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Prospective multicentre study of upper respiratory mucosal transcriptomics reveals two major endotypes of critically ill COVID-19 patients

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Abstract

Background Severe COVID-19 is associated with dysregulated immune responses. Immune responses heterogeneity was previously reported during the first waves of the pandemic. We aimed to characterise mucosal transcriptomic profiles in critically-ill patients during the Omicron era.

Methods This prospective multicentre study included 94 critically-ill COVID-19 patients between May 2022 and August 2023. Upper respiratory tract mucosal transcriptomes were obtained from nasopharyngeal swabs and clustered based on KEGG cytokine-cytokine receptor interaction pathways using an unsupervised algorithm. Differential transcript expression, cell population abundance and gene set enrichment analyses were performed.

Results Here we show that in 56 critically ill COVID-19 patients, transcriptomic clustering reveals two distinct COVID-19 Immune Transcriptomic Respiratory Profiles (CITRP), including CITRP-1 and CITRP-2, characterised by differential expression of cytokine and immune response pathways. Patients in the CITRP-2 group display a more pronounced immune and inflammatory response, involving specific innate immune pathways, neutrophil degranulation and T-helper 2 cytokines (e.g., IL-1, IL-4 and IL-13), and a significantly higher proportion of neutrophils than patients in the CITRP-1 group. No significant differences are observed between the two transcriptomic clusters in clinical, biological and virological characteristics at ICU admission or in patient outcomes.

Conclusions This study highlights the heterogeneity of the immune response in critically-ill COVID-19 patients in the Omicron era, identifies two endotypes from the analysis of upper airway mucosal transcriptomics. Our findings suggest the existence of two distinct pathogenic mechanisms and the detrimental role of neutrophil and Th2 helper cell-mediated inflammation in a subset of patients with severe disease. They support the need for personalised treatment strategies targeting neutrophil-mediated lung damage and/or specific cytokine production in a subset of critically-ill COVID-19 patients.

Plain language summary

Severe forms of COVID-19 are associated with an immune response that behaves irregularly. While previous studies have demonstrated that this response can vary significantly between patients, most of this research was conducted prior to the emergence of the Omicron variant. The aim of this study was to explore whether this diversity in immune responses also exists in critically ill patients infected with Omicron. We analysed the immune systems in samples taken from the upper airways of critically ill patients and grouped them based on similarities in their immune response. Two distinct categories of immune response were identified. One group showed a stronger immune response and more inflammation, but no clear differences were observed in how symptoms or disease progression presented between the two groups. These findings emphasise the diversity of immune responses in critically ill patients infected with the Omicron variant, highlighting the need for more personalised treatment strategies.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which emerged in late 2019, is a highly transmissible and pathogenic human coronavirus responsible for Coronavirus Disease 2019 (COVID-19)[1]. Its severity has been attributed mainly to pulmonary involvement, potentially leading to acute hypoxemic respiratory failure requiring intensive care unit (ICU) admission[2]. In addition to the inherent cytopathic effects of the virus, severe cases are driven by a complex and dysregulated immune and inflammatory response, characterised by a lack of coordination between the innate and adaptive immune systems[3].

Since late 2020, SARS-CoV-2 variants of concern (VOC) have emerged and circulated widely[4]. These VOCs differ by amino acid substitutions located mainly in the spike protein, some of which confer competitive

e-mail: pierre.bay@aphp.fr

advantages to the virus, such as increased transmissibility, enhanced replication efficiency, or immune evasion[4]. This genetic evolution has been accompanied by a clinical phenotypic shift in patient populations, particularly during the Omicron era, with a higher proportion of immunocompromised patients[5]. According to the CDC, COVID-19 hospitalisation rates and disease severity in 2024 remained at least as high as other major seasonal respiratory viruses such as influenza and RSV (https://www.cdc.gov/respiratory-viruses/).

Current pharmacological management of patients hospitalised with severe or critical SARS-CoV-2 Omicron-related pneumonia is predominantly based on systemic corticosteroid therapy with dexamethasone, the use of IL-6 receptor blockers and baracitinib[6]. However, a number of studies have shown that a standardised pharmacological approach may not be the optimal strategy[7], suggesting the need for more personalised approaches[8], particularly those based on the patient's immune profile[7,9]. Transcriptomic analysis of the upper respiratory tract mucosa provides a robust[10] and promising approach to investigate the heterogeneity of the immune response in patients infected with respiratory viruses[11], in particular SARS-CoV-2[12,13].

Several studies have previously investigated the heterogeneity of the clinical phenotype and the immune response during SARS-CoV-2 infection in different contexts, including in critically ill patients during the early waves of the pandemic[14–16] and in the general population following infection[17]. Omics approaches, including transcriptomics, proteomics, and metabolomics, have increasingly been used to stratify COVID-19 patients[18,19], particularly those requiring ICU admission[16], and more broadly to phenotype complex syndromes in the context of precision medicine[20]. However, to the best of our knowledge, no study has specifically investigated this heterogeneity in patients admitted to the ICU with critical COVID-19 during the Omicron era.

The aim of this work was to identify endotypes using transcriptomics of the upper respiratory mucosal immune response in clinically homogeneous patients admitted to the ICU with critical SARS-CoV-2 pneumonia, in order to identify potential targets for specific therapeutic intervention. Based on upper respiratory tract mucosal transcriptomic analysis, we identify two distinct immune transcriptomic respiratory profiles in critically ill patients infected with Omicron. One cluster is characterised by a more pronounced immune and inflammatory response, involving innate immune pathways, neutrophil degranulation, and T helper 2–associated cytokines, while the other exhibits a more attenuated immune profile. These transcriptomic profiles are not associated with significant differences in clinical presentation or outcomes. Together, these findings emphasise the heterogeneity of the immune response to severe SARS-CoV-2 infection during the Omicron era, indicating the presence of different pathogenic mechanisms and supporting the development of more personalised therapeutic strategies.

Methods

Patients and clinical data

This study is a prospective multicenter observational cohort study. Patients admitted between May 1st, 2022 and August 31st, 2023 to one of the 36 participating ICUs (including 19 in the Greater Paris area) were eligible for inclusion in the SEVARVIR cohort study (NCT05162508) if they met the following inclusion criteria: age ≥18 years, SARS-CoV-2 infection confirmed by a positive reverse transcriptase-polymerase chain reaction (RT-PCR) in nasopharyngeal swab samples (NPS), admission in the ICU for acute respiratory failure (i.e., peripheral oxygen saturation (SpO2) ≤ 90% and need for supplemental oxygen or any kind of ventilator support), patient or next of kin informed of study inclusion. Patients with SARS-CoV-2 infection but without acute respiratory failure or with a RT-PCR cycle threshold (Ct) value > 32 in NPS were not included. The study was approved by the Comité de Protection des Personnes Sud-Méditerranée I (N° EudraCT/ID-RCB: 2021-A02914-37). Informed consent was obtained from all patients or their relatives. The NPS were stored at −80 °C.

Demographics, clinical and laboratory variables were recorded upon ICU admission and during their stay in ICU. Patients' frailty was assessed using the Clinical Frailty Scale[21]. The disease severity at ICU admission was evaluated using the World Health Organisation (WHO) 10-point ordinal scale[22], the Sequential Organ Failure Assessment (SOFA) score[23], and the Simplified Acute Physiology Score (SAPS) II score[24]. Acute respiratory distress syndrome (ARDS) was defined in accordance with the Berlin definition[25].

