

# Diagnostische workflow van CMMML

Critically appraised topic

Auteur: Tomas Gajdos

Supervisoren: Dr. Claeys, dr. Peeters en dr. Hendrickx

---

**JESSA**  
Z I E K E N H U I S

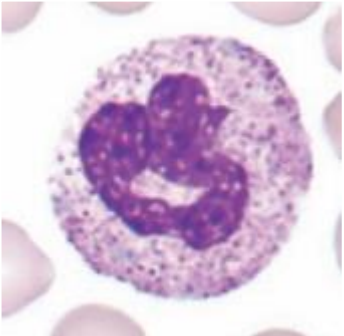
# Inhoud

- Inleiding
  - Monocytose
  - Morfologie
  - WHO classificatie CMML
  - Moeilijkheden bij diagnose
- Onderzoeksvragen
- Conclusie
- To do

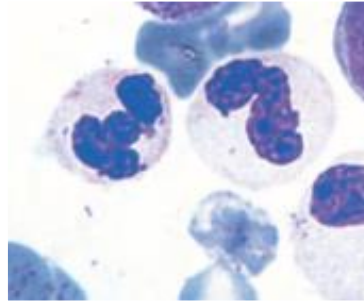
# Monocytose

Reactief vs. neoplastisch

Reactief	Neoplastisch
Transiënt	Persisterend
Mature monocyten	Promonocyten (+ blasten)
Reactieve veranderingen	Dysplasie



↓  
Toxische korreling  
Döhle bodies  
Vacuolisatie



↓  
Hypogranulatie  
Bizarre kernvormen  
Aberrante kernsegmentatie

...

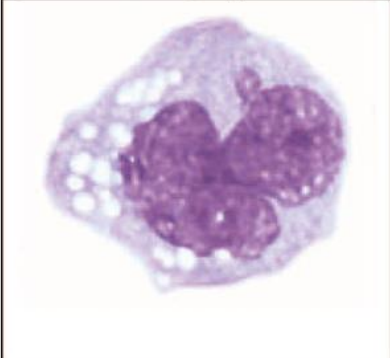
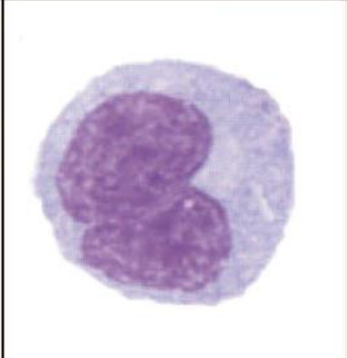
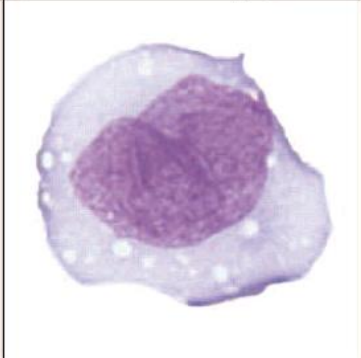
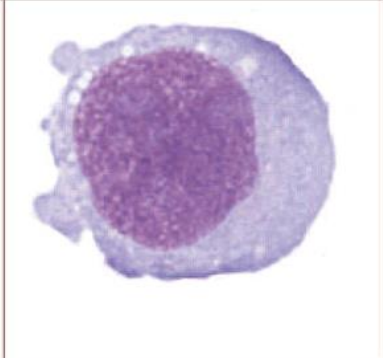
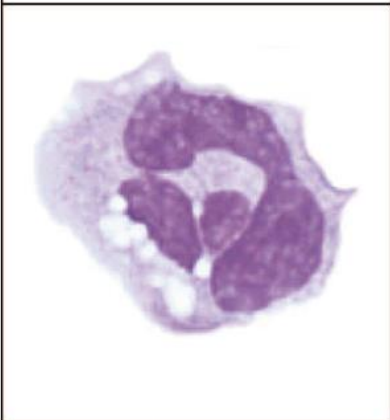
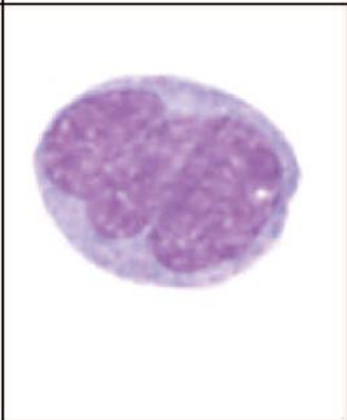
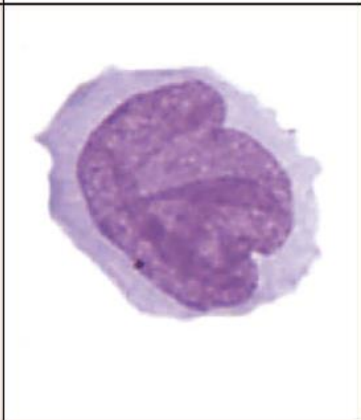
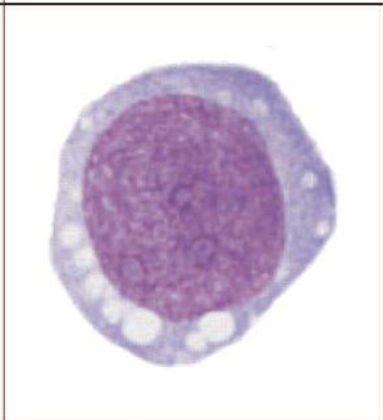
# Monocytose

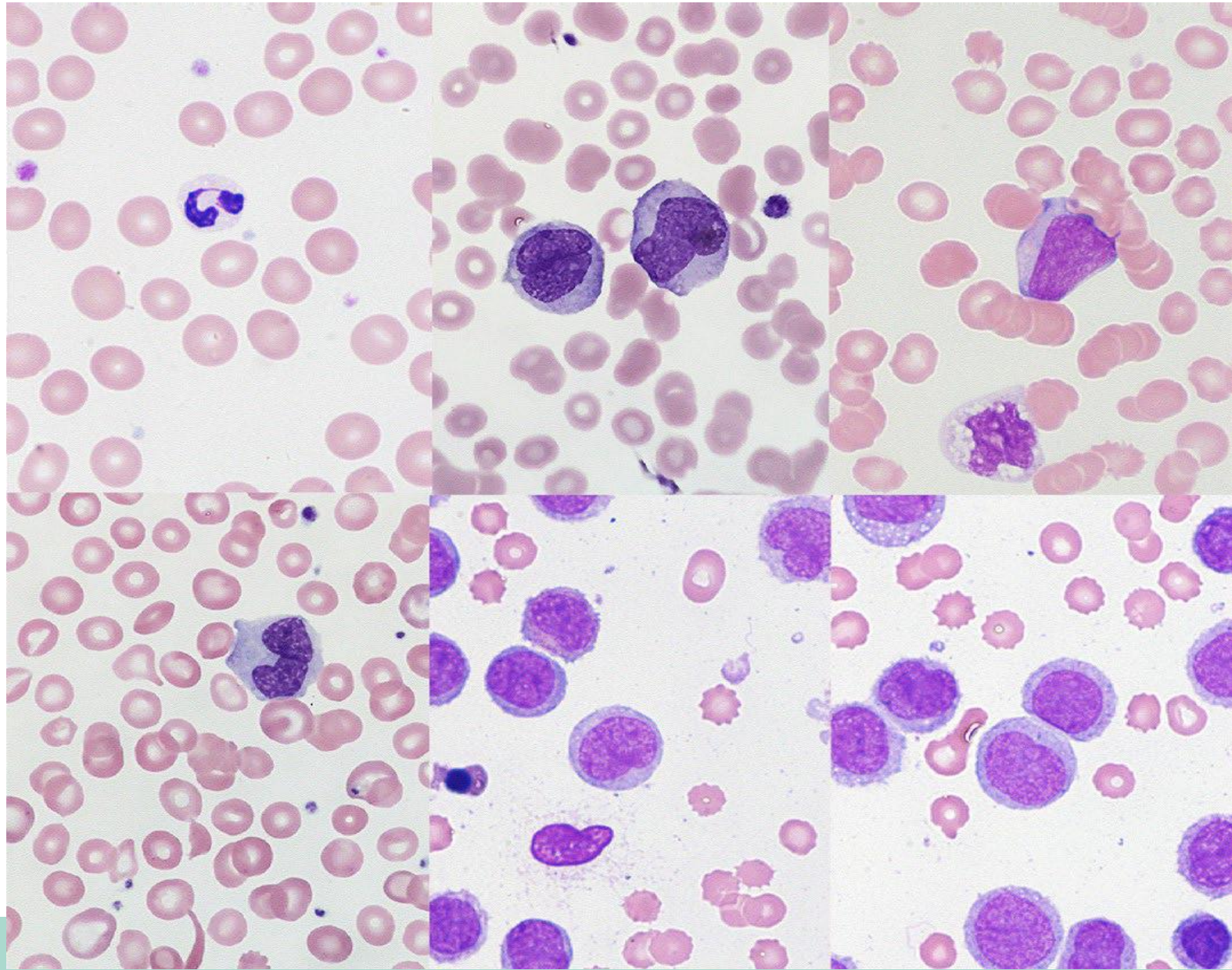
## Oorzaken

<b>Reactief</b>	<b>Neoplastisch</b>
Virale infectie	CMML
Chronische ontsteking	JMML
Auto-immuun pathologie	aCML
Chronische leverziekten	AML
...	...

