Potential hemocytometric parameters for diagnosis of COVID-19

Klaas Dewaele

Promotors:
Prof. Apr. D. Kieffer
Prof. Dr. J-L Rummens
Dr. A. Hendrickx

FAST TRACK

Prediction models for diagnosis and prognosis of covid-19: systematic review and critical appraisal

Laure Wynants,[1,2] Ben Van Calster,[2,3] Gary S Collins,[4,5] Richard D Riley,[6] Georg Heinze,[7] Ewoud Schuit,[8,9] Marc M J Bonten,[8,10] Darren L Dahly,[11,12] Johanna A Damen,[8,9]

ventilation, intubation, or length of hospital stay. The most frequent types of predictors included in the covid-19 prediction models are vital signs, age, comorbidities, and image features. Flu-like symptoms are frequently predictive in diagnostic models, while sex, C reactive protein, and lymphocyte counts are frequent prognostic factors. Reported C index estimates from the strongest form of validation available per model ranged from 0.71 to 0.99 in prediction models for the general population, from 0.65 to more than 0.99 in diagnostic models, and from 0.54 to 0.99 in prognostic models. All models were rated at high or unclear risk of bias, mostly because of non-representative selection of control patients, exclusion of patients who had not experienced the event of interest by the end of the study, high risk of model overfitting, and unclear reporting. Many models did not include a description of the target population (n=27, 12%) or care setting (n=75, 32%), and only 11 (5%) were externally validated by a calibration plot. The Jehi diagnostic model and the 4C mortality score were identified as promising models.

Hemocytometrie voor COVID-19 diagnose

- Snelle screeningstool: **triage op Spoed?**
- Goedkoop en algemeen beschikbaar: **bruikbaar in LRS?**
- Hematologische afwijkingen bij COVID-19 ⇔ andere virale infecties?

Hemocytometrie voor COVID-19 diagnose

1. **Literatuurstudie** (narratieve review — zie Appendix A)
2. Diagnostische performantie?
3. Analytische variabelen?

} **retrospectieve cohort studie**

Study design (zie Appendix C)

- 257 COVID-19 patiënten
- 1261 controlepatiënten
  - **Infectieus (n = 752)**
    - Influenza A/B: n = 178
    - RSV A/B: n = 69
    - CMV/EBV: n = 61
    - Negatief respiratoir panel: n = 301
    - Positief respiratoir panel: n = 105 (niet influenza of RSV)
    - Bacteriële sepsis: n = 38
  - **'Niet-infectieus' (n = 509)**
    - Spoed, niet-opgenomen (n = 103)
    - Spoed, ICU-admitted (n = 100)
    - Spoed, opname interne geneeskunde (n = 51)
    - Spoed, opname geriatrie (n = 52)
    - Spoed, opname hemato-onco (n = 100)
    - Spoed, opname heelkunde (n = 102)

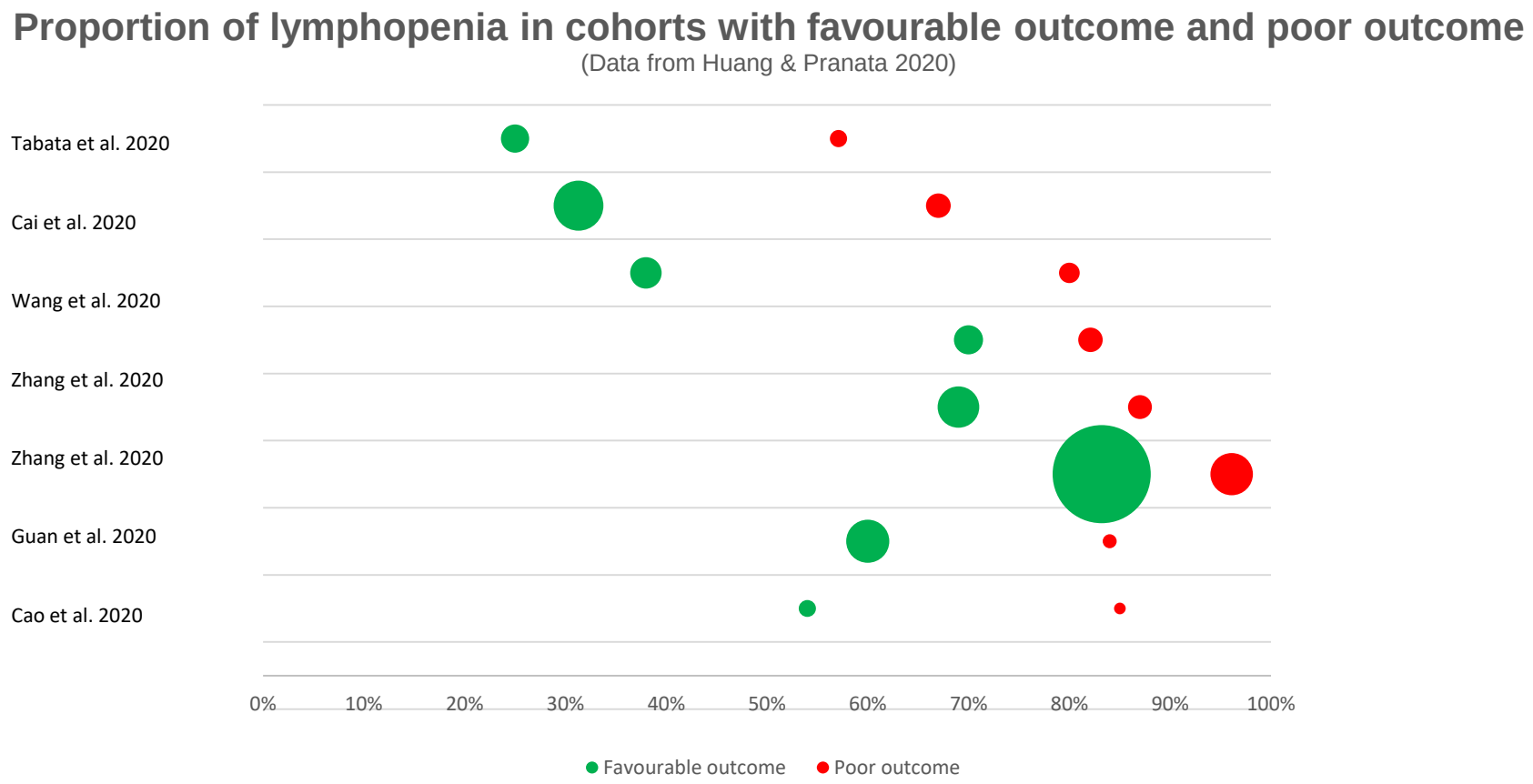
+ stratificatie ICU-opgenomen of niet

Literatuur: hematologische merkers COVID-19

Zie CAT-tekst

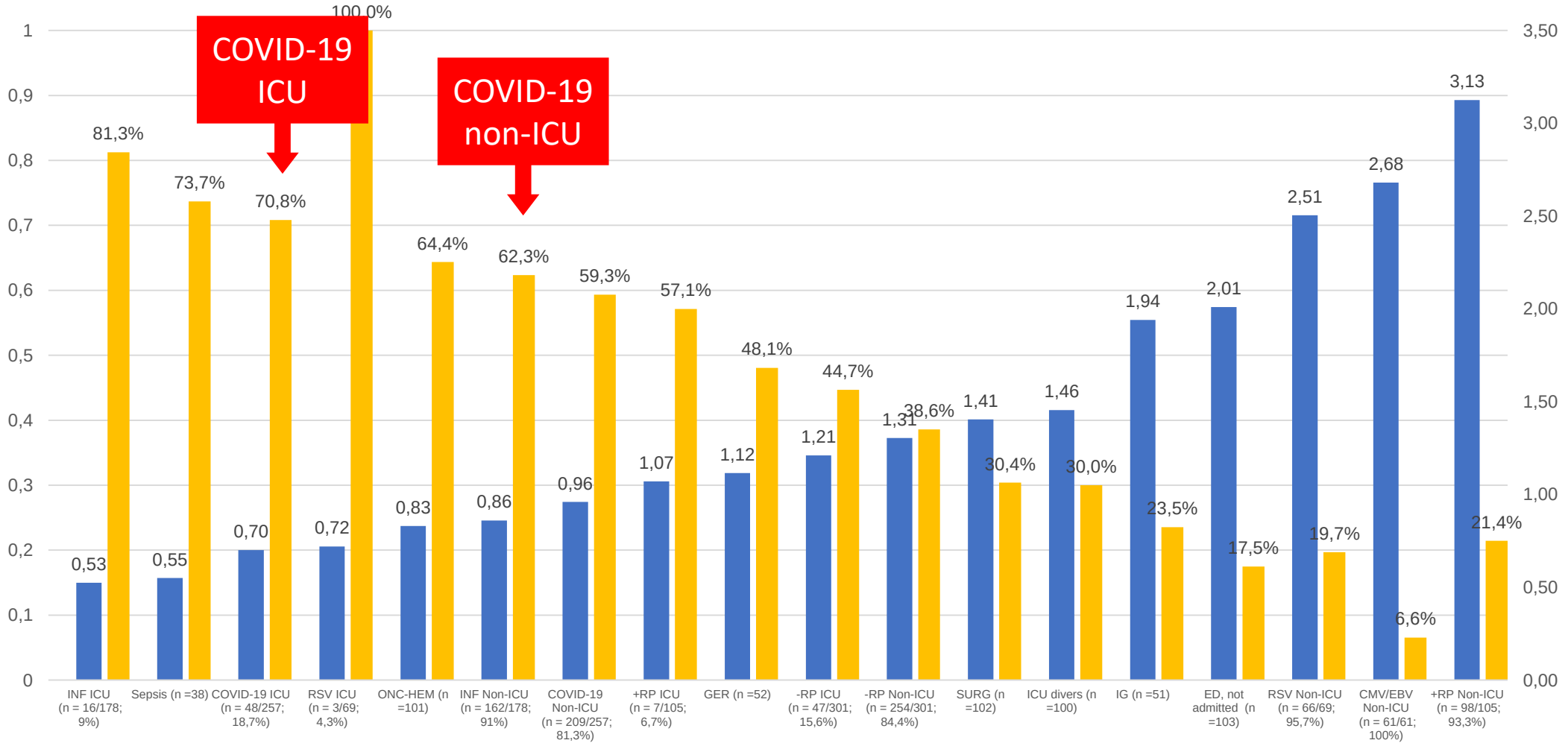
- **Lymfopenie**
- Eosinopenie
- Monocytopenie, lymfocyt-monocyt-ratio
- MECOR score
- PARIS score
- Reactionele lymfocyten, HFLC, AS-LYMP, RE-LYMP