SARS-CoV-2 variant identification

The ull-length SARS-CoV-2 genomes from all the patients included in the study were sequenced using next-generation sequencing. Briefly, viral RNA was extracted from NPS in viral transport medium using the NucliSENS® easyMAG kit on an EMAG device (bioMérieux, Marcy-l'Étoile, France). Sequencing was performed using Illumina® COVIDSeq Test (Illumina, San Diego, California), which employs 98-target multiplex amplifications along the entire SARS-CoV-2 genome. Libraries were sequenced using the NextSeq 500/550 High Output Kit v2.5 (75 Cycles) on a NextSeq 500 instrument (Illumina). The sequences were demultiplexed and assembled as full-length genomes using the DRAGEN COVIDSeq Test Pipeline on a local DRAGEN server (Illumina). Lineages and clades were interpreted using Pangolin and NextClade, before submission to the international GISAID database (https://www.gisaid.org).

RNAseq procedure

The original in-house ISO15189-accredited shotgun metagenomics procedure used in this study (MetaMIC) has been described previously[12,26]. Briefly, a pre-extraction combining mechanical, enzymatic and chemical procedures was performed prior to extraction using the QIAsymphony DSP DNA Midi Kit on a QiaSymphony instrument (Qiagen, Hilden, Germany). The extracts were used for library preparation using the TruSeq Stranded Total RNA kit (Illumina, San Diego, California) and for pair-end sequencing using the NovaSeq6000 S1 reagent Kit v1.5 (300 cycles) on a NovaSeq6000 instrument (Illumina).

Statistics and Reproducibility

The data were filtered based on quality (phred score) and the sequences were mapped against several custom databases derived from the NT database. Human sequences were counted and collected in a separate Fastq file. Transcript expression analysis was performed according to previously published guidelines[27]. Briefly, after quality filtering with Trimmomatic v0.39, RNA transcripts were aligned to the GRCh38 (release 84) human genome using HISAT2 v2.2.1[28]. Expression levels of all genes were estimated using Stringtie v2.1.6[29]. Differential expression was calculated using gene count data and DESeq2 version 1.30.1[30] in the Rstatistical programming environment. The Wald test combined with Benjamini and Hochberg procedure was used to test the significance of the differences in transcript expression. Statistically significant differential expression was defined as an adjusted p-value < 0.05 and an absolute $\log_2$ (fold change) >1.5.

An unsupervised analysis was conducted on the KEGG pathway "Cytokine-cytokine receptor interaction" (hsa04060). Clustering of respiratory transcriptomic profiles was performed using hierarchical clustering with the R package pheatmap (version 1.0.7). Euclidean distances were calculated to compute the dissimilarity matrix, and the hierarchical clustering was performed using the Ward's clustering algorithm, a standard and appropriate approach for clustering analysis in moderately sized cohorts[31]. The number of clusters was set at two, as this was identified as the optimal choice for clustering based on the analysis.

Principal component and heatmap analyses were performed with RStudio software (version 4.2.0). Differentially expressed genes with Benjamini-Hochberg adjusted p-values below 0.05 were used as input to perform gene set enrichment analysis (GSEA) using the STRING database (https://string-db.org/)[32], with Reactome canonical pathways and Gene

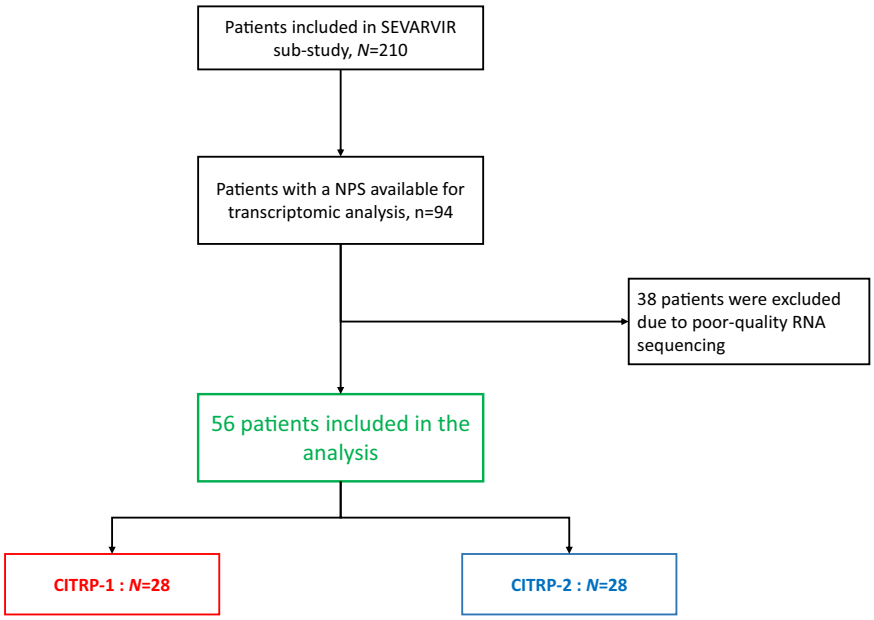

Fig. 1 | Study flow chart. NPS nasopharyngeal swab.

Ontology as the pathway databases. A normalised gene count was used to identify enriched signalling pathways. MCP-counter was used to determine the relative abundance of immune cell populations[33]. Descriptive results are presented as means (± standard deviation [SD]) or medians (1st–3rd quartiles) for continuous variables, and as numbers with percentages for categorical variables. Two- sided p values < 0.05 were considered statistically significant. Unadjusted comparisons by transcriptome profile group were performed using chi-squared or Fisher's exact tests for categorical variables, and t-tests or Mann-Whitney tests for continuous variables, as appropriate. Correlations between continuous variables were tested using Spearman's correlation. Sensitivity analyses were performed in the following subpopulations: (i) patients under 70 years old; (ii) patients above or over 70 years old; (iii) patients with a WHO 10-point scale <7 and (iv) patients with a WHO 10-point scale ≥ 7. Analyses were performed with RStudio software (version 4.2.0).

Results

Patient selection and clustering

Over the study period, a total of 210 patients were admitted to one of the 36 participating ICUs and enroled in the study. Of these, 94 patients had an NPS stored at −80 °C for transcriptomic analysis, of whom 38 were excluded from the study because the number of high-quality RNA transcripts was considered insufficient ($\leq 10^5$ and/or ≤3000 protein-coding genes identified), leaving 56 patients for the current analysis (Fig. 1). The 298 genes of the KEGG pathway "Cytokine-cytokine receptor interaction" (hsa04060) were used for hierarchical clustering. They divided the cohort into two mutually exclusive groups named "CITRP" (for COVID-19 Immune Transcriptomic Respiratory Profile): CITRP-1 ($N = 28$) and CITRP-2 ($N = 28$) (Supplementary Fig. S1). Supplementary Fig. S2 shows the unsupervised transcriptome analysis of the whole cohort, plotted by principal component analysis according to the clustering groups.

Differences between respiratory transcriptomic profiles

We evaluated the differences in gene expression according to the transcriptomic profiles (CITRP-1 vs. CITRP-2 patients). Using an adjusted p-value cut-off point of 0.05, we identified 127 differentially expressed genes (Fig. 2A). Among the 298 genes of the KEGG pathway "Cytokine-cytokine receptor interaction" (hsa04060), 12 were significantly more expressed in the CITRP-2 group (Figs. 2A and 2B), including genes related to IL-1 (e.g., *IL1RN, IL1B, IL1R2*), IL-8, IL-10 (e.g., *CXCL8, TNFRSF1B*) and IL-4 and IL-13 (e.g., *IL4R, OSM, CXCL8*) signalling pathways. The gene with the largest expression difference between CITRP-1 and CITRP-2 was CXCL8, which encodes IL-8.