# Monocytose

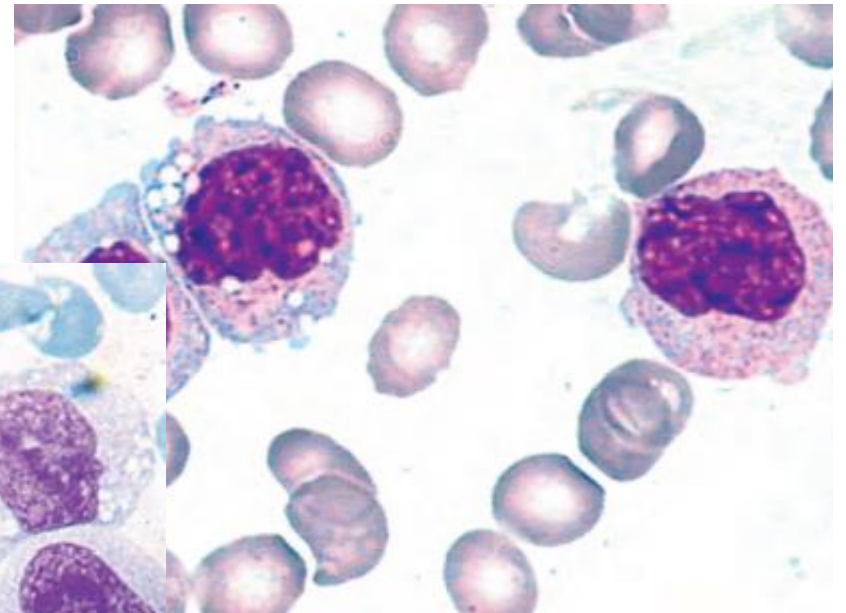
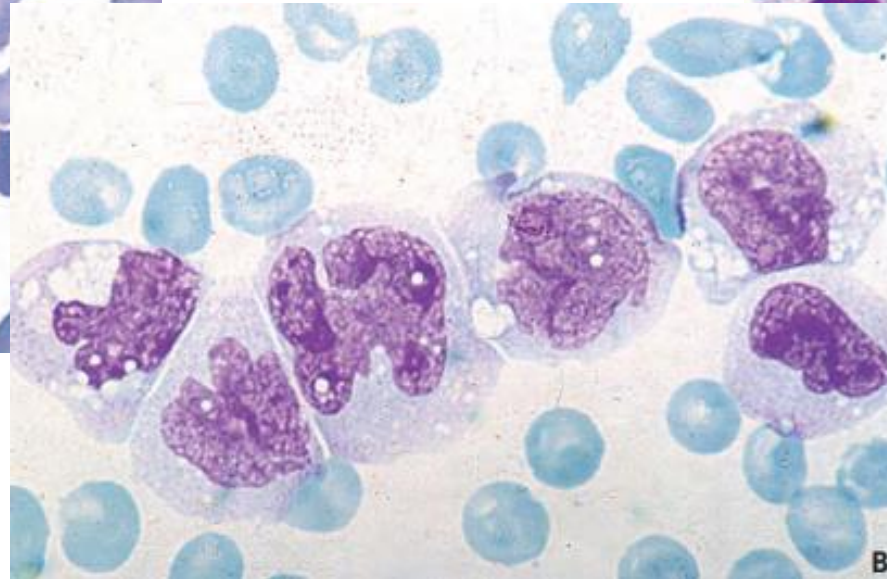
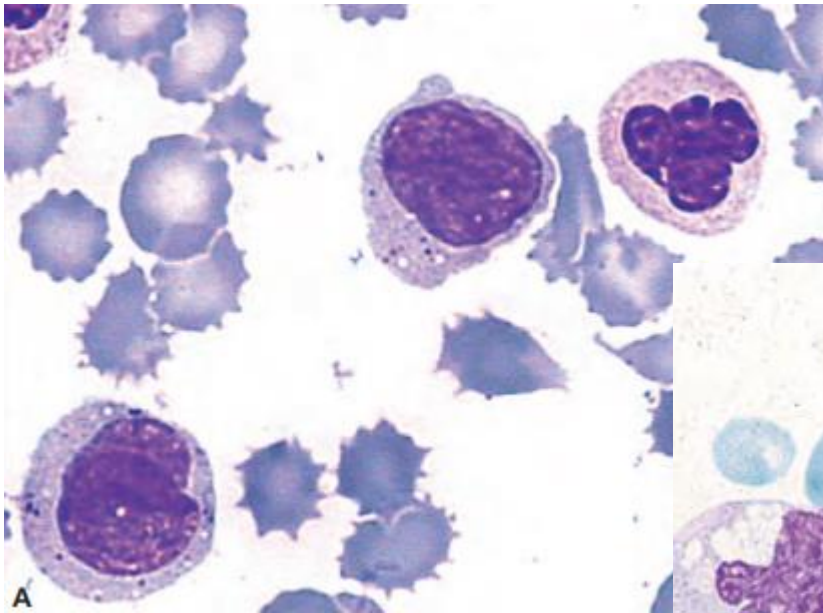
## Morfologie

Monocyte	Immature	Promonocyte	Monoblast
			
			



# CMML

Morfologie



# 2016 WHO classificatie

CMML

Persisterende monocytose  $\geq 1 \times 10^9/L$  en  $\geq 10\%$  van de WBC

+

Geen BCR-ABL1, geen PDGFRA, PDGFRB of FGFR1, geen PCM-JAK2, ETV6-JAK2 of BCR-JAK2 **EN** geen WHO criteria voor PV, ET en PMF

+

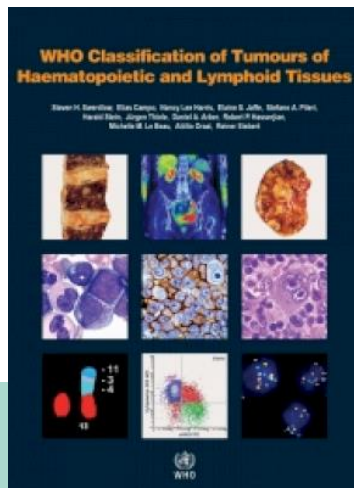
<20% blasten en promonocyten in PB of BM

+

Dysplasie in  $\geq 1$  myeloïde reeksen

**OF**

klonale cytogenetisch of moleculaire afwijkingen **OF** monocytose  $\geq 3$  maanden met uitsluiting van andere oorzaken





# 2016 WHO classificatie

## CMML

- CMML-0: <2% blasten in PB en <5% blasten in BM
- CMML-1: 2-4% blasten in PB en 5-9% blasten in BM
- CMML-2: 5%-19% blasten in PB en 10%–19% blasten in BM OF aanwezigheid van Auerstaven