Literatuur: COVID-19 en lymfopenie

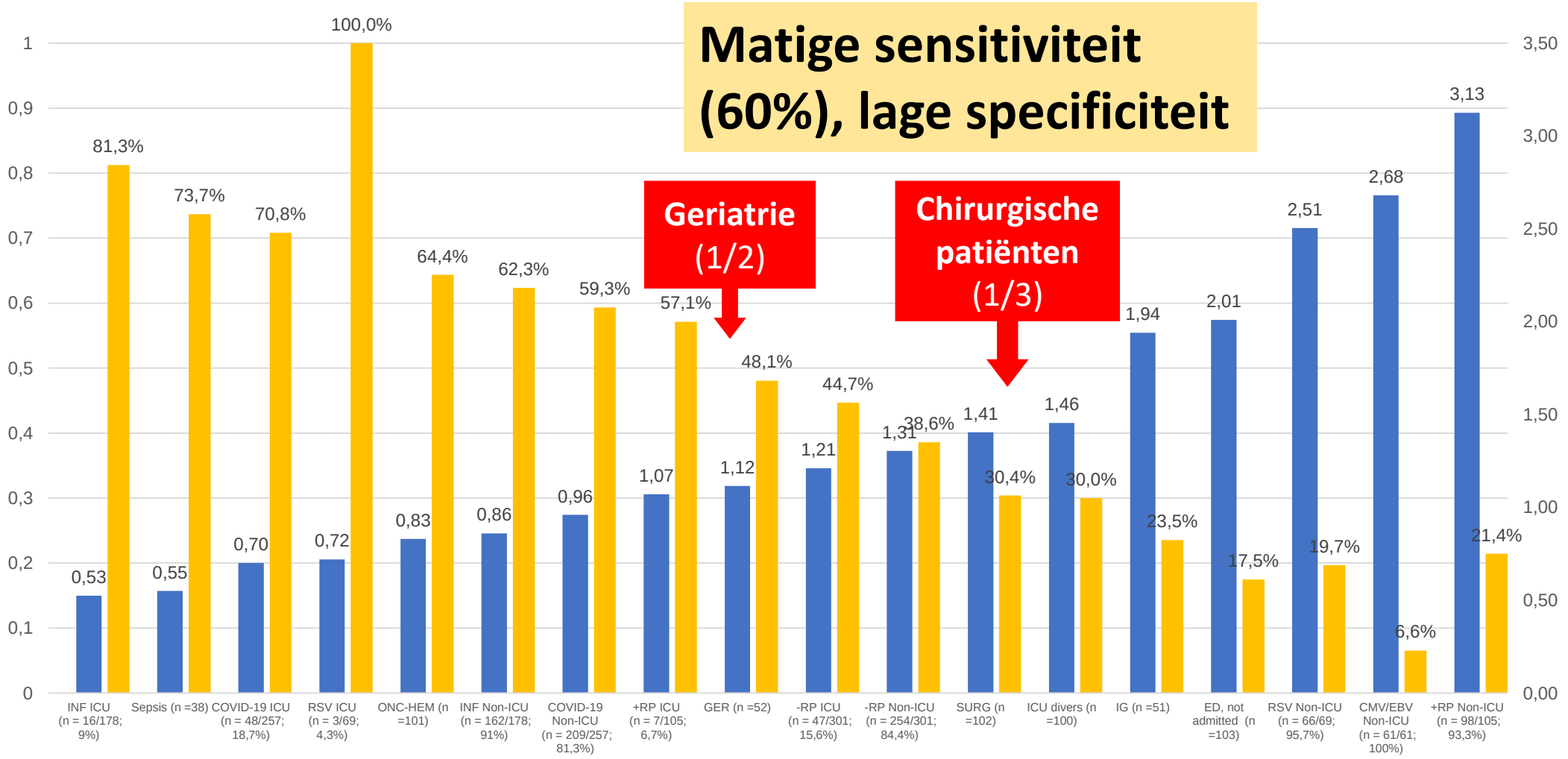


% Lymfopenie erg variabel; disease severity = confounder

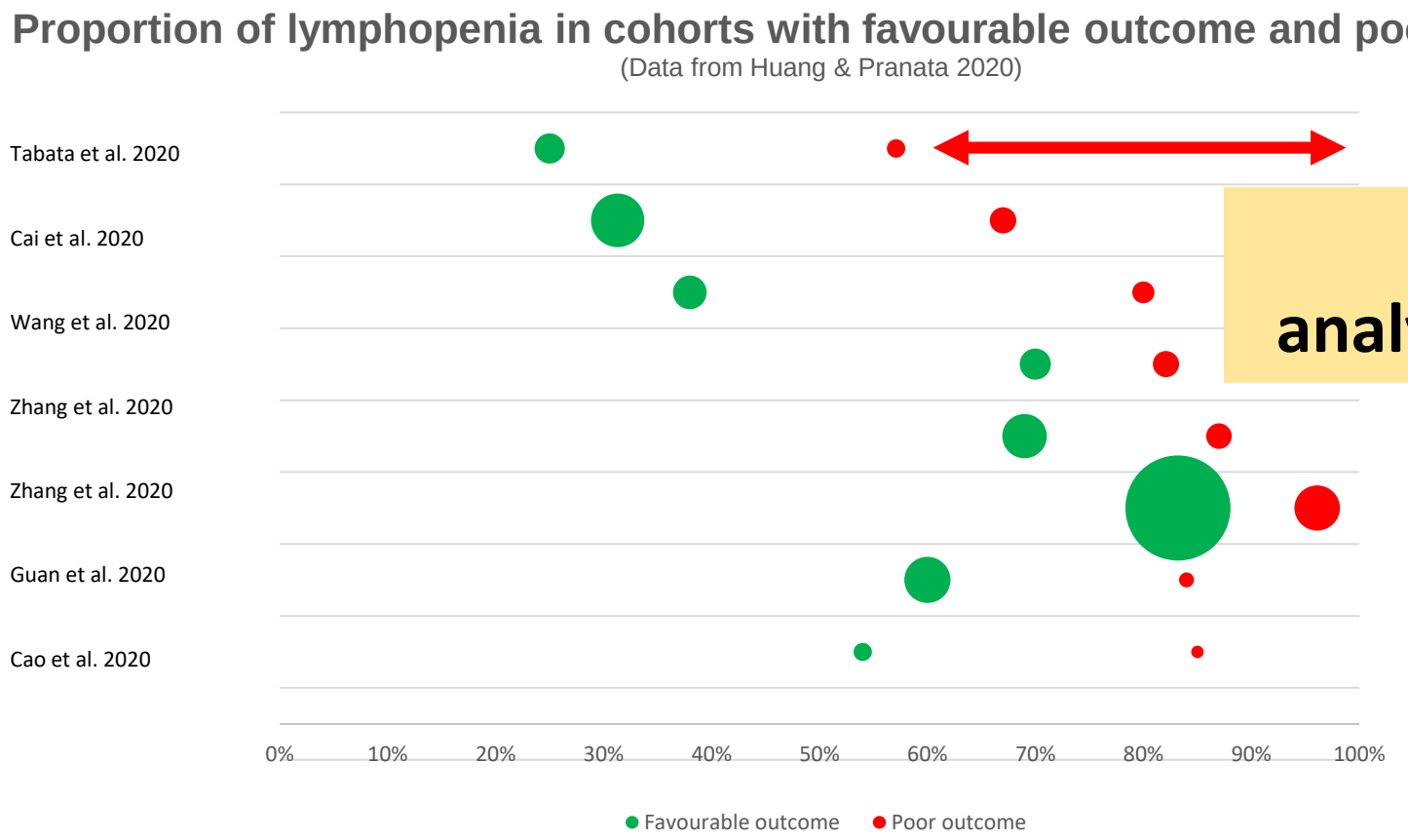
% Lymphopenia in COVID-19 and control cohorts



% Lymphopenia in COVID-19 and control cohorts



Literatuur: COVID-19 en lymfopenie

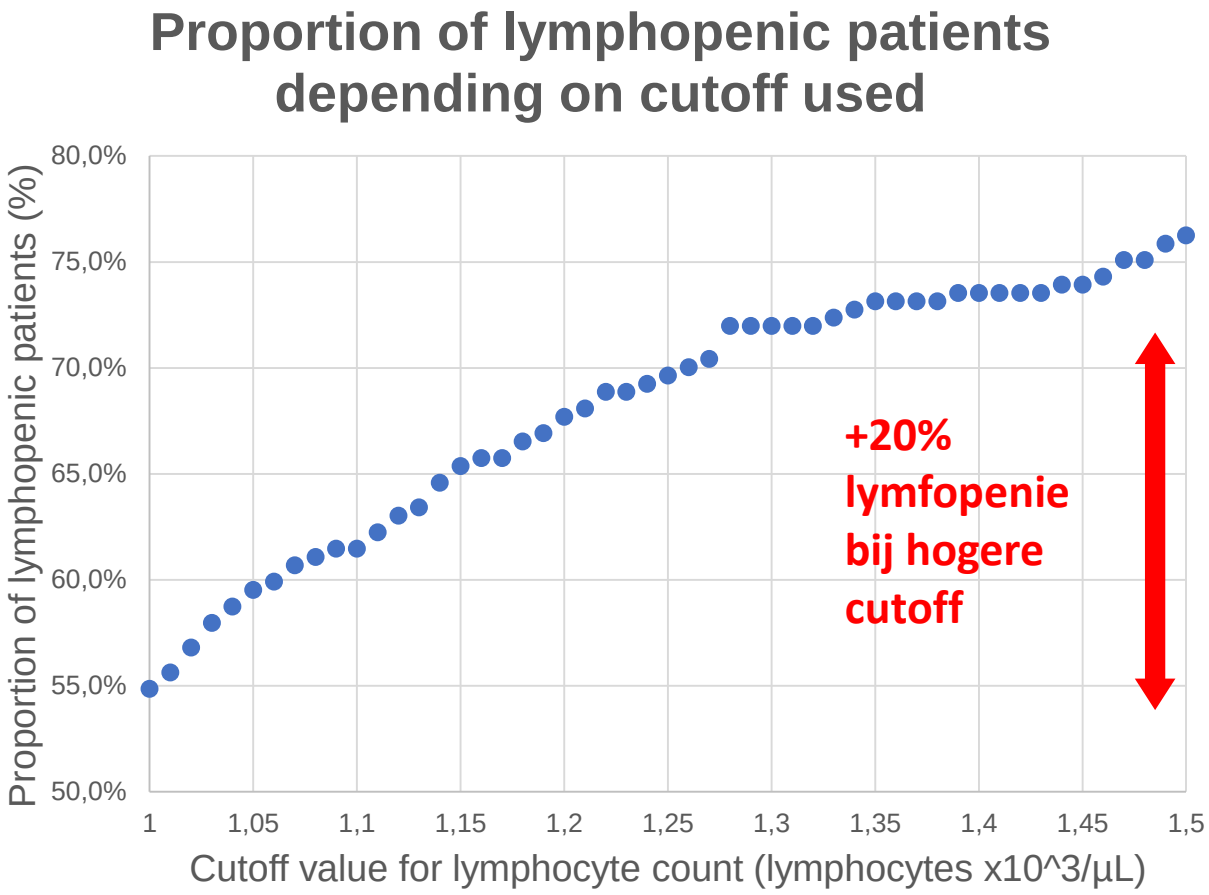


Een rol voor analytische factoren?

Analytische factoren: cutoff lymfopenie

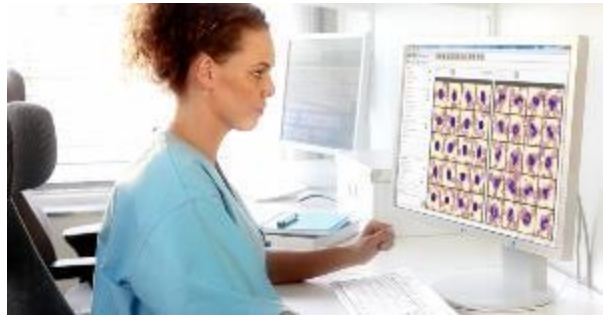
Cutoffs in gebruik:

- ≤ 1.0 x10^3 ly/µL
- ≤ 1.1 x10^3 ly/µL
- ≤ 1.2 x10^3 ly/µL
- ≤ 1.5 x10^3 ly/µL



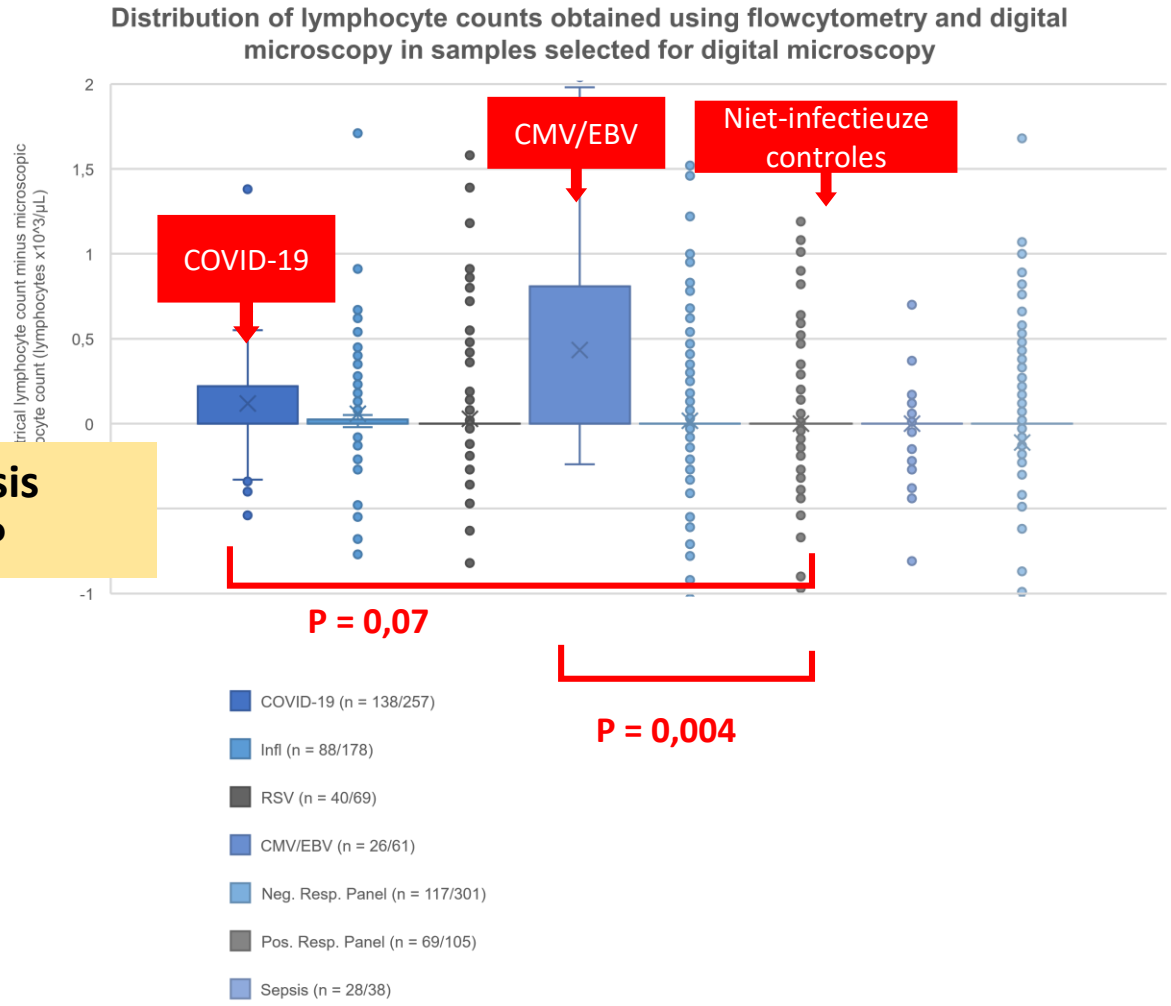
Analytische factoren: DI-60 versus XN

DI-60/Cellavision



XN

Gating errors? Eerder **lysis reactionele lymfocyten?**



Literatuur: hematologische merkers COVID-19

- Lymfopenie
- **Eosinopenie**
- Monocytopenie, lymfocyt-monocyt-ratio
- MECOR score
- PARIS score
- Reactionele lymfocyten, HFLC, AS-LYMP, RE-LYMP

Literatuur: eosinopenie

- **Diagnostische bruikbaarheid?** (AUCs 0.97, 0.85, 0.84)

Received: 1 October 2020 | Revised: 29 October 2020 | Accepted: 20 November 2020
DOI: 10.1111/ijlh.13425

ORIGINAL ARTICLE

ISLH International Journal of Laboratory Hematology WILEY

Evaluation of eosinopenia as a diagnostic and prognostic indicator in COVID-19 infection

Mamta Soni

Conclusions: Eosinopenia on admission is a reliable and convenient early diagnostic marker for COVID-19 infection, helping in early identification, triaging and isolation of the patients till nucleic acid test results are available. Role of eosinopenia as a prognostic indicator is insignificant.