GSEA identified several biological processes enriched in CITRP-2 compared to CITRP-1 patients, involved in the immune and inflammatory responses, including: (i) innate immune pathways including neutrophil degranulation, phagocytosis and Toll Like Receptors signalling cascade; (ii) cytokine signalling pathways, including IL-4/IL-13, IL-1 and IL-10 pathways (Fig. 3A); and (iii) adaptive immunity including antigen processing-cross presentation and ER-phagosome pathway. The expression of pathways associated with interferon responses was not significantly different between the two groups. Figure 3 shows the network combining pathways and genes with a statistically significant differential expression between the CITRP-1 and CITRP-2 groups involving innate (Fig. 3B) and adaptive (Fig. 3C) immunity, respectively. Supplementary Fig. S3 shows the top 30 enriched biological processes in CITRP-2 group using Gene Ontology analysis. The expression of the differentially expressed transcripts in the neutrophil degranulation and cytokine signalling pathways are shown in the heatmaps displayed in Fig. 4A and Fig. 4B, respectively.

Transcriptomics-based quantification of immune cells

We used MCPcounter to estimate the proportions of immune and epithelial cell types in the NPS based on gene expression signatures[33]. The scores for the different cell lineages according to cluster type are shown in Fig. 5A. The estimated relative abundance of cytotoxic T lymphocytes was significantly higher in patients from the CITRP-1 group compared to the CITRP-2 group ($p = 0.044$). Conversely, the estimated relative abundance of neutrophils was significantly higher in patients from the CITRP-2 group compared to the CITRP-1 group ($p < 0.001$). The expression of the different transcripts associated with neutrophil and cytotoxic T lymphocyte lineages is shown in the heatmap in Fig. 5B. No significant correlation was found between the blood lymphocyte count at ICU admission and the estimated relative abundance of different lymphocyte lineages in the upper airway mucosa (Supplementary Fig. S4).

Baseline characteristics and outcome

Comparison of baseline characteristics at ICU admission between the two clusters, CITRP-1 and CITRP-2, showed no statistically significant differences in age, sex, comorbidities, vaccination status, Omicron sublineage or

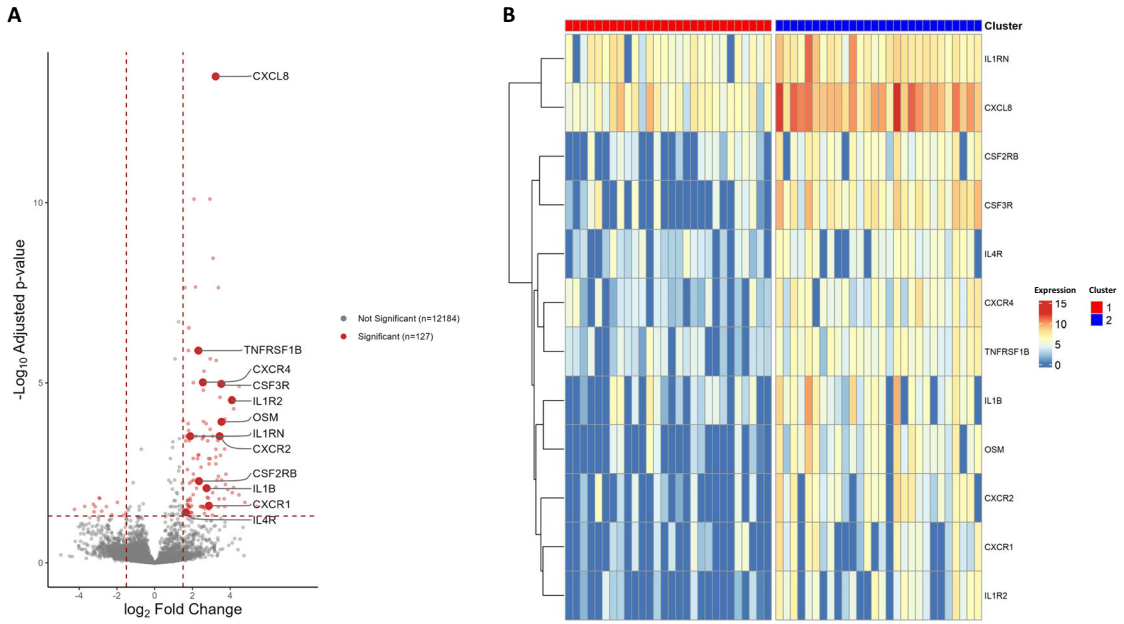

Fig. 2 | Differentially expressed transcripts between COVID-19 respiratory transcriptomic profile (CITRP). A Volcano plot showing the fold change for each transcript and its significance level. Genes with an adjusted two-sided p-value < 0.01 and an absolute $\log_2$ (fold change) >1.5 are coloured in red. The 12 transcripts with a significant differential expression among 298 genes of the KEGG pathway "Cytokine-cytokine receptor interaction" (hsa04060) are shown as bold red circles and labelled on the figure. **B** Heatmap analysis of the 12 transcripts with a significant differential expression (i.e., with an adjusted two-sided p-value < 0.05 and an absolute $\log_2$ (fold change) >1.5) between CITRP-1 and -2.

severity of illness at ICU admission, as reflected by the SOFA and SAPS II scores and the WHO 10-point ordinal scale (Table 1), highlighting the fact that our cohort of critically ill patients was clinically homogeneous. The only baseline difference observed was a significantly lower blood lymphocyte count at ICU admission in patients in the CITRP-2 group compared to those in the CITRP-1 group (0.300 G/L [0.175; 0.500] vs 0.800 G/L [0.450; 1.150], $p < 0.01$). No significant differences were observed for other biological variables at ICU admission. During the ICU stay, there was no significant difference in the management of organ failure symptoms or patient prognosis between the two identified clusters (Table 2). Notably, one third of patients required invasive mechanical ventilation and day 28 mortality was 30% ($N = 17/56$). A sensitivity analysis was performed on patients aged under 70 (Supplementary table 1) and showed that patients in the CITRP-2 group were significantly more likely to be infected with the BA.2 sublineage than those in the CITRP-1 group ($N = 9/15$ (60%) vs $N = 3/13$ (23%), $p = 0.049$). No other significant differences were observed in baseline characteristics or biological variables at ICU admission, management, or outcome during the ICU stay (Supplementary table 2). Sensitivity analyses conducted on patients aged ≥ 70 years (Supplementary tables 3 and 4), with a WHO 10-point scale <7 (Supplementary tables 5 and 6), and patients with a WHO 10-point scale ≥ 7 (Supplementary tables 7 and 8) also showed no significant differences between the two clusters.

Discussion

During the first waves of the COVID-19 pandemic, the severity of COVID-19-associated pneumonia was shown to be partly due to an exaggerated and inappropriate immune response[3], which could be controlled to some extent by immunomodulatory treatments, such as dexamethasone[34] and/or tocilizumab[35]. Several studies have highlighted the heterogeneity of the immune and inflammatory responses in patients admitted to the ICU for COVID-19[14–16], which may affect the efficacy of the aforementioned therapies[7,9]. These studies were mainly conducted prior to the Omicron era, during which changes were observed in both the clinical phenotype of patients[5] and the associated immune responses[13]. This study investigates the heterogeneity of airway mucosal immune responses in critically ill patients in the current Omicron era.

