# Blood cell counts and lymphocyte subsets of patients admitted during the COVID-19 pandemic: a prospective cohort study

Aberrant blood cell counts, most prominently lymphopenia, have been identified as markers of the degree of severity of coronavirus disease 2019 (COVID-19).[1–5] However, most of these studies lack comparison to a COVID-19-negative contemporary control group. Additionally, the longitudinal composition and dynamics of lymphocyte subsets in patients with COVID-19 are insufficiently characterised. We performed a prospective cohort study, in which we compared blood cell and lymphocyte subset counts between (i) hospital-admitted patients with COVID-19 and a large contemporary COVID-19-negative hospitalised control group, and (ii) non-critically and critically ill patients with COVID-19. In addition, we evaluated the impact of these parameters on the in-hospital mortality rate of COVID-19. Finally, we assessed the dynamics of lymphocyte reconstitution in patients with COVID-19 over time.

## Results

From 1 March until 1 May 2020, all adult patients who were admitted to our specialised COVID-19 ward on suspicion of COVID-19 and tested by polymerase chain reaction (PCR) for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) were included. Follow-up lasted until discharge, death or data lock. A total of 551 patients were enrolled. COVID-19 was diagnosed in 197 patients, either by PCR (158/197, 80%) or by high-resolution computed tomography imaging (39/197, 20%). Based on intensive care unit admission or death during hospital admission, 82/197 (42%) of these patients with COVID-19 were retrospectively labelled as 'critically ill'. COVID-19 was ruled out in the remaining 354 patients (controls). The definitive diagnoses in the control group were heterogeneous, ranging from bacterial infections to congestive heart failure and cancer. Baseline characteristics and outcomes of the included patients are presented in Table S1.

Complete routine blood analysis was performed on the day of admission for 349/354 controls, 114/115 non-critically and 78/82 critically ill patients with COVID-19 (Table 1, Table S2). Compared to the controls, patients with COVID-19 had a lower prevalence of anaemia and leucocytosis ($>11 \times 10^9$/l) and a higher prevalence of thrombocytopenia ($<150 \times 10^9$/l) (Fig 1A–C). Furthermore, anaemia was significantly more prevalent in critically ill patients with COVID-19, compared to non-critically ill patients with COVID-19. Leucocyte differentiation was performed within the first few days of admission for 152/354 controls, 76/115 non-critically and 46/82 critically ill patients with COVID-19 (Table 1, Table S2). The median lymphocyte counts were not significantly different between the controls and the patients with COVID-19, or between non-critically and critically ill patients with COVID-19 (Fig 1D). Correspondingly, the prevalence of lymphopenia ($<1·0 \times 10^9$/l) was similar between the controls and patients with COVID-19, and between non-critically and critically ill patients with COVID-19. Lymphopenia, defined by a less stringent threshold ($<1·5 \times 10^9$/l), was present in most patients, but more prevalent in patients with COVID-19. The controls, compared to the patients with COVID-19, had a higher mean neutrophil count (Figure S1A). Additionally, compared to non-critically ill patients with COVID-19, the mean neutrophil count was higher in critically ill patients with COVID-19.

In 25/354 controls, 39/115 non-critically and 40/82 critically ill patients with COVID-19, lymphocyte subsets were quantified by immunophenotyping during admission (Table 1, Table S2). A list of diagnoses in the 25 controls from whom immunophenotyping data were obtained is presented in Table S3. The median CD4^{+}- and CD8^{+}-T-lymphocyte counts were similar between the controls, non-critically and critically ill patients with COVID-19 (Fig 1D,F). The majority of both controls and patients with COVID-19 had diminished CD8^{+}-T-cell counts ($<200$/mm$^2$), but this proportion was larger in the COVID-19 group. Compared to non-critically ill patients with COVID-19, critically ill patients with COVID-19 had a higher mean CD4/CD8 ratio (Figure S1C) and a lower median natural killer (NK) cell count (Figure S1D).

To assess the impact of blood cell and lymphocyte subset counts on the in-hospital mortality risk in patients with COVID-19, we performed univariable survival analyses (Figure S2). Patients with thrombocytopenia or leucocytosis at admission had an increased risk of in-hospital mortality (Figure S2A,B). Although lymphopenia (stringently or leniently defined) was not predictive of in-hospital mortality for COVID-19 (not shown), the presence of CD4^{+}-T-lymphopenia ($<300$/mm$^2$) was associated with an increased risk of in-hospital mortality (Figure S2C).