Sun, S. *et al.* Abnormalities of peripheral blood system in patients with COVID-19 in Wenzhou, China. *Clin. Chim. Acta* **507**, 174–180 (2020). Outh, R. *et al.* Eosinopenia <100/μL as a marker of active COVID-19: An observational prospective study. *J. Microbiol. Immunol. Infect.* **54**, 61–68 (2021). Soni, M. Evaluation of eosinopenia as a diagnostic and prognostic indicator in COVID-19 infection. *Int. J. Lab. Hematol.*

Literatuur: eosinopenie

- **Specifiek voor COVID-19?**

Eosinopenia and COVID-19

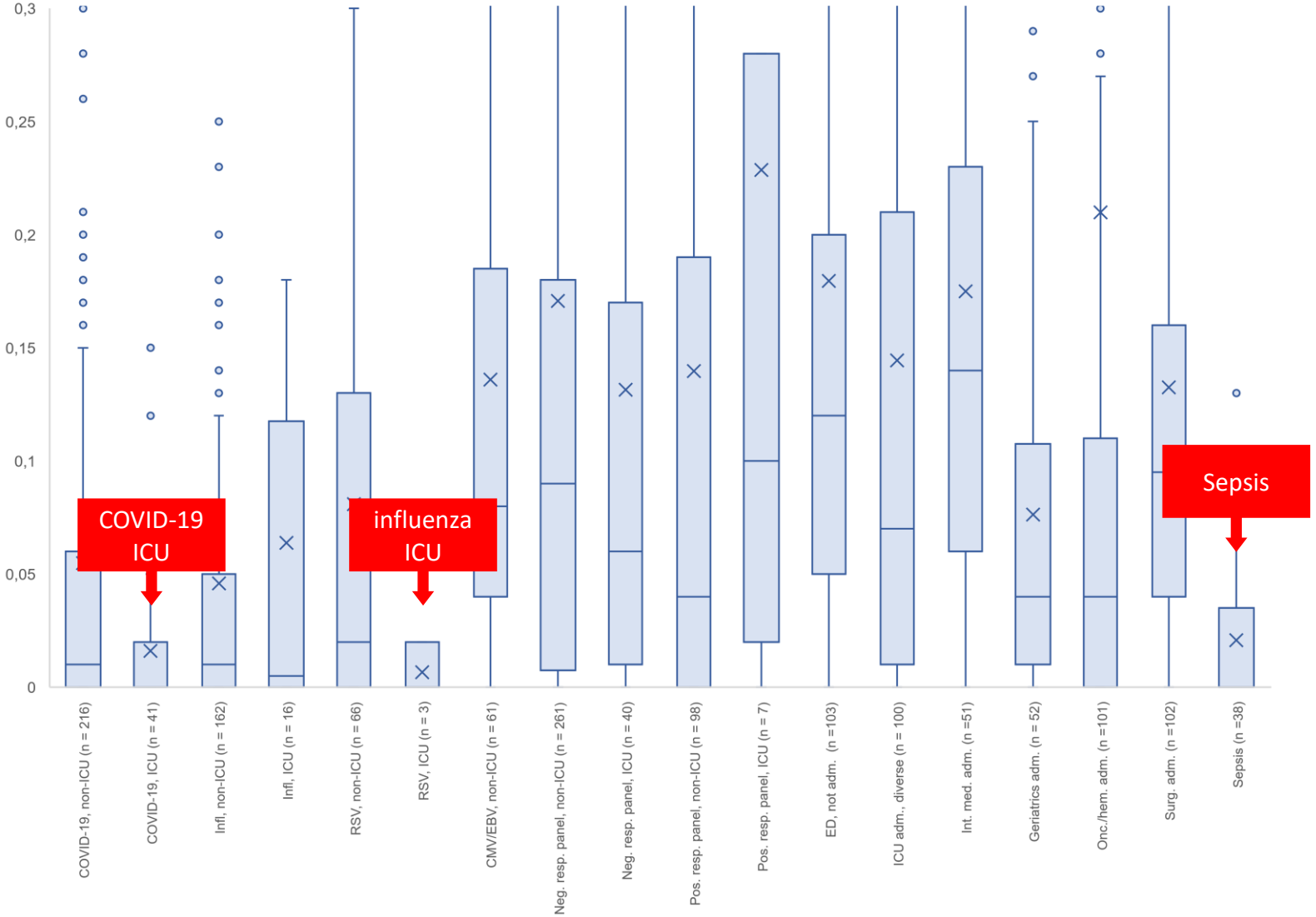
Fahmina Tanni, DO; Eleonora Akker, DO; Muhammad M. Zaman, MD; Nilka Figueroa, MD; Biju Tharian, PharmD; Kenneth H. Hupart, MD

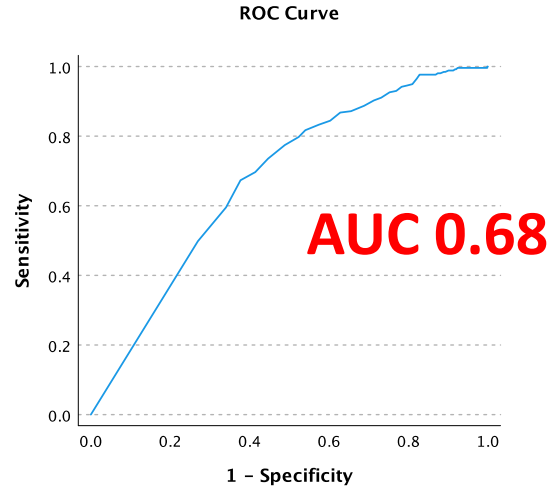
Results: On the day of presentation, 30 patients in the COVID-19 group (60%) and 8 patients in the influenza group (16%) had an eosinophil count of 0. An additional 14 patients in the COVID-19 group had 0 eosinophils during the following 2 days; the total number of patients in the COVID-19 group who had 0 eosinophils on admission or during the ensuing 2 days was 44 (88%). In addition, 18 of 21 (86%) deceased patients in the COVID-19 group who initially presented with eosinopenia remained eosinopenic compared with 13 of 26 (50%) survivors.

Eosinopenie in 60% van COVID-19 patiënten en 16% van influenza patiënten

⇔ Kleine studie, cohortes niet gematcht voor ziekte-ernst!

Distribution of eosinophil counts in COVID-19 and control cohorts





Literatuur: eosinopenie

- **Prognostische bruikbaarheid?** Merker voor ziekte-ernst?

Received: 30 May 2020 | Accepted: 19 January 2021
DOI: 10.1111/ijcp.14047

SHORT REPORT
INFECTIOUS DISEASES

THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE WILEY

Eosinopenia is a reliable marker of severe disease and unfavourable outcome in patients with COVID-19 pneumonia

Massimo Cazzaniga[1] | Luca A. M. Fumagalli[1] | Luciano D'angelo[1] | Mario Cerino[1] | Giulia Bonfanti[2] | Riccardo M. Fumagalli[3,4] | Gianpaolo Schiavo[1] | Cristina Lorini[1] | Elisa Lainu[1] | Sabina Terragni[1] | Marco Chiarelli[1] | Claudio Scarazzati[1] | Claudio Bonato[1] | Mauro Zago[1]

Conclusions: Absolute eosinopenia is associated with clinical outcomes in patients with COVID-19 pneumonia and might be used as a marker to discriminate patients with unfavourable prognosis.

CORRESPONDENCE

Response to: Eosinophil count in coronavirus disease 2019: more doubts than answers

G. Lippi [1], F. Sanchis-Gomar[2] and B.M. Henry[3]

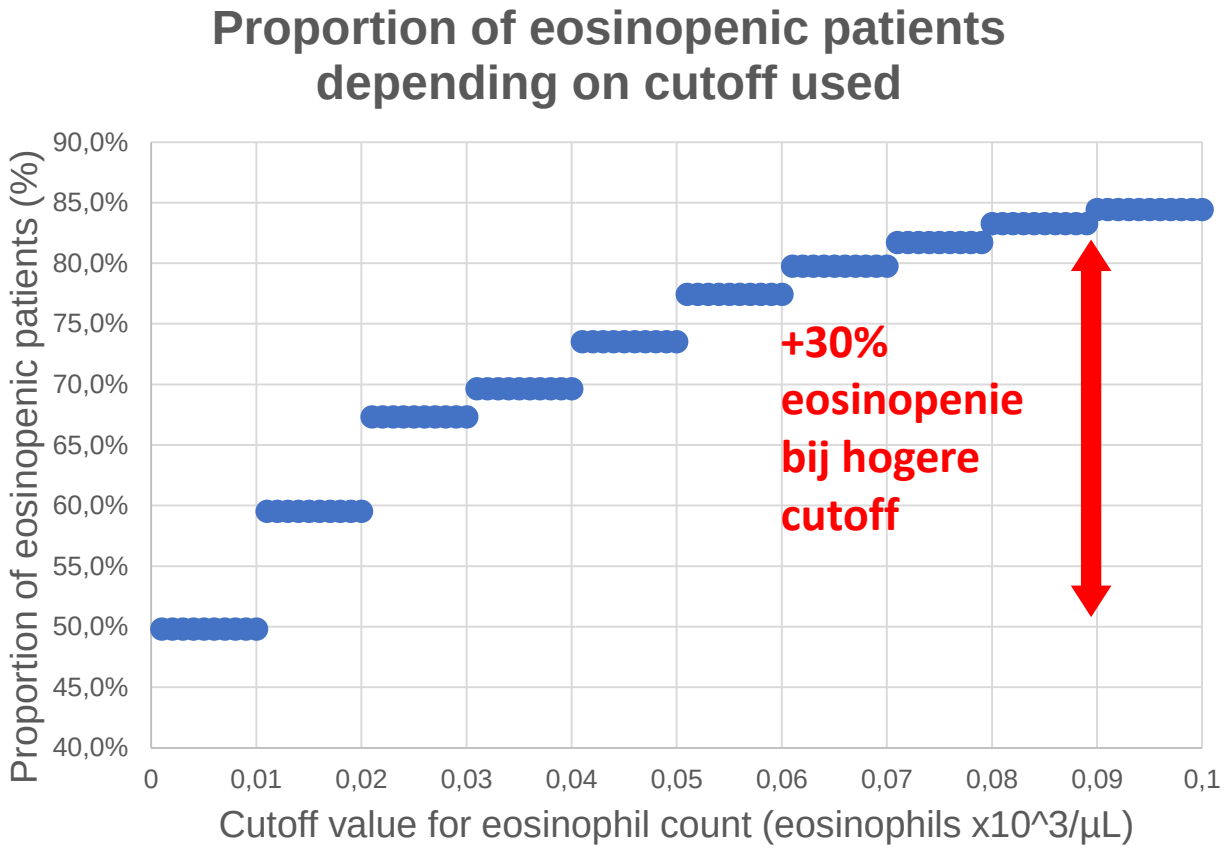
were found to be only marginally significant (i.e. $P = 0.032$ and $P = 0.049$, respectively), the differences remain largely comprised within the between-subject biologic variation of the eosinophil count, which is as high as 76%.[5] Moreover, although the instrument used for obtaining the eosinophil count in patients with COVID-19 has not been clearly described by Roca *et al.*,[1] the analytical goal of this parameter has been set at 26%[6] a threshold which almost overlaps with the differences reported by Roca *et al.*[1] in their study, and which would hence make the significance of their data clinically ambiguous.

Interindividuele biologische variatie tot 76%!!

Analytische factoren: cutoff eosinopenie

Cutoffs in gebruik:

- ≤ 0 eo/µL
- ≤ 10 eo/µL
- ≤ 20 eo/µL
- ≤ 40 eo/µL
- ≤ 45 eo/µL
- ≤ 50 eo/µL
- ≤ 100 eo/µL



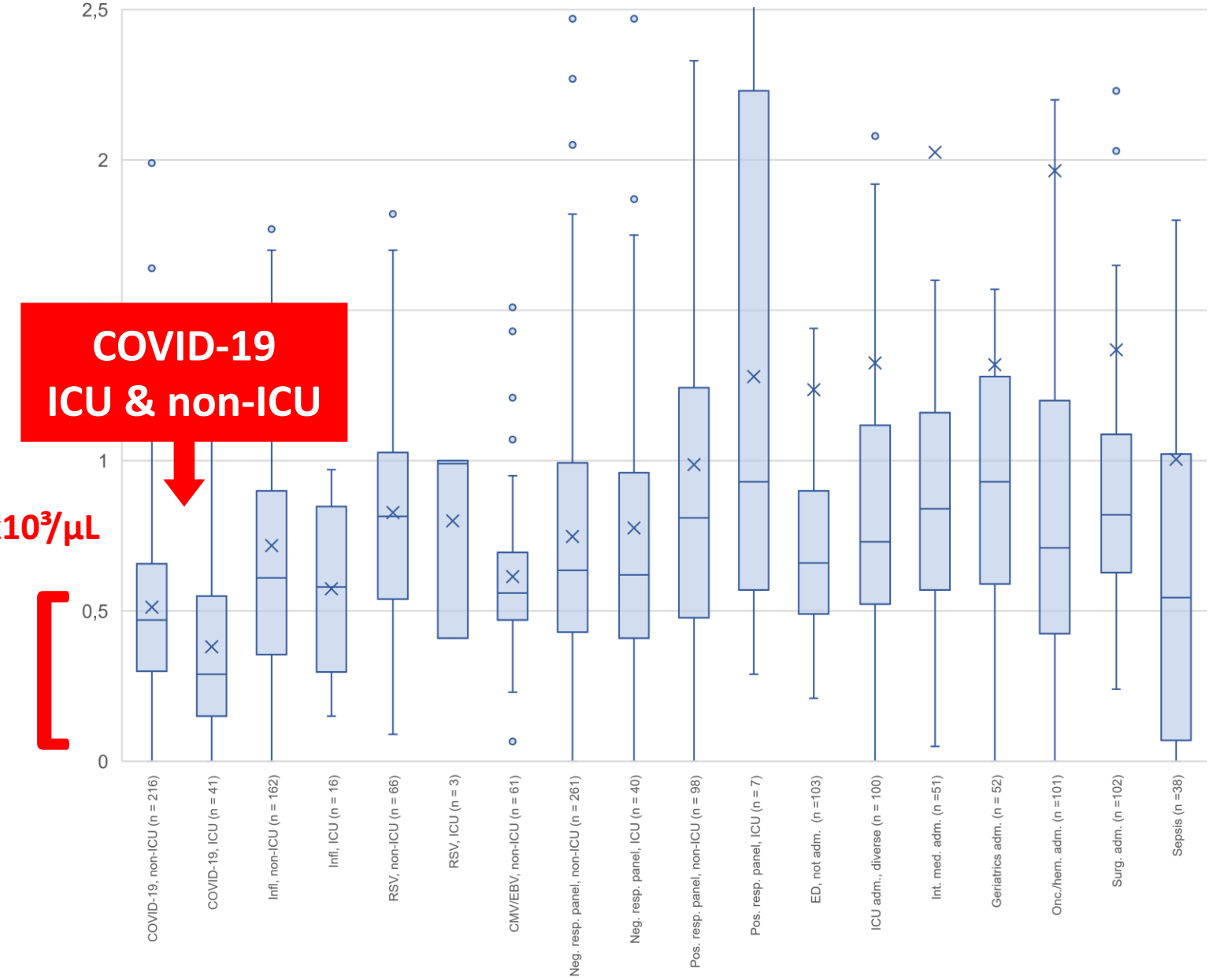
Literatuur: hematologische merkers COVID-19

- Lymfopenie
- Eosinopenie
- **Monocytopenie, lymfocyt-monocyt-ratio**
- MECOR score
- PARIS score
- Reactionele lymfocyten, HFLC, AS-LYMP, RE-LYMP

Literatuur: monocytentelling

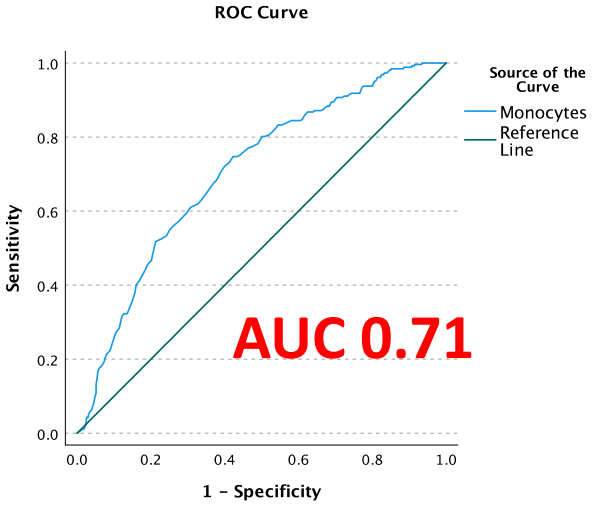
- Normaal, gestegen of gedaald bij COVID-19 (Khartabil et al.)

