






## ORIGINAL RESEARCH

# Pathophysiology of acute respiratory syndrome coronavirus 2 infection: a systematic literature review to inform EULAR points to consider

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## ABSTRACT

**Background** The SARS-CoV-2 pandemic is a global health problem. Beside the specific pathogenic effect of SARS-CoV-2, incompletely understood deleterious and aberrant host immune responses play critical roles in severe disease. Our objective was to summarise the available information on the pathophysiology of COVID-19.

**Methods** Two reviewers independently identified eligible studies according to the following PICO framework: P (population): patients with SARS-CoV-2 infection; I (intervention): any intervention/no intervention; C (comparator): any comparator; O (outcome) any clinical or serological outcome including but not limited to immune cell phenotype and function and serum cytokine concentration.

**Results** Of the 55 496 records yielded, 84 articles were eligible for inclusion according to question-specific research criteria. Proinflammatory cytokine expression, including interleukin-6 (IL-6), was increased, especially in severe COVID-19, although not as high as other states with severe systemic inflammation. The myeloid and lymphoid compartments were differentially affected by SARS-CoV-2 infection depending on disease phenotype. Failure to maintain high interferon (IFN) levels was characteristic of severe forms of COVID-19 and could be related to loss-of-function mutations in the IFN pathway and/or the presence of anti-IFN antibodies. Antibody response to SARS-CoV-2 infection showed a high variability across individuals and disease spectrum. Multiparametric algorithms showed variable diagnostic performances in predicting survival, hospitalisation, disease progression or severity, and mortality.

**Conclusions** SARS-CoV-2 infection affects both humoral and cellular immunity depending on both disease severity and individual parameters. This systematic literature review informed the EULAR 'points to consider' on COVID-19 pathophysiology and immunomodulatory therapies.

## INTRODUCTION

The SARS-CoV-2 pandemic has led to the scientific and global communities facing an unprecedented challenge.<sup>1</sup> The rapid spread

## Key messages

### What is already known about this subject?

- The SARS-CoV-2 pandemic is a global health issue and disease pathogenesis along with mechanisms leading to severe COVID-19 are yet poorly understood.
- A deleterious excessive and aberrant non-effective host immune response may play an important role throughout the course of severe disease.

### What does this study add?

- Cytokine profiles, cellular and humoral immune response are highly heterogeneous across individuals and specific patterns are associated with the evolution to severe COVID-19 and a poor prognosis.
- Failure to maintain high interferon (IFN) levels is characteristic of severe forms of COVID-19 and could be related to loss-of-function mutations in the IFN pathway and/or the presence of anti-IFN antibodies.
- Immune and non-immune-mediated mechanisms play an important role in COVID-19 thrombotic manifestations.
- Multiparametric algorithms including clinical and biological features can predict poor outcomes in SARS-CoV-2 infected individuals.

### How might this impact on clinical practice?

- The emerging knowledge on immune pathways and severe SARS-CoV-2 infection indicate distinct cytokine pathway perturbations compared with other rheumatological disorders including the interleukin-6 and type I IFN pathway.
- Significant knowledge gaps exist that will stimulate further research.

of the virus along with the lack of effective antiviral drugs to treat COVID-19 has so far resulted in more than 65 000 000 confirmed cases and 1 500 000 deaths (COVID-19.who.int/; 15 December 2020).<sup>2</sup> SARS-CoV-2

infection encompasses a broad spectrum of clinical phenotypes, from asymptomatic or mild diseases with little or no respiratory symptoms to severe COVID-19 with life-threatening manifestations such as acute respiratory distress syndrome (ARDS) leading to multiorgan failure and death.<sup>3</sup> Lung damage in severe COVID-19 is linked to inflammatory alveolar and interstitial immune cell infiltration and activation.<sup>4</sup> The cellular and humoral immune response to SARS-CoV-2 appears to inadequately control viral spread or may be evident in tissue where there is no detectable virus with both scenarios being potentially deleterious consequent to severe inflammation.<sup>5</sup> Excessive production and release of proinflammatory mediators, including interleukin (IL)-1 $\beta$ , IL-6, tumour necrosis factor- $\alpha$  and monocyte chemoattractant protein 1 (MCP-1) and many other molecules, occurs in more patients with severe COVID-19.<sup>6</sup> In severe cases, these features resemble other systemic severe inflammatory states such as macrophage activation syndrome (MAS) or secondary haemophagocytic lymphohistocytosis.<sup>6,7</sup>

A massive research effort to better understand the complex viral–host interactions has resulted in an extremely high volume of publications in a very short timeframe. The high heterogeneity and variety in the quality of the literature require a systematic appraisal; in order to propose a synthesis of existing evidence towards improved COVID-19 understanding and therapy. This systematic literature review (SLR) aimed to summarise the available information on the pathogenesis of SARS-CoV-2 infection from the rheumatological perspective, given that this specialty is intimately involved in investigation of aberrant and severe immunological reactions in many organ systems and in heterogeneous autoimmune and autoinflammatory disorders. An SLR addressing therapeutic aspects on the repurposing of rheumatic drugs as potential COVID-19 therapy is addressed elsewhere.<sup>8</sup> This SLR informed the EULAR points to consider (PtC) on COVID-19 pathophysiology and immunomodulatory therapies.<sup>9</sup>

## METHODS

### Search methodology

The scope of the systematic literature search on pathophysiology according to the Population, Intervention, Comparator and Outcome (PICO) approach was determined by the EULAR task force aiming at developing PtCs on COVID-19 pathophysiology and immunomodulatory therapies (online supplemental text S1).<sup>10</sup> Three separate searches (online supplemental text S2, S3 and S4) were performed, one for studies on pathophysiology of COVID-19, the second on studies on COVID-19 treatment and the third on COVID-19 and rheumatic and musculoskeletal diseases (RMDs), with this SLR reporting on pathophysiology. The databases explored were MEDLINE, Embase, The Cochrane Database of Systematic Reviews, CENTRAL and CINAHL. Hand

search for individual original research studies and cross-check for references from specific *Rheumatology*, *Haematology* and *Immunology* journals were selected as described in the online supplemental material.

### Study selection, data collection and assessment of risk of bias

Two reviewers (AA and AN) independently assessed titles and abstracts of the retrieved papers. General eligibility criteria were described as follows: original research articles, published in peer-reviewed journals in English language, on adult and paediatric patients with proven SARS-CoV-2 infection according to the reference standard (nucleic acid amplification tests such as RT-qPCR) presenting with signs/symptoms of COVID-19 or asymptomatic and no diagnosis of RMDs prior to SARS-CoV-2 infection. In addition, different predetermined eligibility criteria were set according to the research questions (online supplemental text S5). Among other, unsupervised clustering methods (defined as multiparametric flow cytometry, mass cytometry, multiplex-luminex technologies, single cell RNA seq) were a pre-requisite for cells population, chemokines and cytokines assessment. In addition, for humoral response assessment, only studies using validated commercially available antibodies testing kits were included. For multiparametric algorithm studies, a minimum size of 200 patients was chosen. The agreement between reviewers, calculated with the Cohen's kappa, was 0.95. Discrepancies were resolved by discussion. The task force methodologist (PMM) was consulted in the case of uncertainties. Data on patients' characteristics, scientific methods, parameters assessed and outcomes were extracted. The risk of bias was calculated with validated tools according to the study design (online supplemental text S6). The structure of reporting this SLR follows the structure of the PtCs,<sup>8</sup> as decided by the task force members following a consensus process.

## RESULTS

Of the 55 496 records yielded by the three searches, 290 were selected for detailed review. Of these, 84 articles met the inclusion criteria for the research questions on the pathogenesis of COVID-19 (online supplemental table S1 and S2).

### Genetic variants and SARS-CoV-2 severity

As far as genes involved in the immune response are concerned, Zhang *et al* demonstrated that known variants of toll-like receptor 3 (TLR3)–and interferon regulatory factor 7 (IRF7)–dependent type I interferon (IFN) immunity associated with life-threatening influenza are present in a subset of patients with life-threatening COVID-19 (table 1).<sup>11</sup> In addition, new TLR3 variants have been identified in life-threatening COVID-19 and linked to hampered IFN immunity *in vivo* and *in vitro*.<sup>11</sup> Variants of the IFN-related genes were also identified by a study sequencing and genotyping interferon-induced transmembrane protein 3 (IFITM3) rs12252 sequence