A variety of clustering methods have previously been used to explore homogeneous phenotypes in critically ill COVID-19 patients[7,9,14]. In our study, the clustering approach based on mucosal transcriptomic analysis has several notable advantages, including its non-invasive nature (unlike bronchoalveolar lavage, for example), its relevance given the compartmentalised immune response described in COVID-19[36], and the established power of respiratory transcriptomics using NPS for immune profiling[10,12,13]. In addition, the choice to perform clustering on the KEGG pathway "Cytokine-cytokine receptor interaction" (hsa04060) was intended to direct the clustering towards the immune and inflammatory response, which was the primary objective of this study. Using this approach, we identified two major respiratory transcriptome clusters, CITRP-1 and CITRP−2, which differed significantly in terms of cytokine signalling and innate and adaptive immune responses, despite having similar clinical profiles.

Innate immunity plays a critical role in combating SARS-CoV-2 infection[37], through several signalling pathways, including the TLR2 pathway, the activation of which leads to inflammasome activation and the release of pro-inflammatory cytokines such as IL-1β. Interestingly, our analyses revealed a differential expression of this pathway between the two clusters identified: IL-1β was among the over-expressed transcripts in the CITRP-2 group, as was the "MyD88:MAL (TIRAP) cascade initiated on plasma membrane" signalling pathway, a MyD88 adaptor-like adaptor, the first known downstream component of TLR4 and TLR2 signalling[38]. Both TLR2 and MYD88 overexpression have been associated with disease severity in COVID-19 and are thought to trigger the cytokine storm. In addition, blocking TLR2 signalling in vivo provided protection against the severity of SARS-CoV-2 infection[39]. The IL-1 pathway is pro-inflammatory and has been implicated in both inflammatory and immunological disorders, as well as in the response to infection[40]. In particular, IL-1β appeared to be the main factor explaining the differences observed between the two groups. Its transcription results from the activation of the NLRP3 inflammasome, whose pathogenicity has been well documented during SARS-CoV−2 infection[37]. Although evaluated in several therapeutic trials, IL-1 inhibitors are not yet part of the conventional therapy for COVID-19[6].

Neutrophil degranulation is another important component of innate immunity and intracellular pathogen clearance, through the fusion of

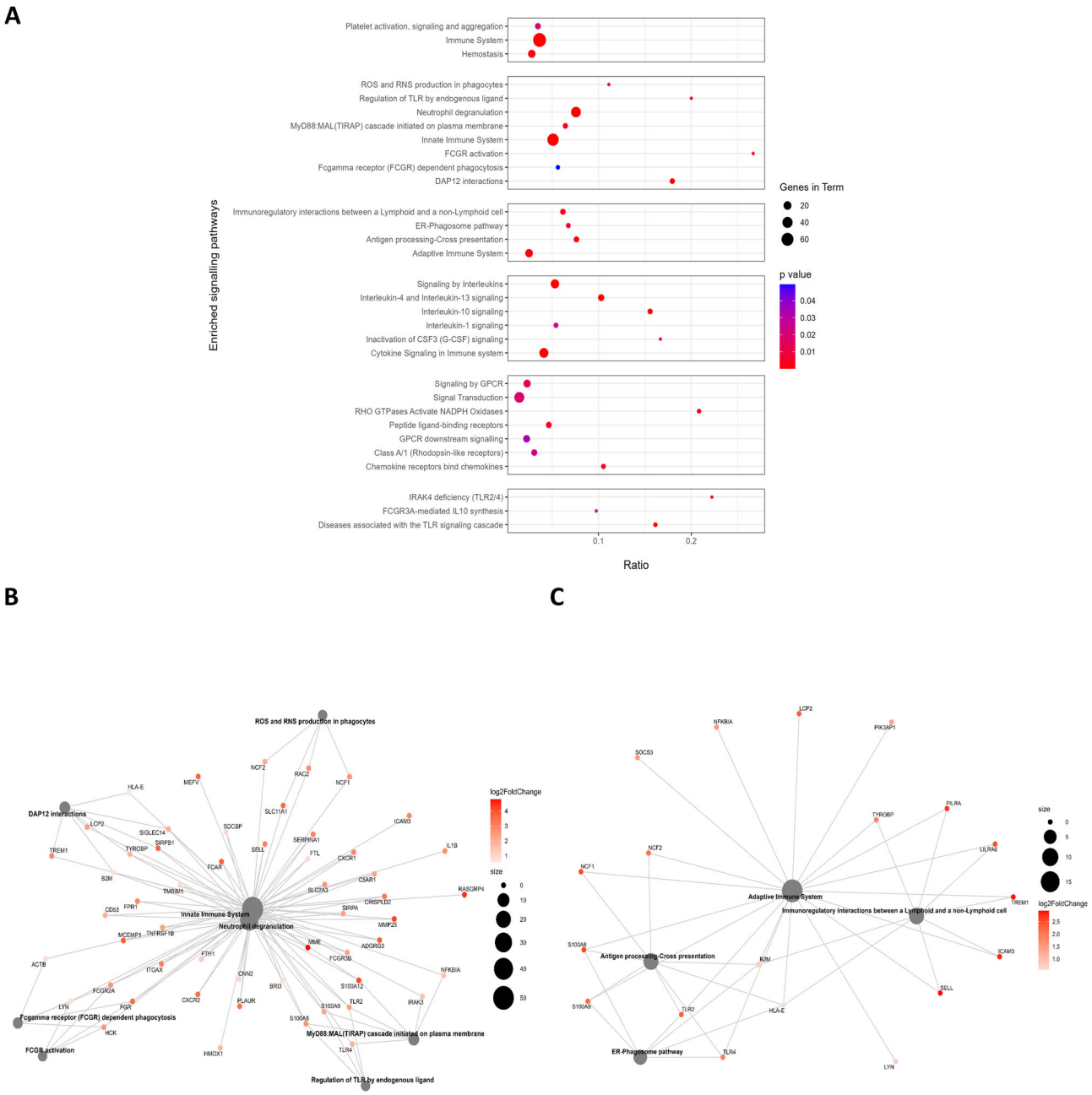

Fig. 3 | Gene set enrichment analysis using the STRING database with Reactome canonical pathways as the pathway database. A dot plot of GSEA showing the enriched pathways. **B**,**C** Networks combining pathways and transcripts with differential expression between clusters, involving **B** innate immunity and **C** adaptive immunity.

granules and phagosomes. However, the neutrophil response can also act as a "double-edged sword". Dysregulated neutrophil degranulation can be pathogenic by releasing toxic granule contents and generating neutrophil extracellular traps (NETs) with potentially associated tissue damage[41,42]. Notably, infection with the Omicron variant was associated with lower NET levels compared with other SARS-CoV-2 strains[43]. In our study, the neutrophil degranulation pathway was significantly enriched in the CITRP2 group, suggesting that neutrophils play a central role in a subset of Omicron-infected patients with acute respiratory failure (i.e., the CITRP2 group), a finding consistent with their previously described role during the early stages of the pandemic[12,42].

In addition, the cytokine with the greatest differential expression between the two groups was CXCL8, a chemokine that acts on CXCR1 and CXCR2 receptors[44]. CXCL8 is a pro-inflammatory chemokine that is considered to be one of the major chemoattractants for neutrophils[44]. Interestingly, previous studies have also shown that elevated levels of CXCL8 correlate with the severity of SARS-CoV-2 infection[12,45]. In addition to the data on enriched pathways identified in our study, evaluation of the abundance of various immune cell lineages with use of MCP counter revealed a significantly higher proportion of neutrophils in the CITRP-2 group. This finding was consistent with the hypothesis of a stronger immune response, particularly a more intense innate response, and highlighted the critical role of CXCL8 in the recruitment of these neutrophils.