Distribution of monocyte counts in COVID-19 and control cohorts



COVID-19 ICU & non-ICU

Ref 0-0,51 x10³/µL



Literatuur: hematologische merkers COVID-19

- Lymfopenie
- Eosinopenie
- Monocytopenie, lymfocyt-monocyt-ratio
- **MECOR score**
- **PARIS score**
- Reactionele lymfocyten, HFLC, AS-LYMP, RE-LYMP

Samengestelde scores: MECOR score

diagnostics

MDPI

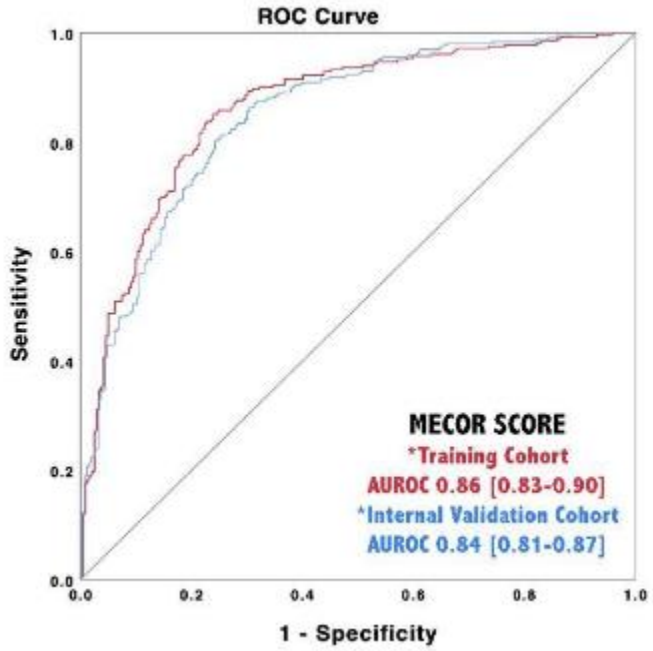
Article

The Model for Early COvid-19 Recognition (MECOR) Score: A Proof-of-Concept for a Simple and Low-Cost Tool to Recognize a Possible Viral Etiology in Community-Acquired Pneumonia Patients during COVID-19 Outbreak

Gianluca Sambataro [1,*,†], Mauro Giuffrè [2,3,†], Domenico Sambataro [4,5], Andrea Palermo [6],

$$\text{MECOR Score} = 18.47 - 3.23 \times \log_e\left(\text{WBC}\left[\text{cells/mm}^3\right]\right) - 0.76 \times \log_e\left(\text{LYM}\left[\text{cells/mm}^3\right]\right) + 11.94 \times \arctan\left(\frac{\text{MON}\left[\text{cells/mm}^3\right] \times \text{NEUT}\left[\text{cells/mm}^3\right]}{\text{PLT}\left[\text{cells/}\mu\text{L}\right]}\right)$$

AUC 0.84



COVID-19 patients n = 135; controles n = 115 CAP (viraal of bacterieel?)

Samengestelde scores: PARIS score

RESEARCH ARTICLE

Pre-test probability for SARS-Cov-2-related infection score: The PARIS score

Mickael Tordjman[1]*, Ahmed Mekki[2], Rahul D. Mali[3], Ines Saab[1],

Table 3. Pre-test diagnostic probability of COVID-19 infection: PARIS score.

Variables	Points
Eosinophils < 0.06 G/L	1
Lymphocytes < 1.3 G/L	2
Neutrophils <5G/L	1
Basophils <0.04G/L	1
Score = 0–1 → Low probability	
Score = 2–3 →Intermediate probability	
Score ≥4 → High probability	

https://doi.org/10.1371/journal.pone.0243342.t003

AUC 0.92

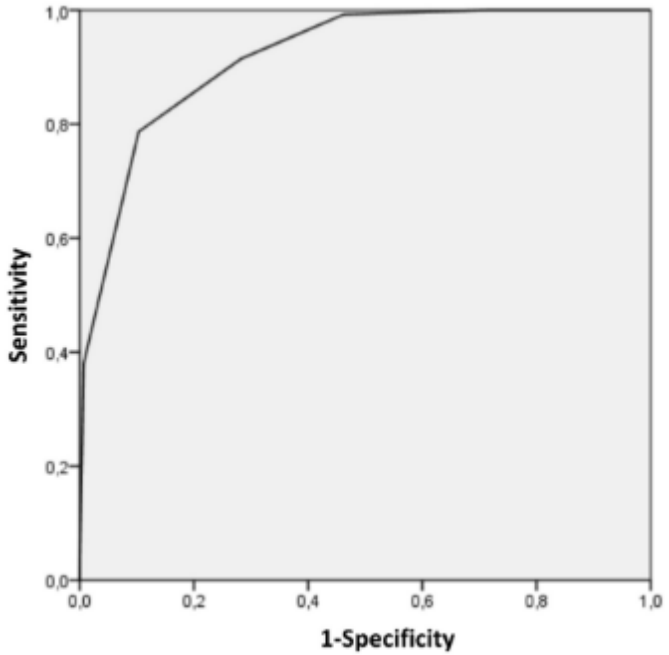


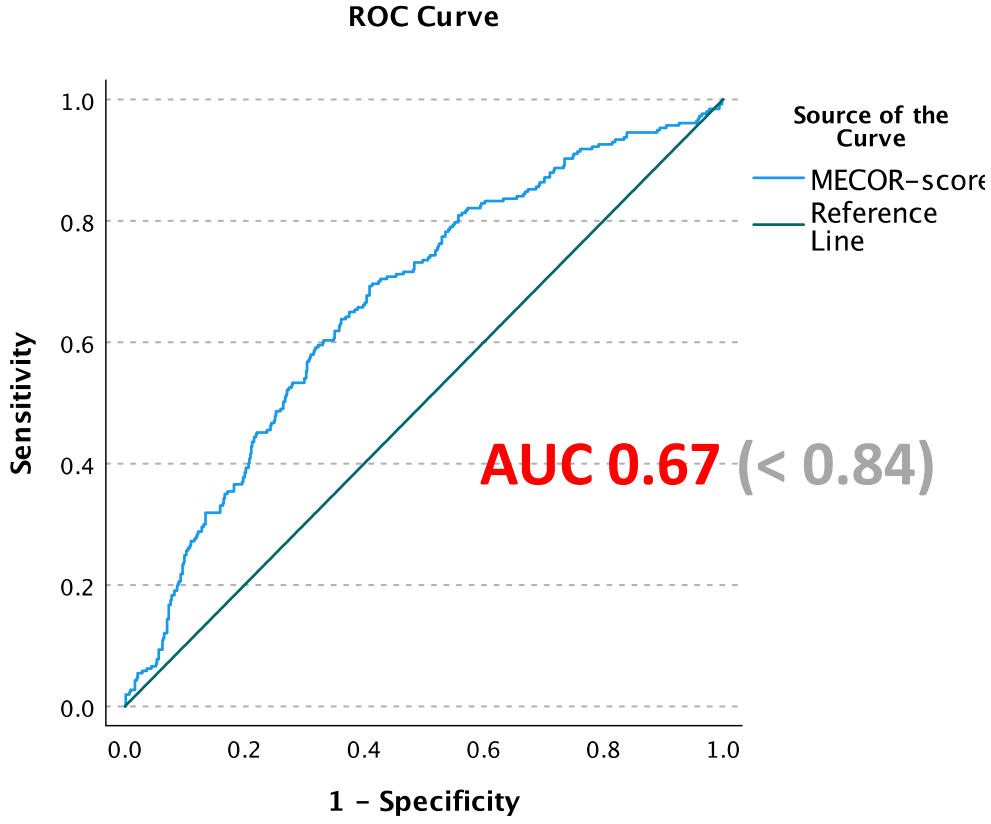
Fig 2. Receiver operating characteristic (ROC) curve of the PARIS score for the validation cohort. Area under the curve = 0.919.

https://doi.org/10.1371/journal.pone.0243342.g002

COVID-19 patients n = 261; controles n = 144 (slechts 1/144 controles virale luchtweginfectie)

MECOR score

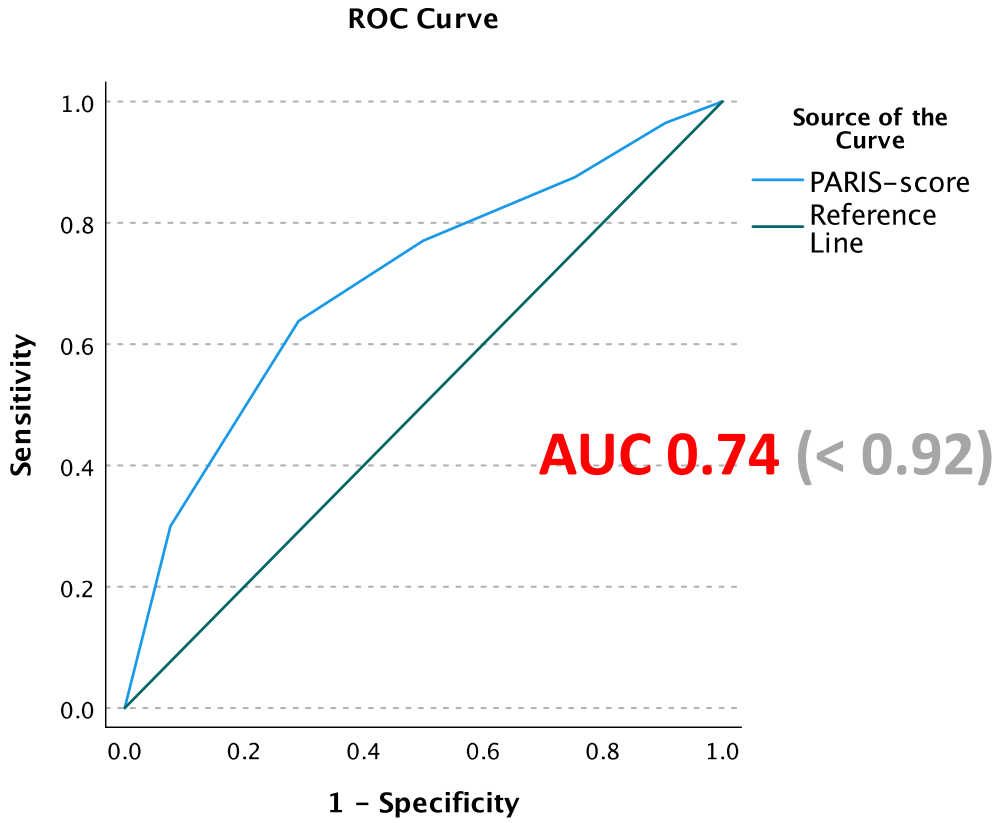
257 COVID-patiënten versus 653 controles
met (vermoeden van) respiratoire infectie



AUC 0.67 (< 0.84)

PARIS score

257 COVID-patiënten versus 1261 controles
(variabele etiologie)



AUC 0.74 (< 0.92)

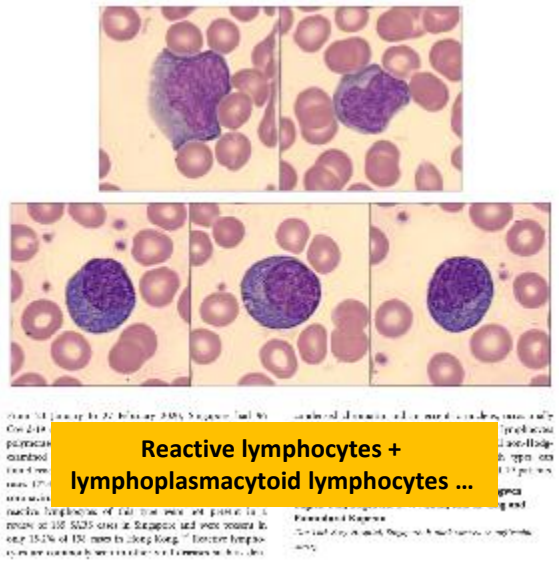
Literatuur: hematologische merkers COVID-19

- Lymfopenie
- Eosinopenie
- Monocytopenie, lymfocyt-monocyt-ratio
- MECOR score
- PARIS score
- **Reactionele lymfocyten, HFLC, AS-LYMP, RE-LYMP**

Literatuur: ‘reactionele’ lymfocyten

- **Reactionele/plasmacytoïde lymfocyten op uitstrijkje?**

Reactive lymphocytes in patients with Covid-19

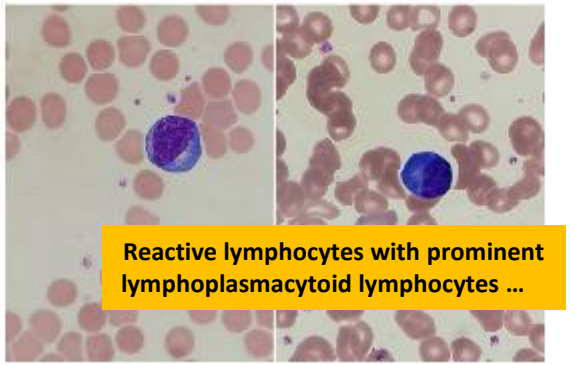


Reactive lymphocytes + lymphoplasmacytoid lymphocytes …

N = 16/23
69%

Plasmacytoid lymphocytes in SARS-CoV-2 infection (Covid-19)

David Foldes[1] | Richard Hinton[1] | Siamak Arami[1] | Barbara J. Bain[2]

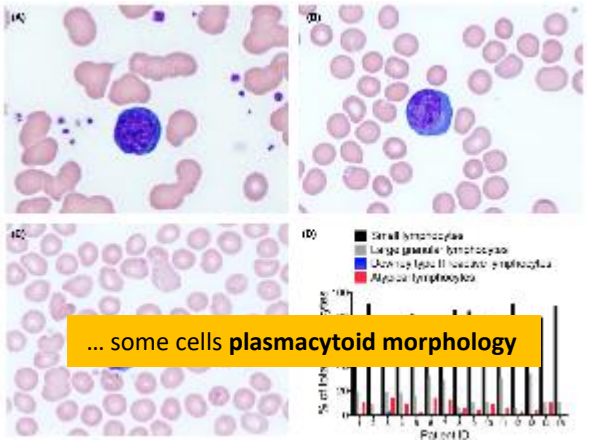


Reactive lymphocytes with prominent lymphoplasmacytoid lymphocytes …

N = 1/1

Atypical lymphocytes in peripheral blood of patients with COVID-19

Samuel B. Weinberg
Amir Tahdal
Feng Ji



… some cells **plasmacytoid morphology**

N = 14/15
93,3%

Literatuur: ‘reactionele’ lymfocyten

‘**Plasmacellen**’ in 371 van 2301 patiënten (16.1%)

CLINICAL RESEARCH STUDY

THE AMERICAN JOURNAL of MEDICINE®

Peripheral Plasma Cells Associated with Mortality Benefit in Severe COVID-19: A Marker of Disease Resolution

Mary Boulanger, MD,[a] Emily Molina, MD,[a] Kunbo Wang, MS,[b] Thomas Kickler, MD,[c] Yanxun Xu, PhD,[b] Brian T. Garibaldi, MD[a,d]

[a]Department of Medicine, Johns Hopkins Hospital, Baltimore, Md; [b]Department of Applied Mathematics and Statistics, Johns Hopkins University, Baltimore, Md; [c]Department of Pathology, Johns Hopkins Hospital, Baltimore, Md; [d]Division of Pulmonary and Critical Care, Johns Hopkins University School of Medicine, Baltimore, Md.