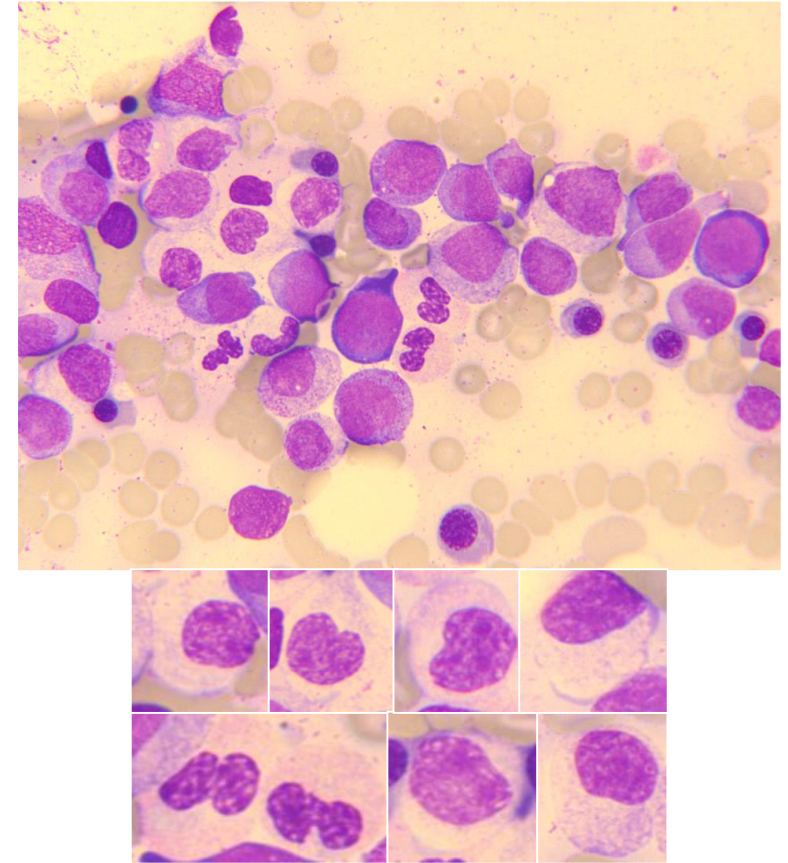
# Moeilijkheden

## Diagnose

- Dysplasie is niet in elke cellijn.
- Promonocyten zijn moeilijk te identificeren.
- Moeilijk onderscheid:  
dysplastische hypogranulaire myeloïde cellen vs. monocytën.
- Blasten zijn zeldzaam (80% van de gevallen afwezig)



Mono-dysplasie score



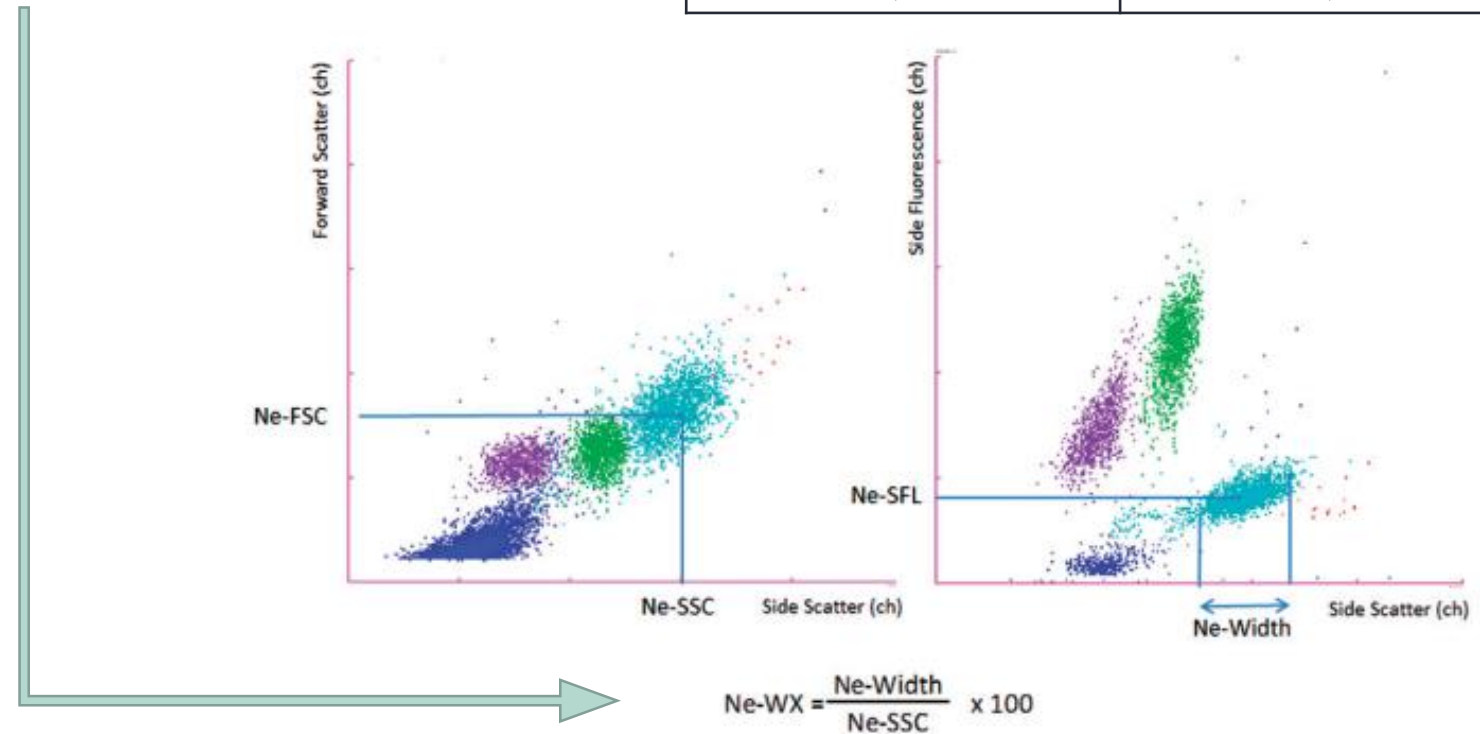
# Mono-dysplasie score

(Cfr. Schillinger et al)

- Formule: 
$$\frac{1}{1+e^{-(-11,623+0,026*Ne-WX-1,385*\frac{Ne}{Mo}+2,714*AbsMono)}}$$

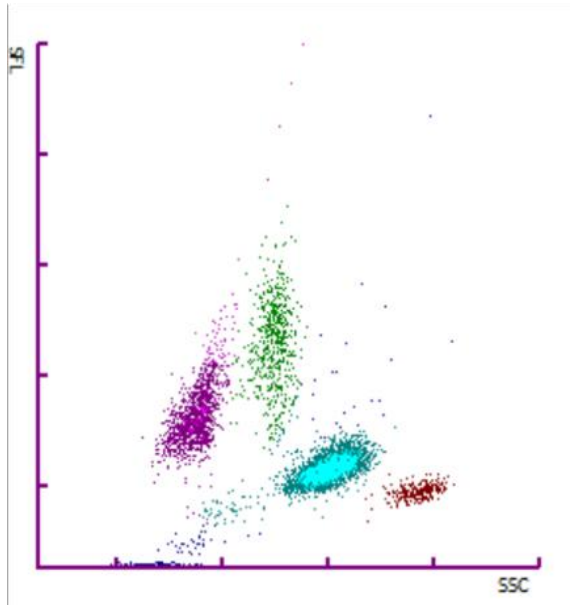
- >0,160 = positief
- Gestandardiseerd
- Operator onafhankelijk
- Onmiddellijk berekend

Sensitiviteit	specificiteit
92,3%	93,6%

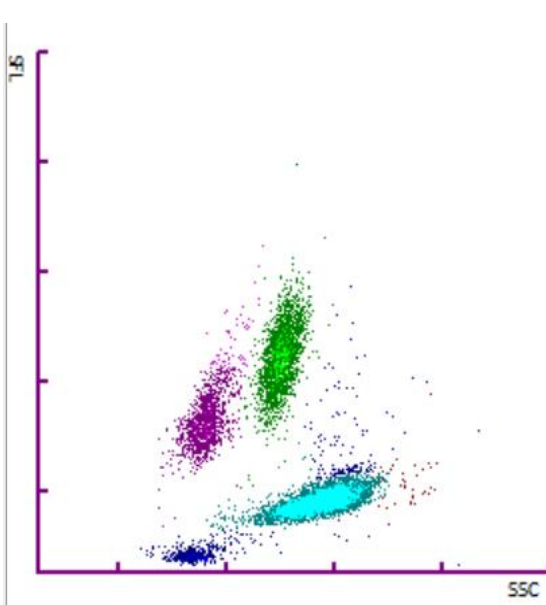


# Mono-dysplasie score

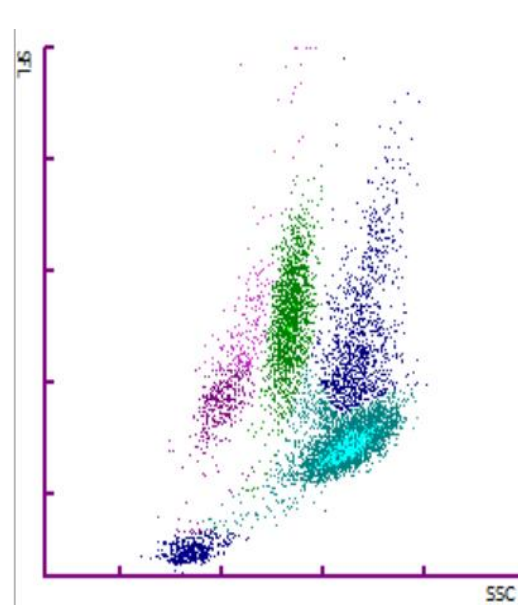
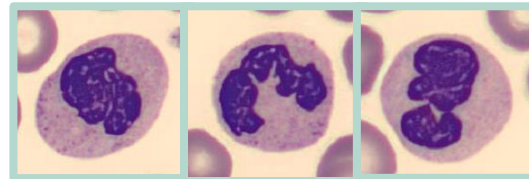
WDF-kanaal