**Table 1** Genetic variants and disease severity

Author	Study type	Population	Blood type distribution	Other findings	RoB
<b>Rhesus and ABO</b>					
Ellinghaus <i>et al</i> <sup>17</sup>	GWAS	1980 severe COVID-19 vs 2381 HD Italian Spanish	NA	<ul style="list-style-type: none"> <li>▶ rs657152 A or C SNP at locus 9q34.2 (OR for the A allele 1.32; 95% CI 1.20 to 1.47; p&lt;0.0001)</li> <li>▶ Higher risk of severe COVID-19 in blood group A vs other blood groups (OR 1.45; 95% CI 1.20 to 1.75; p=0.0148)</li> <li>▶ Lower risk of severe COVID-19 in blood group O vs other blood groups (OR 0.65; 95% CI 0.53 to 0.79; p&lt;0.0001)</li> <li>▶ No significant difference in blood group distribution between patients receiving supplemental oxygen only and those receiving mechanical ventilation of any kind</li> </ul>	Low
<b>HLA</b>					
Novelli <i>et al</i> <sup>16</sup>	Sequencing and genotyping of HLA genes	99 COVID-19 vs 1017 normal Italian subjects	Haplotypes more prevalent in COVID-19 B*27:07 DRB1*15:01 DQB1*06:02	p value vs HD 0.004 0.048 0.016	Unclear
Ellinghaus <i>et al</i> <sup>17</sup>	GWAS	1980 severe COVID-19 vs 2381 HD Italian Spanish	<ul style="list-style-type: none"> <li>▶ No SNP association signals at the HLA complex that met the significance threshold of suggestive association</li> <li>▶ No significant allele associations with either COVID-19 infection or disease severity</li> </ul>	High	
<b>Genes encoding molecules involved in the host immune response</b>					
Zhang <i>et al</i> <sup>12</sup>	Sequencing and genotyping of IFITM3 rs12252 sequence	80 COVID-19 (56 mild, 24 severe) vs Beijing population (International Genome Sample Resource)	<ul style="list-style-type: none"> <li>▶ Association between homozygosity for the C allele (CC vs CT/TT) and disease severity (OR 6.37; p&lt;0.0001)</li> <li>▶ 2 of the 3 patients who died carried the CC genotype</li> <li>▶ The frequency of CC genotype in mild patients is similar to that in the general Beijing population</li> </ul>	Unclear	
Cabrera-Marante <i>et al</i> <sup>14</sup>	Sequencing and genotyping of PRF1 rs35947132 (A91V) sequence	22 severe young COVID-19 vs 22 HD (14 Latin-American, 7 Spanish and 1 Polish)	<ul style="list-style-type: none"> <li>▶ Both A91V-positive patients died</li> </ul>	High	
Ellinghaus <i>et al</i> <sup>17</sup>	GWAS	1980 severe COVID-19 vs 2381 HD Italian Spanish	<ul style="list-style-type: none"> <li>▶ Cross-replicating associations with rs11385942 at locus 3p21.31 (spanning the genes <i>LZTFL1</i>, <i>CCR9</i>, <i>FYCO1</i>, <i>CXCR6</i> and <i>XCR1</i>)</li> </ul>	High	
Zhang <i>et al</i> <sup>11</sup>	RNA Seq	659 life-threatening COVID-19 pneumonia vs 534 asymptomatic or benign SARS-CoV-2 infection	<ul style="list-style-type: none"> <li>▶ Known variants of Toll-like receptor 3 (TLR3)–and interferon regulatory factor 7 (IRF7)–dependent type I interferon (IFN) immunity associated with life-threatening influenza are present in a subset of patients with life-threatening COVID-19</li> <li>▶ New variants within the same loci have been identified in life-threatening COVID-19</li> <li>▶ Patients showing these variants have hampered IFN immunity in vivo and in vitro</li> </ul>	Low	
Pairo-Castineira <i>et al</i> <sup>13</sup>	GWAS	2244 critical (ICU) COVID-19 vs 11220 HD	<ul style="list-style-type: none"> <li>▶ Significant associations in a gene cluster encoding antiviral restriction enzyme activators (OAS1, OAS2, OAS3) near the gene encoding tyrosine kinase 2 (TYK2) and in the interferon receptor gene <i>IFNAR2</i></li> <li>▶ Using Mendelian randomisation, evidence in support of a causal link from low expression of <i>IFNAR2</i>, and high expression of <i>TYK2</i>, to life-threatening disease</li> <li>▶ Transcriptome-wide association in lung tissue revealed that high expression of the monocyte/macrophage chemotactic receptor <i>CCR2</i> is associated with severe COVID-19</li> </ul>	Low	

**ACE-2**

Continued

Table 1 Continued

Author	Study type	Population	Blood type distribution	Other findings	RoB
Benetti <i>et al</i> <sup>19</sup>	Whole exome seq	131 COVID-19 vs 258 HD		<ul style="list-style-type: none"> <li>▶ Different distributions of variants vs controls</li> <li>▶ Lower ACE2 allelic variability vs controls (p value&lt;0.029)</li> </ul>	Unclear
Novelli <i>et al</i> <sup>16</sup>	Whole exome seq	131 hospitalised COVID-19 vs 1000 HD		<ul style="list-style-type: none"> <li>▶ No evidence of consistent association of ACE2 variants with COVID-19 severity</li> </ul>	Unclear
Gomez <i>et al</i> <sup>20</sup>	ACE2 gene seq and SNP assessment	204 COVID-19 (137 non-severe and 67 severe) vs 536 HD		<ul style="list-style-type: none"> <li>▶ Low ACE2 allelic variability</li> <li>▶ ACE I/D DD phenotype more prevalent in severe diseases but not significant at multivariable analysis</li> <li>▶ The ACE2 rs2285666 alleles did not differ based on severity nor vs controls</li> <li>▶ Both polymorphisms are associated with hypertension</li> </ul>	Unclear
Ellinghaus <i>et al</i> <sup>17</sup>	GWAS	1980 severe COVID-19 vs 2381 HD Italian Spanish		<ul style="list-style-type: none"> <li>▶ No evidence identified in ACE-2 loci</li> <li>▶ Cross-replicating associations with rs11385942 at locus 3p21.31 (spanning the <i>SLC6A20</i> gene among others)</li> </ul>	High

GWAS, genome-wide association study; HD, healthy donor; HLA, human leucocyte antigens; JAK, Janus kinase; NA, not available; RoB, risk of bias; SNP, single nucleic acid polymorphism; TYK, tyrosine kinase.

that observed an association between homozygosity for the C allele (CC vs CT/TT) and disease severity (OR 6.37;  $p < 0.0001$ ).<sup>12</sup> A first genome-wide association study (GWAS) conducted in 1980 patients with severe COVID-19 identified cross-replicating associations with rs11385942 at locus 3p21.31 spanning genes involved in the immune response such as *CCR9*, *CXCR6* and *CXCR1*.<sup>4</sup> While writing this manuscript, an important GWAS study came to our attention.<sup>13</sup> Although outside the review period, we highlight it due to its relevance and the exceptionality of the rapid pace of publications on the topic of this SLR. This GWAS made on 2244 critically ill patients revealed association with single nucleic acid polymorphism (SNP) involved in the IFN pathway (*IFNAR2*, *TYK2*, *OAS*) and *CCR2*. Mendelian randomisation supported a causal link from low expression of *IFNAR2* and high expression of *TYK2* to life-threatening disease, and high expression of *CCR2* as well.<sup>13</sup> Sequencing and genotyping of *perforin* rs35947132 (*A9IV*) sequence in patients with severe COVID-19 was also performed showing that both patients carrying the sequence died.<sup>14</sup> Of interest, previous studies reported a higher prevalence of the *A9IV* variant in patients with haemophagocytic lymphohistocytosis,<sup>15</sup> suggesting a possible common mechanism. Data on human leucocyte antigen (HLA) haplotypes are scarce and only showed a higher prevalence of some haplotypes (*B\*27:07*, *DRB1\*15:01* and *DQB1\*06:02*) in 99 COVID-19 patients versus 107 healthy donors.<sup>16</sup> In addition, the only available GWAS failed to identify any SNP association signals at the HLA complex that met the significance threshold of suggestive association or any significant allele associations with either COVID-19 infection or disease severity (1980 and 2381 patients, respectively).<sup>17</sup>

Other genes that are not directly involved in the immune response but may be related to SARS-CoV-2 infection have been explored. The ACE-2 facilitates

SARS-CoV-2 entry in human cells by binding of the virus spike protein.<sup>18</sup> Low ACE2 allelic variability has been reported,<sup>19,20</sup> along with a different distribution of variants versus controls.<sup>19</sup> However, no solid association between ACE-2 variants and disease severity has been demonstrated.<sup>17,19-21</sup> Finally, with regard to blood type, the only available data come from a GWAS study which identified the rs657152 A or C SNP at locus 9q34.2 (OR for the A allele 1.32; 95% CI 1.20 to 1.47;  $p < 0.0001$ ) and estimated a higher risk of severe COVID-19 in blood group A versus other blood groups and a lower risk of severe COVID-19 in blood group O versus other blood groups.<sup>17</sup> All data pertaining to this research question are reported in table 1.

### Myeloid cellular response to SAR-CoV-2 infection according to disease phenotype

Innate and adaptive cellular immune response has been thoroughly assessed. It is worth noting that only a few studies used unsupervised clustering approaches (single cell RNA seq, Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq)) while most used multiparametric flow cytometry. Since different gating strategies were used, different 'unique' subsets were reported in several studies and are shown in table 2. Data detailed in the text were reported in at least two individual studies. Neutrophils were reported to be overall increased in patients with COVID-19 regardless of disease severity versus healthy donor (HD).<sup>22,23</sup> Of interest, circulating immature neutrophils were reported to be increased, similarly to bacterial sepsis.<sup>22</sup> The monocyte compartment was affected by SARS-CoV-2 infection in different manners. A shift towards classical CD14+ inflammatory monocytes producing TNF $\alpha$  and IL-1 $\beta$  was observed in all patients with COVID-19 versus HD.<sup>22-25</sup> In addition, the expression of HLA-DR was strongly reduced,