Our results also highlighted differences in the Th2-mediated inflammatory response in the upper airway mucosa, with the expression of the IL4/13 pathway being more pronounced in patients from the CITRP-2 group. Th2 has been implicated in the pathophysiology of COVID-19, particularly in a study investigating the inflammatory response in the nasal mucosal[46]. Post-mortem lung analysis of patients with COVID-19 has shown increased expression of IL-4 and M2 macrophages, while IL-13 has been associated

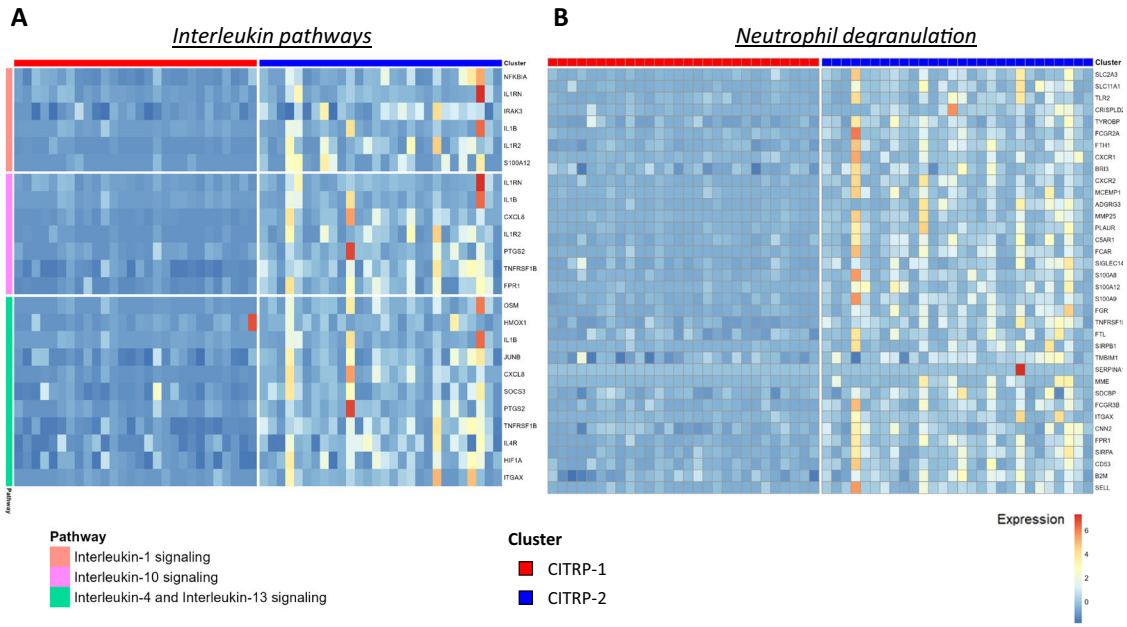

Fig. 4 | Heatmap analysis of the differentially expressed transcripts between COVID-19 respiratory transcriptome profiles-1 and -2, involving **A** interleukins pathways and **B** neutrophil degranulation.

with the need for mechanical ventilation in clinical trials[47]. The IL-10 pathway, classically known for its anti-inflammatory and immunoregulatory properties[48], was also found to be enriched in the CITRP2 group. Interestingly, several studies have shown that critically ill COVID-19 patients with ARDS caused by SARS-CoV−2 have elevated levels of IL-10[49], with prognostic implications. These findings suggest a paradoxical pro-inflammatory activity (in particular by activating CD8 + T lymphocytes and mast cells) and/or an impairment of its anti-inflammatory capacity (i.e., IL-10 "resistance")[50].

Several studies, summarised in Table 3, have investigated the impact of different SARS-CoV-2 variants on the host immune response[13,51–57]. Overall, these studies suggest that the Omicron variant elicits a different immune response to earlier variants (i.e., ancestral, Alpha, and Delta). In vitro and ex vivo studies generally show consistent results, indicating that Omicron sublineages, particularly the early sublineages BA.1 and BA.2, are associated with reduced innate immune evasion[52–57]. This results in stronger innate and interferon responses, as well as reduced viral replication, particularly in the lungs. In vivo data, particularly from critically ill patients, are scarce and difficult to interpret. One study found that the respiratory immune response, including interferon signalling and neutrophil degranulation, was more pronounced in patients infected with the Omicron variant than in those infected with earlier variants[13]. Another study found that patients infected with the Omicron variant exhibited a lower systemic inflammatory response, yet a stronger T-cell response[51]. Taken together, these findings suggest that an Omicron infection may be characterised by a more effective local mucosal immune response, thereby limiting an excessive systemic inflammatory reaction. However, it is particularly challenging to assess the adaptive immune response because of the substantial influence of pre-existing immunity, whether induced by a previous infection or vaccination. In our study, the transcriptomic profile was not associated with a specific sublineage, except in the sensitivity analysis conducted on younger patients. In this subgroup, patients in the CITRP-2 cluster (i.e., those with a stronger inflammatory signature) were significantly more frequently infected with the BA.2 sublineage than with XBB sublineages. This finding is consistent with previous reports indicating that only the early Omicron sublineages (i.e., BA.1 and BA.2) exhibit reduced innate immune evasion, resulting in stronger interferon and pro-inflammatory responses compared to both pre-Omicron variants and later Omicron sublineages[52].

Patients in the CITRP-1 and CITRP-2 groups did not have different clinical phenotypes or clinical outcomes. This may be explained by the fact that our population was homogeneously composed of individuals with very severe disease in the ICU. Relationships between disease severity and transcriptomic profiles might be easier to find by including additional groups of patients with less severe disease. The limited sample size of our study may also account for these results. Our findings highlight the complexity of identifying biomarkers associated with prognosis in this population with very severe disease. They also highlight the added value of transcriptomic analysis in capturing the complexity of the immune response and providing insights beyond traditional clinical and biological assessments. In the future, our findings will need to be validated in larger, more clinically diverse cohorts to stratify patients based on their immune profiles and to identify panels of biomarkers that could inform personalised treatment strategies. Indeed, the heterogeneity of the immune response observed in our study reinforces the notion that a "one-size-fits-all" therapeutic approach will not be sufficient[7–9], particularly in the setting of ICU patients. As the immune response to SARS-CoV-2 varies between individuals, a more personalised approach, guided by the identification of biomarker candidates (e.g., IL-8, NETs, Th2 inflammatory biomarkers …) combined in endotypes could help tailor interventions to the specific needs of each patient[58]. Transcriptomic profiling will also remain necessary in research settings, given the continuous evolution of SARS-CoV-2 variants that may continue to influence the immune responses and clinical outcomes[5,13]. Furthermore, additional data is required to assess the specific impact of therapeutic interventions and co-infections on the immune response of critically ill patients with COVID-19.