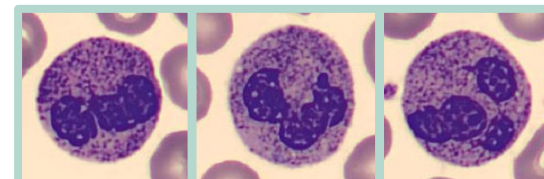
Normaal



CMML



Reactief



# Monocytose vlaggen

## Morfologie

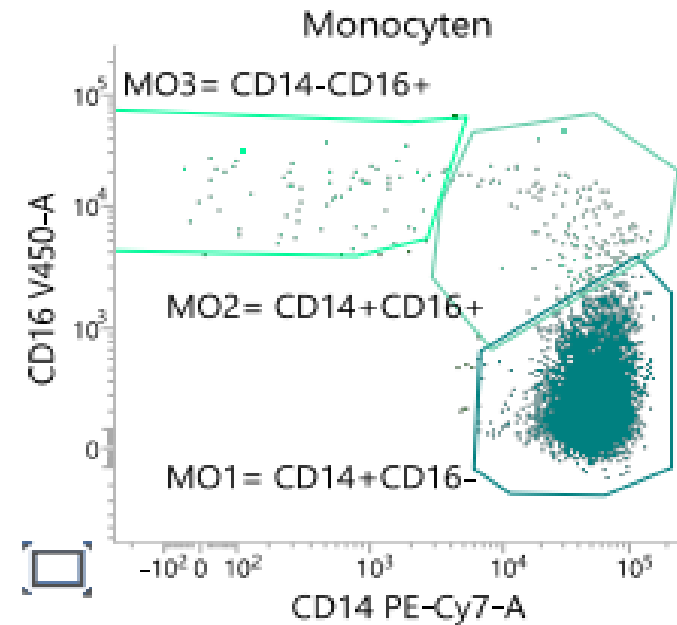
Totaal = 202	Monodysplasie-score positief (n=124)		Twijfelachtige monocytose (n=64)		Extreme monocytose (n=14)	
	aantal uitstrijkjes	percentage	aantal uitstrijkjes	percentage	aantal uitstrijkjes	percentage
Toxische korreling	24	19,4	26	40,6	4	28,6
Hypersegmentatie neutrofielen	8	6,5	0	0,0	1	7,1
Döhle bodies	0	0,0	0	0,0	1	7,1
Degranulatie neutrofielen	21	16,9	8	12,5	1	7,1
Neutrofielen kernafwijking	16	12,9	11	17,2	2	14,3
Vacuolisatie neutrofielen	2	1,6	1	1,6	0	0,0
Jonge monocyten	1	0,8	2	3,1	0	0,0
Dysplastische kenmerken	29	23,4	14	21,9	2	14,3
Diagnose CMML	14	11,3	4	6,3	0	0,0

	aantal uitstrijkjes	percentage
Dysplasie + CMML	7	38,9
Afwezigheid dysplasie + CMML	11	61,1
<b>Totaal</b>	<b>18</b>	<b>100</b>

# Flowcytometrie

Doel CMMML panel (cfr. **Selimoglu-Buet et al**)

- Drie populaties monocyten in perifereer bloed:
  1. **Klassieke monocyten** (CD14+/CD16-)
  2. **Intermediaire monocyten** (CD14+/CD16+)
  3. **Niet klassieke monocyten** (CD14-/CD16+)



>94% = indicatie voor CMMML: Onafhankelijk van mutaties, subtype, of van dysplasie vs. proliferatieve eigenschappen

Sensitiviteit	specificiteit
90,6%	95,1%

# Flowcytometrie

Oud vs. nieuw panel

Oud	HV450	HV500	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
CMML	CD14 2.5 µL	CD45 2.5 µL	CD16 10 µL	CD56 10 µL	CD2 10 µL	CD15 1 µL	CD33 2.5 µL	CD24 1 µL



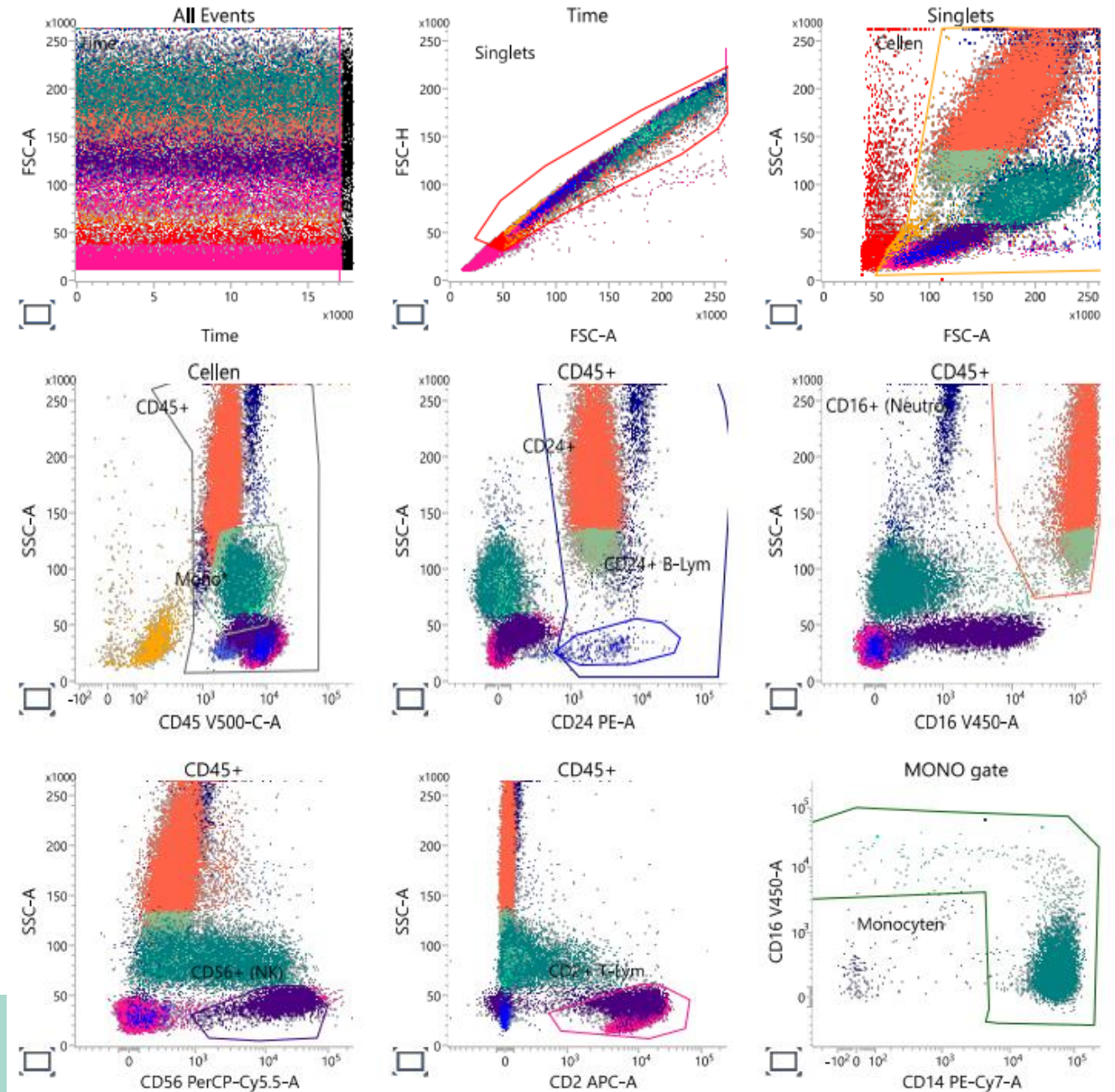
Nieuw	PE	PERCP Cy5.5	PE Cy7	APC	V450	V500-C
CMML	CD24 5 µl	CD56 2.5 µl	CD14 2.5 µl	CD2 2.5 µl	CD16 2.5 µl	CD45 2.5 µl