**Table 2** Cellular immune response to SARS-CoV-2 infection according to disease phenotype

	Author	Study type	Patients N	Control N	Cells	RoB
<b>COVID-19 (mild, moderate and/or severe) vs healthy donors</b>						
<b>Neutrophils</b>	Schulte-Schrepping <i>et al</i> <sup>22</sup>	CytoF, single cell RNA seq, flow cytometry	58 (40 COVID-19, 8 influenza)	10 HD	↑ LDN, FUT4(CD15)+ CD63+ CD66b+ pro-neutrophils, and ITGAM(CD11b)+ CD101+ pre-neutrophils, reminiscent of emergency myelopoiesis, ↑ CD274(PD-L1)+ ZC3H12A+ mature neutrophils reminiscent of gMDSC-like cells	Unclear
	Silvin <i>et al</i> <sup>23</sup>	CytoF, single cell RNA seq	13 COVID-19 (mild 5, severe 8)	25 HD	↑ neutrophils ↑ CD10LowCD101+ neutrophils in patients with mild disease, whereas ↑ CD10LowCD101- neutrophils in patients with severe disease	Unclear
<b>Monocytes</b>	Arunachalam <i>et al</i> <sup>27</sup>	CytoF+Bulk RNA-seq CITE-seq PBMCs	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 influenza (ATL)	45 HD (HK) 24 HD (ATL)	↓ HLA-DR and expression of proinflammatory cytokines. Impaired response to stimulation with a bacterial or viral ligand cocktail	High
	Kuri-Cervantes <i>et al</i> <sup>28</sup>	Multiparametric flow cytometry	35 (7 moderate, 28 severe), 7 recovered	12 HD	↓ HLA-DR expression in severe patients	High
	Lucas <i>et al</i> <sup>26</sup>	Multiparametric flow cytometry	113 (moderate 80, severe 33)	108 HD	↓ reduction of HLA-DR monocytes	Low
	Wen <i>et al</i> <sup>24</sup>	Multiparametric flow cytometry	10 recovered (5 early and 5 late)	5 HD	↑ CD14++ IL1β+ monocytes and IFN-activated monocytes	High
	Lee <i>et al</i> <sup>25</sup>	ScRNA seq PBMCs	8 COVID-19 (severe, mild, asymptomatic), 5 Influenza	4 HD	The TNF/IL-1β-driven inflammatory response was dominant in COVID-19 across all types of cells among PBMCs	High
	Silvin <i>et al</i> <sup>23</sup>	Cy-TOF, single cell RNA seq	13 COVID-19 (mild 5, severe 8)	25 HD	↑ CD14HighCD16High intermediate monocytes in patients with mild COVID-19 vs severe or HD	High
	Schulte-Schrepping <i>et al</i> <sup>22</sup>	Cy-TOF, single cell RNA seq, flow cytometry	58 (40 COVID-19, 8 influenza)	10 HD	↑ inflammatory HLA-DRhiCD11chi CD14+ monocytes with an interferon-stimulated gene signature in mild forms ↑ HLA-DRhiCD11chi monocytes in severe forms ↓ expression of CD11c and HLA-DRA and HLA-DRB1 early and sustained ↑ CD226+ CD69+ monocytes Dysfunctional HLA-DRloCD163hi and HLA-DRloS100Ahi CD14+ in severe forms	High
<b>Dendritic cells</b>	Arunachalam <i>et al</i> <sup>27</sup>	Cy-TOF+Bulk RNA-seq CITE-seq PBMCs	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 influenza (ATL)	45 HD (HK) 24 HD (ATL)	↓ pDCs pool reduced Impaired mTOR signalling and IFN-α production in response to the TLR stimuli and TNF response.	High
	Zhou <i>et al</i> <sup>32</sup>	Multiparametric flow cytometry Patients DC cultures	Acute COVID-19 (6 severe and 11 mild) Convalescent COVID-19 (2 severe and 22 mild)	HD	↑ monocytic myeloid-derived suppressive cells in acute patients vs HD ↓ CD11c+ cDCs decreased in convalescent patients ↓ CD86 expression vs HD but not HLA-DR	High
<b>T cells</b>	Weistemeier <i>et al</i> <sup>30</sup>	Multiparametric flow cytometry PBMCs	30 mild	10 HD	↓ CD4+ No difference in any of the subsets (naïve (N) (CD45RO- CCR7+ CD28+), central memory (CM) (CD45RO+ CCR7+ CD28+), transitional memory (TM) (CD45RO+ CCR7- CD28+), effector memory (EM) (CD45RO+ CCR7- CD28-), and terminally differentiated effector (E) (CD45RO- CCR7- CD28-)) ↓ CD8+ (↓ naïve, ↑ effector, effector memory and transitional memory cells) ↑ cytotoxic molecules secretion granzyme A in effector, effector memory, and transitional memory cells and granzyme and perforin in effector memory, and transitional memory cells More multifunctional effector and effector memory T cells	High
	Kuri-Cervantes <i>et al</i> <sup>28</sup>	Multiparametric flow cytometry PBMCs	35 (7 moderate, 28 severe), 7 recovered	12 HD	=across all groups	High
	Lucas <i>et al</i> <sup>26</sup>	Multiparametric flow cytometry PBMCs	113 (moderate 80, severe 33)	108 HD	↓ CD4+ and CD8+	Low
	Wang <i>et al</i> <sup>31</sup>	CytoF PBMCs	12 (4 mild, 5 severe, 3 critical)	12 HD	↑ CD4+ CD8+ double-positive T cells ↑ naïve CD4+ T cells ↑ TGF-β+ CD28- naïve CD4+ T cells	Unclear

Continued

Table 2 Continued

	Author	Study type	Patients N	Control N	Cells	RoB
	Wen <i>et al</i> <sup>24</sup>	Multiparametric flow cytometry	10 recovered (5 early and 5 late)	5 HD	↓ CD8+ T cells ↓ effector memory CD8+ T cell ↑ CD4+ T cells, the ratio of central memory CD4+ T cells was significantly higher ↓ naïve CD4+ T cells, Tregs and effector memory CD4+ (especially in the early recovery group) T cell expansion decreased in the early recovery group	High
	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	↓ CD4+ and CD8+ =TCR $\alpha/\beta$ - and $\gamma/\delta$ -positive T lymphocytes, across groups ↑ T central memory (CD45RA- CCR7+) cells =naïve (CD45RA+ CCR7+), T effector memory (CD45RA- CCR7-), T effector memory CD45RA+ (CD45RA+ CCR7-), and HLA-DR+ cells across groups ↓ naïve (CD45RA+ CCR7+) and T central memory (CD45RA-CCR7+) cells ↑ T effector memory (CD45RA+ CCR7-) and senescent (CD57+) CD8+ T cells =T effector memory (CD45RA- CCR7-) and HLA-DR+ CD8+ T cells across groups ↑ IL-2-producing CD4+ T cells and ↓ IL-2-producing CD8+ T lymphocytes and IFN- $\gamma$ -producing CD4+ and CD8+ T cells ↓ IL-2+ IFN- $\gamma$ + TNF- $\alpha$ + and IL-2+ IFN- $\gamma$ + TNF- $\alpha$ - CD8+ (polyfunctional) T cell	Unclear
	Odak <i>et al</i> <sup>34</sup>	Multiparametric flow cytometry	30 (15 severe)	None	↑ CD4+ effector/effector memory (CD45RA- CD62L-), ↓ CD4+ terminally differentiated cells (CD45RA+ CD62L-) ↑ CD8 naïve T cells ↓ CD8 effector/effector memory cells, CD8 central memory and CD8t effector memory ↑ naïve-like $\gamma\delta$ ( $\gamma\delta$ naïve-) cells ↓ in effector-like $\gamma\delta$ ( $\gamma\delta$ eff-) cells	High
	Song <i>et al</i> <sup>33</sup>	Multiparametric flow cytometry	41 (29 mild, 12 severe)	None	↑ activated CD38+ CD8+ T cells, HLA-DR+ CD8+ T cells and CD38+ HLA-DR+ CD8+ T cells =CD38+ CD4+ T cells and HLA-DR+ CD4+ T cells among groups	High
	Zhou <i>et al</i> <sup>32</sup>	Multiparametric flow cytometry Patients DC cultures	Acute COVID-19 (6 severe and 11 mild) Convalescent COVID-19 (2 severe and 22 mild)	HD	↑ PD-1 expression in CD4 T-cell central memory and effector memory CD4 T cells have ↓ polyfunctionality for releasing both IFN- $\gamma$ and TNF- $\alpha$ in vitro in acute patients effector memory and CD45RA+ effector CD8 T cells ↓ polyfunctionality for releasing both IFN- $\gamma$ and TNF- $\alpha$ effector memory and CD45RA+ effector CD8 T cells ↓ for granzyme B and perforin	High
<b>NK cells</b>	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	↓ NK cells ↓ perforin and granzyme A	Unclear
	Wen <i>et al</i> <sup>24</sup>	Multiparametric flow cytometry	10 recovered (5 early and 5 late)	5 HD	↓ NK cells	High
<b>B cells</b>	Wen <i>et al</i> <sup>24</sup>	Multiparametric flow cytometry	10 recovered (5 early and 5 late)	5 HD	Plasma cells ↓ naïve B cells	High
	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	= naïve (IgD+ CD27-), memory-nonswitched (IgD+ CD27+), memory-switched (IgD- CD27+), and B lymphocytes and plasmablasts (CD27hiCD38hi) ↓ transitional (IgMhiCD38hi) B lymphocytes	Unclear
<b>COVID-19 severe vs healthy donors</b>						
<b>Monocytes</b>	Lee <i>et al</i> <sup>25</sup>	Single cell RNA seq PBMCs	8 COVID-19 (severe, mild, asymptomatic), 5 influenza	4 HD	↑ classical monocytes ↓ DCs, non-classical monocytes, intermediate monocytes IFN-I-driven signatures in addition to TNF/IL-1 $\beta$ -driven inflammation	High
	Silvin <i>et al</i> <sup>23</sup>	Cy-TOF, single cell RNA seq	13 COVID-19 (mild 5, severe 8)	25 HD	↓ non-classical CD14LowCD16High monocytes ↓ the expression of HLA-DR on classical monocytes	High