Immunophenotyping was not performed at a standardised time point for each patient. Additionally, immunophenotyping
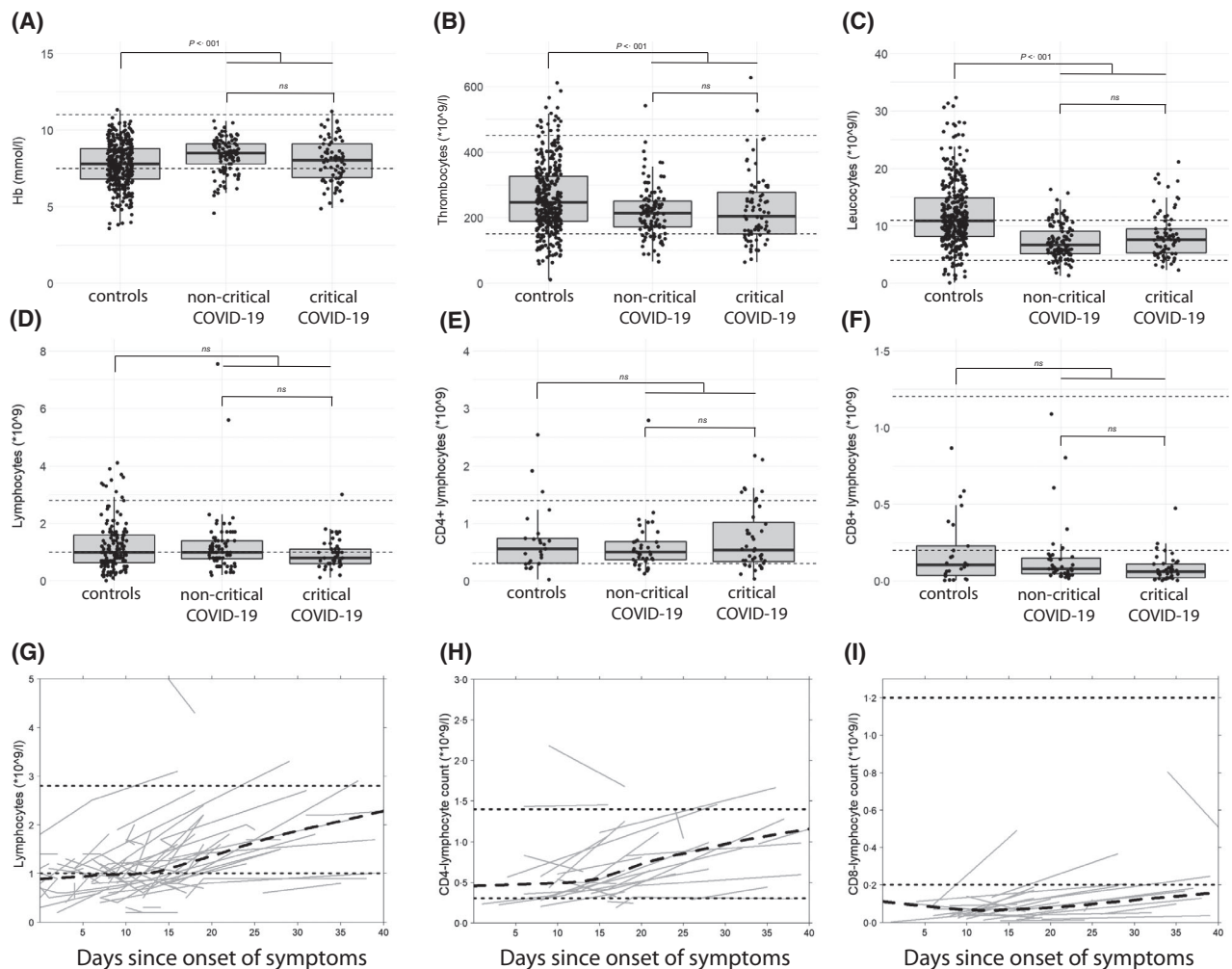
**Fig 1.** Routine blood analysis, leucocyte differentiation and immunophenotyping. (A–F) Dot plots with routine blood analysis, leucocyte differentiation and immunophenotyping results for controls, non-critically and critically ill patients with COVID-19. Each dot represents a single measurement. Upper and lower hinges represent the IQR, whereas the whiskers extend 1·5-times the IQR. Dotted lines represent the normal reference range. (A–C) routine blood analysis results at day of admission. Significance tested with Student's $t$-test. (D) total lymphocyte counts, quantified by leucocyte differentiation within the first few days of admission. Significance tested with Mann–Whitney–Wilcoxon test. (E,F) $CD3^+CD4^+$ and $CD3^+CD8^+$-T-lymphocyte counts, quantified by immunophenotyping. Significance tested with Mann–Whitney–Wilcoxon test. (G, H) Spaghetti plots illustrating lymphocyte subset counts over time in COVID-19-positive patients, with $t_0$ defined as start of symptoms. Grey lines represent repeated measurements in individual patients, the black dashed line represent the summarised trend of all individual patients and the black dotted lines denote the upper and lower limit of the normal reference range, uncorrected for age. (G) Total lymphocyte counts in patients withCOVID-19 over time in patients with repeated measurements, including both leucocyte differentiation and immunophenotyping results. (H,I) lymphocyte subset counts over time in COVID-19-positive patients. Only patients with repeated immunophenotyping measurements were included. COVID-19; coronavirus disease 2019; Hb, haemoglobin; IQR, interquartile range; ns, not significant.