# Flowcytometrie

## Gatingstrategie

- Hoofdoel: Monocyten opzuiveren
  - CD24: neutrofielen en B-lymfocyten
  - CD16: rijpe granulocyten
  - CD56: NK-cellen
  - CD2: T- en NK-lymfocyten

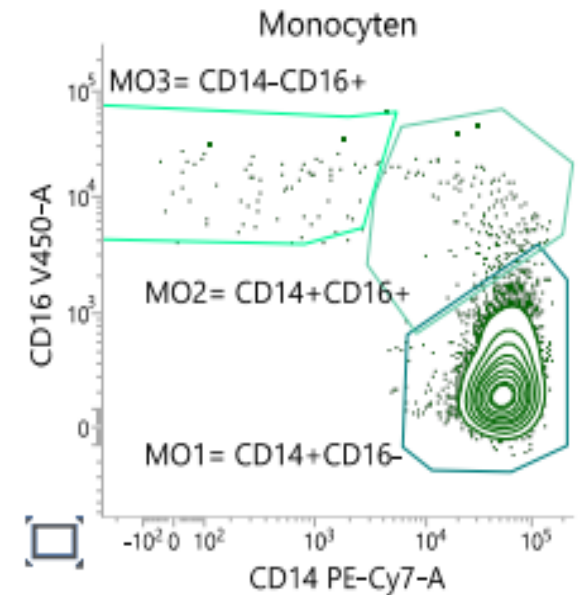
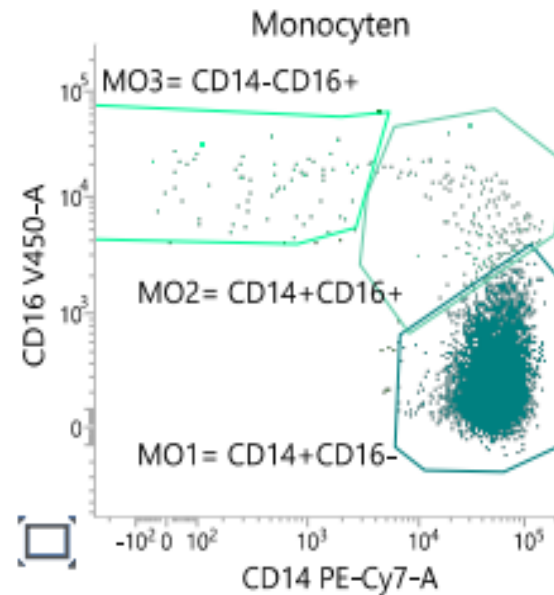




# Flowcytometrie

## Gatingstrategie

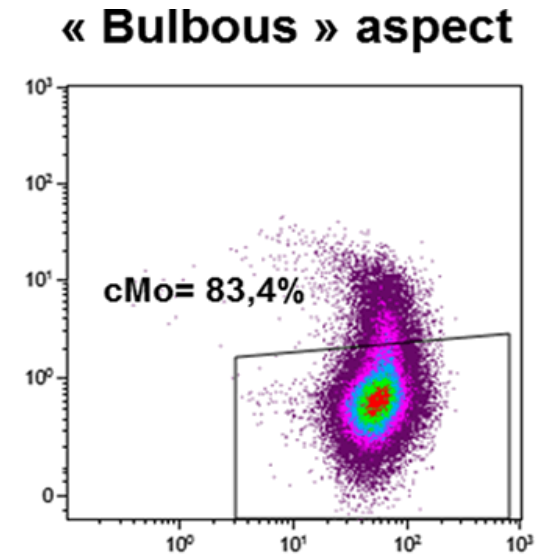
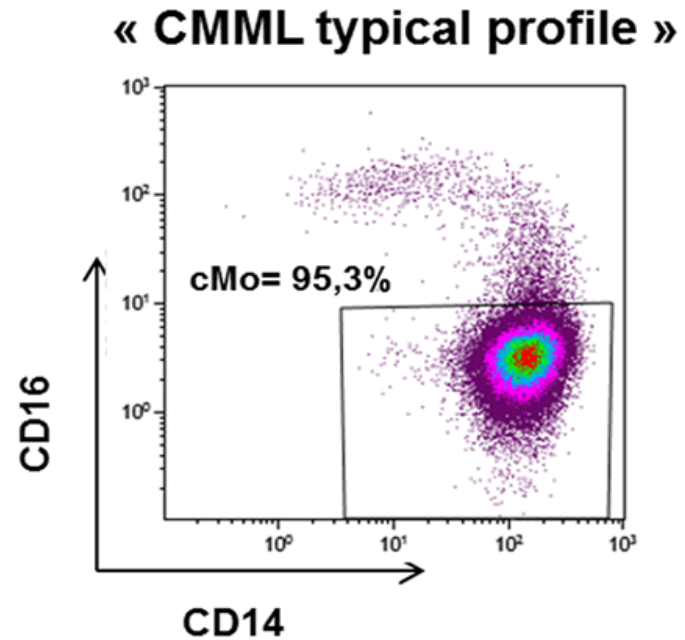
- CD16/CD14 (**MONOCYTEN**)
  - Contour plot:
    - Relatieve frequentie van data
    - Betere visualisatie
    - Expressie patronen & clusters



# Flowcytometrie

## Pitfalls

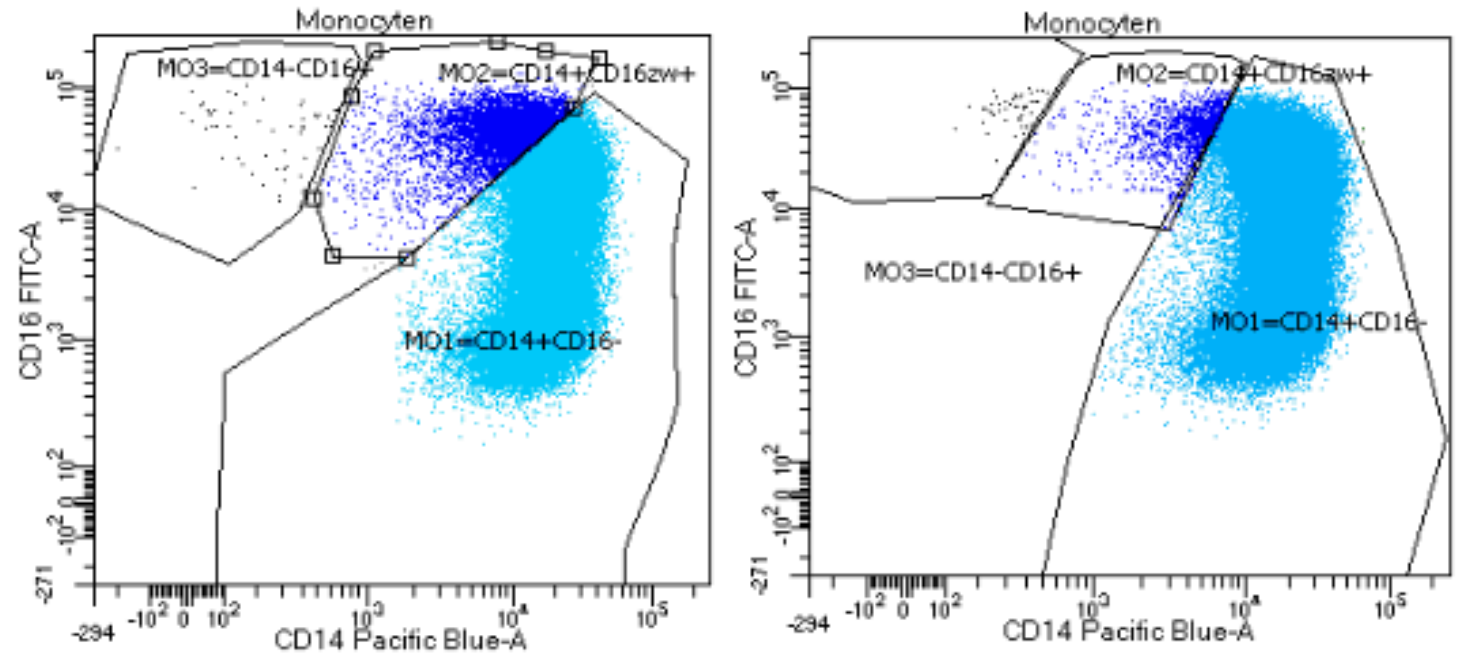
- CMML + reactief:
  - ↓ klassieke monocytenuitendeling
  - Traanvormig aspect
  - Ontbreken van CD16+ staart