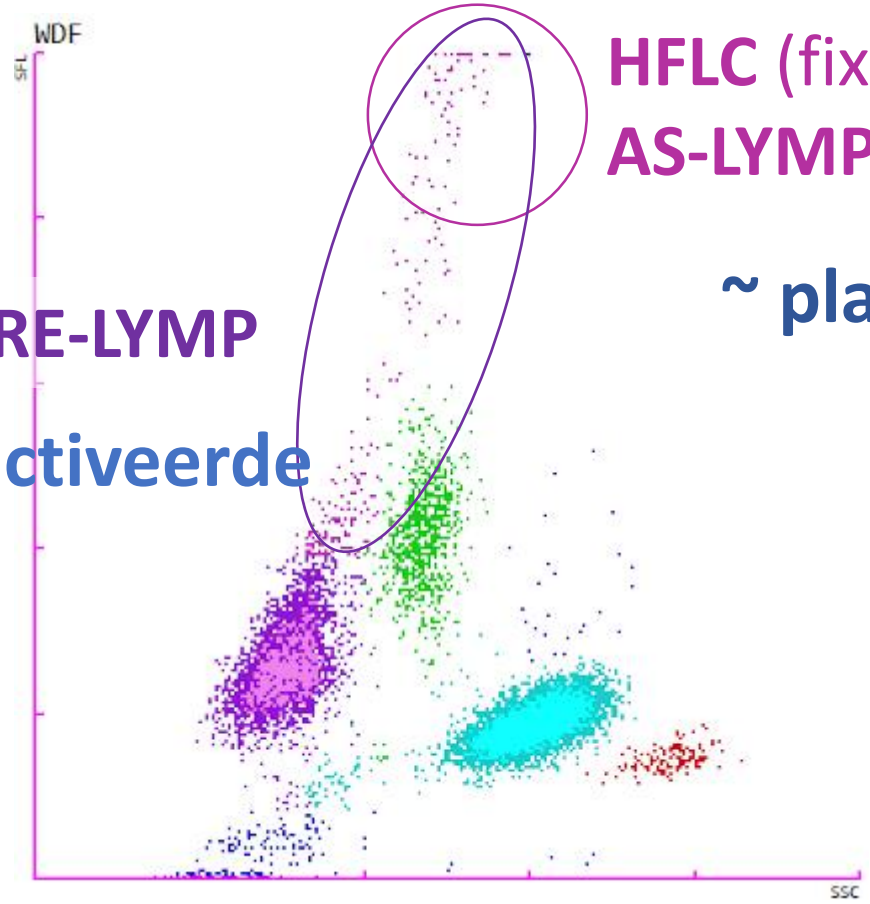
geen gestandaardiseerde morfologische criteria

ABSTRACT

BACKGROUND: Cytokines seen in severe coronavirus disease 2019 (COVID-19) are associated with proliferation, differentiation, and survival of plasma cells. Plasma cells are not routinely found in peripheral blood, though may produce virus-neutralizing antibodies in COVID-19 later in the course of an infection.
METHODS: Using the Johns Hopkins COVID-19 Precision Medicine Analytics Platform Registry, we identified hospitalized adult patients with confirmed severe acute respiratory coronavirus 2 (SARS-CoV-2) infection and stratified by presence of plasma cells and World Health Organization (WHO) disease severity. To identify plasma cells, we employed a sensitive flow cytometric screening method for highly fluorescent lymphocytes and confirmed these microscopically. Cox regression models were used to evaluate time to death and time to clinical improvement by the presence of plasma cells in patients with severe disease.
RESULTS: Of 2301 hospitalized patients with confirmed infection, 371 had plasma cells identified. Patients with plasma cells were more likely to have severe disease, though 86.6% developed plasma cells after onset of severe disease. In patients with severe disease, after adjusting for age, sex, body mass index, race, and other covariates associated with disease severity, patients with plasma cells had a reduced hazard of death (adjusted hazard ratio: 0.57; 95% confidence interval: 0.38-0.87; *P* value: .008). There was no significant association with the presence of plasma cells and time to clinical improvement.
CONCLUSIONS: Patients with severe disease who have detectable plasma cells in the peripheral blood have improved mortality despite adjusting for known covariates associated with disease severity in COVID-19. Further investigation is warranted to understand the role of plasma cells in the immune response to COVID-19.
 • The American Journal of Medicine (2021) 000:1–5

KEYWORDS: Convalescent plasma; COVID-19; Mortality; Plasma cells; plasmacytosis

‘reactionele’ lymfocyten: flowcytometrie



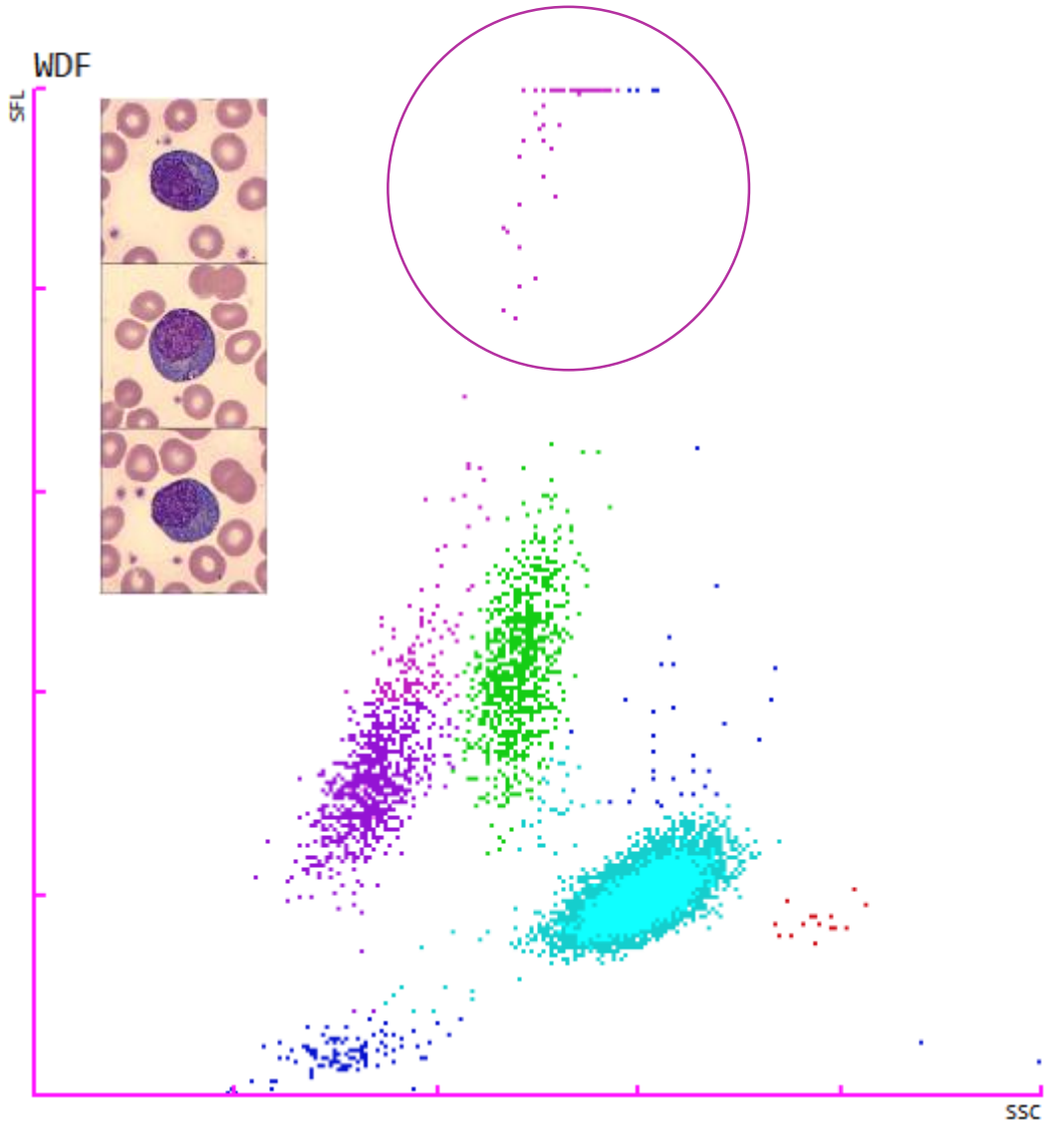
HFLC (fixed gating)
AS-LYMP (flexible gating)