Our study has limitations. First, several patients could not be analysed due to poor sequencing quality, probably due to the choice of the procedure (i.e., NPS). The exclusion of these patients may have introduced a selection bias that may have affected the generalisability of our findings, despite the fact that our study was prospective and multicentre study. The relatively

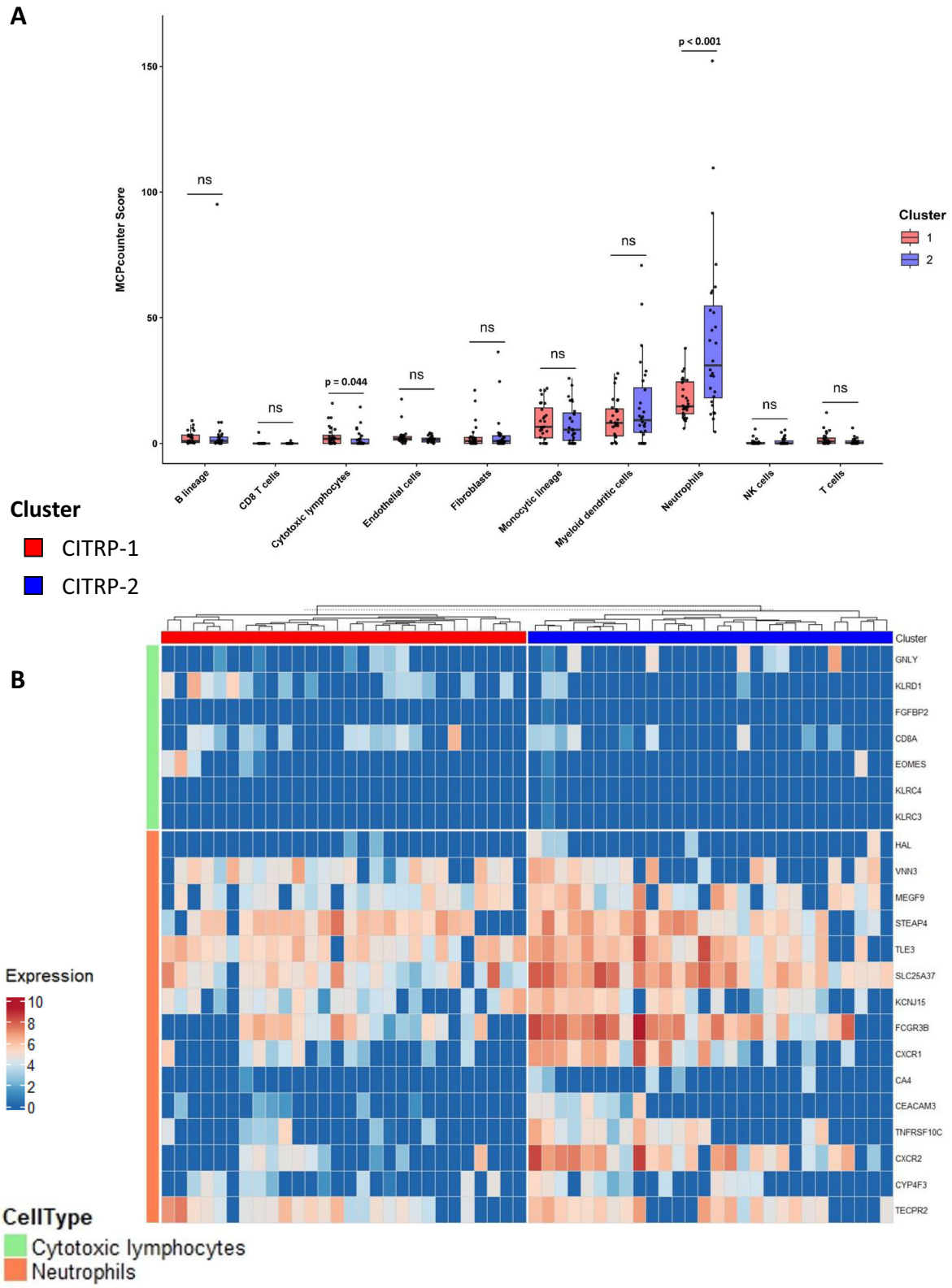

Fig. 5 | A Relative abundance (immune score) of cell lineages according to COVID-19 respiratory transcriptome profile status, as estimated from transcriptomics data using MCP-counter. The y-axis shows MCP-counter scores for each cell lineage. The data distribution is shown by box plots, each point represents one patient. Two-sided p-values were calculated using Wilcoxon's rank test: ns, not significant. **B** Heatmap analysis of the transcripts involved in neutrophil and cytotoxic T lymphocyte MCP-counter estimation.

Table 1 | Characteristics of patients on admission to the intensive care unit according to CITRP status

Variable	CITRP-1, n = 28	CITRP-2, n = 28	P
Demographics and comorbidities			
Sex, females	9 (32)	12 (43)	0.41
Age, years	71 [61–75]	69.7 [62.1; 74.6]	0.48
Diabetes	15 (54)	18 (67)	0.32
Obesity	5 (19)	6 (22)	0.38
Chronic heart failure	2 (7.1)	3 (11)	0.67
Hypertension	17 (61)	16 (59)	0.91
Chronic respiratory failure	6 (21)	10 (37)	0.2
Chronic renal failure	8 (29)	5 (19)	0.38
Cirrhosis	1 (3.6)	0 (0)	1
Immunosuppression	11 (39)	12 (43)	0.92
None	17 (61)	16 (57)	
Solid organ transplant	5 (18)	4 (15)	
Onco-haematological malignancies	4 (14)	5 (19)	
Others[1]	2 (7.1)	3 (11)	
Number of comorbidities	2.00 [1.00; 3.00]	2.00 [1.00; 3.00]	0.98
Clinical frailty scale	4.00 [3.00; 4.75]	3.00 [2.00; 4.25]	0.4
SARS-CoV-2 infection and Vaccination			
Previous SARS-CoV-2 infection	3 (11)	2 (8.3)	1
SARS-CoV-2 vaccination	26 (96)	24 (92)	0.61
SARS-CoV-2 serology at ICU admission			0.68
Unavailable	17 (61)	17 (61)	
Negative[2]	4 (14)	2 (7.1)	
Positive	7 (25)	9 (32)	
First symptoms-ICU admission, days	6.00 [3.00; 8.50]	5.00 [2.50; 10.0]	0.86
SARS-CoV-2 RNA detection in nasopharyngeal swabs, Ct	18.0 [16.0; 22.0]	18.0 [16.0; 20.5]	0.88
SARS-CoV-2 Omicron lineage			0.41
BA.2	9 (32)	12 (43)	
XBB	19 (68)	16 (57)	
Patients severity upon ICU admission and biological features			
WHO 10-point scale	6.00 [6.00; 6.25]	6.00 [6.00; 6.25]	0.98
SAPS II score	42.5 [34.0; 60.0]	39.0 [32.0; 43.8]	0.35
SOFA score	4.50 [3.25; 8.75]	4.00 [2.75; 6.25]	0.46
PaO2/FiO2 ratio, mmHg	147 [109; 192]	154 [78.8; 250]	0.8
Arterial lactate level, mM	1.45 [1.12; 2.20]	1.50 [1.00; 2.15]	0.62
Blood leucocytes, G/L	8.85 [6.60; 13.2]	11.2 [6.62; 14.8]	0.67
Blood lymphocytes, G/L	0.800 [0.450; 1.15]	0.300 [0.175; 0.500]	**<0.01**
Blood platelets, G/L	176 [123; 221]	206 [134; 292]	0.25
Serum urea level, mM	9.00 [5.00; 14.5]	7.50 [6.00; 17.2]	0.58
Serum creatinine level, µM	92.5 [56.8; 155]	77.0 [53.0; 185]	0.85
Oxygen/ventilatory support			0.92
Oxygen	3 (11)	4 (14)	
High flow oxygen	12 (43)	13 (46)	
NIV/C-PAP	6 (21)	4 (14)	
Invasive MV	7 (25)	7 (25)	
Vasopressor support	6 (23)	6 (21)	0.88
Bacterial co-infection	7 (25)	5 (18)	0.51

Results are N (%), means (± standard deviation) or medians (interquartile range, i.e., quartile 1; quartile 3).
CITRP COVID-19 respiratory transcriptomic profile, *ICU* intensive care unit, *Ct* cycle threshold, *WHO* World Health Organisation, *SOFA* Sequential Organ Failure Assessment, *SAPS II* Simplified Acute Physiology Score II, *NIV* non-invasive ventilation, *C-PAP* continuous-positive airway pressure, *MV* mechanical ventilation.
[1]Includes HIV infection, long-term corticosteroid treatment, and other immunosuppressive treatments.
[2]Defined as <30 Binding Antibody Units (BAU)/mL
Two-tailed p-values come from unadjusted comparisons using Chi-square or Fisher's exact tests for categorical variables, and t-tests or Mann–Whitney tests for continuous variables, as appropriate. No adjustment for multiple comparisons was performed. Bolded p-values are significant at the $p < 0.05$ level.