# Flowcytometrie

## Pitfalls

- Slecht afgelijnde populaties
  - Dot plot als hulpmiddel

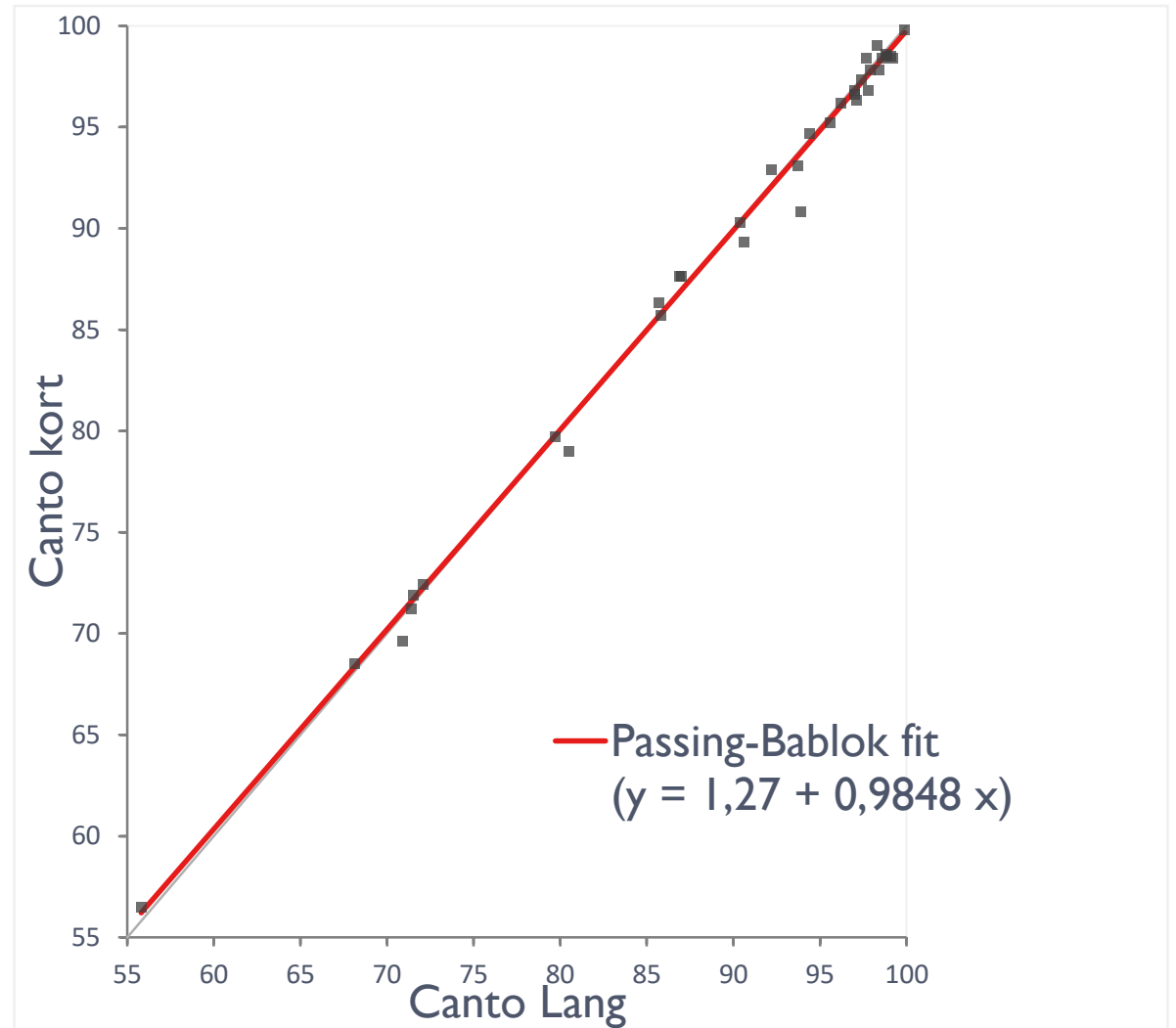


# Flowcytometrie

Oud vs. nieuw panel

- 35 stalen
  - 20 CMMML
  - 15 reactioneel

Correlatie	Passing Bablok	Gem% verschil
0,99	$Y = 1,27 + 0,9848X$	-0,23



# Flowcytometrie

Oud vs. nieuw panel

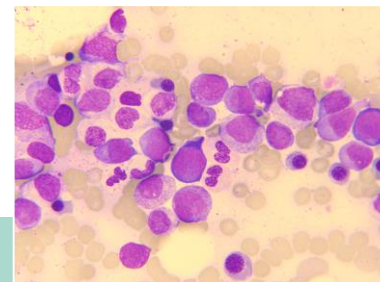
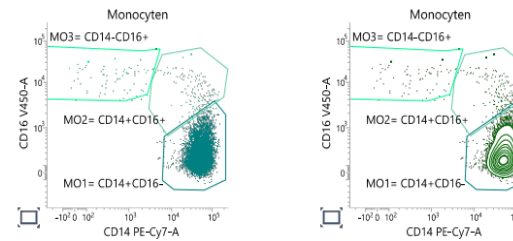
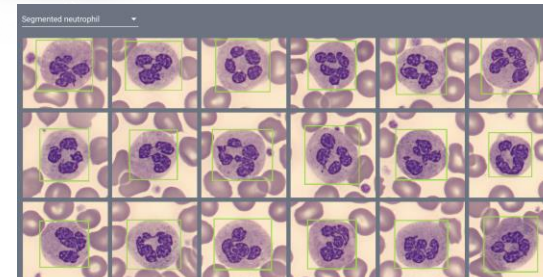
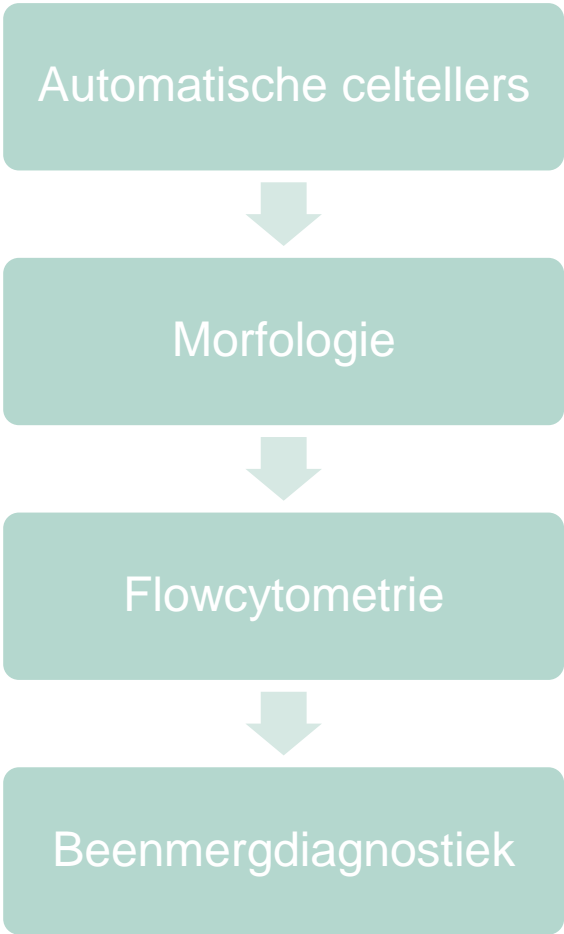
Canto	Bevestigde diagnose NGS/APO/Andere		Totaal
	Positief	Negatief	
MO1 (CD14+,CD16-) $\geq$ 94%	22	3	25
MO1 (CD14+,CD16-) $<$ 94%	1	30	31
Totaal	23	33	56

