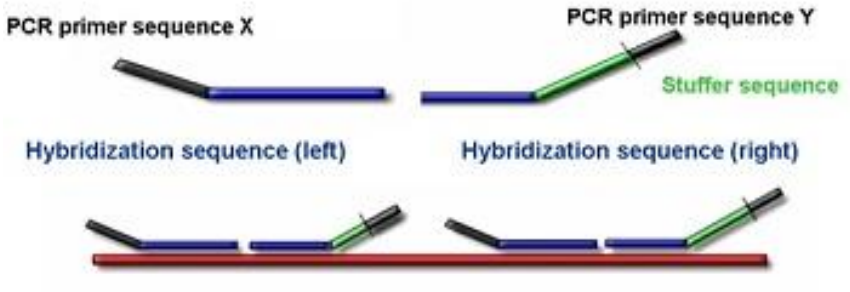
Result Laboratorium

MLPA

Multiplex ligation-dependent probe amplification

Principe MLPA

1. Denaturation and Hybridization

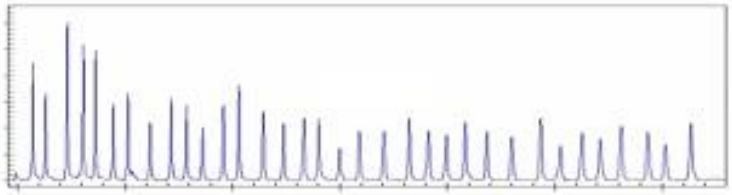


2. Ligation

3. PCR with universal primers X and Y

exponential amplification of ligated probes only

4. Fragment analysis



Result
Laboratorium

MLPA

Voordelen

Hoge gevoeligheid (tot 0,1%)

Kan goed als screening worden ingezet.

Beperking

Afhankelijkheid van de soort probes die in de mix zitten

Probes in MPN kits

P-520

- CALR deletie (type I)
- CALR insertie (type II)
- MPL W515K
- MPL W515L
- JAK2 exon 12 (2 mutaties)
- JAK2 V617F
- KIT D816V

Table 1. SALSA MLPA P520-A1 MPN mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position Reference	Target	Mutation details
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA			
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation			
100	X-fragment: Specific for the X chromosome			
105	Y-fragment: Specific for the Y chromosome			
115	Reference probe S0973-L26704	4p13		
124 §	**CALR probe** S0999-L26702		19p13.2	**L367fs*46**=c.1092_1143del52
130 §	**CALR probe** S1001-L26517		19p13.2	**K385fs*47**=c.1154_1155insTTGTC
136	Reference probe 16316-L25926	3q21.3		
142	Reference probe 07387-L26769	12q13.11		
148	Reference probe 10663-L11245	6p12.2		
154	Reference probe 13781-L15275	11p14.1		
160	Reference probe 17621-L21665	10q22.2		
167 § *	**JAK2 probe** 16924-L21237		9p24.1	**N542_E543del**=c.1624_1629delAATGAA
172 § *	**JAK2 probe** 16924-L21238		9p24.1	**E543_D544del**=c.1627_1632delGAAGAT
175 § Ж	**MPL probe** S1048-SP0405-L21261		1p34.2	**W515K**=c.1543_1544TG>AA
182 § Ж	**MPL probe** S1048-SP0405-L20503		1p34.2	**W515L**=c.1544G>T
190	Reference probe 11556-L26717	5q31.2		
199 § Ж	**KIT probe** 17722-SP0542-L23707		4q12	**D816V**=c.2447A>T
215 § Σ	**JAK2 probe** 05672-L21576		9p24.1	**V617F**=c.1849G>T
230	Reference probe 17130-L26574	11p11.2		
239	Reference probe 05386-L26770	12p11.21		
245	Reference probe 13572-L26953	1q23.2		
265	Reference probe 12434-L26073	14q24.3		
276	Reference probe 16270-L26771	20q11.23		
288	Reference probe 05713-L20268	2p11.2		
297	Reference probe 04570-L20036	16q13		
313	Reference probe 04833-L20693	5p13.2		
328	Reference probe 13397-L26608	6q12		
338	Reference probe 12785-L15496	2q12.3		

§ Mutation-specific probe. This probe will generate a signal when the mutation is present.
Ж This probe consists of three parts and has two ligation sites.
* When both probe signals at 167 nt and 172 nt have a similar height to each other it is indicative for JAK2 E543_D544del mutation. When only the probe signal at 167 nt is present, it is indicative for JAK2 N542_E543del mutation.

Σ This probe can have very low unspecific background signal detected also in healthy patient samples. This back ground signal has never exceeded the signal detected with JAK2 V617F samples with 1% allele burden in the internal quality tests at MRC-Holland. See Figure 2 for further information on this.

Note: Please notify us of any mistakes: info@mlpa.com. The identity of the genes detected by the reference probes is available in Table 2b.