~ plasma(cytoïde) cellen

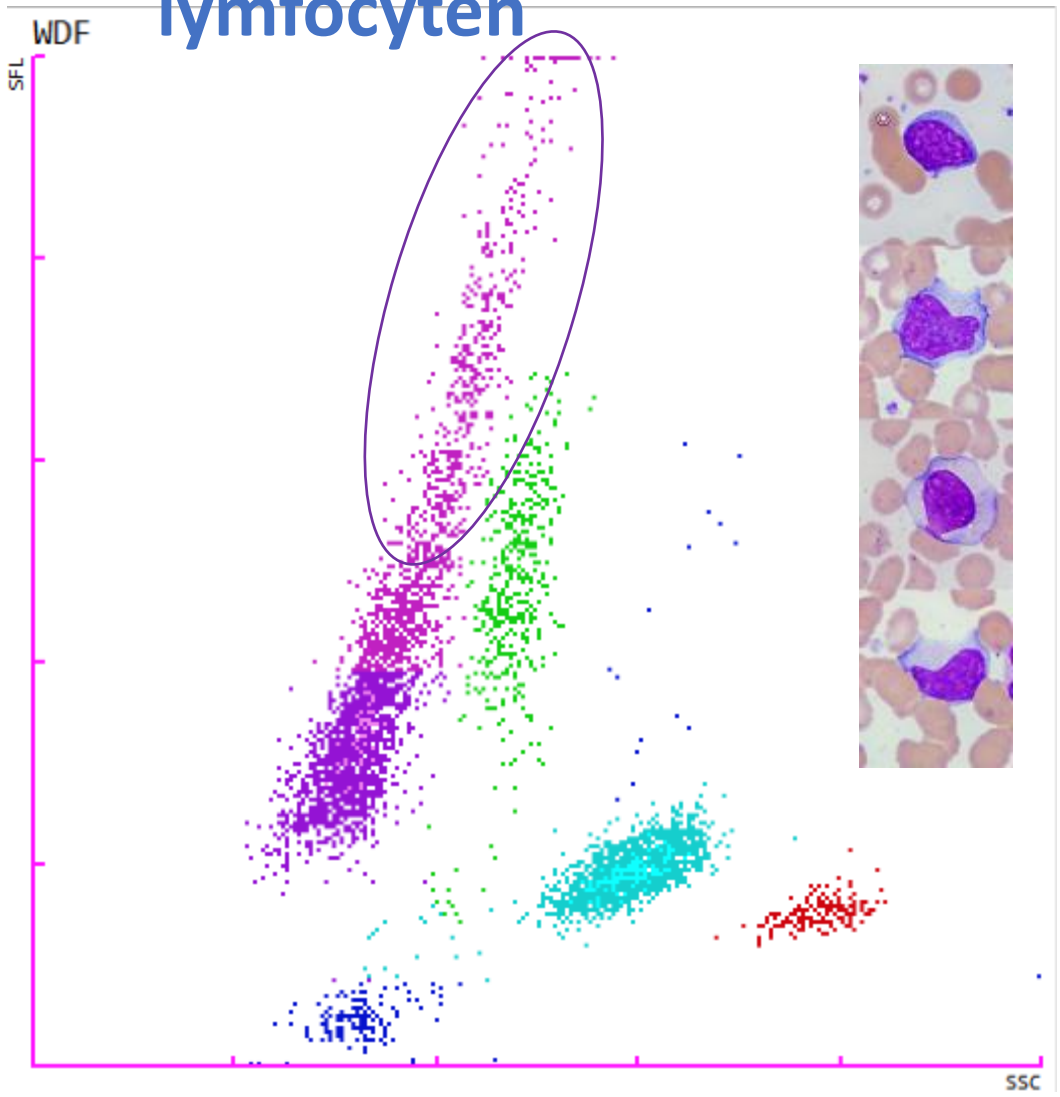
RE-LYMP

~ polymorfe geactiveerde lymfocyten

~ plasma(cytoïde) cellen



~ polymorfe geactiveerde lymfocyten



Literatuur: ‘reactionele’ lymfocyten

<u>Diagnostische bruikbaarheid RE-LYMP, HFLC?</u>

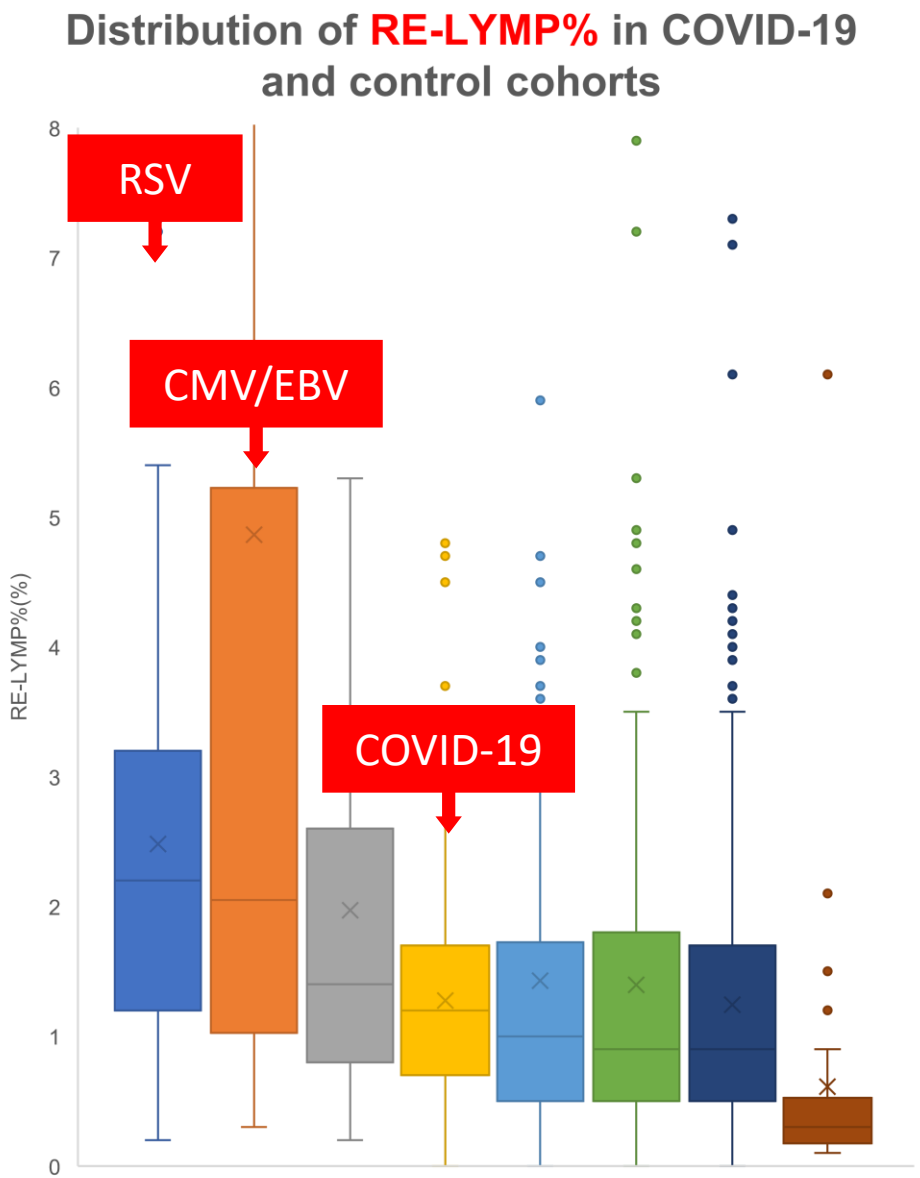
Hoger in COVID-19 dan negatieve controles (Martens et al., Yun et al.)

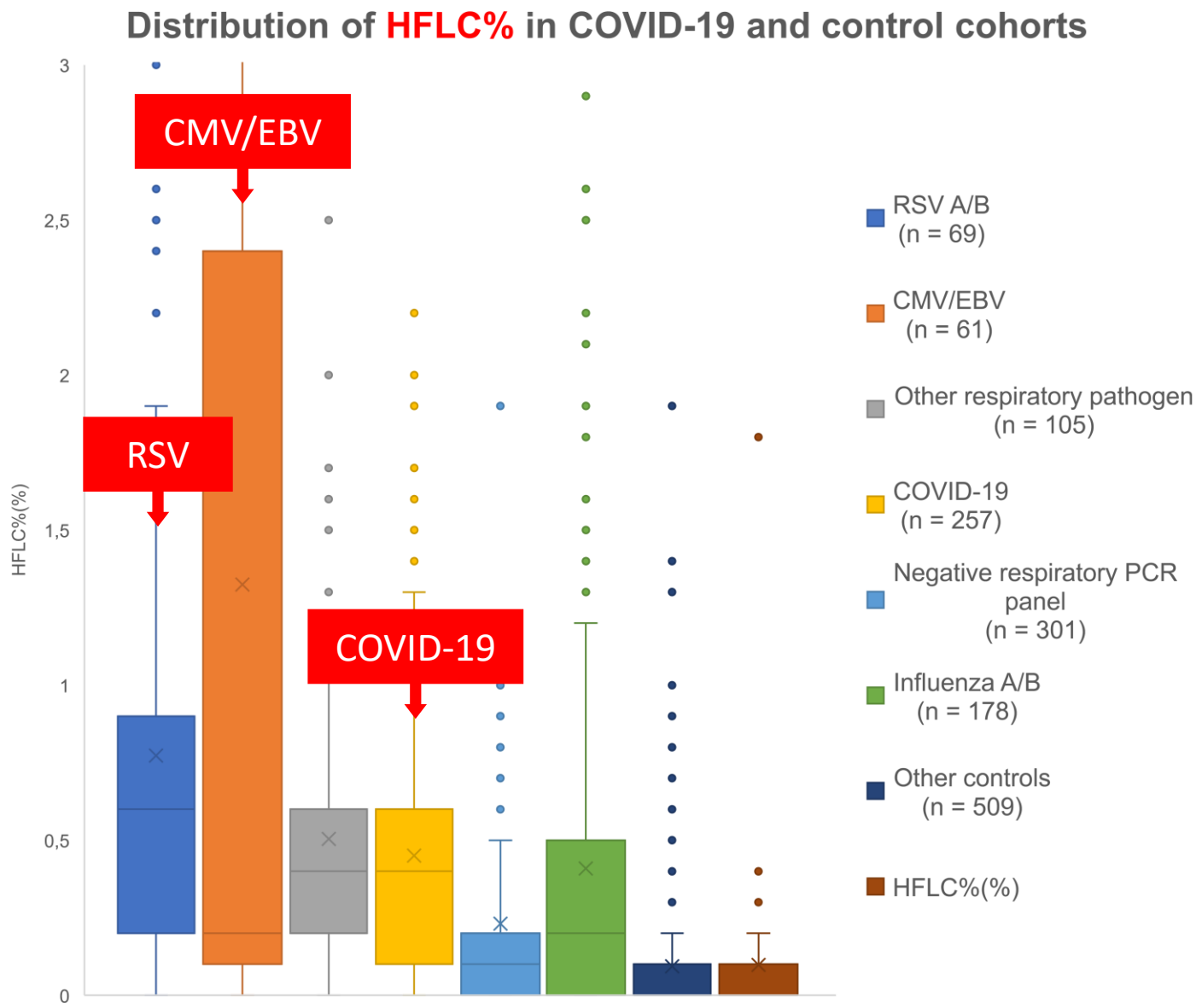
Maar lager in COVID-19 dan andere virale aandoeningen? (Rutkowska et al.)

<u>Prognostische bruikbaarheid RE-LYMP, HFLC?</u>

Gestegen waarden wisselend **geassocieerd met**

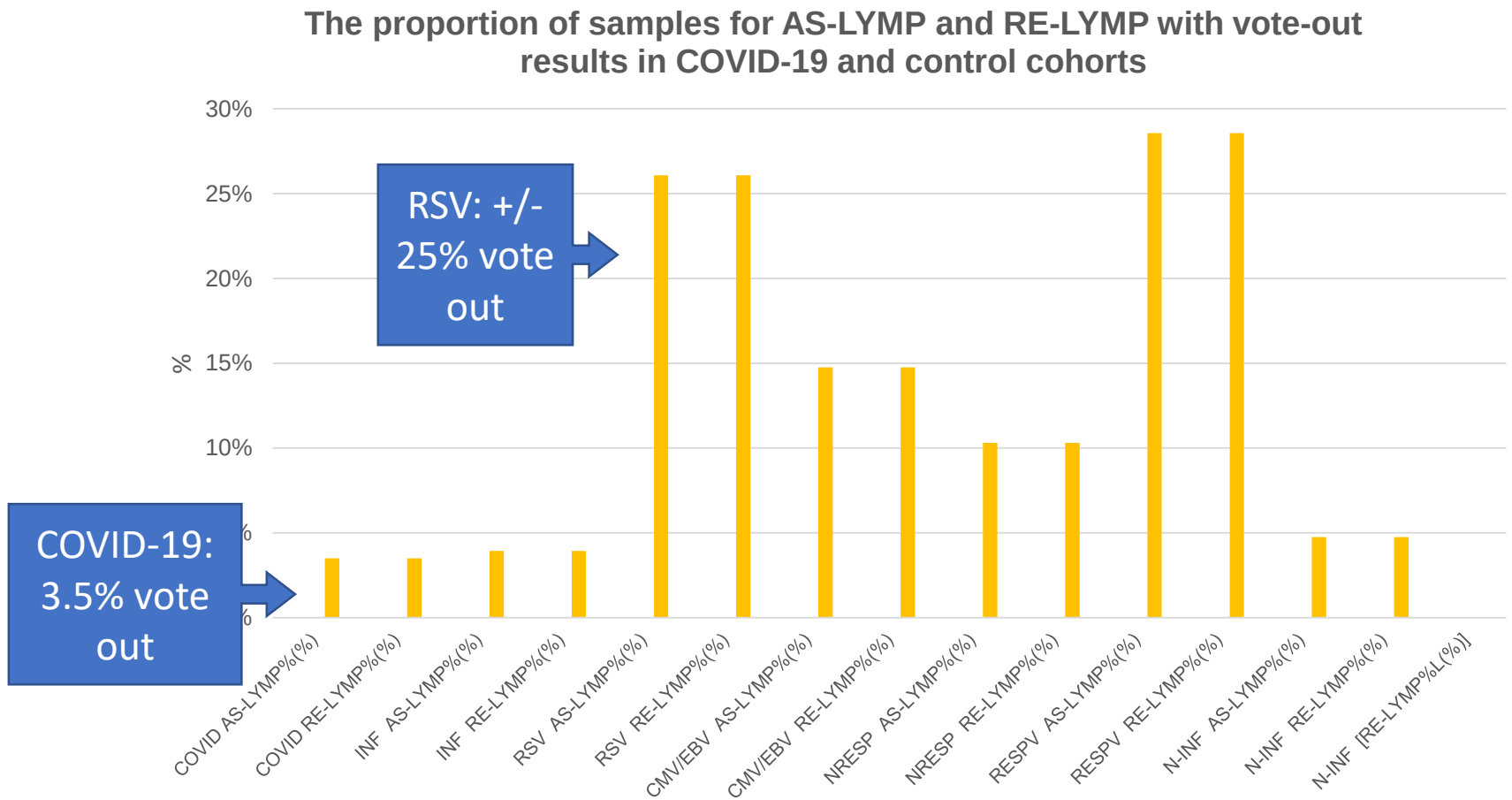
- **ernstigere ziekte/slechtere outcome** (Lin et al., Yip et al., Martens et al., Linssen et al.)
- of **juist betere overleving?** (Boulanger et al., Wang et al.)





HFLC en RE-LYMP relatief aspecifiek

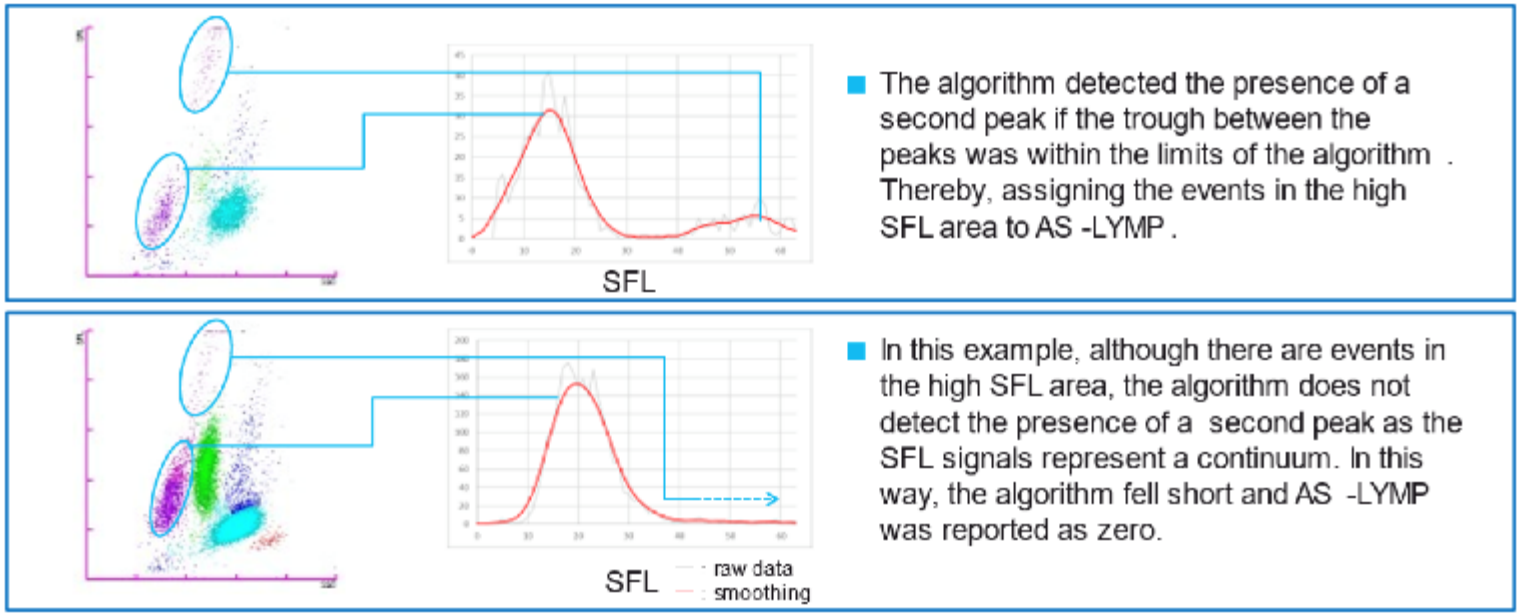
AS-LYMP: ‘vote out’ resultaten



AS-LYMP: vals negatieve resultaten IPU 22.08

Valse meting AS-LYMP ‘0’ ondanks waarschijnlijke aanwezigheid plasmacytoïde cellen (IPU 22.08)