Continued

**Table 2** Continued

	Author	Study type	Patients N	Control N	Cells	RoB
<b>T cells</b>	Kuri-Cervantes <i>et al</i> <sup>28</sup>	Multiparametric flow cytometry	35 (7 moderate, 28 severe), 7 recovered	12 HD	↓ CD4+ and CD8+ ↓ CD8+ mucosal-associated invariant T cells (MAIT cells) ↓ innate lymphoid cells (ILCs) =in recovered and non-recovered populations	High
<b>NK cells</b>	Kuri-Cervantes <i>et al</i> <sup>28</sup>	Multiparametric flow cytometry	35 (7 moderate, 28 severe), 7 recovered	12 HD	↓ NK cells especially of both CD56brightCD16– and CD56dimCD16 populations in severe patients and ↓ circulating CD16+ NK cells	High
	Odak <i>et al</i> <sup>34</sup>	Multiparametric flow cytometry	30 (15 severe)	None	↓ NK, NKT, γδ- T cells	High
<b>B cells</b>	Kuri-Cervantes <i>et al</i> <sup>28</sup>	Multiparametric flow cytometry	35 (7 moderate, 28 severe), 7 recovered	12 HD	↑ plasmablasts (p<0.0001) In the non-plasmablast B cell population: ↓ CD21+ CD27+ ↑ CD21– CD27– non-plasmablasts Profound oligoclonal expansion Same in both recovered and non-recovered patient populations	High
<b>COVID-19 severe vs mild</b>						
<b>T cells</b>	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	↓ CD4+ without differences in subpopulation =CD8+ total populations but ↓ T effector memory (CD45RA–CCR7–) and ↑ T effector memory CD45RA+ CCR7) cells other subpopulations = =polyfunctional T cells	Unclear
	Odak <i>et al</i> <sup>34</sup>	Multiparametric flow cytometry	30 (15 severe)	None	↓ CD8+ ↓ Treg ↓ effector/effector memory (CD45RA– CD62L–)	High
	Song <i>et al</i> <sup>33</sup>	Multiparametric flow cytometry	41 (29 mild, 12 severe)	None	↓ CD4+ and CD8+ T cells ↑ PD-1+ CD8+ T cells in severe patients ↑ TIM-3+ CD8+ T cells and TIM-3+ CD4+ T cells ↑ PD-1 expression on CD38+ HLA-DR+ CD4+ T and CD38+ HLA-DR+ CD8+ T cells	Low
<b>NK cells</b>	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	↓ NK cells ↓ granzyme A	Unclear
	Odak <i>et al</i> <sup>34</sup>	Multiparametric flow cytometry	30 (15 severe)	None	↓ NK, NKT, γδ- T cells	High
<b>COVID-19 severe vs mild to moderate</b>						
<b>Dendritic cells</b>	Lee <i>et al</i> <sup>25</sup>	Single cell RNA seq PBMCs	8 COVID-19 (severe, mild, asymptomatic), 5 influenza	4 HD	↓ DCs in the severe group	High
	Kuri-Cervantes <i>et al</i> <sup>28</sup>	Multiparametric flow cytometry	35 (7 moderate, 28 severe), 7 recovered	12 HD	↓ conventional (CD11c+ CD123lo/–) and plasmacytoid (CD11c– CD123+) compared with moderate disease and HDs	High
<b>COVID-19 active vs recovered</b>						
<b>T cells</b>	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	↓ CD4+ without differences in subpopulation =CD8+ total populations but ↓ T effector memory (CD45RA–CCR7–) and ↑ T effector memory CD45RA+ CCR7) cells other subpop = =polyfunctional T cells	Unclear
	Odak <i>et al</i> <sup>34</sup>	Multiparametric flow cytometry	30 (15 severe)	None	↓ CD8+ ↓ Treg ↓ effector/effector memory (CD45RA– CD62L–)	High
<b>NK cells</b>	Mazzoni <i>et al</i> <sup>29</sup>	Multiparametric flow cytometry	30 (13 severe)	None	↓ NK cells ↓ granzyme A	Unclear
	Odak <i>et al</i> <sup>34</sup>	Multiparametric flow cytometry	30 (15 severe)	None	↓ NK, NKT, γδ T cells	High
<b>COVID-19 Convalescent severe vs convalescent mild</b>						
<b>T cells</b>	Zhang <i>et al</i> <sup>101</sup>	Multiparametric flow cytometry	5 severe and 4 mild	12 HD	↑ CD8+ effector memory (TEM) cells vs HD ↓ MAIT cells ↑ CD8+ T effector memory cells re-expressing CD45RA (named CD8+ terminal effector cells in severe disease)	High
<b>Immunotypes associated with disease severity</b>						

Continued

Table 2 Continued

Author	Study type	Patients N	Control N	Cells	RoB
Mathew <i>et al</i> <sup>25</sup>	Multiparametric flow cytometry	125 hospitalised, 36 recovered	60 HD	Immunotype 1: Activated CD4 and CD8 T effector memory cells, ↓ circulating follicular helper cells, hyperactivated or exhausted CD8 T cells and plasmablasts Positively correlated with disease severity Immunotype 2: Not correlated with disease severity Immunotype 3: No activated T or B cells. Negatively correlated with disease severity.	

ATL, Atlanta; CITE-seq, Cellular Indexing of Transcriptomes and Epitopes by Sequencing; CyTOF, cytometry by time of flight; gMDS, granulocytic myeloid derived suppressor cells; HD, healthy donor; HK, Hong-Kong; HLA-DR, Human Leucocyte Antigen – DR isotype; IFN, interferon; IL, interleukine; ITGAM, integrin alpha M; LDN, low density neutrophils; mTOR, mechanistic target of rapamycin; NK, natural killer; PBMCs, peripheral blood mononuclear cells; RNA, ribonucleic acid; RoB, risk of bias; TLR, Toll-like receptor; TNF, tumour necrosis factor.

especially in severe patients and monocytes response to stimulation *in vitro* with bacterial or viral ligand cocktail was impaired.<sup>23 26–28</sup> The dendritic cells (DCs) pool was decreased in all patients with COVID-19 compared with HD<sup>27</sup> and especially severe patients compared with both HD and moderate forms.<sup>25 28</sup>

### Lymphoid cellular response to SAR-CoV-2 infection according to disease phenotype

The lymphoid compartment was also affected by SARS-CoV-2 infection. Lymphopenia was frequently reported with both CD4+ and CD8+ lymphocytes consistently reduced compared with HD.<sup>26 27 29</sup> The same results were observed in mild<sup>30</sup> or severe<sup>28</sup> versus HD and in recovered patients<sup>24</sup> versus HD. Other studies showed various modulation of T-cell subsets as detailed in table 2.<sup>24 31</sup> Blood CD8+ T cell cytotoxicity was decreased in mild<sup>30</sup> or all COVID-19 patients compared with HD,<sup>32</sup> as shown by a reduction in perforin, granzyme A and B production. An increase in PD-1 expression by CD8+ T cells was reported in severe patients.<sup>33</sup> To summarise, two major abnormalities were described in the lymphoid compartment: a relative percentage increase of both central memory CD4+ cells and terminal effector CD8+ cells expressing PD1 suggesting a possible exhausted phenotype. NK cells were decreased in COVID-19 patients versus HD, and in severe COVID-19 versus both HD and mildly affected individuals.<sup>24 28 29 34</sup>

Finally, results regarding recovered versus active COVID-19 were conflicting, with one study reporting no differences in the lymphoid population,<sup>28</sup> while two other studies showed a reduction of NK cells and of different T lymphocyte populations in acutely infected patients followed by a recovery in lymphocytes level during the convalescent phase.<sup>29 34</sup> B cells were less often studied but an increase in circulating plasmablasts was reported, while other results were inconsistent.<sup>24 28 29</sup> In addition, one study identified immunotypes associated with disease severity.<sup>35</sup> More specifically, the Immunotype 1 associating activated CD4 and CD8 T effector memory cells, along with a reduction of circulating follicular helper cells, hyperactivated or exhausted CD8 T cells and plasmablasts was associated with severe diseases, while Immunotype 3 lacking activated T and B cells was associated with milder forms.<sup>35</sup>

### Circulating and tissue neutrophil extracellular traps during SARS-CoV-2 infection

Five studies assessed serum and tissue neutrophil extracellular traps (NETs) release and the results are detailed in table 3. All of them reported an increase in circulating NETs in COVID-19 regardless of disease severity, when compared with healthy donors or convalescent COVID-19 patients.<sup>36–39</sup> Moreover, NETs levels in tracheal fluid were higher than plasma levels<sup>36 37</sup> and large NETs infiltrating area were reported within the lung tissue of deceased patients, along with small vessel clot occlusion with material composed of Cit-H3+ MPO+ cells and NETs.<sup>36 38 40</sup> Functionally, neutrophils isolated from COVID-19 patients with displayed a higher baseline production of NETs *in vitro*.<sup>36 37</sup> Circulating platelet-neutrophil aggregates were also observed. It has been suggested that they contribute to the hypercoagulability state observed in COVID-19 and so offer insights into the extensive pulmonary and systemic immunothrombosis that emerges in severe COVID-19.<sup>36 38</sup>