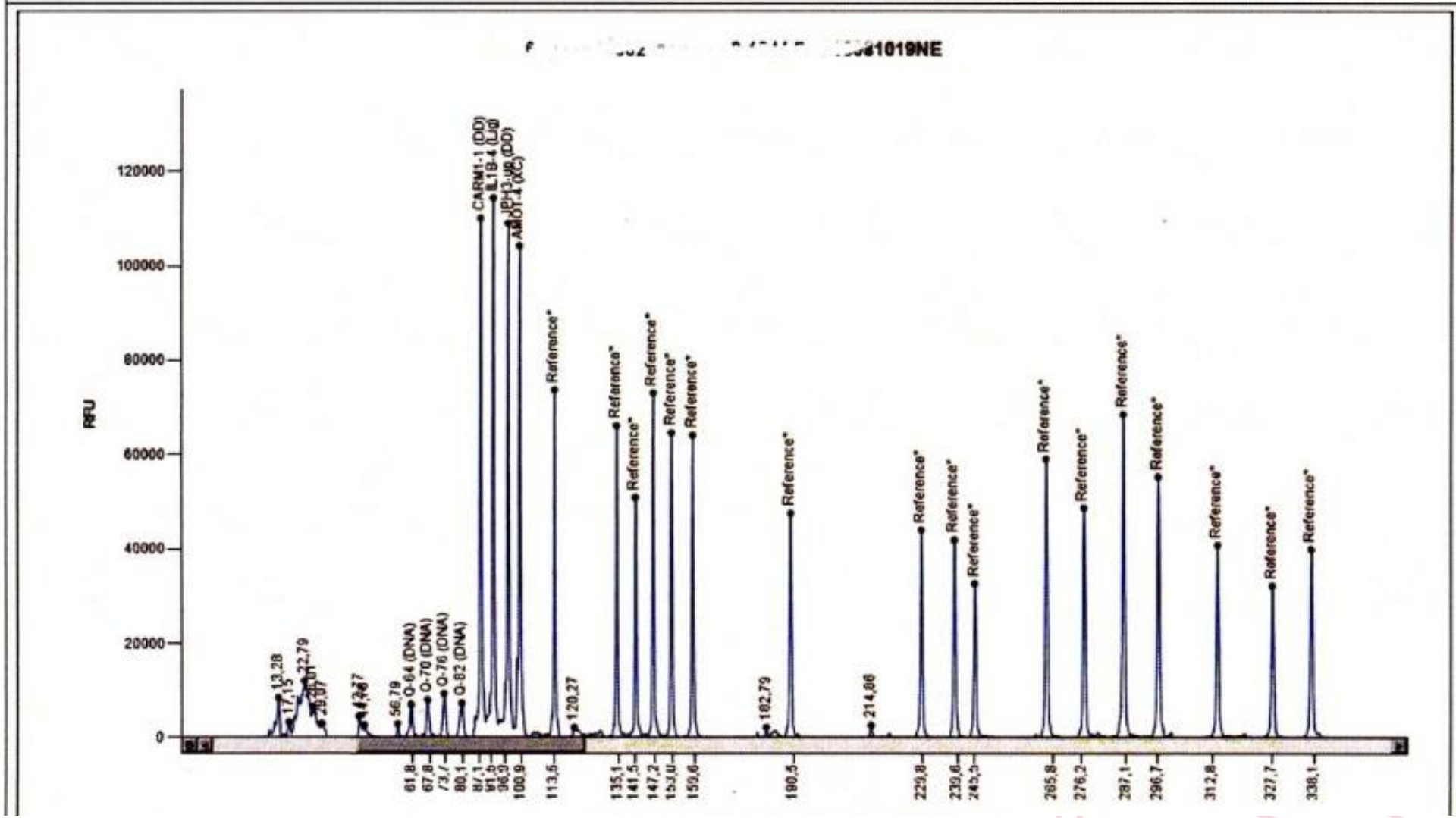
Table 2a. P520-A1 MPN mix 2 probes arranged according to their chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon / Mutation details	Ligation site	Partial sequence (24 nt adjacent to ligation site)	MV location (HG18)
MPL gene, at 1p34.2, indicated ligation sites are in NM_005373.2. Mutations in the thrombopoietin receptor (MPL) gene, most commonly W515L and W515K mutations in exon 10, are detected in patients with ET and PMF, which can support diagnosis when JAK2 is not mutated. W515L and W515K are reported to be the predominant MPL mutations in MPNs (Ma W et al. 2011, *Diagn Mol Pathol*, 20:34-9).					
182 § Ж	S1048-SP0405-L20503	**MPL,** exon 10 **W515L=c.1544G>T**	1589-1590 and 25nt after ex 10	GCTGCTGATGTT-46nt spanning oligo-TGGCGGTGGACC	01-043.587.563
175 § Ж	S1048-SP0405-L21261	**MPL,** exon 10 **W515K=c.1543_1544TG>AA**	1589-1590 and 25nt after ex 10	GCTGCTGAGGAA-46nt spanning oligo-TGGCGGTGGACC	01-043.587.563
KIT gene, at 4q12, indicated ligation sites are in NM_000222.2. Somatic activating KIT D816V mutation is present in more than 97% of systemic mastocytosis patient samples (Erben P et al. 2014, *Ann Hematol*, 93:81-8). This D816V mutation results in ligand-independent activation of KIT tyrosine kinase and detection of this point mutation aids in prediction of response to tyrosine kinase inhibitor (TKI) therapy (imatinib).					
199 § Ж	17722-SP0542-L23707	**KIT,** exon 17 **D816V=c.2447A>T**	2496-2495 and 2535-2534 *reverse*	CATTCTTGATGA-38nt spanning oligo-CCATGAGTAAGG	04-055.293.998
JAK2 gene, at 9q24.1, indicated ligation sites are in NM_004972.3. Discovery of a JAK2 mutation common to MF, PV and ET has linked these diseases on a molecular level and diagnostic criteria for MPN include detection of a clonal marker e.g. JAK2 V617F mutation in exon 14 or exon 12 mutations. JAK2 is mutated in 97% of all patients with PV, 55% of patients with ET and 65% of patients with PMF (Tefferi A, 2010, *Leukemia*, 24:1128–38). The most common JAK2 mutations in exon 12 are the following 6-bp deletions: N542-E543del and E543-D544del.					
167 § £	16924-L21237	**JAK2,** exon 12, **N542-E543del =c.1624_1629delAATGAA**	2126-2127	AAAATCAGAGAT-TTGATATTTGTA	09-005.059.976
172 § £	16924-L21238	**JAK2,** exon 12, **E543-D544del =c.1627_1632delGAAGAT**	2126-2127	AAAATCAGAAAT-TTGATATTTGTA	09-005.059.976
215 § Σ	05672-L21576	**JAK2,** exon 14 **V617F=c.1849G>T**	2343-2342 *reverse*	GTCTCCACAGAA-ACATACTCCATA	09-005.063.725
CALR gene, at 19p13.2, indicated ligation sites are in NM_004343.3. Majority of patients with ET or PM that are negative for JAK2 and MPL carry a somatic mutation in CALR gene. A 52-bp deletion (type 1=L367fs*46) and 5-bp insertion (type 2=K385fs*47) are the most common mutations found in CALR (53% and 32%, respectively), resulting in a frameshift to an alternative reading frame (Klampfl T et al. 2013, *N Engl J Med*, 369:2379-90; Nangalia J et al. *N Engl J Med*, 369:2391-405).					
130 §	S1001-L26517	**CALR,** exon 9 **K385fs*47=c.1154_1155insTTGTC**	1234-1235	CAGAGGACAA**TT-GTC**GGAGGATGA	19-012.915.595
124 §	S0999-L26702	**CALR,** exon 9 **L367fs*46=c.1092_1143del52**	1224-1223 *reverse*	CTTGTCCTCTGC-TCCTCGTCCTGT	19-012.915.528

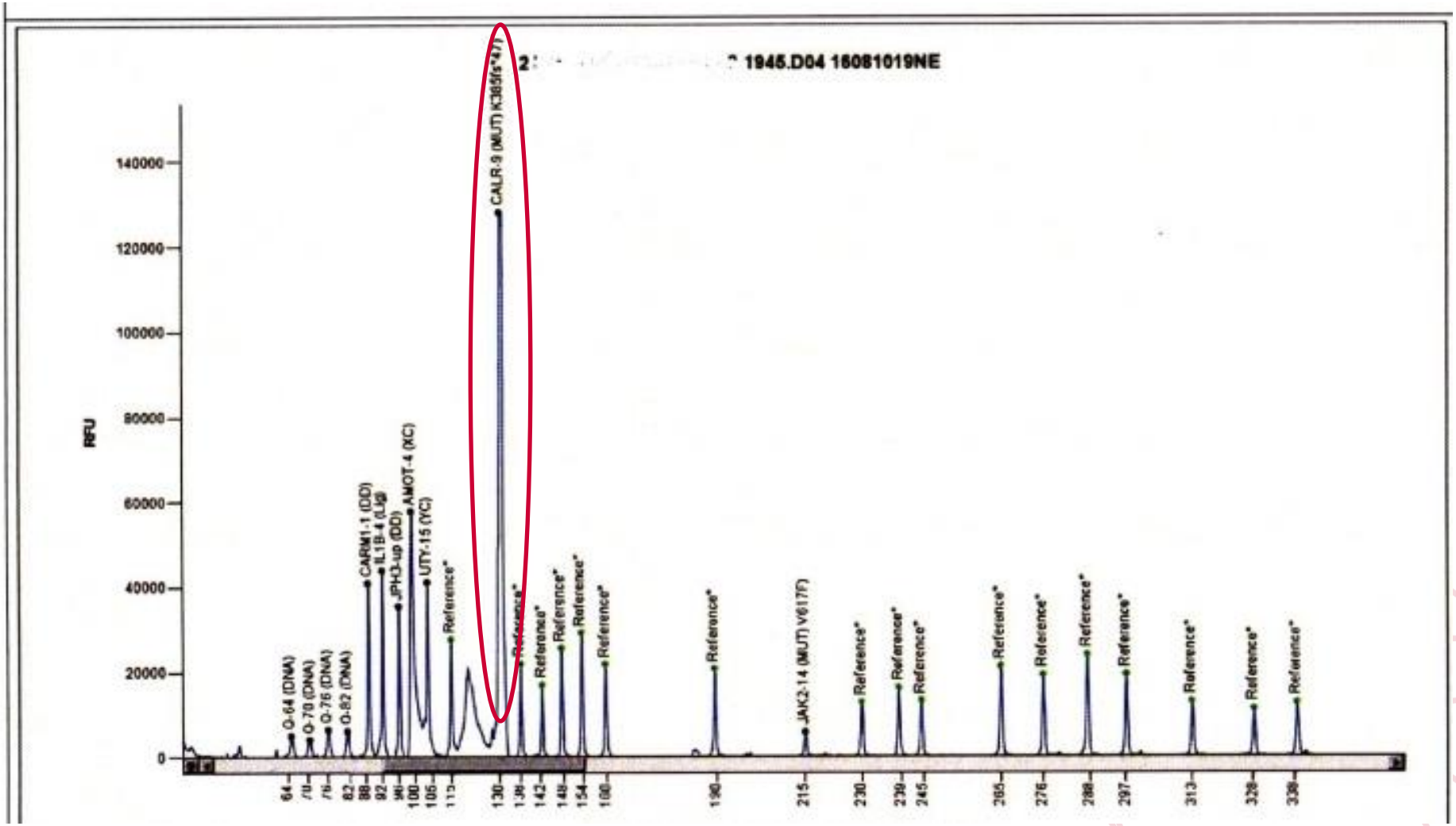
§ Mutation-specific probe. This probe will generate a signal when the mutation is present.
Ж This probe consists of three parts and has two ligation sites.
Σ This probe can have very low unspecific background signal detected also in healthy patient samples. This back ground signal has never exceeded the signal detected with JAK2 V617F samples with 1% allele burden in the internal quality tests at MRC-Holland. See Figure 2 for further information on this.
£ When both probe signals at 167 nt and 172 nt have a similar height to each other it is indicative for JAK2 E543_D544del mutation. When only the probe signal at 167 nt is present, it is indicative for JAK2 N542_E543del mutation.