Niet in rekening genomen in verschillende studies…



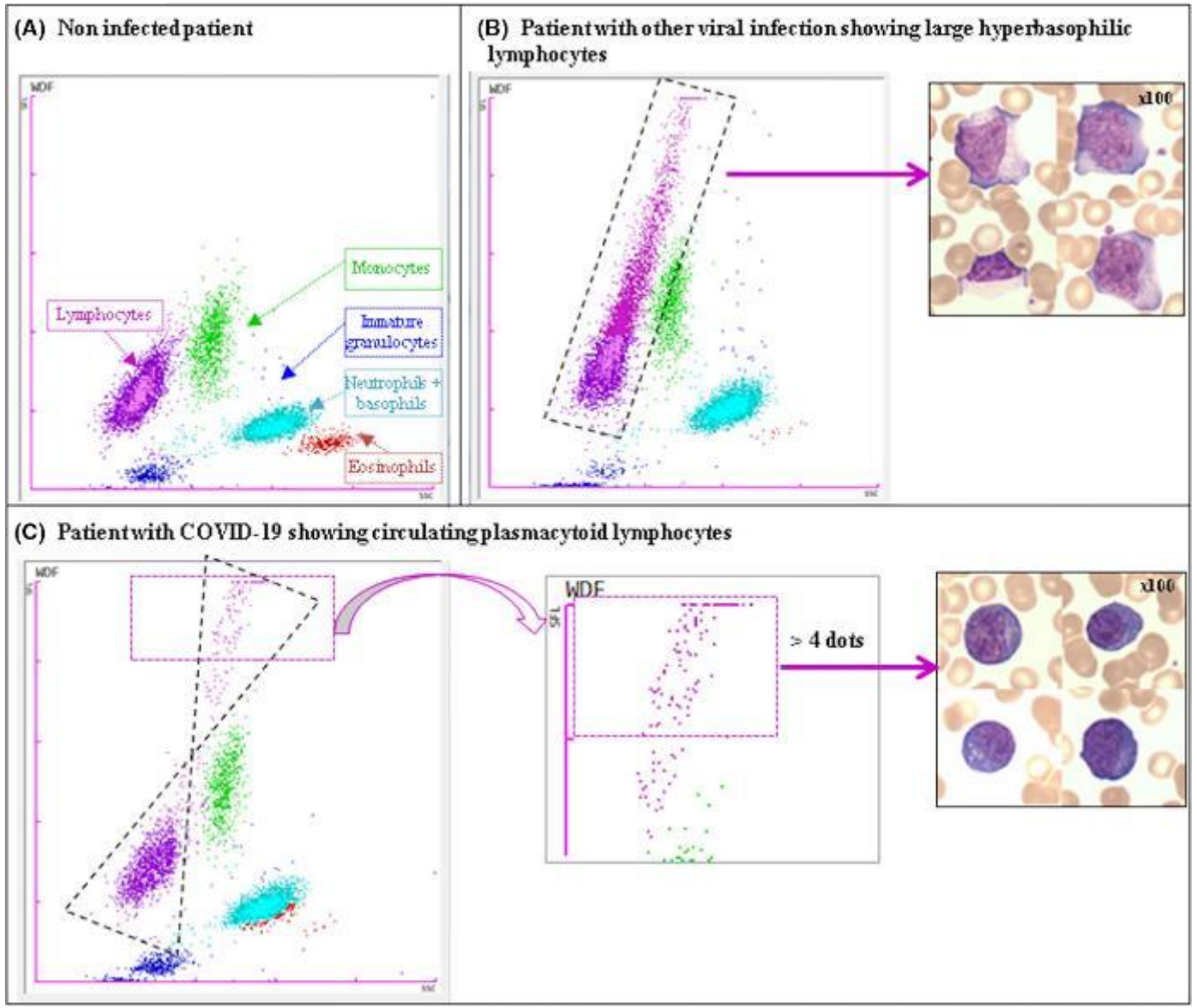
bjh short report

Rapid screening of COVID-19 patients using white blood cell scattergrams, a study on 381 patients

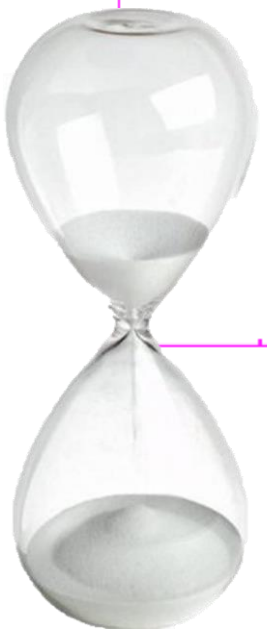
Zandloperpatroon én ≥4 dots in hoogste-fluorescentie regio:

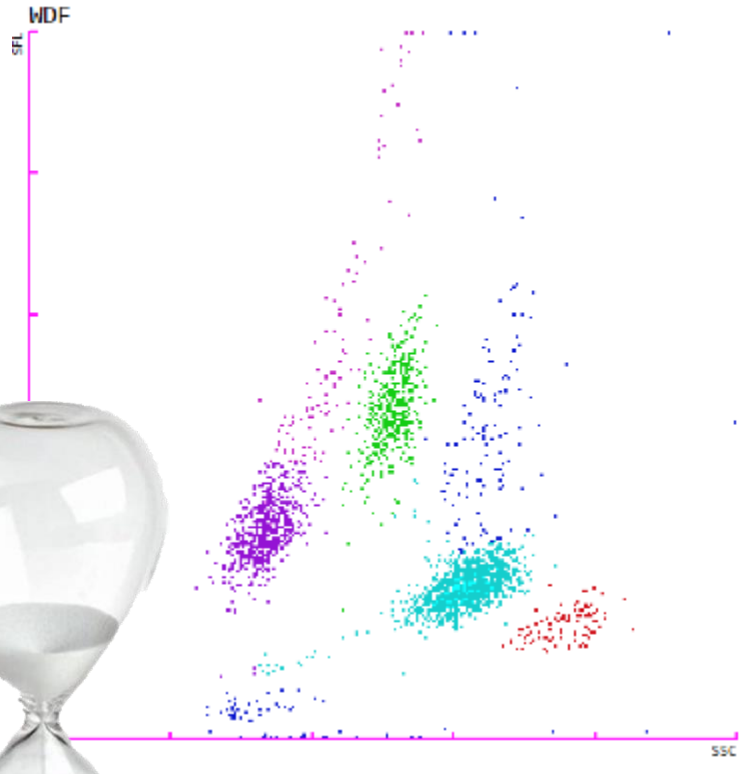
Sens 85.9%, spec 83.5%

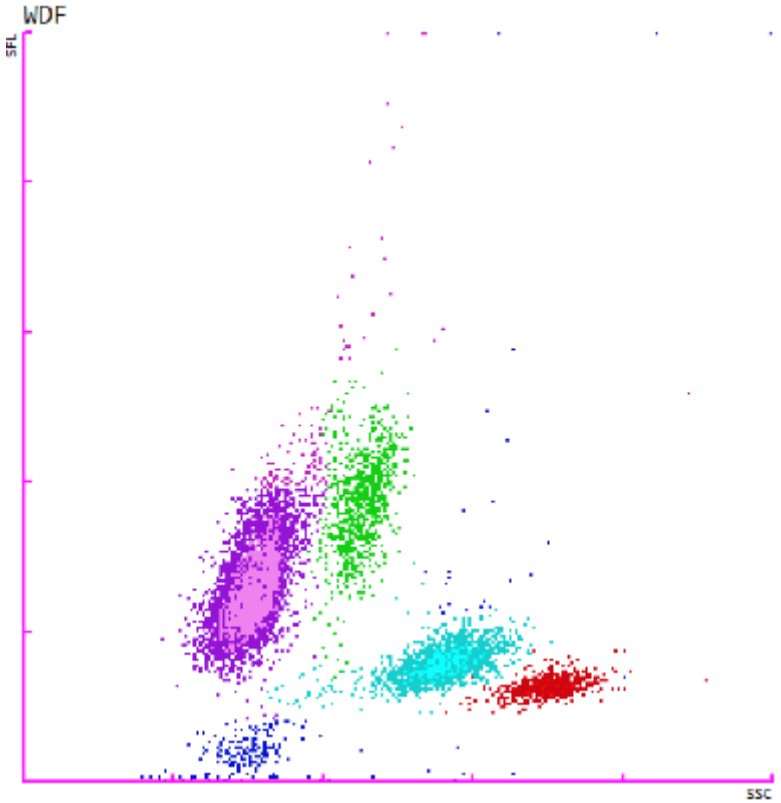
85 COVID-19 patients and 85 patients with influenza, EBV, *Mycoplasma pneumoniae* and parvovirus infections

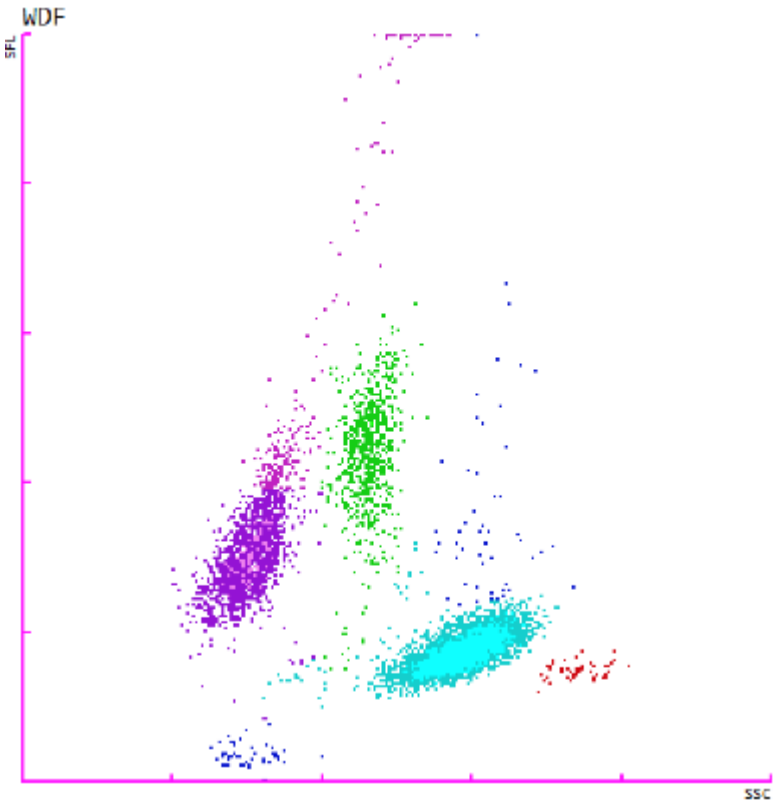


?





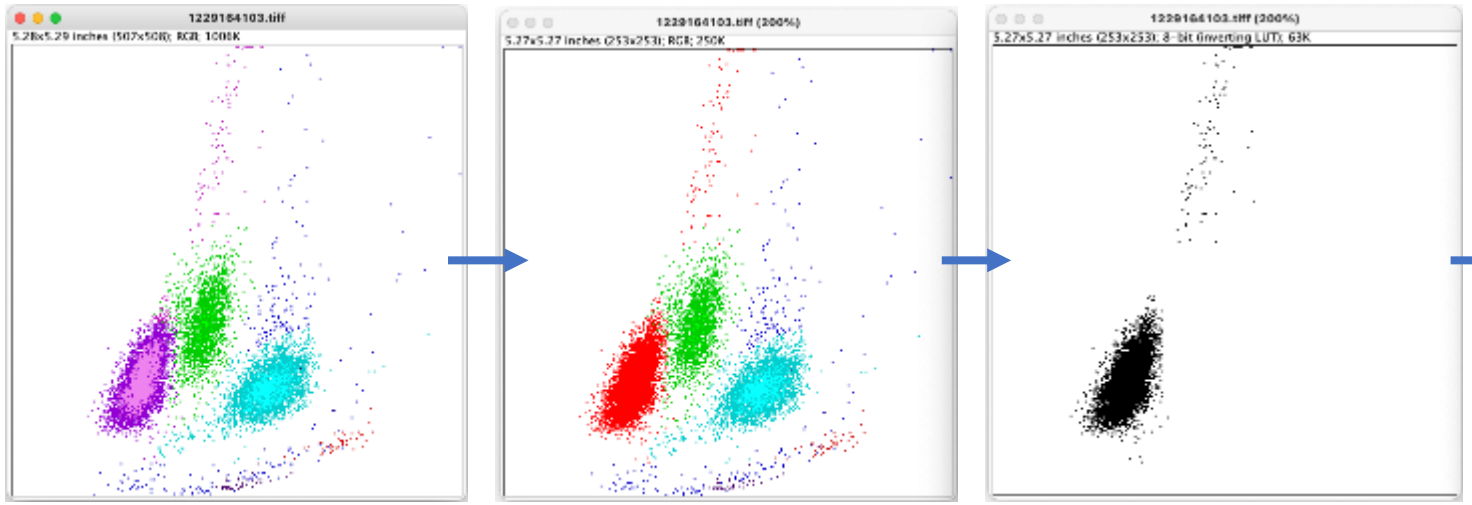




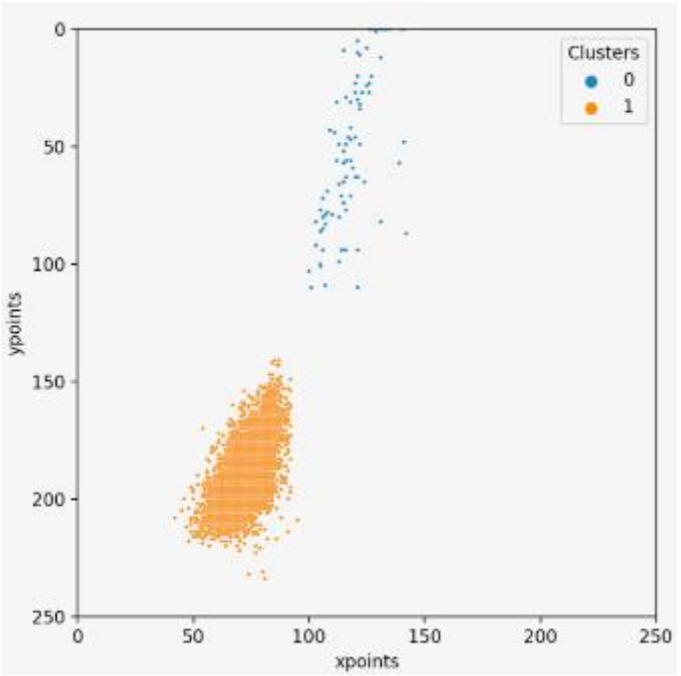
Subjectief… performantie zandloperpatroon niet gerepliceerd door andere groepen