Diagnostische specificiteit	90,9
Diagnostische sensitiviteit	95,7
Positieve predictieve waarde	88,0
Negatieve predictieve waarde	96,8

merkers oud panel	kleur	firma	Kloon	catalogogr	Prijs/stuk	#testen/stuk	prijs per test
CD45	HV500	BD Biosciences	HI30	560777	360,99	100	1,8
CD24	APC-Vio770	Miltenyi Biotec	REA832	130-112-659	308	100	1,54
CD2	PerCP-Cy5,5	BD Biosciences	RPA-2.10	560643	181,44	50	7,26
CD56	PE	BD Biosciences	MY31	345810	286,65	100	1,43
CD33	APC	BD Biosciences	P67.6	345800	345,35	100	1,73
CD15	PE-Vio770	Miltenyi Biotec	VIMC6	130-114-012	131	30	2,18
CD16	FITC	BD Biosciences	CLB	656146	243,65	50	2,44
CD14	HV450	BD Biosciences	MφP9	560350	147,52	177,76	2,46
						TOTAAL	20,84 €
merkers nieuw panel	kleur	firma	Kloon	catalogogr	Prijs/stuk	#testen/stuk	prijs per test
CD45	V500-C	BD Biosciences	2D1	655873	378,11	200	1,89
CD24	PE	BD Biosciences	ALB9	306069	550,6	400	1,38
CD2	APC	BD Biosciences	39C1.5	304608	517,38	100	1,29
CD56	PerCPCy5.5	BD Biosciences	N901	B49189	512,96	200	2,56
CD16	V450	BD Biosciences	3G8	644489	371,96	100	1,86
CD14	PE-Cy7	BD Biosciences	RM052	147220	544,04	60	1,36
						TOTAAL	10,35 €

# Diagnostische workflow

CMML



Hematologische  
celteller

$\geq 1.0 \times 10^9/L$  EN  
10% monocytyn

Ja

Mono-dysplasie score?

Positief

Negatief

Type monocytose  
vlag

Positieve mono-dysplasie  
score

Twijfelachtige  
monocytose

$\geq 3.0 \times 10^9/L$   
monocytyn

Reactieve monocytose

Te ondernemen  
actie

- Uitstrijkje screenen op dysplasie\* en afwijkingen\*\* bij de monocytyn
- WDF-scattergram nakijken

- Uitstrijkje screenen op dysplasie\* en afwijkingen\*\* bij de monocytyn
- WDF-scattergram nakijken