Note: Exon numbering may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

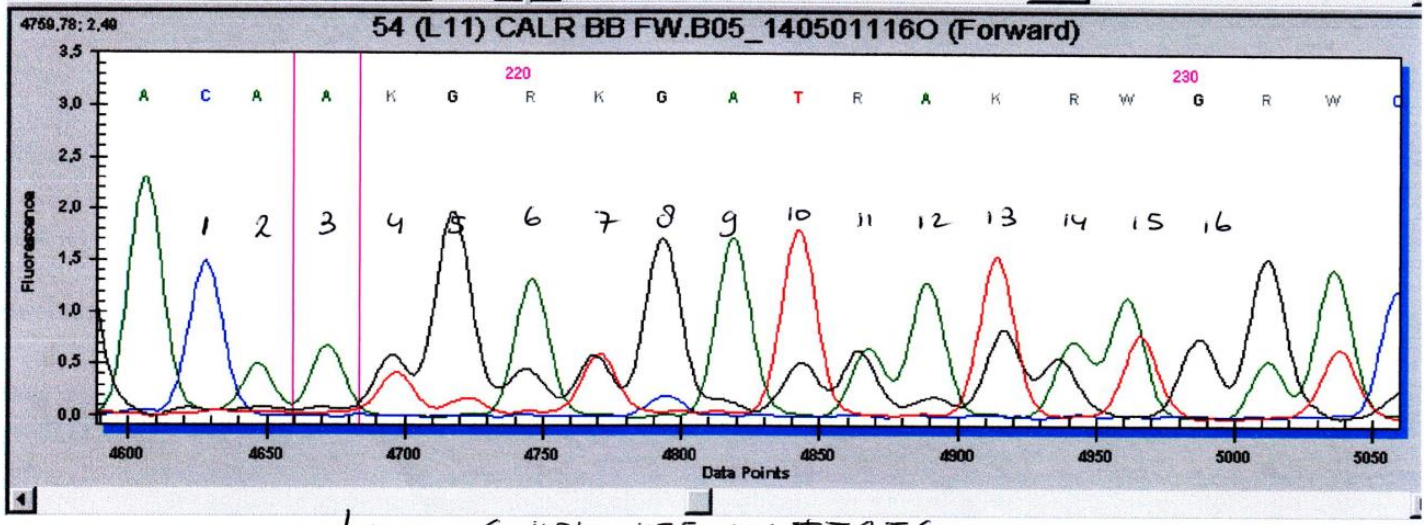
Result Laboratorium



MLPA CALR mut type 2



Base Number	310			320				330				340	
Codon Number	02	103	104	105	106	107	108	109	110	111	112	113	114	1
Reference AA Translation		E	D	K	E	D	D	E	D	K	D	E	D	E
CALR BB FW+ CALR AA Rev.txt	CA	GAG	GAC	AAG	GAG	GAT	GAT	GAG	GAC	AAA	G-AT	GAG	GAT	G
54 (L11) CALR BB FW.B05_140501	CA	GAG	GAC	AAK	GRK	GAT	RAK	RWG	RWC	AAR	GAMT	RAG	RWT	R
Differences				?	??		?	?	??	??	?	<+?	?	??	?
Consensus	CA	GAG	GAC	AAK	GRK	GAT	RAK	RWG	RWC	AAR	GAMT	RAG	RWT	R
Consensus AA Translation		E	D	KN	*	D	*	*	*	K	DE X	*	X	



L11 = C.1154_1155 ins TTgTC

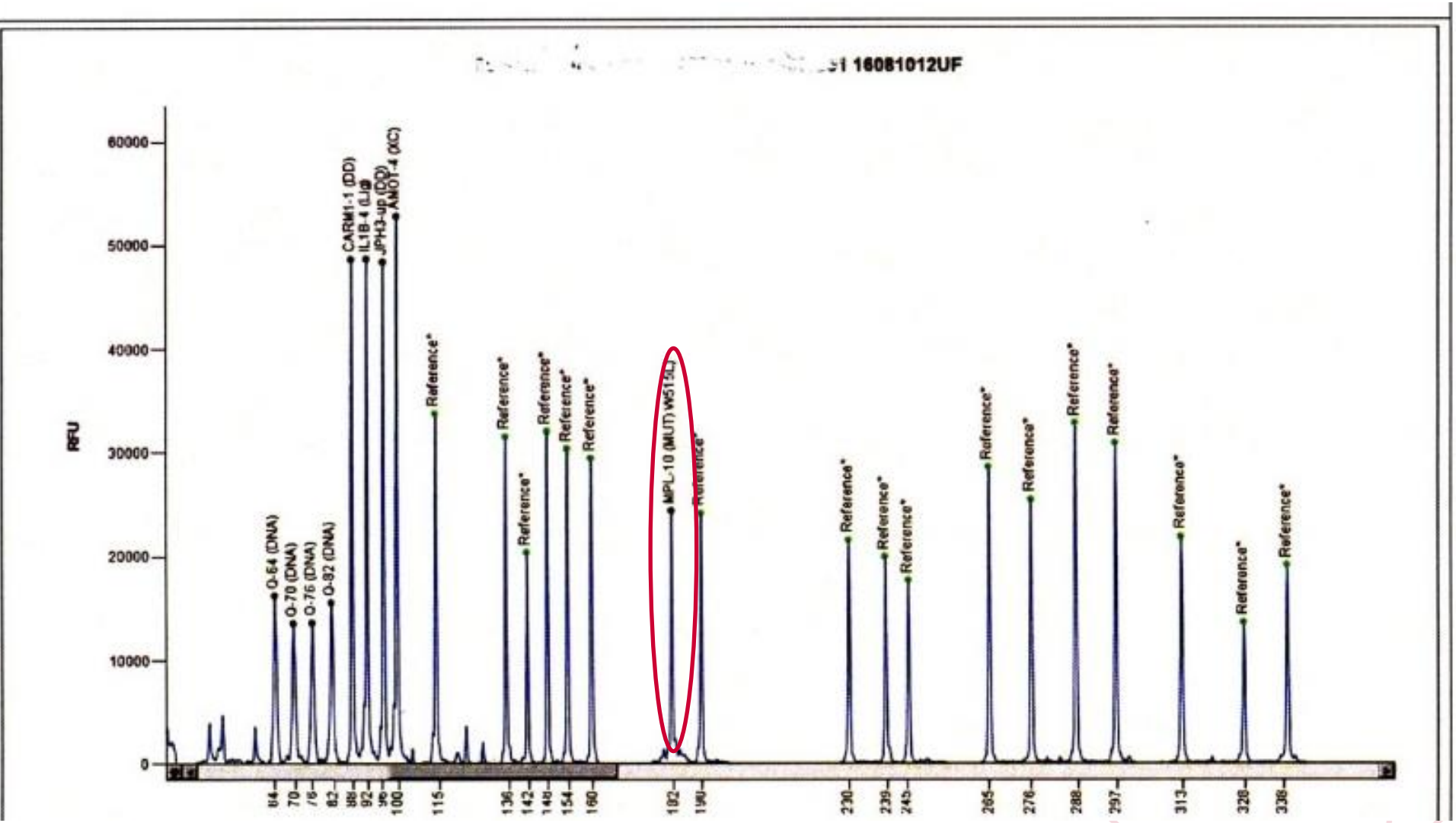
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