Table 2 | Management and outcomes of patients with severe SARS-CoV-2 infection during intensive care unit stay according to the CITRP status

Variable	CITRP-1, n = 28	CITRP-2, n = 28	p
Invasive MV	9 (32)	11 (39)	0.58
Prone positioning	2 (7.7)	7 (28)	0.075
MV duration, days	9.00 [5.75; 14.8]	12.0 [3.50; 25.0]	0.93
Live-ventilator-free days at day-28	28.0 [0; 28.0]	27.5 [0; 28.0]	0.87
Vasopressor support	8 (29)	9 (32)	0.77
Duration of vasopressors, days	1.00 [0; 4.00]	1.00 [1.00; 6.00]	0.52
Renal Replacement Therapy	4 (14)	7 (25)	0.31
Ventilator-acquired pneumonia[a] (among IMV)	5 (62)	2 (18)	0.074
Number of VAP episodes Median (IQR)	1.00 [0; 1.00]	0 [0; 0]	0.12
Dexamethasone	13 (52)	13 (59)	0.63
Tocilizumab	4 (14)	2 (7.1)	0.67
Monoclonal antibodies	2 (7.4)	0 (0)	0.24
Day-28 mortality	9 (32)	8 (29)	0.77
Duration of ICU stay, days	6.50 [4.00; 13.2]	9.50 [6.75; 22.2]	0.17

Results are *N* (%), means (± standard deviation) or medians (interquartile range, i.e., quartile 1; quartile 3). *ICU* intensive care unit, *CITRP* COVID-19 Immune Transcriptomic Respiratory profile, *MV* mechanical ventilation, *VAP* ventilator-acquired pneumonia, *IMV* invasive mechanical ventilation.
[a]VAP episodes were recorded per definition in patients under IMV since more than 48 h.
Two-tailed *p*-values come from unadjusted comparisons using Chi-square or Fisher's exact tests for categorical variables, and *t*-tests or Mann–Whitney tests for continuous variables, as appropriate. No adjustment for multiple comparisons was performed. Bolded p-values are significant at the $p < 0.05$ level.

small sample size analysed should be taken into consideration when interpreting the results. Another limitation is the use of bulk transcriptomics rather than single-cell RNA sequencing. While bulk RNA sequencing provides valuable insights into overall gene expression, it lacks the resolution to study individual cell populations in depth. Single-cell RNA sequencing would have provided a more detailed understanding of immune responses at the cellular level, potentially revealing additional heterogeneity within the immune cell types involved. In addition, our study focused on transcriptomics from NPS rather than peripheral blood. Although validated, this approach may not fully capture the systemic immune response. Nevertheless, several previous studies have demonstrated the efficacy of respiratory transcriptomics performed on NPS in investigating the immune response to respiratory virus infections[10,12,13]. Finally, larger validation cohorts incorporating blood-based immunological data are needed to confirm the clustering of critically ill patients infected with the Omicron lineage of SARS-CoV-2.

Conclusion

In conclusion, our study highlights the heterogeneity and the complexity of the local immune response in critically ill patients with COVID-19 in the Omicron era. Transcriptomic analysis of the upper airway mucosa identified two distinct endotypes that were not captured by standard clinical and biological assessments. These findings support the need for personalised treatment strategies based on the immune response in critically ill patients with COVID-19.

Table 3 | Characteristics of studies investigating the immune response to the Omicron variant compared with previous SARS-CoV-2 variants

Author, year, reference	Type of study	Main analyses	Innate Immunity	Adaptative immunity	Cytokine signalling	Viral proteins	Other findings
Bay et al., 2023[13]	Prospective, monocentric study with critically ill COVID-19 patients (*N* = 88). Ancestral strain, VOCs Alpha, Delta and Omicron	Nasopharyngeal transcriptomic Omicron (BA.1 and BA.2) versus patients infected with Alpha and delta variants / ancestral strain	↗ Inflammatory response, neutrophil degranulation, IFN signalling	↗ T-cell activation	↗ IL-4/-13 and TNF pathways	n.a	↗ neutrophils and monocytes relative abundance
Shahbaz et al., 2023[51]	Prospective, monocentric study with critically ill COVID-19 patients (*N* = 140). Ancestral strain, VOCs Delta and Omicron	Blood samples with flow cytometry Cytokines, chemokines and immune checkpoints measurements	n.a	↗ T-cell activation (vs. Delta variant)	↘ Inflammatory cytokines (e.g., IFN-γ, IL-8, TNF-α, IL-1β …)	n.a	↘ levels of plasma galectins (Gal-3, Gal-9)
Bouhaddou et al., 2025[52]	Infection of lung epithelial cells	Comparative proteomic and genomic analyses of VOCs	IFN and pro inflammatory responses: ↗ in earlier Omicron sublineages (BA.1 and BA.2) / ↘ in BA.4 and BA.5 sublineages	n.a	↗ IFN-β and CXCL-10	BA.1 and BA.2: ↘ Orf6 and Orf9b BA.5: ↗ Orf6	Greater rescue of viral replication with Ruxolitinib (vs. Alpha and Delta)
Savellini et al., 2023[53]	Infection of lung epithelial cells	IFN response (IFN-β quantification and gene expression) and viral replication	↗ IFN-β in BA.1 (transcriptional level) and BA.2	n.a	n.a	Impact of Orf6 D61L substitution on IFN antagonism	n.a
Alfi et al., 2022[54]	Ex-vivo infection (human nasal and lung tissues)	Cell culture, RNA quantification (Omicron vs. Delta variant and ancestral strain)	↗ IFN response	n.a	n.a	n.a	↘ viral replication in the lung tissues, restored by Ruxolitinib
Rong et al., 2025[55]	Infected mice (Omicron vs. ancestral strain)	Blood and lung single-cell RNA sequencing	↗ neutrophils in the lungs (immature: wound healing pathway, mature: immune response)	n.a	↘ CXCL10, CXCL1, CCL5, CXCL2, TNF, IL-1 in hyper-inflammatory monocytes and macrophages in the lungs	↘ IL-17 and NF-κB signalling pathways in Ly6c2+ monocytes and interstitial macrophages	↘ oxidative stress pathway
Yuan et al., 2022[56]	Infected Syrian hamsters (Omicron BA.1 vs Delta variant)	Clinical signs, viral burden, and cytokine and chemokine profiles	n.a	n.a	↘ Inflammatory cytokine and chemokine genes	n.a	↘ Body weight loss and clinical severity ↘ Lung viral replication
Purwono et al., 2024[57]	Infection of lung epithelial cells (ancestral and VOCs)	Growth kinetics, measurement of cytokines and chemokines	n.a	n.a	↗ TNF-α ↘ IL-1β and IL-2	n.a	Omicron: lower infectivity rate, slower replication kinetics, and fewer syncytia formations

Abbreviations: *COVID-19* Coronavirus disease 2019, *IFN* interferon, *IL* interleukin, *RNA* ribonucleic acid, *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2, *TNF* tumour necrosis factor, *VOC* variant of concern.