- Uitstrijkje screenen voor blasten en myeloproliferatieve kenmerken

- Resultaten correleren met klinische bevindingen en infectieparameters

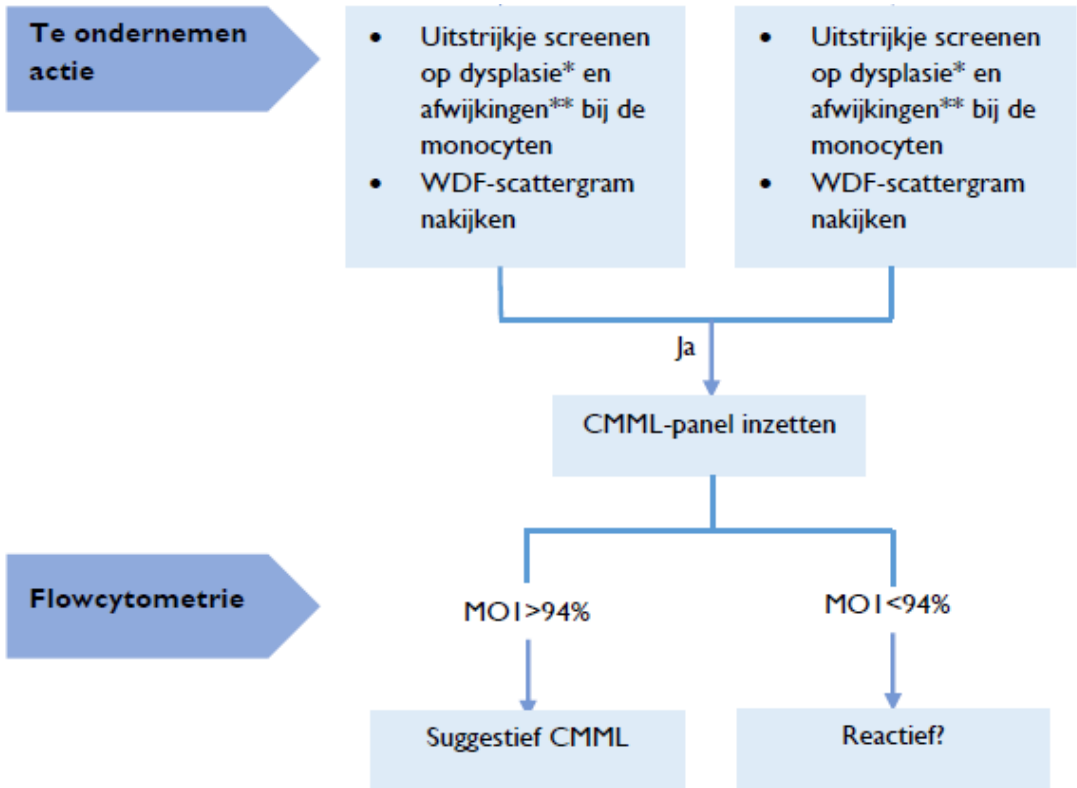
Ja

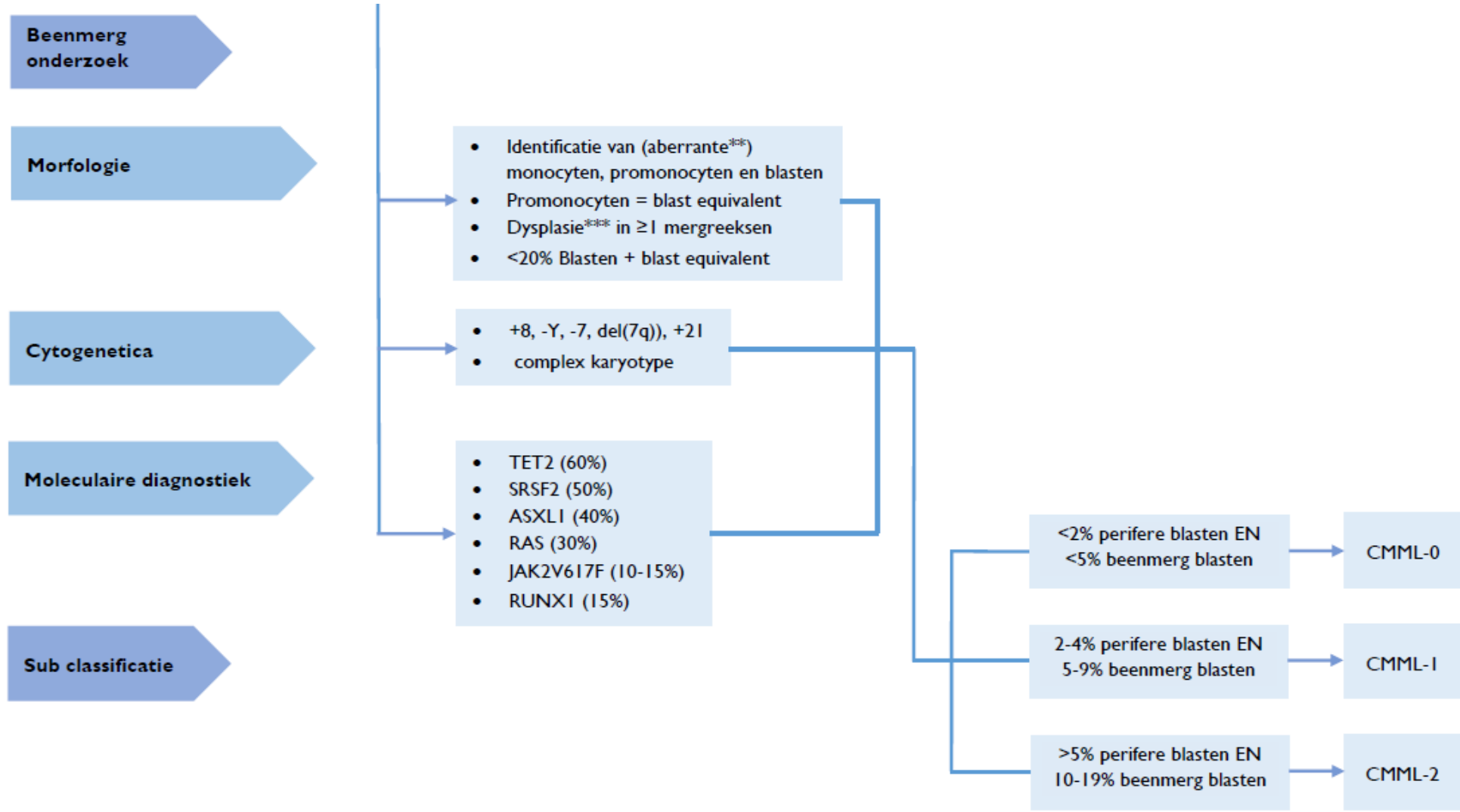
CMML-panel inzetten

Ja

Beenmergonderzoek

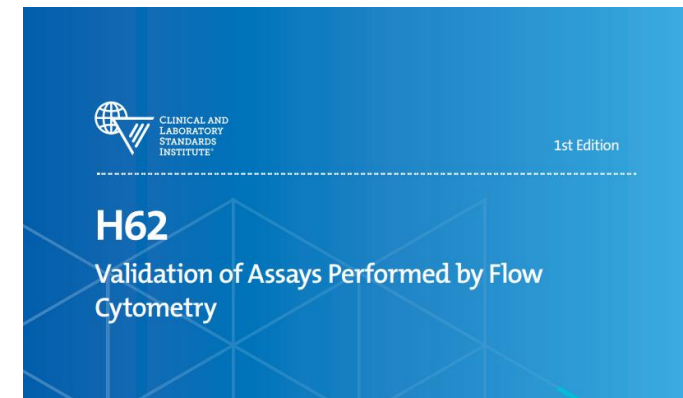






# To do

- Workflow toepassen + herevalueren
- MLT's bijscholen:
  - Nieuwe monoklonale antistoffen
  - Gating strategie
  - Pitfalls
- Validatie CMML-panel volgens CLSI H62-document



# Referenties

1. Valent P, Orazi A, Savona MR, Patnaik MM, Onida F, Van De Loosdrecht AA, et al. Proposed diagnostic criteria for classical chronic myelomonocytic leukemia (CMML), CMML variants and pre-CMML conditions. *Haematologica*. 2019;104(10):1935–49.
2. [http://www.islh.org/web/consensus\\_rules.php](http://www.islh.org/web/consensus_rules.php)
3. Geneviève, F., Mercier-Bataille, D., Wagner-Ballon, O., Trimoreau, F., Fenneteau, O., Schillinger, F., Leymarie, V., Girard, S., Settegrana, C., Daliphard, S., Soenen-Cornu, V., Cividin, M., Châtelain, B., Troussard, X., & Bardet, V. (2014). Smear microscopy revision: propositions by the GFHC.
4. Renneville, A., Patnaik, M. M., Chan, O., Padron, E., & Solary, E. (2021). Increasing recognition and emerging therapies argue for dedicated clinical trials in chronic myelomonocytic leukemia. In *Leukemia* (Vol. 35, Issue 10, pp. 2739–2751). Springer Nature. <https://doi.org/10.1038/s41375-021-01330-1>
5. Patnaik, M. M., & Tefferi, A. (2020). Chronic Myelomonocytic leukemia: 2020 update on diagnosis, risk stratification and management. *American Journal of Hematology*, 95(1), 97–115. <https://doi.org/10.1002/ajh.25684>.
6. Hunter, A. M., Zhang, L., & Padron, E. (2018). Current Management and Recent Advances in the Treatment of Chronic Myelomonocytic Leukemia. *Current Treatment Options in Oncology*, 19(12). <https://doi.org/10.1007/s11864-018-0581-6>
7. Arber, D. A., & Orazi, A. (2019). Update on the pathologic diagnosis of chronic myelomonocytic leukemia. In *Modern Pathology* (Vol. 32, Issue 6, pp. 732–740). Nature Publishing Group. <https://doi.org/10.1038/s41379-019-0215-y>
8. Lynch DT, Hall J, Foucar K. How I investigate monocytosis. *Int J Lab Hematol*. 2018;40(2):107–14.
9. Sangiorgio VFI, Arber DA, Orazi A. How I investigate chronic myelomonocytic leukemia. *Int J Lab Hematol*. 2020;42(2):101–8.
10. Itzykson R, Fenaux P, Bowen D, Cross NCP, Cortes J, De Witte T, et al. Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults. *HemaSphere*. 2018;2(6):e150.
11. Patnaik MM, Lasho TL, Vijayvargiya P, Finke CM, Hanson CA, Ketterling RP, et al. Prognostic interaction between ASXL1 and TET2 mutations in chronic myelomonocytic leukemia. *Blood Cancer J* [Internet]. 2016;6(1):e385–5. Available from: <http://dx.doi.org/10.1038/bcj.2015.113>
12. Padron, E., Garcia-Manero, G., Patnaik, M. M., Itzykson, R., Lasho, T., Nazha, A., Rampal, R. K., Sanchez, M. E., Jabbour, E., al Ali, N. H., Thompson, Z., Colla, S., Fenaux, P., Kantarjian, H. M., Killick, S., Sekeres, M. A., List, A. F., Onida, F., Komrokji, R. S., Solary, E. (2015). An international data set for CMML validates prognostic scoring systems and demonstrates a need for novel prognostication strategies. *Blood Cancer Journal*, 5(7). <https://doi.org/10.1038/bcj.2015.53>
13. Cervera, N., Itzykson, R., Coppin, E., Prebet, T., Murati, A., Legall, S., Vey, N., Solary, E., Birnbaum, D., & Gelsi-Boyer, V. (2014). Gene mutations differently impact the prognosis of the myelodysplastic and myeloproliferative classes of chronic myelomonocytic leukemia. *American Journal of Hematology*, 89(6), 604–609. <https://doi.org/10.1002/ajh.23702>

# Referenties

14. Ricci, C., Fermo, E., Corti, S., Molteni, M., Faricciotti, A., Cortelezzi, A., Deliliers, G. L., Beran, M., & Onida, F. (2010). RAS mutations contribute to evolution of chronic myelomonocytic leukemia to the proliferative variant. *Clinical Cancer Research*, 16(8), 2246–2256. <https://doi.org/10.1158/1078-0432.CCR-09-2112>
15. Schillinger F, Sourdeau E, Boubaya M, Baseggio L, Clauser S, Cornet E, et al. A new approach for diagnosing chronic myelomonocytic leukemia using structural parameters of Sysmex XNTM analyzers in routine laboratory practice. *Scand J Clin Lab Invest* [Internet]. 2018 Apr 3;78(3):159–64. Available from: <https://doi.org/10.1080/00365513.2018.1423702>
16. Kocaturk E, Kusku Kiraz Z, Uskudar Teke H, Demir SS, Alatas IO. A new approach for diagnosing hematological malignancies using monocytosis workflow optimization and abnormal lymphocyte/blast flag of Sysmex XN series of blood count analyzers. *Int J Lab Hematol*. 2020;42(6):744–9.
17. Zhu J, Sourdeau E, Aubert H, Clauser S, Maillon A, Capron C, et al. A hierarchical approach in the diagnostic workflow of chronic myelomonocytic leukemia: Pivotal role of the “Mono-dysplasia-score” combined with flow cytometric quantification of monocyte subsets. *Int J Lab Hematol*. 2019;41(6):782–90.
18. Selimoglu-Buet D, Wagner-Ballon O, Saada V, Bardet V, Itzykson R, Bencheikh L, et al. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. *Blood*. 2015;125(23):3618–26.
19. Hudson CA, Burack WR, Leary PC, Bennett JM. Clinical Utility of Classical and Nonclassical Monocyte Percentage in the Diagnosis of Chronic Myelomonocytic Leukemia. *Am J Clin Pathol*. 2018;150(4):293–302.
20. Tarfi S, Harrivel V, Dumezy F, Guy J, Roussel M, Mimoun A, et al. Multicenter validation of the flow measurement of classical monocyte fraction for chronic myelomonocytic leukemia diagnosis. *Blood Cancer J*. 2018;8(11):0–9.
- 21.razi A, Bennett JM, Germing U, et al., et al. Chronic myelomonocytic leukaemia. In: Swerdlow SH, ed. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th ed.)*. Lyon, France: IARC; 2017:82–86.
22. Monocytosis workflow management – a smart solution. Sysmex. 2021.
23. <https://webapps.riziv-inami.fgov.be/Nomen/nl/search>
24. N. Boeckx, Monocytien, CMML en ASXL1, Laboratoriumgeneeskunde, UZ Leuven, 2016,

Vragen?



JESSA  
ZIEKENHUIS