C A A g g A g g A T g A T g A g

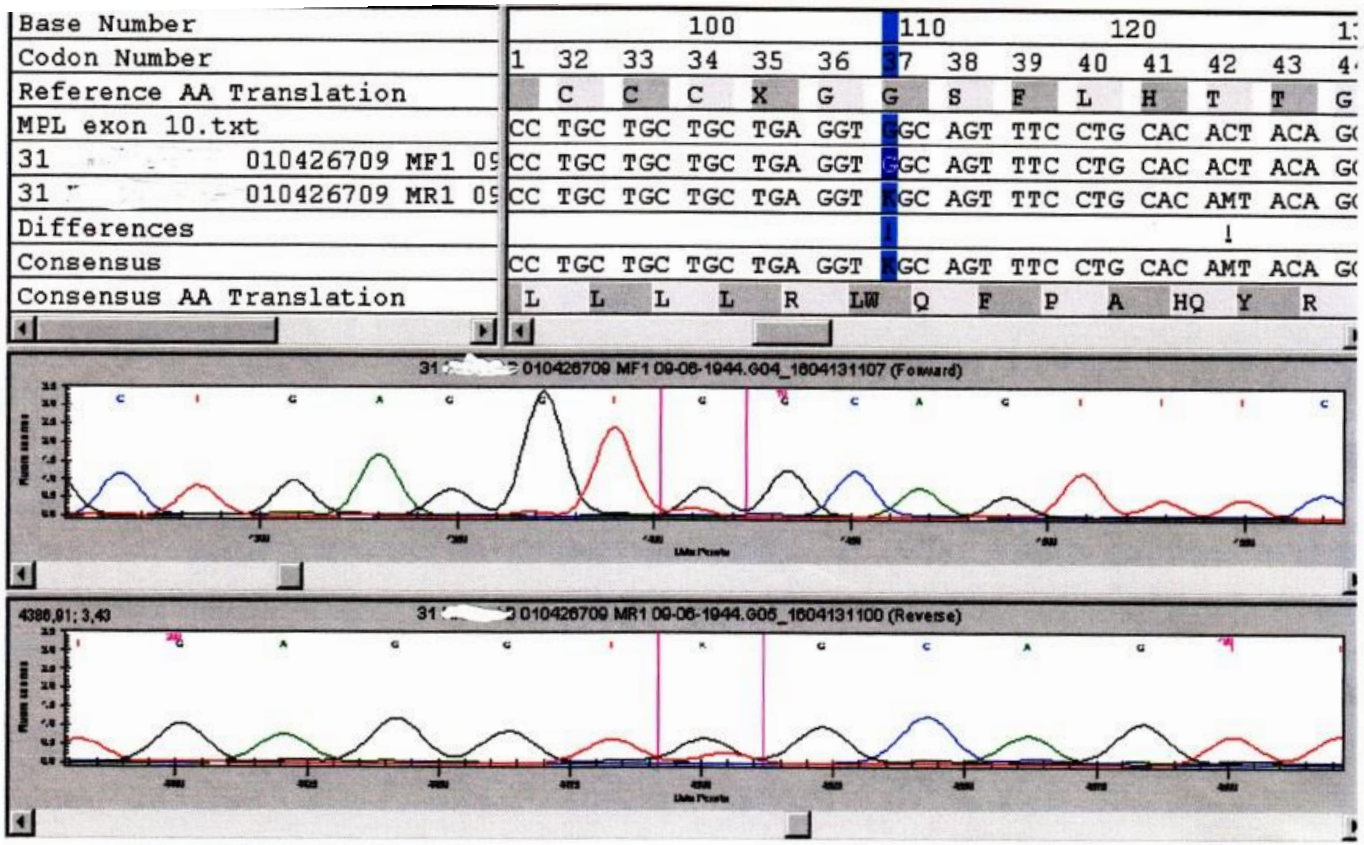
C A A T T g T C g g A g g A T g

insertie.