### Cytokine and chemokine profiles associated with COVID-19 severity

Studies using unbiased approaches such as mass cytometry or assessing several cytokines through Multiplex or Luminex techniques were included. Five studies assessing the cytokine release in COVID-19 regardless of disease severity showed consistent (reported in at least two manuscript) increase of IL-1 $\alpha$ , IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, IL-10, IL-17, IL-18, TNF- $\alpha$ , IFN- $\alpha$  2, IFN- $\gamma$ , G-CSF, M-CSF, TRAIL, FGF, VEGF and PGDF when compared with HDs.<sup>26 27 41–44</sup> The following chemokines were also reported to be consistently increased: Eotaxin, MCP3, MIP-1 $\alpha$ . Additional components were reported to be increased or decreased only in one study and are reported in table 4. Although few disparities in cytokine profiles were highlighted in COVID-19 compared with other infections (sepsis, ARDS or influenza), no cytokines were consistent reported to be differentially expressed.<sup>27 45</sup> Variations in cytokines and chemokines released were also reported; depending on disease severity when comparing mild with moderate disease,<sup>27 42</sup> mild or moderate vs severe.<sup>26 27 41 42 46</sup> Of interest, patients with severe COVID-19 displayed higher levels of IFN- $\gamma$ , IL-1RA, IL-6, IL-10, M-CSF, MCP-1, MCP-3 and ENRAGE when compared with milder forms.<sup>26 27 42 46</sup>



**Table 3** Circulating and tissue neutrophil extracellular traps (NETs) during SARS-CoV-2 infection

Author	Patients N	Control	RoB	
Middleton <i>et al</i> <sup>36</sup>	33 COVID-19 (n=28 hospitalised, n=5 convalescent)	17 HD	↑ circulating NETs (MPO-DNA complexes) in patients vs HD and convalescent. NET levels in tracheal aspirate fluid>in plasma samples Plasma NETs levels=in HD and recovered patients ↑ baseline NETs levels in PMNs isolated from COVID-19 patients ↓ in PMN granularity vs HD ↑ circulating platelet-neutrophil aggregates	High
Veras <i>et al</i> <sup>37</sup>	32 COVID-19 (17 critical and 15 severe)	19 HD	↑ circulating nets ↑ NETs in the tracheal aspirate from COVID-19 patients NET levels in tracheal aspirate fluid>in plasma samples ↑ baseline NET levels in PMNs isolated from COVID-19 patients ↑ branch lengths of the released NETs	Unclear
Zuo <i>et al</i> <sup>39</sup>	51 COVID-19 (27 severe, 24 mild)	30 HD	↑ cell-free DNA, MPO-DNA complexes, citrullinated histone H3 COVID-19 sera trigger control neutrophils to release NETs	High
Leppkes <i>et al</i> <sup>38</sup>	71 COVID-19	None	↑ PMNs with low buoyant density, activation pattern (low L-selectin, CD62L) and partial degranulation (increased CEACAM-8, CD66b) resembling low-density granulocytes. ↑ circulating platelet-neutrophil aggregates Exhausted phenotype with ↓ spontaneous oxidative burst ↑ MPO-DNA complexes and NE-DNA complexes ↑ NE activity in the blood more than 30-fold and 60-fold	High

DNA, desoxyribonucleic acid; HD, healthy donor; MPO, myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; PMNs, polymorphonuclear neutrophils.

In addition, one study showed that IL-1 $\alpha$ , IL-1 $\beta$ , IL-17A, IL-12 p70 and IFN- $\alpha$  were decreasing steadily after 10 days in patients with moderate forms of COVID-19, while severe patients maintained higher levels.<sup>26</sup> Del Valle *et al* have also shown that high serum IL-6 and TNF- $\alpha$  levels at the time of hospitalisation were strong and independent predictors of patient survival (p<0.0001 and p=0.0140, respectively), adjusted on prognostic factors in a large cohort of patients.<sup>47</sup>

### Interferon response to SARS-CoV-2 infection at the transcriptional and protein level

Three studies explored IFN response in patients with COVID-19 using CyTOF<sup>48</sup> or multiparametric flow cytometry<sup>32</sup> (table 5). Of interest, type I IFN responses were not sustained over time in severe and critical patients.<sup>32</sup> In one study, plasma levels of IFN- $\alpha$ 2 protein and IFN activity were significantly reduced in severe and critical patients compared with patients with mild-to-moderate disease.<sup>48</sup> In another study, impaired mechanistic target of rapamycin (mTOR) signalling and IFN- $\alpha$  production by plasmacytoid DCs was shown, and single-cell RNA sequencing revealed a lack of type I IFNs in patients with severe COVID-19 and transient expression of IFN-stimulated genes.<sup>32</sup> The failure to maintain high IFN production in severe forms of COVID-19 could also be related to loss-of-function mutations in the interferon pathway<sup>49</sup> and/or the presence of anti-IFN antibodies associated with more severe forms of the disease.<sup>13</sup> Noting the aforementioned loss of function in IFN

pathways, the data were contradictory regarding IFN production by monocytes, while an IFN signature was reported in classical inflammatory monocytes in one study, a reduction of IFN production was reported in another study.<sup>22 25</sup> Similarly, IFN- $\alpha$  and IFN- $\beta$  production in response to stimulation in vitro were impaired in acute COVID-19 patients' DCs, while in convalescent patients, DCs could only produce IFN- $\beta$ . Conversely, serum levels of IFN- $\alpha$  and IFN- $\gamma$  were increased in another study, and a correlation between viral load and IFN levels was reported.<sup>26</sup> However, methods used for cytokines measurement (Simoa or Luminex) and timing of samples (early vs late timepoints) were different between studies. In addition, cytokines were assessed at both transcriptional or protein levels depending on the study and this could partly explain the observed differences.

### Humoral immune response to SAR-CoV-2 infection according to disease phenotype

Five longitudinal studies assessing anti-SARS CoV-2 IgM and IgG using commercially available assays were included (table 6).<sup>50-54</sup> Three studies used ELISA,<sup>50 51 53</sup> while two studies used chemiluminescence immunoassays (CLIA)<sup>52</sup> targeting various SARS-CoV-2 antigens. A variable timing of appearance for IgM within the first 2 weeks after symptom onset has been described and one study reported that patients with mild COVID-19 did not show any IgM response up to 4 weeks after symptom onset.<sup>50</sup> As far as IgGs are concerned, studies using ELISA agreed that these antibodies appear by the second/third week

**Table 4** Cytokine and chemokine profiles associated with COVID-19 severity

	Author	Technique	Patients N	Control N	Cytokines	RoB
<b>COVID-19 (mild, moderate and/or severe) vs control</b>	Chi <i>et al</i> <sup>42</sup>	Multiplex (48 cytokines)	70 (22 mild, 36 moderate, 8 severe, 4 convalescent)	4 HC	↑ IL-1β, IL-1Ra, IL-2, IL-2Ra, IL-6, IL-7, IL-8, IL-9, IL-10, IL-1, IL-15, IL-17, IL-18 ↑ TNF-α, IFN-α2, IFN-γ, G-CSF, M-CSF, TRAIL, FGF, PGDF, Eotaxin, CXCL1/GRO-α ↑ MCP3, MIP-1α, MIG, MCP-1	High
	Xu <i>et al</i> <sup>43</sup>	Multiplex (48 cytokines)	(7 mild, 6 severe, 10 fatal)	4 HD	↑ 20 cytokines, chemokines and growth factors, including IL-1α, IL-1β, IL-4, IL-5, IL-7, IL-12 p40, IL-13, IL-16, TNF-α, TRAIL, IFN-α2, CXCL1/GRO-α, CXCL12/SDF-1α, CCL11/Eotaxin, CCL27/CTACK, G-CSF, LIF, MIF, SCGF and VEGF	High
	Fraser <i>et al</i> <sup>41</sup>	Multiplex (57 cytokines)	10 severe	10 HD	↑ elastase 2, HSP-70, IL-1RA, IL-6, IL-8, ↑ MCP-1 monokine induced by γ-IFN, MMP8. ↑ resistin, TNF, IL-10, IL-18, M-CSF, granzyme B, thrombospondin-1, MIP-1β, MMP-2 ↑ neutrophil gelatinase-associated lipocaline, IL-15, IFN-γ	Low
	Arunalacham <i>et al</i> <sup>27</sup>	Multiplex (17 cytokines)	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 influenza (ATL)	45 HD (HK) 24 HD (ATL)	↑ IL-6, MCP-3, TNFα, EN-RAGE, TNFSF14 and Oncostatin M	High
	Lucas <i>et al</i> <sup>26</sup>	Multiparametric flow cytometry	113 (moderate 80, severe 33)	108 HD	↑ IL-1α, IL-1β, IL-17A, IL-12 p70 and IFN-α	Low
<b>COVID-19 vs other diseases</b>	Wilson <i>et al</i> <sup>45</sup>	Luminex (76 cytokines)	15 (9 critical) vs 16 critical sepsis and 12 critical ARDS	None	↑ thymic stromal lymphopoietin lower in moderate and severe COVID-19 compared with ARDS and sepsis IL-16 lower in moderate COVID-19 compared with ARDS and sepsis	High
	Arunalacham <i>et al</i> <sup>27</sup>	Multiplex (17 cytokines)	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 influenza (ATL)	45 HD (HK) 24 HD (ATL)	↑ TNFSF14 in COVID-19 patients vs other pulmonary diseases	High
<b>Severe COVID-19 vs control</b>	Sims <i>et al</i> <sup>44</sup>	Multiplex (184 cytokines)+ IL-19 assay	25 (6 mild, 4 moderate, 8 severe, 7 critical)	20 HD	↑ 21-fold IFN-γ, 18-fold IL-6, IL-10, MCP-1, MCP-2, 12-fold MCP-3, 10-fold CXCL10, 2-fold MCP-3 IL-19 (p<0.001)	High
	Lucas <i>et al</i> <sup>26</sup>	Multiparametric flow cytometry	113 (moderate 80, severe 33)	108 HD	IL-1α, IL-1β, IL-6, IL-10, IL-18 and TNF-α are correlated with severity	Low
	Del Vallee <i>et al</i> <sup>47</sup>	ELISA	1484	HD CAR T-cell treated patients with or without cytokine release syndrome	↑ IL-6 (mean 332 pg/mL) (p<0.0001), IL-8 ((mean 110 pg/mL) (p<0.0001) and TNF-α (mean 28 pg/mL) (p<0.0001) Strong predictors of disease severity	High
<b>Mild COVID-19 vs severe</b>	Chi <i>et al</i> <sup>42</sup>	Multiplex (48 cytokines)	70 (22 mild, 36 moderate, 8 severe, 4 convalescent)	4 HD	↑ IL-6, IL-7, IL-10, G-CSF, M-CSF IP-10, MCP-3, MIP-1α, MIG, MCP-1	High
	Sims <i>et al</i> <sup>44</sup>	Multiplex (184 cytokines)+ IL-19 assay	25 (6 mild, 4 moderate, 8 severe, 7 critical)	20 HD	↑ IFN-γ, IL-1RA, IL-6, IL-10, IL-19, MCP-1, MCP-2, MCP-3, CXCL9, CXCL10, CXCL5, EN-RAGE, and poly(ADP-ribose) polymerase 1 (p<0.001)	High
	Xu <i>et al</i> <sup>43</sup>	Multiplex (48 cytokines)	(7 mild, 6 severe, 10 fatal)	4 HD	↑ 16 cytokines, chemokines and growth factors, including HGF, CXCL8/IL-8, CCL7/MCP-3, CCL2/MCP-1, CXCL9/MIG, CXCL10/IP-10, IL-6, IL-18, IL-2, MCSF, IL-1Ra, IL-2Ra/CD25, IFN-γ, CCL3/MIP-1α, basic FGF and SCF were significantly higher in fatal than severe and/or mild COVID-19 patients	High
	Arunalacham <i>et al</i> <sup>27</sup>	Multiplex (17 cytokines)	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 Influenza (ATL)	45 HD (HK) 24 HD (ATL)	↑ IL-6, MCP-3, EN-RAGE, TNFSF-14, and oncostatin M	High