was performed two- or three-times in 29 and four patients with COVID-19 respectively. This allowed us to perform a longitudinal analysis in which we included all patients with repeated measurements and defined the date of first symptom as $t_0$. Although patients with COVID-19 generally had lymphopenia at admission (Fig 1D), the lymphocyte counts gradually increased to reference values around disease day 14 (Fig 1G). $CD4^+$-T lymphocytes experienced a similar rise after 2 weeks (Fig 1H), but $CD8^+$-T-lymphocyte reconstitution was much slower, and average $CD8^+$-T-cell counts did not reach normal limits even after 40 days (Fig 1I).

## Discussion and conclusions

The core strength of our present study is the inclusion of a large, contemporary, COVID-19-negative control population, which allowed us to critically reappraise the haematological features of patients with COVID-19. At admission, anaemia, leucocytosis and neutrophilia were more prevalent in the controls than in the patients with COVID-19. In agreement with recent reports, thrombocyte counts were lower in patients with COVID-19, and thrombocytopenia was associated with an increased risk of in-hospital mortality.[6,7]

Table 1. Blood cell and lymphocyte subset counts of the patients with COVID-19 and the controls.

| Variable | Controls (no COVID-19), $n = 354$ | Patients with COVID-19, $n = 197$ | $P$ |
|---|---|---|---|
| Haemoglobin, mmol/l, mean (95% CI) | 7·8 (7·6–7·9) | 8·2 (8·0–8·4) | <0·001 |
| Anaemia, $n$ (%) | 153 (43) | 58 (30) | 0·003 |
| N/A, $n$ | 2 | 4 | |
| Thrombocyte count, $\times 10^9$/l, mean (95% CI) | 262 (251–274) | 224 (210–237) | <0·001 |
| Thrombocytopenia, $n$ (%) | 46 (13) | 39 (20) | 0·03 |
| N/A, $n$ | 5 | 5 | |
| Leucocyte count, $\times 10^9$/l, mean (95% CI) | 12·0 (11·4–12·6) | 8·2 (7·1–9·2) | <0·001 |
| Leucocytosis, $n$ (%) | 167 (48) | 34 (18) | <0·001 |
| N/A, $n$ | 3 | 4 | |
| Neutrophil count, $\times 10^9$/l, mean (95% CI) | 8·9 (8·0–9·8) | 6·2 (5·6–6·8) | <0·001 |
| Neutrophilia, $n$ (%) | 82 (54) | 36 (30) | <0·001 |
| N/A, $n$ | 202 | 75 | |
| Lymphocyte count, $\times 10^9$/l, median (95% CI) | 1·1 (1·0–1·3) | 1·0 (0·9–1·1) $\times 10^9$/l | 0·26 |
| Lymphopenia <1·0 $\times 10^9$/l, $n$ (%) | 77 (51) | 73 (60) | 0·13 |
| Lymphopenia <1·5 $\times 10^9$/l, $n$ (%) | 107 (70) | 101 (83) | 0·02 |
| N/A, $n$ | 202 | 75 | |
| CD4$^+$-T-lymphocyte count, /mm$^2$, median (95% CI) | 589 (444–861) | 581 (486–696) | 0·86 |
| Below reference range, $n$ (%) | 5 (20) | 16 (20) | 0·98 |
| N/A, $n$ | 329 | 118 | |
| CD8$^+$-T-lymphocyte count, /mm$^2$, median (95% CI) | 133 (72–278) | 81 (64–101) | 0·25 |
| Below reference range, $n$ (%) | 17 (68) | 70 (89) | 0·02 |
| N/A, $n$ | 329 | 118 | |
| CD4/CD8 ratio, mean (95% CI) | 14·9 (5·8–23·9) | 12·7 (9·0–16·3) | 0·55 |
| B-lymphocyte count, /mm$^2$, median (95% CI) | 143 (86–204) | 143 (119–174) | 0·85 |
| Below reference range, $n$ (%) | 10 (40) | 33 (42) | 0·88 |
| N/A, $n$ | 329 | 118 | |
| Igκ/Igλ light chain ratio, median (95% CI) | 1·6 (1·4–2·1) | 1·5 (1·4–1·6) | 0·55 |
| NK cell count, /mm$^2$, median (95% CI) | 196 (138–259) | 164 (132–198) | 0·33 |
| Below reference range, $n$ (%) | 7 (28) | 23 (29) | 0·86 |
| N/A, $n$ | 329 | 118 | |

Routine blood counting, leucocyte differentiation and immunophenotyping results of patients with COVID-19 and COVID-19-negative controls. $P$ values were calculated using Welch's $t$-test and a Mann–Whitney–Wilcoxon test for normally distributed and non-normally distributed continuous variables, respectively, and using a chi-square test for categorical variables. 95% CI, 95% confidence interval; COVID-19, coronavirus disease 2019; Ig, immunoglobulin; N/A, not available; NK cell, natural killer cell.