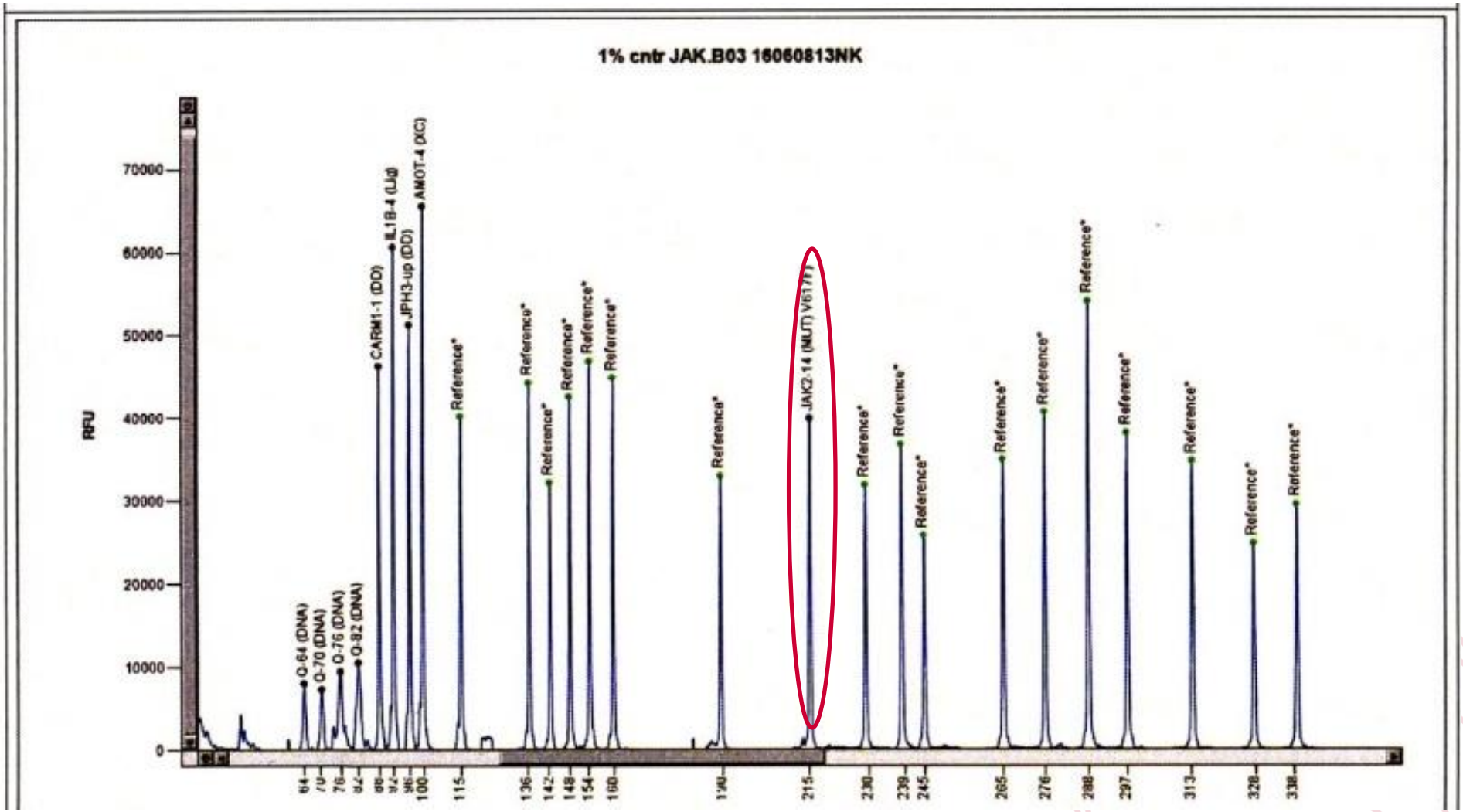
MLPA MPL mutatie



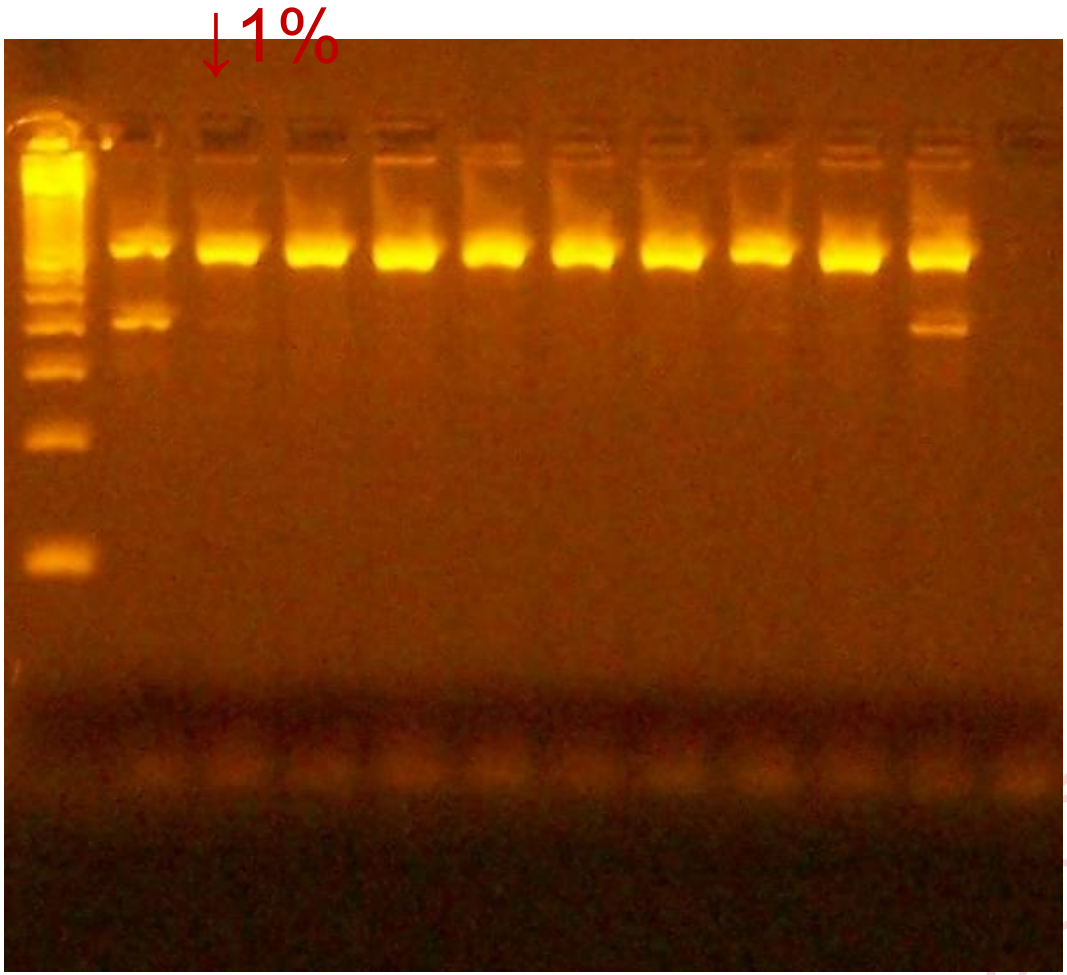
Sequentie analyse MPL



MLPA JAK V617F 1% load



PCR JAK-2 V617F



PAL: nieuwe locatie DNA

Niveau één

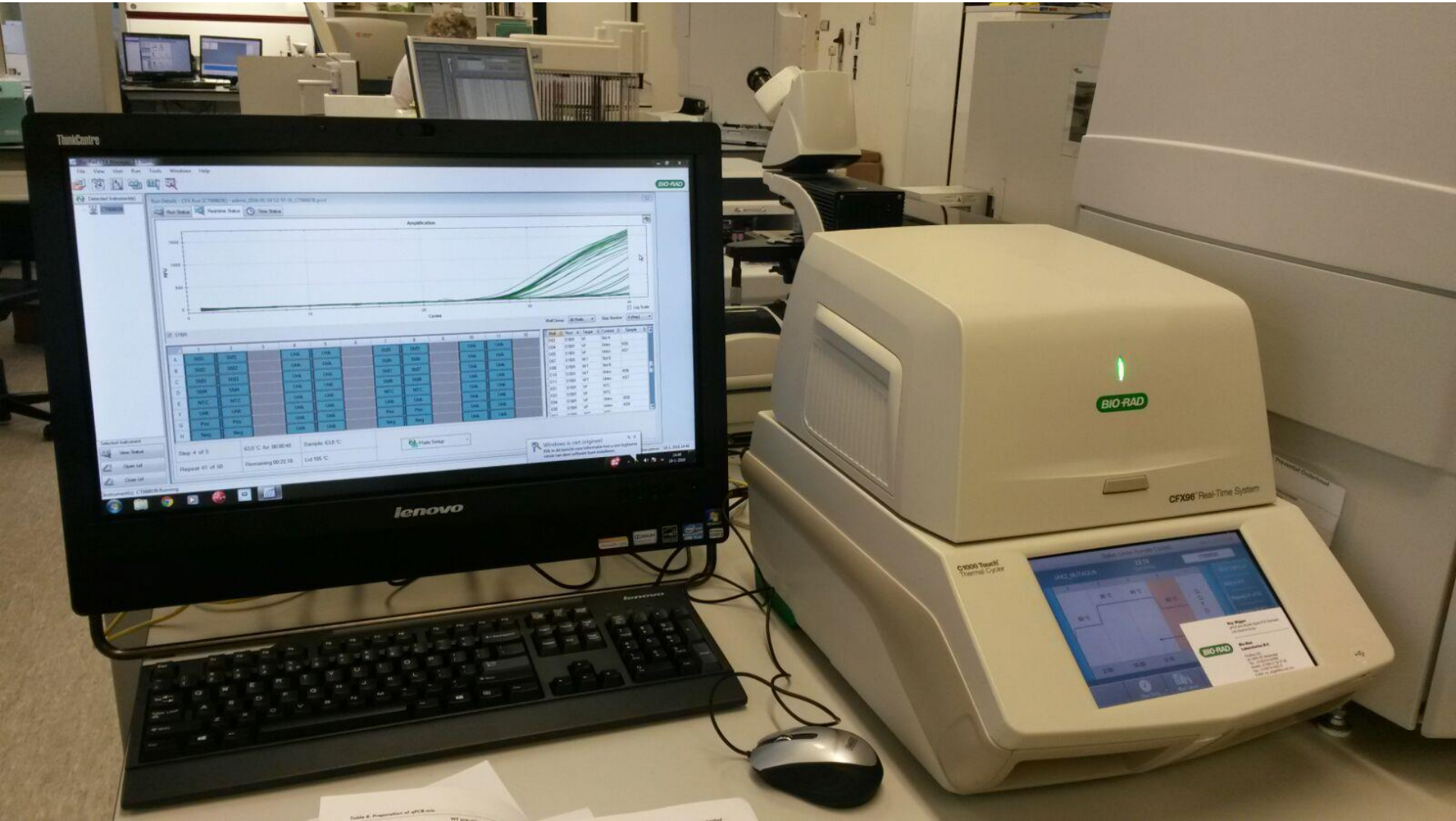
Result Laboratorium

Niveau twee

Niveau drie

Nieuw realtime PCR apparaat.

Result Laboratorium



Casus Marjan

Result Laboratorium

Hematologie/serologie

Hemoglobine	H	12.5	mmol/L	(8.5 - 11.0)
Hematocriet	H	0.59	L/L	(0.41 - 0.51)
Erytrocyten		5.93	10e12/L	(4.60 - 6.20)
MCV		100	fL	(80 - 100)
RDW		14.5	%	(11.0 - 16.0)
vitamine B12		692	pmol/L	(130 - 700)
foliumzuur		7	nmol/L	(5 -)
Ferritine		38	ug/L	(25 - 250)
IJzer		26.3	umol/L	(14 - 28)
Transferrine		2.98	g/L	(2.00 - 3.60)
TIJBC		75	umol/L	(45 - 80)
Verzadigingspercentage		35	%	(20 - 60)
Trombocyten		277	10e9/L	(150 - 400)
Leukocyten		6.2	10e9/L	(4.3 - 10.0)

Machine diff

Basofielen		< 0.1	10e9/L	(0.0 - 0.15)
Eosinofielen		0.2	10e9/L	(- 0.7)
Lymfocyten		1.2	10e9/L	(1.0 - 4.0)
Neutrofielen		4.2	10e9/L	(1.8 - 7.3)
Monocyten		0.6	10e9/L	(0.2 - 1.0)
JAK2 V617F		Zie Opm. → neg		
Zie uitslagbrief.				

Klinische Chemie algemeen

Creatinine		78	umol/L	(59 - 104)
MDRD (e-GFR)		> 60	mL/min	(60 -)
Urinezuur	H	0.48	mmol/L	(0.20 - 0.42)
Natrium		139	mmol/L	(135 - 145)
Kalium		3.7	mmol/L	(3.5 - 5.0)
Calcium		2.37	mmol/L	(2.20 - 2.65)
Albumine		42	g/L	(35 - 50)
Bilirubine	H	24	umol/L	(- 17)
Gamma GT		18	E/L	(- 50)
Alkalische fosfatase		100	E/L	(30 - 100)
ASAT		36	E/L	(- 37)
LD		380	E/L	(- 450)
Testosteron		10.9	nmol/L	(5.0 - 16.5)
Vrij testosteron index		32		(20 - 96)
DHEAS		3.9	umol/L	(0.0 - 9.9)
SHBG		34	nmol/L	(10 - 57)

Eiwitten en Merkers

CRP		< 5	mg/L	(0 - 10)

Verzendbepalingen

Erytropoetine	L	<1	IU/L	(4.0 - 29.0)

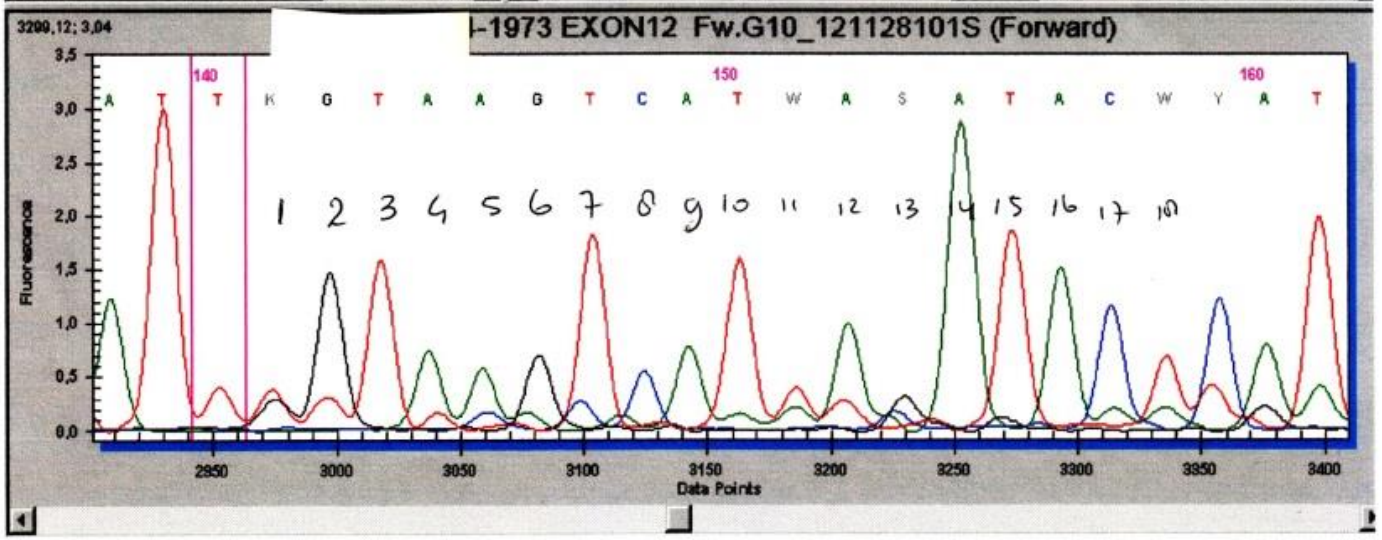
Man

69 Jaar

Wat valt op?