Continued

**Table 4** Continued

	Author	Technique	Patients N	Control N	Cytokines	RoB
	Lucas <i>et al</i> <sup>26</sup>	Multiparametric flow cytometry	113 (moderate 80, severe 33)	108 HD	↑ thrombopoietin, IL-33, IL-16, IL-21, IL-23, IFN-λ, Eotaxin and Eotaxin 3	Low
<b>Mild COVID-19 vs moderate</b>	Chi <i>et al</i> <sup>42</sup>	Multiplex (48 cytokines)	70 (22 mild, 36 moderate, eight severe, 4 convalescent)	4 HD	↑ IL-18, M-CSF, IP-10	High
	Arunalacham <i>et al</i> <sup>27</sup>	Multiplex (17 cytokines)	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 influenza (ATL)	45 HD (HK) 24 HD (ATL)	↑ EN-RAGE, TNFSF14 and Oncostatin M	High
<b>Moderate COVID-19 vs severe</b>	Chi <i>et al</i> <sup>42</sup>	Luminex	70 (22 mild, 36 moderate eight severe, 4 convalescent)	4 HD	↑ MCP-3, MIG and MIP-1α	High
	Wilson <i>et al</i> <sup>45</sup>	Luminex (76 cytokines)	15 (9 critical) vs 16 critical sepsis and 12 critical ARDS	None	↑ PDGF-BB	High
	Wilson <i>et al</i> <sup>45</sup>	Luminex (76 cytokines)	15 (9 critical) vs 16 critical SEPSIS and 12 critical ARDS	None	No difference between four groups in IL-1β, IL-1RA, IL-6, IL-8, IL-18 and TNF-α and another 64 cytokines	High
	Arunalacham <i>et al</i> <sup>27</sup>	Multiplex (17 cytokines)	36 (HK, 27 mild, 5 moderate, 4 severe) 40 (ATL) 24 influenza (ATL)	45 HD (HK) 24 HD (ATL)	↑ IL-6, oncostatin M	High
	Lucas <i>et al</i> <sup>26</sup>	Multiparametric flow cytometry	113 (moderate 80, severe 33)	108 HD	After day 10, the following markers declined: IL-1α, IL-1β, IL-17A, IL-12 p70 and IFN-α in patients with moderate disease, while patients with severe COVID-19 maintained elevated levels Following day 10, IFNα, IFNλ, IL-1β, IL-1Ra, IL-18, IL-33, Eotaxin-2 remained high in severe while decreased in moderate	Low
<b>Mild/severe vs critical</b>	Xu <i>et al</i> <sup>43</sup>	Multiplex (48 cytokines)	(7 mild, 6 severe, 10 fatal)	4 HD	↑ 16 cytokines, chemokines and growth factors, including HGF, CXCL8/IL-8, CCL7/MCP-3, CCL2/MCP-1, CXCL9/MIG, CXCL10/IP-10, IL-6, IL-18, IL-2, MCSF, IL-1Rα, IL-2Rα/CD25, IFN-γ, CCL3/MIP-1α, basic FGF and SCF Among these IFN-γ, IL-1Rα, IL-2, IL-2Rα, IL-6, CXCL8, IL18, CCL2, CCL3, SCF, HGF and basic FGF were upregulated to similar levels at the early stages and then upregulated significantly in fatal patients at day 14	High

ARDS, acute respiratory distress syndrome; CCL, C-C motif chemokine ligand; CEACAM-8, CEA cell adhesion molecule; CXCL, chemokine (C-X-C motif) ligand; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; HGF, Hepatocyte Growth Factor; HSP, heat shock protein; IFN, interferon; IL, interleukine; IP-10, interferon gamma-induced protein 10; LIF, leukaemia inhibitory factor; MCP, monocyte chemotactic protein; M-CSF, macrophage colony stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by gamma-interferon; MIP, monocyte chemotactic protein; MMP-2, matrix metalloproteinase; PDGF-BB, platelet-derived growth factor; SCF, stem cell factor; SCGFβ, stem cell growth factor beta; SDF-1α, stromal cell-derived factor 1; CAR T-cell, chimeric antigen receptor T cells; TNF, tumour necrosis factor; TNFSF14, tumour necrosis factor ligand superfamily member 14; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

after symptom onset while those using CLIA identified IgGs as early as week 1.<sup>52 54</sup> IgGs were still detectable up to 6–8 weeks after symptom onset.<sup>50 52–54</sup> The studies assessing neutralising antibodies (Nab) provided highly heterogeneous data and since assays were not standardised, comparison across studies was not possible.<sup>50 55–57</sup> The SLR did not retrieve any article identifying a role of antibody dependent enhancement and detrimental effect of anti-SARS-CoV-2 antibodies.

### Platelets, endothelial dysfunction and thrombosis and SARS-CoV-2 infection

A clear pathophysiological link between lung inflammation in COVID-19 and extensive immunothrombosis that has been associated with severe disease and mortality exists pointing towards potential involvement of platelets and endothelial cells. One study sequenced total RNA from platelets isolated from SARS-CoV-2 infected individuals identifying specific clusters of expression in

**Table 5** Interferon response to SARS-CoV-2 infection

Author	Study type	Patients N	Control N	Populations	Cells	RoB
Hadjadj <i>et al</i> <sup>48</sup>	CytoF Simoa immunoassay RT-qPCR PBMCs	50 (15 mild to moderate, 17 severe and 18 critical patients)	HD 18	COVID-19 (mild, moderate and/or severe) vs HD  Severe COVID-19 vs HD  Severe COVID-19 vs mild/moderate	↓↓↓ IFN-β mRNA and protein  ↑ genes involved in type I IFN signalling (such as <i>IFNAR1</i> , <i>JAK1</i> and <i>TYK2</i> ) ↓ IFN-stimulated genes (such as <i>MX1</i> , <i>IFITM1</i> and <i>IFIT2</i> )  ↓↓ plasma levels of IFN-α2 protein and IFN activity significantly Lower type I IFN response in severe vs critical patients	Low
Zhu <i>et al</i> <sup>46</sup>	Multiparametric flow cytometry Patients' DC cultures	Acute COVID-19 (6 severe and 11 mild) Convalescent COVID-19 (2 severe and 22 mild)	HD	COVID-19 (mild, moderate and/or severe) vs HD	After stimulation: IFN-α was not induced in 3/3 active patients and 3/4 convalescent patients IFN-β was not increased in 3/3 active patients, rather only slightly elevated in 3/4 convalescent patients indicating a reduced capacity of making antiviral interferon	High

HD, healthy donor; IFN, interferon; JAK, Janus kinase; MCP, monocyte chemoattractant protein; TYK, tyrosine kinase.

