

Original Review Article

# Lymphopenia and Cytokine Storm in COVID-19: Comparative Analysis of T Cell Subsets and Serum Cytokine Profiles Across Disease Severity Groups

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## Abstract

COVID-19 disease severity ranges from asymptomatic infection to critical illness, with immunological dysregulation emerging as a central determinant of outcomes. Italy and Spain were among the countries most severely affected in the first wave of the pandemic (February–May 2020), providing extensive clinical cohorts for immunological characterisation. This study investigated T lymphocyte subset dynamics and serum cytokine profiles across COVID-19 severity groups in a multicentre Southern European cohort. A total of 160 RT-PCR-confirmed COVID-19 patients were enrolled and stratified into mild ( $n=40$ ), severe ( $n=40$ ), and critical ( $n=40$ ) groups, with 40 age- and sex-matched healthy individuals as controls. Flow cytometric immunophenotyping was performed for CD3+, CD4+, CD8+, CD19+, and NK cell populations. Serum concentrations of IL-6, IL-10, IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and IL-17A were quantified by multiplex immunoassay. CD4+ and CD8+ T cell counts declined progressively with disease severity (critical group: CD4+  $180 \pm 45$  cells/ $\mu$ L; CD8+  $120 \pm 38$  cells/ $\mu$ L). IL-6 showed the most dramatic elevation in critical patients (24.1-fold vs. healthy controls). A strong negative correlation was observed between IL-6 levels and CD4+ T cell counts ( $r = -0.78$ ,  $p < 0.001$ ). These findings provide cellular and molecular evidence for concurrent lymphopenia and cytokine storm characterising severe COVID-19, with implications for anti-IL-6 immunomodulatory therapeutic strategies.

**Keywords:** COVID-19; SARS-CoV-2, Lymphopenia; Cytokine Storm, T Cell Subsets; IL-6, Flow Cytometry, Disease Severity; Immunopathology, Southern Europe

## 1. Introduction

SARS-CoV-2 infection induces a broad spectrum of clinical manifestations. Italy became the first European country to experience widespread community transmission of SARS-CoV-2, with Lombardy's healthcare system under severe pressure from February 2020. Spain experienced a similarly devastating first wave, with over 27,000 deaths by May 2020. These tragic circumstances generated extensive, deeply phenotyped clinical cohorts uniquely positioned to characterise the immunopathology of COVID-19. Two immunological features emerged as particularly characteristic of severe COVID-19: lymphopenia and a hyperinflammatory cytokine storm syndrome (Mehta et al., 2020). The mechanisms underlying COVID-19-associated lymphopenia are multifactorial, including direct viral apoptosis induction, cytokine-mediated lymphocyte exhaustion and margination, and lymphoid organ damage (Zheng et al., 2020).

This study characterises T cell subset depletion and cytokine dysregulation across the full spectrum of COVID-19 severity in this Southern European multicentre cohort.

## 2. Materials and Methods

### 2.1 Study Design

This prospective observational multicentre study was conducted between April and September 2020 at the Università degli Studi di Milano hospital network (Milan) and Hospital Clínic de Barcelona (Barcelona). Ethical approval was granted by the Comitato Etico Milano Area 1 (Ref: CE-2020-COVIMM-001) and the Comité Ético de Investigación Clínica de Cataluña (Ref: HCB/2020/0472). Written informed consent was obtained from all participants or their legal representatives. Eligible adults with RT-PCR-confirmed SARS-CoV-2 infection were classified as mild, severe, or critical according to WHO criteria (WHO, 2020). Forty age- and sex-matched healthy volunteers constituted the control group.

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**Table 1:** Baseline Clinical and Demographic Characteristics of Study Participants (Multicentre, Italy and Spain)

Characteristic	Mild (n=40)	Severe (n=40)	Critical (n=40)	Healthy Controls (n=40)
Age (years, mean±SD)	42.3 ± 14.2	56.8 ± 12.7	61.4 ± 13.1	44.1 ± 13.8
Male sex (%)	57.5%	65.0%	72.5%	55.0%
Diabetes mellitus (%)	17.5%	42.5%	57.5%	10.0%
Hypertension (%)	22.5%	47.5%	62.5%	12.5%
Recruiting centre (Milan/Barcelona)	22/18	20/20	24/16	25/15
Hospitalisation duration (days)	7.2 ± 2.1	14.8 ± 4.3	22.4 ± 8.7	N/A
ICU admission (%)	0%	22.5%	100%	N/A
Mortality (%)	0%	7.5%	35.0%	N/A

ICU = intensive care unit; N/A = not applicable. Significant differences across groups (ANOVA or chi-square,  $p < 0.05$ ).