Lymphopenia, previously presented as a hallmark of COVID-19,[1,8,9] is a common feature of critical illness in general.[10] Indeed, in our present cohort, neither the median lymphocyte counts nor the prevalence of lymphopenia (<1·0 $\times 10^9$/l) were different between the patients with COVID-19 and the controls. This suggests that lymphopenia per se, at least stringently defined, is not a discriminatory feature of COVID-19.

Unfortunately, we were only able to perform immunophenotyping in a proportion of patients. Furthermore, immunophenotyping could not be performed at a standardised time point, which bears the risk of comparing patients at different stages of their disease. Nevertheless, the range in timing of the immunophenotyping, in combination with repeated sampling in some patients, allowed us to demonstrate that in patients with COVID-19, reconstitution of CD4$^+$-T lymphocytes starts around disease day 14, whereas CD8$^+$-T-lymphocyte reconstitution was much slower. This possibly reflects the functional exhaustion reported in these cells in the context of COVID-19.[8]

In conclusion, we demonstrate that red cell, white cell, and platelet counts are significantly different between COVID-19-positive and -negative patients, whereas lymphopenia is not a distinguishing feature of COVID-19. Our present data help to identify patients at higher risk of a critical disease course, who could potentially most benefit from pre-emptive interventions.

## Methods

For the methods section, please see the supporting material.

## Acknowledgements

## Conflict of Interest

The authors do not have any conflict of interest to report.

**Paul J. Hengeveld**[1,2] (iD)
**Aaram O. Khader**[1]
**Linda H. A. de Bruin**[3]
**Inge G. P. Geelen**[1] (iD)
**Eske A. van Baalen**[1]
**Eva Jansen**[1]
**Nathalie I. Bouwer**[1] (iD)
**Ömer Balak**[4]
**Jurgen A. Riedl**[3]
**Anton W. Langerak**[2] (iD)
**Peter E. Westerweel**[1] (iD)
**Mark-David Levin**[1] (iD)

[1]*Department of Internal Medicine, Albert Schweitzer Hospital, Dordrecht,* [2]*Laboratory Medical Immunology, Erasmus MC, Rotterdam,* [3]*Result Laboratory, Albert Schweitzer Hospital and* [4]*Department of Pulmonology, Albert Schweitzer Hospital, Dordrecht, the Netherlands.*
*E-mail: p.j.hengeveld@asz.nl*

**Keywords:** COVID-19, lymphocytes, lymphopenia, subsets, immunophenotyping

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig S1.** Routine blood analysis, leucocyte differentiation and immunophenotyping.

**Fig S2.** Survival analysis in COVID-19-positive patients.

**Table S1.** Baseline clinical characteristics and outcomes.

**Table S2.** Blood cell- and lymphocyte subset counts of non-critically and critically ill patients with COVID-19.

**Table S3.** Definitive diagnoses in controls who underwent immunophenotyping.

**Data S1.** Supplementary methods.

## References

1. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. *Am J Hematol.* 2020;**95**:834–47.

2. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. *Br J Haematol.* 2020;**189**:428–37.

3. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis.* 2020;**221**:1762–9.

4. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* 2020;**8**:475–81.

5. Zhao Q, Meng M, Kumar R, Wu Y, Huang J, Deng Y, et al. Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a systemic review and meta-analysis. *Int J Infect Dis.* 2020;**96**: 131–5.

6. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clin Chim Acta.* 2020;**506**:145–8.

7. Maquet J, Lafaurie M, Sommet A, Moulis G, Alvarez M, Amar J, et al. Thrombocytopenia is independently associated with poor outcome in patients hospitalized for COVID-19. *Br J Haematol.* 2020 [Epub ahead of print]. DOI: https://doi.org/10.1111/bjh.16950

8. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol.* 2020;**17**:533–5.

9. Bermejo-Martin JF, Almansa R, Menéndez R, Mendez R, Kelvin DJ, Torres A. Lymphopenic community acquired pneumonia as signature of severe COVID-19 infection. *J Infect.* 2020;**80**:e23–e24.

10. Hohlstein P, Gussen H, Bartneck M, Warzecha KT, Roderburg C, Buendgens L, et al. Prognostic relevance of altered lymphocyte subpopulations in critical illness and sepsis. *J Clin Med.* 2019;**8**:353.