Data availability

The raw transcriptomic data are provided in Supplementary Data File 1. Source data are provided in the following Supplementary Data Files: Fig. 2a (Supplementary Data 2), Fig. 2b (Supplementary Data 3), Fig. 3a (Supplementary Data 4), Figs. 3b and 3c (Supplementary Data 5), Fig. 4 (Supplementary Date 6) and Fig. 5 (Supplementary Data 7). To ensure the confidentiality of our participants, the individual-level data will not be made publicly available. Additional data supporting the current study are available from the corresponding author (mail to: pierre.bay@aphp.fr) upon reasonable request and will be reviewed within four weeks.

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Author contributions

P.B., N.d.P., and S.F. designed the study; L.B. and P.B.; C.R. and S.C. performed statistical analyses; P.B., S.P., R.F., N.H., T.P., M.T., S.J., A.J., D.C., D.R., A.G., and N.d.P. included the patient and were responsible for clinical data collection; A.S., S.F., M.A., A.C., L.M.-J., A.P., A.H., E.G., Q.L.H., and L.H. were responsible of the management of virological samples; C.R., J.M.P., and S.F. were responsible of virological analyses; C.R., S.F., A.S., M.A., and P.B. performed or supervised key aspects of sample processing, library preparation and/or sequencing.; P.B., N.d.P., and S.F. wrote the first draft of the article; All authors revised and approved the article. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. S.F. is the guarantor.

Competing interests

Slim Fourati reports having served as a speaker for Sanofi, GlaxoSmithKline, AstraZeneca, MSD, Pfeizer, Cepheid and Moderna. Jean-Michel Pawlotsky reports having served as an advisor and/or speaker for Abbott, Abbvie, Diagnostics for the Real World, Gilead and GSK. Christophe Rodriguez reports having served as a speaker for Pfizer and Tecan. Nicolas de Prost reports having served as an advisor or speaker for Moderna and AstraZeneca. All other authors declare no competing interest.

Human Ethics Approval And Consent To Participate

The study was approved by the Comité de Protection des Personnes Sud-Méditerranée I (N° EudraCT/ID-RCB: 2021-A02914-37). Informed consent was obtained from all patients or their relatives.

Additional information

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Correspondence and requests for materials should be addressed to Pierre Bay.

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Pierre Bay[1,2,3,4] ✉, Laure Boizeau[5], Sébastien Préau[6], Raphaël Favory[6], Aurélie Guigon[7], Nicholas Heming[8], Elyanne Gault[9], Tài Pham[2,10,11], Amal Chaghouri[12], Matthieu Turpin[13], Laurence Morand-Joubert[14,15], Sébastien Jochmans[16], Aurélia Pitsch[17], Adrien Joseph[18], Damien Contou[19], Amandine Henry[20], Damien Roux[21,22], Quentin Le Hingrat[23], Antoine Guillon[24],

Lynda Handala[25], Stefano Caruso[3,26], Mohamed Ader[27], Alexandre Soulier[27], Jean-Michel Pawlotsky[3,4,27], Christophe Rodriguez[3,4,5], Nicolas de Prost[1,2,3,4,28] & Slim Fourati[3,4,27,28]

[1]Service de Médecine Intensive Réanimation, DMU Médecine, Hôpitaux Universitaires Henri Mondor, AP-HP (Assistance Publique-Hôpitaux de Paris), Créteil, France. [2]GRC CARMAS, Faculté de Santé de Créteil, UPEC (Université Paris-Est-Créteil), Créteil, France. [3]Université Paris-Est-Créteil (UPEC), Créteil, France. [4]IMRB INSERM U955, Team «Viruses, Hepatology, Cancer», Créteil, France. [5]Plateforme de Génomique, Hôpitaux Universitaires Henri Mondor, AP-HP (Assistance Publique-Hôpitaux de Paris), IMRB (Institut Mondor de Recherche Biomédicale), Créteil, France. [6]U1167 – RID-AGE Facteurs de Risque et Déterminants Moléculaires des Maladies Liées au Vieillissement, University Lille, Inserm, CHU Lille, Institut Pasteur de Lille, Lille, France. [7]Service de virologie, CHU de Lille, Lille, France. [8]Médecine Intensive Réanimation, Hôpital Raymond Poincaré, Assistance Publique – Hôpitaux de Paris (AP-HP), Garches, France. [9]Laboratoire de Virologie, Hôpital Ambroise Paré, Assistance Publique – Hôpitaux de Paris (AP-HP), Boulogne, France. [10]Service de Médecine Intensive-Réanimation, Assistance Publique – Hôpitaux de Paris, Hôpital de Bicêtre, DMU 4 CORREVE Maladies du Cœur et des Vaisseaux, FHU Sepsis, Le Kremlin-Bicêtre, France. [11]Inserm U1018, Equipe d'Epidémiologie respiratoire intégrative, CESP, Villejuif, France. [12]Laboratoire de Virologie, Hôpital Paul Brousse, Assistance Publique – Hôpitaux de Paris, Villejuif, France. [13]Sorbonne Université, Centre de Recherche Saint-Antoine INSERM, Médecine Intensive Réanimation, Hôpital Tenon, Assistance Publique – Hôpitaux de Paris, Paris, France. [14]Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Paris, France. [15]Laboratoire de virologie, Hôpital Saint-Antoine, Assistance Publique – Hôpitaux de Paris, Paris, France. [16]Service de Réanimation Polyvalente, Hôpital Marc Jacquet, Melun, France. [17]Laboratoire de Microbiologie, Hôpital Marc Jacquet, Melun, France. [18]Service de Réanimation médico-chirurgicale, Assistance Publique–Hôpitaux de Paris, Hôpital Ambroise Paré, Boulogne, France. [19]Service de Réanimation, Hôpital Victor Dupouy, Argenteuil, France. [20]Service de Virologie, Hôpital Victor Dupouy, Argenteuil, France. [21]AP-HP, Hôpital Louis Mourier, DMU ESPRIT, , Service de Médecine Intensive Réanimation, Colombes, France. [22]Université Paris Cité, INSERM UMR-S1151, CNRS UMR-S8253, Institut Necker Enfants Malades, Paris, France. [23]Université Paris Cité, IAME INSERM UMR 1137, Service de Virologie, Hôpital Bichat-Claude Bernard, Assistance Publique – Hôpitaux de Paris, Paris, France. [24]Service de Médecine Intensive Réanimation, CHU Tours, Université de Tours, Inserm U1100, Centre d'Etudes des Pathologies Respiratoires (CEPR), Tours, France. [25]INSERM U1259, Université de Tours, Tours, France, CHRU de Tours, National Reference Center for HIV-Associated Laboratory, Tours, France. [26]Département de Pathologie, Hôpitaux Universitaires Henri Mondor, AP-HP (Assistance Publique-Hôpitaux de Paris), Créteil, France. [27]Department of Virology, Hôpitaux Universitaires Henri Mondor, Assistance Publique – Hôpitaux de Paris, Créteil, France. [28]These authors contributed equally: Nicolas de Prost, Slim Fourati. ✉e-mail: pierre.bay@aphp.fr