patients with COVID-19, regardless of severity, compared with normal subjects. In particular, enriched pathways observed in COVID-19 associated with protein ubiquitination, antigen presentation and mitochondrial dysfunction. Of interest, one of the top significantly over-expressed genes was *IFITM3*, whose variants have been associated with disease severity as mentioned above.<sup>11</sup> In addition, a comparison of data obtained in patients with COVID-19 with existing RNA-Seq data in H1N1 influenza and sepsis revealed that numerous gene changes were unique for each disease condition. Of the differentially expressed genes that were shared, >96% changed in the same direction.<sup>58</sup> Data regarding the detection of platelets positive for SARS-CoV-2 RNA revealed that they were present only in a small subset of patients with COVID-19.<sup>59 60</sup> With regard to platelet function, two studies showed higher basal activation in COVID-19 as demonstrated by P-selectin expression, compared with normal subjects,<sup>58 59</sup> with basal hyperactivation and stimulated in vitro responses being more pronounced in severe COVID-19.<sup>58 59</sup> Since P-selectin is also responsible for interaction between platelets and monocytes, it is not surprising that Hottz *et al* also demonstrated that platelets form higher numbers of aggregates with monocytes in severe COVID-19. In addition, while aggregated, platelets

induce monocyte expression of tissue factor (TF) via P-selectin and integrin  $\alpha\text{IIb}/\beta 3$ .<sup>59</sup> Finally, while data on in vitro platelet aggregation are conflicting,<sup>58 61</sup> two studies agreed on a greater adhesion and spreading on fibrinogen and collagen compared with normal subjects<sup>58</sup> and in severe vs mild COVID-19.<sup>60</sup>

Regarding circulating endothelial cells (CECs), a marker of endothelial injury, data are conflicting with two studies reporting increased numbers in COVID-19 vs normal subjects,<sup>62 63</sup> one study reported numbers similar to those of normal controls<sup>64</sup> and one study observed higher numbers of CECs in patients with COVID-19 in intensive care unit (ICU) versus those not admitted to ICU.<sup>65</sup> Only one study investigated circulating endothelial progenitors (CEPs) and observed that they were higher in COVID-19 compared with normal subjects but there was no difference between mild and severe disease. Of interest, apoptotic CEPs/mL positively correlated with the copies of SARS-CoV-2 RNA in severe COVID-19.<sup>64</sup> All data pertaining to these research questions are presented in [table 7](#).



**Table 6** Humoral immune response to SAR-CoV-2 infection according to disease phenotype

Author	Population	Method	IgG	IgM	IgA	Neutralisation assay	RoB
Wang <i>et al</i> <sup>50</sup>	23 COVID-19 vs 48 HD	ELISA*	↑ at D10–15 after onset Remained ↑ for at least for 6W HD: no Abs detected	SEVERE: ↑ within W1-2 and ↓ after W4 MILD: Most patients negative up to W4 HD: no Abs detected	ND	▶ 73.9% of patients generated NAb ▶ Higher NAb titres in severe vs mild	Unclear
Xiang <i>et al</i> <sup>53</sup>	85 COVID-19 vs 60 HD	ELISA†	↑ at D11-12 after onset Remained ↑ for ≥30 HD: 5% positivity	↑ at D9 after onset, Remained ↑ for ≥30D HD: No Abs	ND	ND	Unclear
Zhao <i>et al</i> <sup>51</sup>	173 COVID-19	ELISA‡	↑ at D14 (median)	↑ at D12 (median)	ND	ND	Unclear
Xie <i>et al</i> <sup>52</sup>	56 COVID-19	CLIA§	↑ in W1 Remained ↑ for at least 41D, even after the resolution of infection	↑ in W1 and ↓ at W4-5	ND	ND	Unclear
Zhou <i>et al</i> <sup>54</sup>	52 COVID-19	CLIA*	↑ in W1 100% patients positive at D28 Remained ↑ up to D54	↑ in W1 and peak at W4 (77% of patients)	ND	ND	Unclear

\*Antigen not specified.

†Recombinant nucleocapsid protein.

‡IgM: receptor binding domain of the spike protein; IgG: a recombinant nucleoprotein.

§Envelope (E) protein and nucleocapsid (N) protein.

D, day; HD, healthy donor; M, month; NAb, neutralising antibodies; ND, not detailed; RoB, risk of bias; W, week.

### Multiparametric algorithms for prediction of disease outcome and progression

Several algorithms have been published, using mostly a retrospective design on both inception and validation cohorts (table 8). Most algorithms included clinical parameters such as: demographics (age, race, ethnicity, gender, socioeconomic status, smoking, body mass index), symptoms (fever, fatigue, shortness of breath, diarrhoea, vomiting, haemoptysis, dyspnoea, unconsciousness), comorbidities (asthma, diabetes, hypertension, immunosuppressive disease, cancer history) and treatment (nonsteroidal anti-inflammatory drugs, immunomodulatory therapies). Biological parameters were also included as follows: immune cells (white cell count, neutrophil count, lymphocyte count, neutrophil-to-lymphocyte ratio), inflammatory markers (C reactive protein, ferritin), coagulation markers (platelets, procalcitonin, NT-proBNP, AT) and others (haemoglobin, ALT, AST, direct bilirubin, albumin, chloride, potassium, anion gap, glomerular filtration rate, blood urea nitrogen, myoglobin, troponin, lactate dehydrogenase). Imaging parameters including severe chest X-ray radiographic abnormalities and diffuse pulmonary infiltration on CT that have also been linked to severe disease.

One multiparametric model aimed at predicting the risk of hospitalisation with an area under the curve (AUC) of 0.9,<sup>66</sup> while three studies aimed at predicting survival

with AUC between 0.879 and 0.955,<sup>67–69</sup> and two other aimed at predicting disease mortality with AUC between 0.871 and 0.975.<sup>70–71</sup> Other algorithms were developed, aiming at predicting disease progression towards a severe phenotype with AUC from 0.77 to 0.9.<sup>72–79</sup> Each algorithm is detailed in table 8.

### Difference in pathogenesis of SARS-CoV-2 infection between adults and children

Very few studies compared adult and paediatric patients with SARS-CoV-2 infection and all of them evaluated very small cohorts. Some differences were observed with regard to clinical (eg, diarrhoea and vomiting more frequent in children) and haematological (eg, neutropenia more frequent in children) features. This may hint possible different pathogenic mechanisms in response to SARS-CoV-2 infection; none of the studies specifically explored them.<sup>80–82</sup>

### Gut and SARS-CoV-2 infection

Only four publications about two studies investigating the gut microbiome of patients with COVID-19 were retrieved by the SLR and both of them identified a dysbiosis (online supplemental table S3). A highly heterogeneous configuration that was different according to the faecal SARS-CoV-2 viral load was observed, along with depletion of beneficial commensals and abundance of

**Table 7** Endothelial dysfunction, thrombosis and SARS-CoV-2 infection

Author	Methods	Population	Description of result	Rob
<b>Platelet gene expression</b>				
Manne <i>et al</i> <sup>58</sup>	RNA Seq	10 COVID-19 vs 5 HD	<ul style="list-style-type: none"> <li>▶ Non-ICU and ICU COVID-19 patients clustered together, but separately from healthy controls</li> <li>▶ In COVID-19 enriched pathways associated with protein ubiquitination, antigen presentation and mitochondrial dysfunction</li> <li>▶ One of the top significantly overexpressed genes in COVID-19 is <i>IFITM3</i>, coding for an antiviral protein normally absent in HD and overexpressed in dengue and H1N1</li> <li>▶ No expression of ACE2 mRNA in COVID-19 and HD (nor of ACE2 protein by WB)</li> <li>▶ In 2 ICU COVID-19 mRNA expression of the SARS-CoV-2 N1 was detected</li> <li>▶ A comparison with existing RNA-Seq data in H1N1 and sepsis revealed that numerous gene expression differences between both condition. Of the shared gene expression, &gt;96% changed in the same direction.</li> </ul>	Low
Zaid <i>et al</i> <sup>60</sup>	PCR	49 COVID-19 vs 17 HD	SARS-CoV-2 RNA was detected in 11 COVID-19 (9/38 mild and 2/11 severe) and in no HD. Individuals with positive platelets for SARS-CoV-2 RNA were significantly older but otherwise similar to the negative patients.	Unclear
<b>Platelet activation</b>				
Manne <i>et al</i> <sup>58</sup>	In vitro assays	41 COVID-19 vs 17 HD	<ul style="list-style-type: none"> <li>▶ Higher basal expression of P-sel vs HD</li> <li>▶ Increasing doses of 2MeSADP and PAR1 peptide (SFLLRN or TRAP) induced significantly greater activation (P-sel expression) vs HD</li> <li>▶ P-sel expression in response to low-dose 2MeSADP significantly correlated with COVID-19 severity based on SOFA scores</li> <li>▶ 2MeSADP, SFLLRN (TRAP) and collagen-related peptide resulted in decreased PAC-1 binding vs HD (not due to altered expression of <math>\alpha</math>IIb which was similar in the 2 groups)</li> </ul>	Low
Hottz <i>et al</i> <sup>59</sup>	In vitro assays	37 COVID-19 vs 11 HD	<ul style="list-style-type: none"> <li>▶ Higher basal expression of P-sel vs HD</li> <li>▶ Higher basal expression of P-sel in severe vs mild/asymptomatic COVID-19</li> <li>▶ Similar expression of P-sel in mild/asymptomatic vs HD</li> <li>▶ TXA2 synthesis was increased in platelets from severe but not mild/asymptomatic COVID-19</li> <li>▶ Platelet-derived factors are present in tracheal aspirates from COVID-19 patients</li> <li>▶ P-selectin elevations above HD median was predictive of in-hospital mortality (OR=9.6 (95% CI 1.02 to 90.35); p=0.045).</li> </ul>	Low
Zaid <i>et al</i> <sup>60</sup>	PCR	49 COVID-19 vs 17 HD	Suboptimal concentrations of $\alpha$ -thrombin induced higher expression of p-PCK $\delta$ vs HD, with higher concentration no difference between groups	Unclear
<b>Platelet aggregation</b>				
Manne <i>et al</i> <sup>58</sup>	In vitro assays	41 COVID-19 vs 17 HD	<ul style="list-style-type: none"> <li>▶ In vitro platelet aggregation in response to low-dose agonists (2MeSADP, thrombin and collagen) was significantly increased vs HD</li> <li>▶ Aggregation was more pronounced in ICU COVID-19, especially at lower doses of thrombin</li> <li>▶ Aggregation is associated with increased MAPK pathway in ICU COVID-19</li> <li>▶ PLA2 in upregulated at baseline vs HD and further increases on stimulation with low-dose agonists</li> <li>▶ TXA2 was increases by 2MeSADP in platelets from COVID-19 ICU</li> <li>▶ In vitro aggregation of platelets from COVID-19 ICU could be reduced by pretreatment with high-dose aspirin</li> </ul>	Low