Result Laboratorium

Base Number	190			200			210				220		
Codon Number	64	65	66	67	68	69	70	71	72	73	74	75	76
Reference AA Translation	L	I	F	V	S	H	X	I	L	I	T	V	F
JAK 2 ex12.txt	TTG	ATA	TTT	GTA	AGT	CAT	TAG	ATA	CTC	ATT	ACT	GTC	TT-T
XON12 Fv	TTG	ATA	TTK	GTA	AGT	CAT	WAS	ATA	CWY	ATW	AMT	GTS	WTAT
Differences			?				? ?		??	<?	?	?	? ? +
Consensus	TTG	ATA	TTK	GTA	AGT	CAT	WAS	ATA	CWY	ATW	AMT	GTS	WTAT
Consensus AA Translation	L	I	FL	V	S	H	*	I	HL	I	NT	V	IL



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
R	T	g	T	A	A	g	T	C	A	T	T	A	g	A	T	A	
P	g	T	T	T	C	A	C	A	A	A	A	T	C	A	g	A	

Sequentie met forward primer

TGTTTCACAA AATCAGAAAT GAAGATTTGA TATTGTTTCACAA AATCAGAAAT GAAGATTTGA
TGTTTCACAA AATCAGAAAT GAAGATTTGA TATTT*GTAAGTCA TTAGATA*

TATTT*GTAAGTCA TTAGATA*

Sequentie met reverse primer

TGTTTCACAA AATCAGAAAT GAAGATTTGA TATTGTTTCACAA AATCAGAAAT GAAGATTTGA
ATGAACCAAA TGGTGTTTCACAA AATCAGAAAT GAAGATTTGA

TATTT*GTAAGTCA TTAGATA*
TATTT*GTAAGTCA TTAGATA*

Patiënt
TG TTT CAC AAA ATC AGA AAT GAA GAT TTG ATA TTG TTT CAC AAA ATC AGA
F H K I R N E D L I L F H K I R

AAT GAA GAT TTG ATA TTT *GTAAGTCATTAGATA*
N E D L I F intron

Wild type
TG TTT CAC AAA ATC AGA AAT GAA GAT TTG ATA TT|T *GTAAGTCATTAGATA*
F H K I R N E D L I F intron

Insertie (duplicatie) bij patiënt is onderstreept. Plaats van insertie tov wild type is aangegeven met verticale streep in wild type sequentie.

Beschreven in de literatuur als:
F537-I546dup10, F547L mutatie (eiwit)
1608-1640dupl33 (DNA)

Originele referentie:
Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, et al.
Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood 2008;111:1686-9.

Casus 2: vrouw uit 1964

Hb: 7.0
WBC: 12.1
Trombo's: 236
Reti's: 1.2%

DIFF.	.			
eo's	1		%	0 - 6
baso's	1		%	0 - 2
staven	9	+	%	0 - 4
segmenten	26	-	%	40 - 80
lymfo's	38		%	12 - 46
mono's	8		%	2 - 12
blasten	4	+	%	0 - 0
promyelo's	3	+	%	0 - 0
myelo's	2	+	%	0 - 0
metamyelo	8	+	%	0 - 0
anisocyt.	++			
microcyt.	++			
anisochr.	+			
hypochr.	+++			
bas.punkt.	+			
elliptocyt	+			
polychr.	+			
traandr.cel	+			
erytroblas	3		/100 wbc	
Diff. opmerk	TEKST advies: consult hematoloog (hs)			
Trombocyten	289		10exp 9/l	150 - 400
macro trom.	+			
----------KLINISCHE CHEMIE----------				
Kreatinine	64		umol/l	45 - 90
ASAT	29		u/l	<31
ALAT	25		u/l	<34
LD	914	+	u/l	<250
Alk.fosfat.	109		u/l	40 - 125
Albumine	39		g/l	35 - 52
eGFR CKD-EPI	82		ml/mn/1.73m2	>60
----------KLINISCHE CHEMIE SPECIEEL--------				
CRP	<2.9		mg/l	<10
----------KLIN.CHEM.SPECIEEL, VITAMINE'S---				
Vitamine B12	869	+	pmol/l	130 - 700
25-OH Vit.D3	10	-	nmol/l	50 - 150

Result Laboratorium

Moleculaire diagnostiek

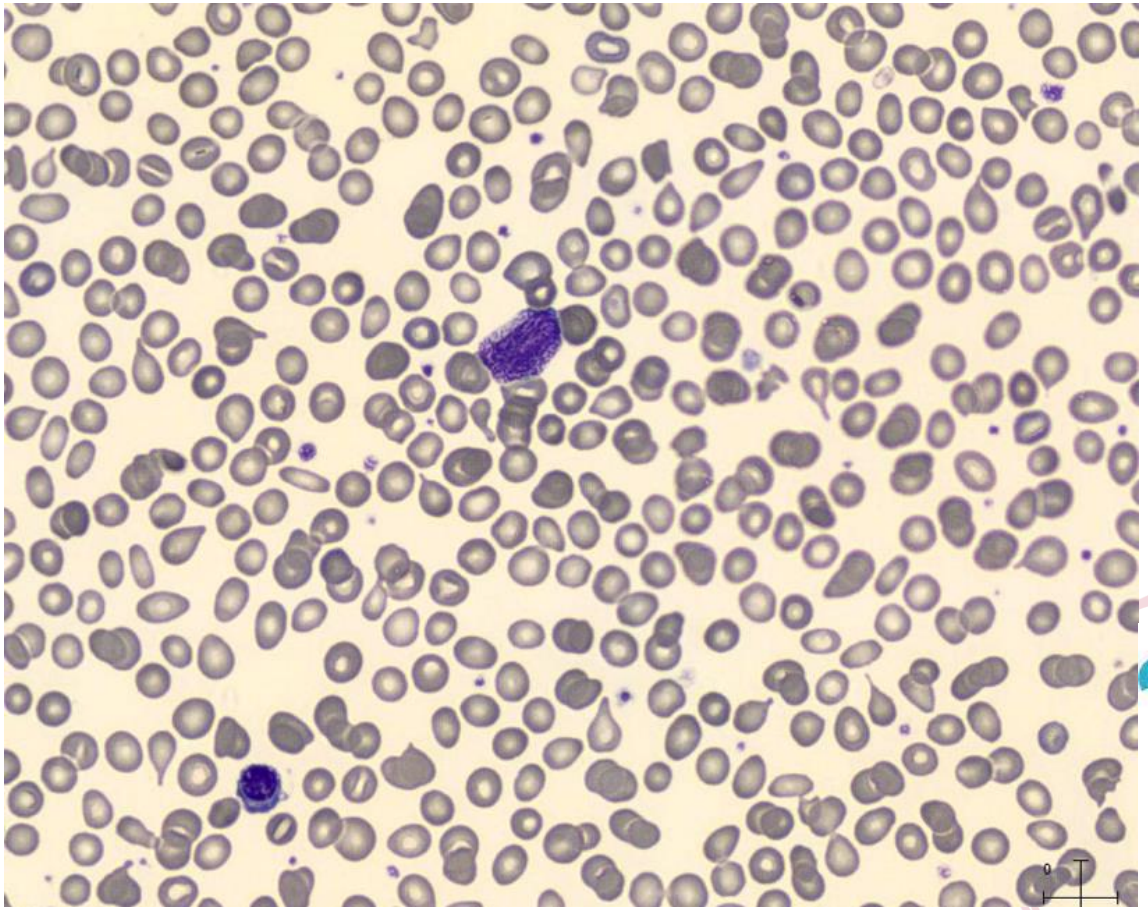
JAK2 V617F: niet aantoonbaar.