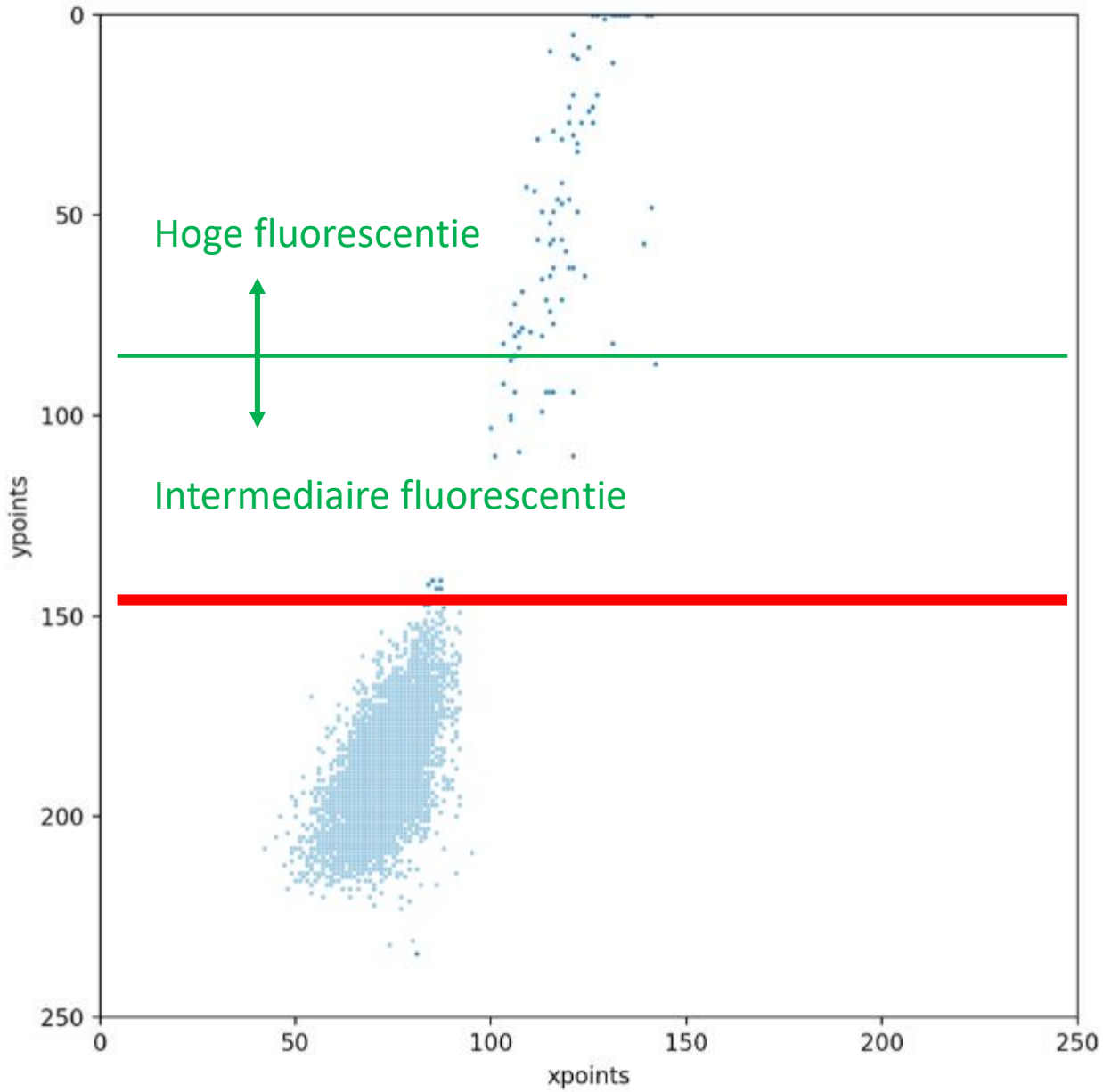
Softwarematige kwantificatie ‘AS-LYMP’

ImageJ
Image Processing & Analysis in Java

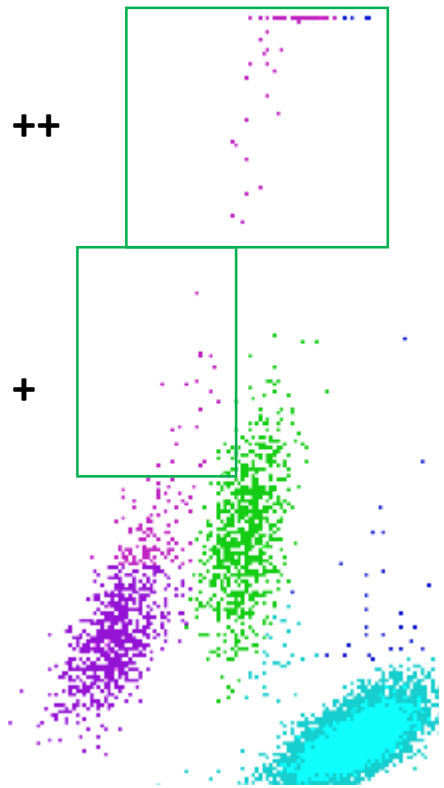


python™

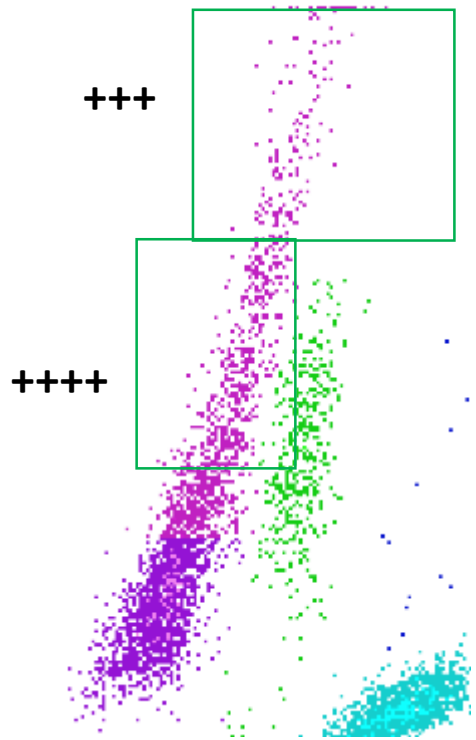




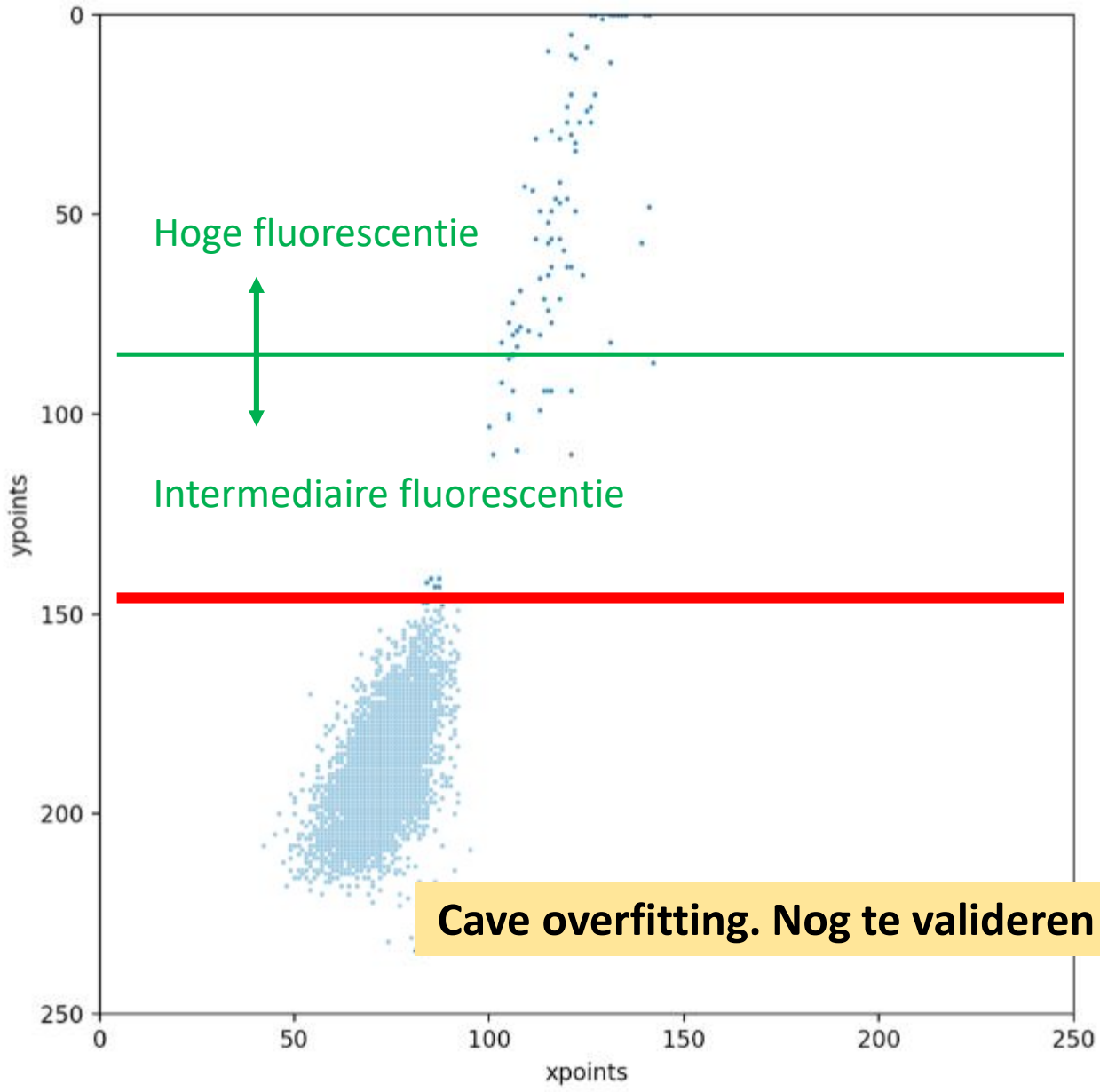
$$ratio = \frac{dots\ boven\ threshold}{dots\ onder\ threshold}$$



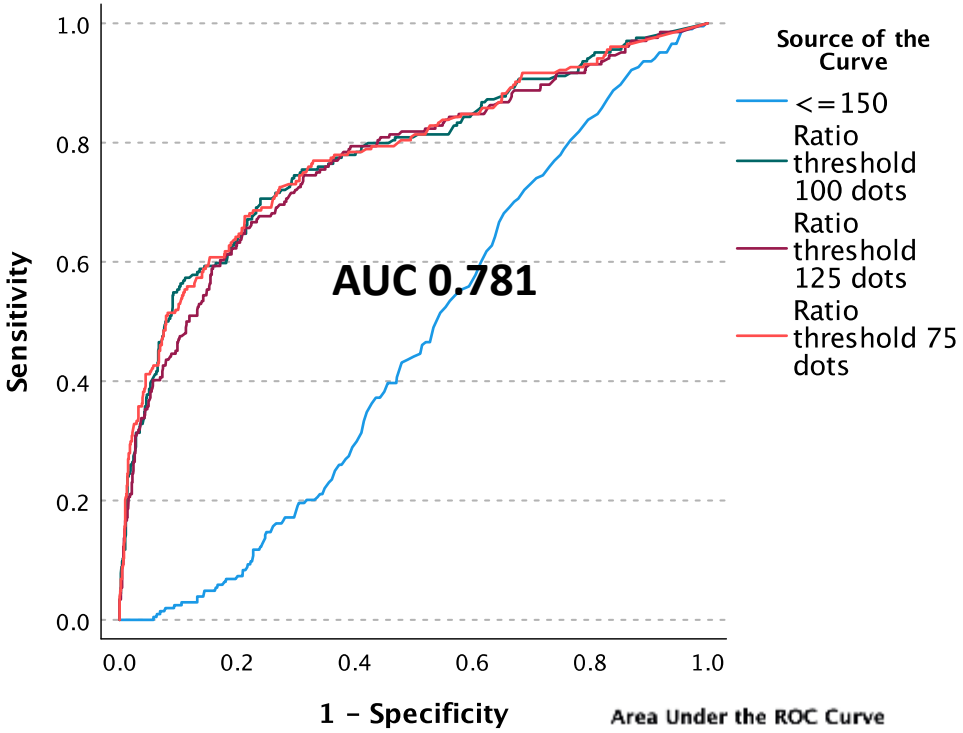
$ratio \uparrow\uparrow$



$ratio = 1\ of\ \downarrow$



$$ratio = \frac{dots\ boven\ threshold}{dots\ onder\ threshold}$$



Cave overfitting. Nog te valideren in onafhankelijke cohort.

Area Under the ROC Curve

Test Result Variable(s)	Area
Ratio threshold 75 dots	.781
Ratio threshold 100 dots	.779
Ratio threshold 125 dots	.768
<=150	.462

The test result variable(s): Ratio threshold 75 dots, Ratio threshold 100 dots, Ratio threshold 125 dots, <=150 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

Besluit

- Literatuur: **vaak optimistisch beeld van performantie hematologische indices**
  - Studies vaak klein
  - Slecht omschreven controlepopulatie
  - Sampling bias (confounders zoals ziekte-ernst)
  - Onvoldoende aandacht voor analytische factoren
- **Ratio hoge/intermediaire fluorescentie lymfocyten toon beste performantie: AUC 0.78**
  - Doch globaal weinig performant: sensitiviteit van 80%: 40% vals positieven
- PCR/antigeentests nu meer algemeen beschikbaar: hematologische indices relatief weinig performant

To do’s

- Combinatie van PARIS + AS-LYMP?
- Systematische vergelijking van hematologische afwijkingen bij diverse respiratoire infecties (bijvoorbeeld: RSV, influenza, COVID-19)
- Met mortaliteitsdata performantie van prognostische scores nagaan
- Temporele trends van hematologische afwijkingen (bijv. AS-LYMP) nagaan

Dank aan **Veerle Geelen**, wetenschappelijk assistent Jessa ZH

Dank aan promotors **prof. Dr Rummens, prof. Apr. Kieffer, dr. A. Hendrickx**

Vragen?