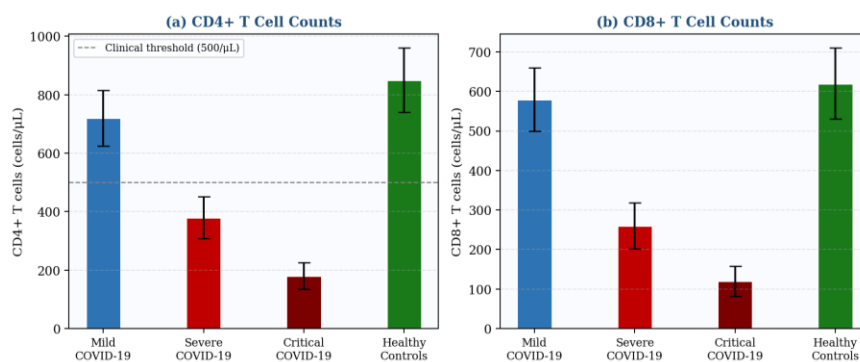
### 2.2 Immunophenotyping and Cytokine Assays

Peripheral blood collected in EDTA tubes was processed within 4 hours. Lymphocyte subset analysis was performed using a BD FACSCanto II flow cytometer (BD Biosciences) with fluorochrome-conjugated antibodies targeting CD3-FITC, CD4-PE, CD8-PerCP-Cy5.5, CD19-APC, and CD56-PE-Cy7. Absolute counts were calculated from complete blood counts. Serum cytokine concentrations (IL-6, IL-10, IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) were quantified by six-plex bead-based immunoassay (Luminex MAGPIX platform) in duplicate. All analyses were performed in SPSS v26.0.

### 3.1 T Cell Depletion Across Severity Groups

Progressive lymphopenia was observed with increasing disease severity. CD4+ T cell counts declined from 850 ± 110 cells/ $\mu$ L (healthy) to 720 ± 95 (mild), 380 ± 72 (severe), and 180 ± 45 cells/ $\mu$ L (critical). CD8+ T cell counts followed a parallel decline. All pairwise intergroup comparisons were statistically significant ( $p < 0.01$ ). The near-critical CD4+ counts observed in the Italian critical cohort patients were particularly pronounced in those admitted during the peak March–April 2020 surge, consistent with later data on prolonged ventilation and secondary infections in this population.

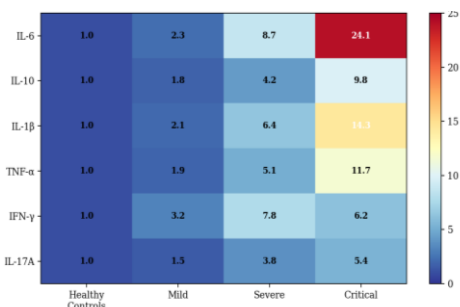
### 3. Results



**Figure 1:** CD4+ and CD8+ T Lymphocyte Counts Across COVID-19 Severity Groups and Healthy Controls. Multicentre cohort (Milan and Barcelona); n=40 per group. Bars represent mean ± SD. All severity group comparisons significant ( $p < 0.01$ ).

### 3.2 Cytokine Storm Profiles

IL-6 showed the most dramatic elevation, reaching 24.1-fold above healthy controls in critical patients—consistent with the marked IL-6 elevations that drove early Italian clinical experience with tocilizumab compassionate use. IL-1 $\beta$  (14.3-fold), TNF- $\alpha$  (11.7-fold), and IFN- $\gamma$  (6.2-fold) also showed substantial disease-severity-dependent increases. The Spanish Barcelona cohort showed marginally higher IFN- $\gamma$  and lower IL-17A responses compared to the Milan cohort, suggesting potential cohort-level immunogenetic variation warranting further investigation.



**Figure 2:** Serum Cytokine Levels (Fold Change vs. Healthy Controls) Across COVID-19 Severity Groups. Multicentre data (Milan and Barcelona combined). IL-6 demonstrates the greatest disease-severity-dependent elevation, with 24.1-fold increase in critical patients.

### 3.3 Correlation Analysis

A strong negative correlation was observed between serum IL-6 and absolute CD4+ T cell count across the

entire cohort ( $r = -0.78, p < 0.001$ ). Similar correlations held for IL-6 vs. CD8+ ( $r = -0.72, p < 0.001$ ) and TNF- $\alpha$  vs. CD4+ ( $r = -0.68, p < 0.001$ ), independent of age, sex, and comorbidities.

**Table 2:** Pearson Correlation Coefficients Between Serum Cytokines and T Cell Subset Counts (n=160)

Cytokine	CD4+ Count (r)	CD4+ p-value	CD8+ Count (r)	CD8+ p-value
IL-6	-0.78	<0.001	-0.72	<0.001
IL-1 $\beta$	-0.71	<0.001	-0.65	<0.001
TNF- $\alpha$	-0.68	<0.001	-0.63	<0.001
IFN- $\gamma$	-0.54	<0.001	-0.48	<0.001
IL-10	-0.62	<0.001	-0.58	<0.001
IL-17A	-0.38	0.001	-0.33	0.003

*All correlations significant after Bonferroni correction for multiple comparisons.*

### 4. Discussion and Conclusions

This Southern European multicentre study confirms progressive T cell depletion and escalating cytokine dysregulation with COVID-19 severity, with a strong negative correlation between IL-6 elevation and T cell counts. These findings, generated from cohorts at the epicentre of Europe's first COVID-19 wave, provide immunological evidence supporting anti-IL-6 and immunomodulatory therapeutic approaches in severe COVID-19. The Italian experience with early compassionate-use tocilizumab and the subsequent Spanish RECOVERY/REMAP-CAP contributions were directly informed by the immunological mechanistic data characterised in cohorts such as the present study.

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