MPL W515K/L: niet aantoonbaar.

CALR exon 9: type 1 mutatie aantoonbaar.

Casus 2: vrouw uit 1964

Perifeer bloed



Beenmergonderzoek

Flowcytometrie: **Perifeer bloed: 3% myeloide blasten.**
Beenmerg gelijk aan perifeer bloed. (?)

Patholoog (biopt): **Myelofibrose graad 3. Geen blasten toename.**

Klinische chemie:

Conclusie:
Perifeer bloed, waarin een leuko-erytroblastair bloedbeeld met traandruppelcellen en morfologisch circa 2% blasten. Beenmergpreparaten van slechte kwaliteit (dry tap?), waarin een van de beschikbare preparaten een zeer forse toename van morfologisch afwijkende megakaryocyten. Beeld allereerst sterk verdacht voor een myeloproliferatieve aandoening: in casu (primaire) myelofibrose.

WHO 4de editie

Result Laboratorium

Table 7. WHO criteria for overt PMF

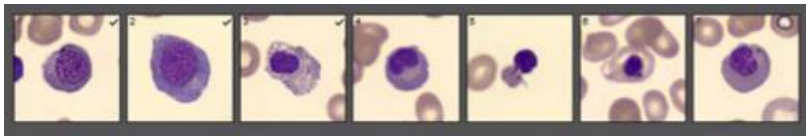
WHO overt PMF criteria
Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*$^{+}$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq 11 \times 10^9$/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

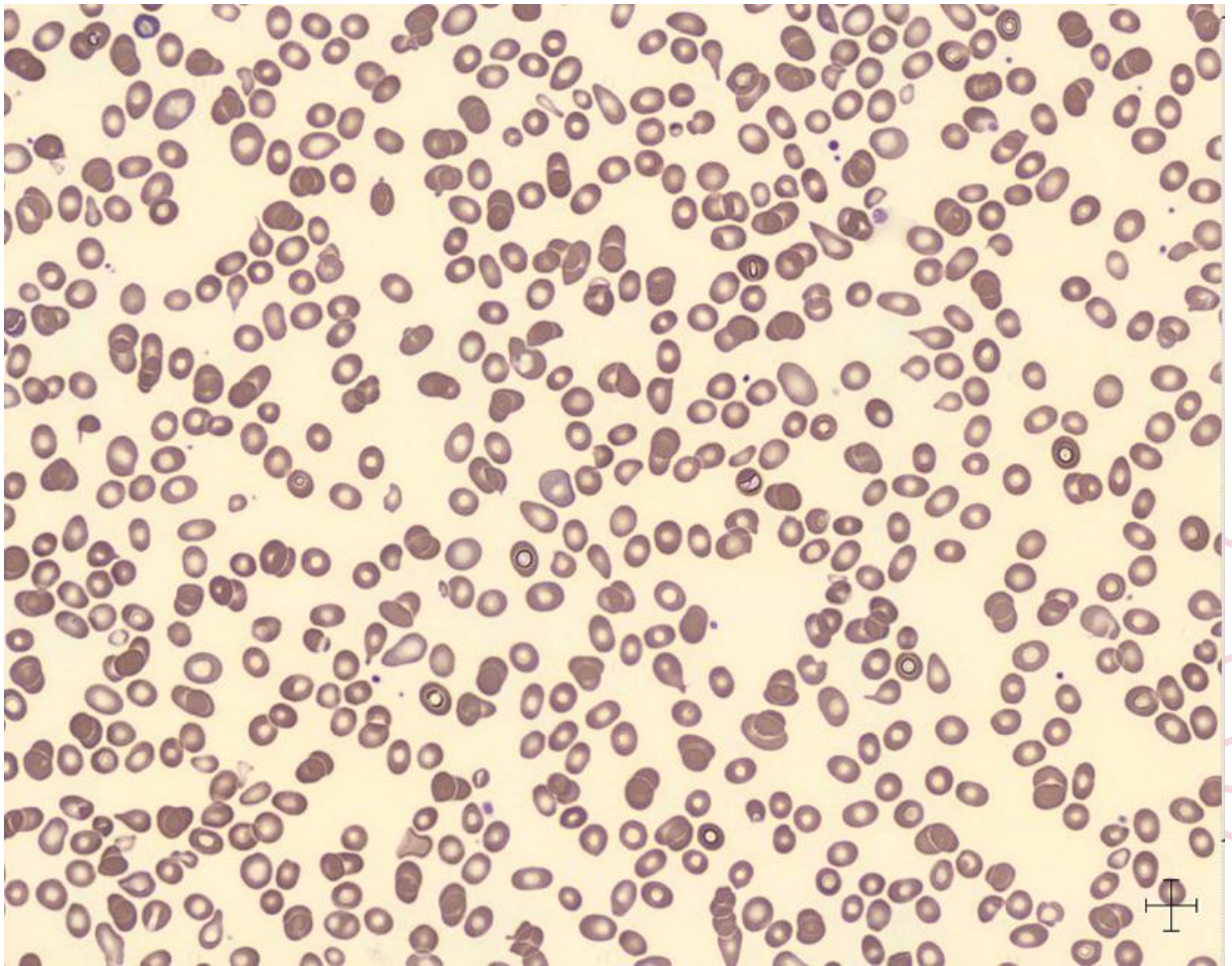
‡BM fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Casus 3: vrouw uit 1964



Hb: 3.2
WBC: 5.5
Trombo's: 90
Reti's: 2.6%

DIFF.	.
eo's	0
baso's	1
staven	1
segmenten	56
lymfo's	29
mono's	7
blasten	2
promyelo's	1
myelo's	2
metamyelo	1
poikilocyt	+
fragmento	+
traandr.cel	++
erytroblas	4
Trombocyten	90
thr.aggr.	afw



Moleculaire diagnostiek

JAK2 V617F: niet aantoonbaar.

MPL W515K/L: niet aantoonbaar.

CALR exon 9: niet aantoonbaar.

Casus 3: vrouw uit 1964

LD	>4000	++	u/l	<250
Bili. totaal	31	+	umol/l	<17
Bili. direct	4		umol/l	<5
Alk.fosfat.	59		u/l	40 - 125
GGT	17		u/l	<38
Albumine	38		g/l	35 - 52
eGFR CKD-EPI	>90		ml/mn/1.73m2	>60
Spijtserum	g8a			
----------KLINISCHE CHEMIE SPECIEEL--------				
IJzer	49	+	umol/l	9 - 30
Transferrine	2.54		g/l	2.0 - 3.6
Transf.verz.	77	+	%	<45
Ferritine	480	+	ug/l	20 - 150
Haptoglobine	<0.08	--	g/l	0.3 - 2.0
----------KLIN.CHEM.SPECIEEL, VITAMINE'S---				
Vitamine B12	44	--	pmol/l	130 - 700
Foliumzuur	13		nmol/l	5 - 36

Beenmergonderzoek

Flowcytometrie: Perifeer bloed met een myeloide blasten populatie met een omvang van circa 1% van de leukocyten. Beenmerg?

Patholoog (biopt): cristabiopt: representatief biopt met zeer celrijk merg (100%) met enige dysmegakaryopoiese en hyperplasie van de erythroide reeks met forse linksverschuiving. Geen significante blastentoename. Geen fibrose. Beeld passend i.h.k.v. vitamine B12 deficiëntie. Geen myeloproliferatieve neoplasie of overtuigende aanwijzingen voor MDS.

Klinische chemie:

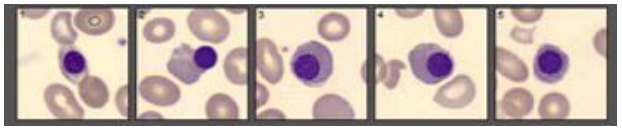
Conclusie:

Perifeer bloed met opvallende vormafwijkingen van de rode bloedcellen. Beenmerg met een verhoogd aantal megakaryocyten en actieve erytropoiese. Vooralsnog past dit bij de ernstige vitamine B12 deficiëntie. Een (prefibrotisch stadium van) myelofibrose lijkt op basis van de morfologie minder waarschijnlijk op dit moment gezien de o.a. relatief normale morfologie van de megakaryocyten. Graag deze uitslag interpreteren in samenhang met de PA.

Casus 3: vrouw uit 1964

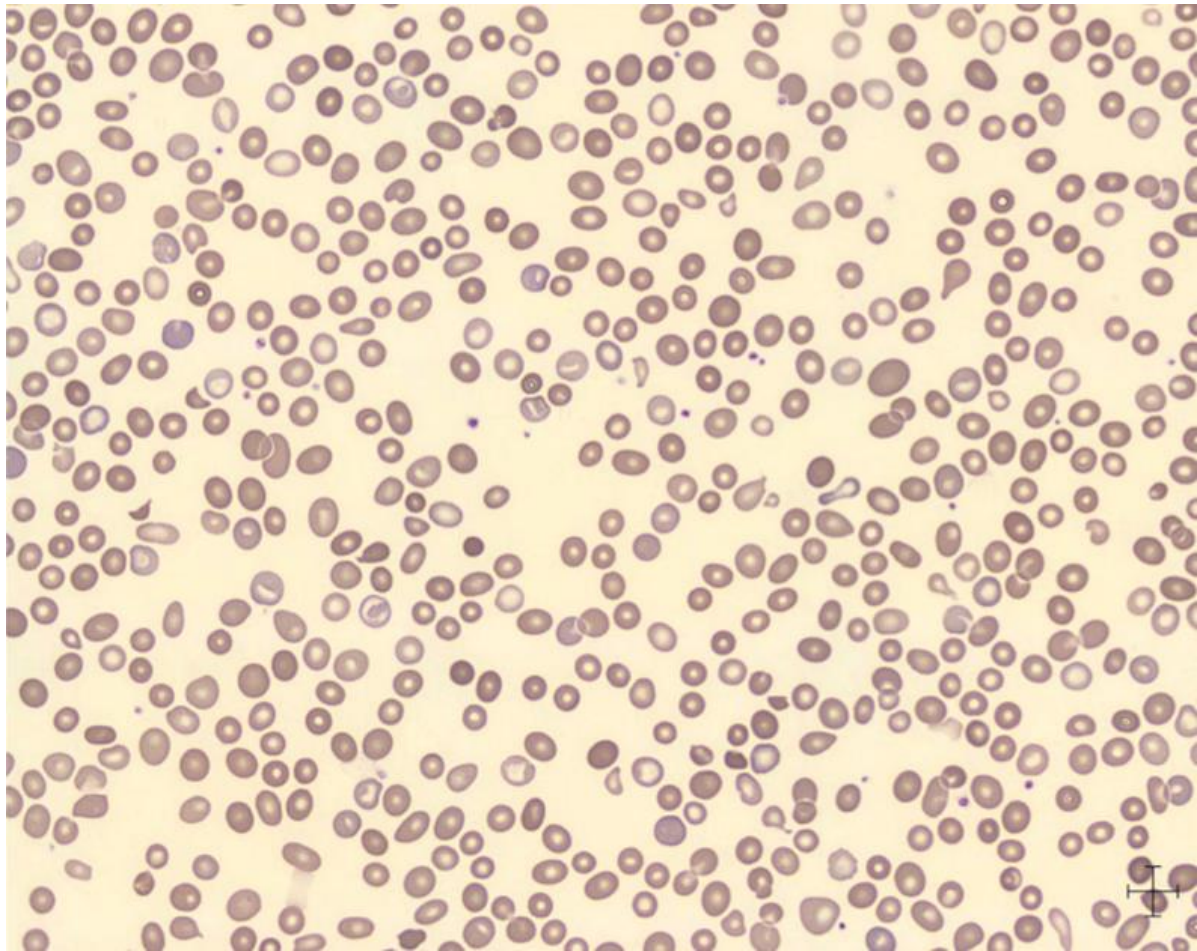
Result Laboratorium

5 dagen na B12 injecties



Hb: 5.7
WBC: 4.5
Trombo's: 117
Reti's: 22%

DIFF.	.
eo's	2
baso's	0
staven	1
segmenten	65
lymfo's	25
mono's	7
bas.punkt.	+
poikilocyt	++
traandr.cel	+
erytroblas	3
Trombocyten	117
macro trom.	+



Casus 3: vrouw uit 1964

Result Laboratorium

Bepaling	Eenheid	Ref. waarde	12-05-2016 08:58 S. Kuipers		12-05-2016 11:24 S. Kuipers		17-05-2016 11:33 S. Kuipers		17-05-2016 11:34 S. Kuipers		31-05-2016 09:52 S. Kuipers	
HEMATOLOGIE												
Hemoglobine	mmol/l	7.5 - 10.0	3.2	L			5.7	L			7.3	L
Hematocriet	l/l	0.35 - 0.45	0.15	L			0.29	L			0.37	
Erytrocyten	10exp12/l	4.00 - 5.00	1.29	L			2.66	L			3.78	L
MCV	fl	80 - 100	116	H			108	H			98	
MCHC	mmol/l	20 - 23	21				20				20	
MCH	amol	1750 - 2100	2460	H			2140	H			1930	
RDW		12.1 - 14.1	20.8	H			23.9	H			18.9	H
Reticulocyt	%	0.5 - 2.5	2.6	H			22.4	H			2.6	H
Leukocyten	10exp 9/l	4.0 - 10.0	5.5				4.5				8.7	
Neutrof.tot	10exp 9/l	1.8 - 7.3	3.32				2.89				5.64	
Eosinofielen	10exp 9/l	0 - 0.7									0.26	
DIFF.			.				.				.	
eo's	%	0 - 6	0				2				3	
baso's	%	0 - 2	1				0				1	
staven	%	0 - 4	1				1					
segmenten	%	40 - 80	56				65				65	
lymfo's	%	12 - 46	29				25				23	
mono's	%	2 - 12	7				7				8	
blasten	%	0 - 0	2	H								
promyelo's	%	0 - 0	1	H								
myelo's	%	0 - 0	2	H								
metamyelo	%	0 - 0	1	H								
poikilocyt			+				++					
fragmento			+									
traandr.cel			++				+				+	
erytroblas	/100 wbc		4				3					
bas.punkt.							+					
Trombocyten	10exp 9/l	150 - 400	90	L			117	L			489	H
thr.aggr.			afw									
macro trom.							+					
Bezinking	mm/uur	<30	9									
BLOEDGROEPENSEROLOGIE												
Bloedgr/Rh			A pos		A pos							
Kruisbloed			<Memo>									
IrregulairAS			neg		neg							
Dir. Coombs			neg									
STOLLING,TROMBOSE,FIBRINOLYSE												
PT (sec)	sec	9 - 14	11									
APTT	sec	ca.35	21									
BEENMERG												
Beenmerg	nr.	.							klaar			
KLINISCHE CHEMIE												
Natrium	mmol/l	135 - 145	139				144				137	
Kalium	mmol/l	3.5 - 5.0	3.3	L			4.1				4.3	
Calcium	mmol/l	2.10 - 2.55	2.21				2.24				2.33	
Kreatinine	umol/l	45 - 90	41	L			35	L			42	L
eGFR CKD-EPI	ml/mn/1.73	>60	>90				>90				>90	
ASAT	u/l	<31	105	H								
ALAT	u/l	<34	39	H								
LD	u/l	<250	>4000	H			2072	H			284	H

Casus 4

Result Laboratorium

Huisarts belt over labuitslagen van man uit 1968.

Bezinking		2	mm/uur	(	-	15	)
Hemoglobine		9.9	mmol/L	(	8.5 -	11.0	)
Hematocriet		0.49	L/L	(	0.41 -	0.51	)
vitamine B12		242	pmol/L	(	130 -	700	)
Trombocyten	H	813	10e9/L	(	150 -	400	)
Leukocyten	H	10.7	10e9/L	(	4.3 -	10.0	)
Machine diff							
Basofielen		0.1	10e9/L	(	0.0 -	0.15	)
Eosinofielen		0.4	10e9/L	(	-	0.7	)
Lymfocyten		2.5	10e9/L	(	1.0 -	4.0	)
Neutrofielen		7.1	10e9/L	(	1.8 -	7.3	)
Monocyten		0.5	10e9/L	(	0.2 -	1.0	)
Creatinine		64	umol/L	(	59 -	104	)
MDRD (e-GFR)		> 60	mL/min	(	60 -		)
Gamma GT		7	E/L	(	-	50	)
Alkalische fosfatase		68	E/L	(	30 -	100	)
ALAT		16	E/L	(	-	41	)
Glucose nuchter plasma		5.2	mmol/L	(	4.0 -	6.1	)
TSH screening							
TSH		2.8	mE/L	(	0.4 -	4.0	)
Vitamine D 25-OH	L	38	nmol/L	(	50 -	200	)
Prostaat Specif. Ag		1.6	ug/L	(	0.0 -	4.0	)

********** < EINDE > **********

Wat vinden we hiervan?
Wat gaan we doen?

http://wavemakers.prezly.com/persbericht-iedereen-geniaal-humane-genetica-in-woorden-en-cartoons