Continued

**Table 7** Continued

Author	Methods	Population	Description of result	Rob
Denorme <i>et al</i> <sup>61</sup>	In vitro assays	11 COVID-19 vs 11 HD	<ul style="list-style-type: none"> <li>▶ In vitro generation of procoagulant platelets in response to dual agonist stimulation with thrombin and convulxin was significantly lower vs HD</li> <li>▶ Procoagulant platelet responses were similarly reduced in non-ICU and ICU COVID-19</li> <li>▶ Dysregulated procoagulant platelet responses in COVID-19 are due to higher mitochondrial ROS levels at baseline and dual agonist stimulation of platelets from COVID-19 patients did not elicit further increases in mitochondrial ROS generation</li> <li>▶ In vitro generation of procoagulant platelets in response to agonist stimulation with Ca<sup>2+</sup> ionophore A23187 was similar to HD</li> </ul>	Unclear
<b>Platelets adhesion</b>				
Manne <i>et al</i> <sup>58</sup>	In vitro assays	41 COVID-19 vs 17 HD	Greater adhesion and spreading on fibrinogen and collagen vs HD	Low
Zaid <i>et al</i> <sup>60</sup>	PCR	49 COVID-19 vs 17 HD	The number of adherent platelets on a collagen-coated surface under flow conditions was significantly higher in severe vs mild	Unclear
<b>Platelets and monocytes interactions</b>				
Hottz <i>et al</i> <sup>59</sup>	In vitro assays	37 COVID-19 VS 11 HD	<ul style="list-style-type: none"> <li>▶ Platelets formed higher numbers of aggregates with monocytes in severe COVID-19</li> <li>▶ While aggregated, platelets induced monocyte expression of tissue factor (TF) via P-sel and integrin <math>\alpha</math>IIb/<math>\beta</math>3 and TF expression was maximal at 2 hour of interaction</li> <li>▶ Only P-selectin, but not <math>\alpha</math>IIb/<math>\beta</math>3, neutralisation was able to reduce platelet-monocyte aggregate formation</li> <li>▶ Abciximab pre-treatment did not affect P-sel expression on platelets from COVID-19 patients that were aggregated with monocytes</li> <li>▶ Aspirin or clopidogrel failed to modify platelet-monocyte aggregate formation or platelet-induced TF expression</li> <li>▶ TF expression in monocytes was increased among non-survivors</li> <li>▶ Platelet-monocyte aggregates did not associate with mortality</li> </ul>	Low
<b>Circulating endothelial cells</b>				
Mancuso <i>et al</i> <sup>64</sup>	Flow cytometry	27 active COVID-19 vs 9 recovered COVID-19 vs 8 HD	<ul style="list-style-type: none"> <li>▶ CECs/mL were similar to HD</li> <li>▶ Less apoptotic CECs/mL vs HD</li> <li>▶ Less apoptotic CECs/mL in recovered COVID-19 vs HD, vs mild COVID-19 vs severe COVID-19</li> <li>▶ Apoptotic CECs/mL inversely correlated with viral RNA copies</li> </ul>	Unclear
Nizzoli <i>et al</i> <sup>63</sup>	Flow cytometry	30 COVID vs 6 HD	<ul style="list-style-type: none"> <li>▶ CECs/mL higher vs HD</li> <li>▶ CECs/mL higher in early phase of disease</li> </ul>	High
Guervilly <i>et al</i> <sup>65</sup>	Immuno magnetic separation	80 no ICU COVID-19 vs 19 ICU COVID-19	<ul style="list-style-type: none"> <li>▶ CECs/mL higher in ICU vs non-ICU COVID-19 independently of all comorbidities</li> <li>▶ CECs/mL directly correlated with length of hospital stay</li> <li>▶ CECs/mL independently associated with CKD</li> </ul>	Unclear
Khider <i>et al</i> <sup>62</sup>	Immuno magnetic separation	66 COVID-19 vs 30 clinically suspected non COVID-19	<ul style="list-style-type: none"> <li>▶ CECs/mL higher vs HD</li> <li>▶ CECs/mL lower in COVID-19 treated with anticoagulation (for any reason) vs untreated</li> <li>▶ In patients treated with ACEi or ARBs the effect of curative anticoagulation on the CEC level was more pronounced</li> </ul>	Unclear
<b>Circulating precursors endothelial cells</b>				
Mancuso <i>et al</i> <sup>64</sup>	Flow cytometry	27 active COVID-19 vs nine recovered COVID-19 vs 8 HD	<ul style="list-style-type: none"> <li>▶ Viable and apoptotic CEPs/ml higher in acute and recovered COVID-19 vs HD</li> <li>▶ No difference between mild and severe COVID-19</li> <li>▶ Apoptotic CEPs/ml positively correlated with the copies of SARS-CoV-2 RNA in severe COVID-19</li> </ul>	Unclear

HD, healthy donor; ICU, intensive care unit; RoB, risk of bias.

**Table 8** Multiparametric algorithms for prediction of disease outcome and progression

Author	Design	Statistical model	Population	Score components	Diagnostic performances	RoB
<b>Prediction of hospitalisation</b>						
Jehi <i>et al</i> <sup>66</sup>	Retrospective	LASSO logistic regression	Training: 2852 Validation: 1684	Age, race, ethnicity, gender, smoking, BMI, socioeconomic status, fever, fatigue, shortness of breath, diarrhoea, vomiting, asthma, diabetes, hypertension, immunosuppressive disease, NSAIDs, immunosuppressive treatment, platelets, AST, chloride, potassium, blood urea nitrogen	Prediction of Hospitalisation Training: AUC=0.9, Scaled Brier Score 42.6% (95% CI 37.8%, 47.4%) Validation: AUC=0.813 Scaled Brier Score 25.6% (19.9%, 31.3%)	High
<b>Prediction of survival</b>						
Wu <i>et al</i> <sup>68</sup>	Retrospective	Univariate and multivariate Cox regression analyses	Training: 210 Validation: 60	Neutrophil count, lymphocyte count, procalcitonin, age and C reactive protein	Survival Training: AUC=0.955 Validation: AUC=0.945	High
Dong <i>et al</i> <sup>67</sup>	Retrospective	LASSO logistic regression and multivariate Cox regression	Training: 369 Validation: 259	Hypertension, higher neutrophil-to-lymphocyte ratio and increased NT-proBNP	Survival Training: AUC=0.92 Validation: AUC=0.92	High
Zhang <i>et al</i> <sup>69</sup>	Retrospective	Multivariate logistic regression	Training: 516 Validation: 186	Age, lactate dehydrogenase level, neutrophil-to-lymphocyte ratio and direct bilirubin level	14 and 28 days Survival Training: C-index=0.886 Validation: C-index=0.879	High
<b>Prediction of mortality</b>						
Wang <i>et al</i> <sup>70</sup>	Retrospective	Multivariate logistic regression	Training: 199 Validation: 44	FAD-85 score age+0.01 * ferritin+D-dimer	28-day mortality Training: AUC=0.871 Validation: AUC=NA Sensitivity 86.4% Specificity 81.8%	High
Weng <i>et al</i> <sup>71</sup>	Retrospective	LASSO logistic regression	Training: 176 Validation: 125	Age, neutrophil-to-lymphocyte ratio, D-dimer and C reactive protein	28-day mortality Training: AUC=0.921 Validation: AUC=0.975 Sensitivity 86.4% SPECIFICITY 81.8%	High
<b>Prediction of disease progression</b>						
Gerotziakas <i>et al</i> <sup>75</sup>	Prospective	Multivariate logistic regression	Training: 310 Validation: 120	COMPASS-COVID-19 score: Obesity, gender, haemoglobin, lymphocyte, and the cDIC-ISTH (International Society on Thrombosis and Haemostasis score for compensated disseminated intravascular coagulation score) including platelet count, prothrombin time, D-dimers, antithrombin and protein C levels	Disease progression Training: AUC=0.77 Validation: AUC=NA Sensitivity 81% Specificity 60%	High
Bartoletti <i>et al</i> <sup>76</sup>	Retrospective	Multivariate logistic regression	Training: 644 Validation: 469	PREDI-CO score: Age, obesity, body temperature, respiratory rate, lymphocyte count, creatinine $\geq$ 1 mg/dL, C reactive protein and lactate dehydrogenase	Disease progression Training: AUC=0.89 Validation: AUC=0.85 Sensitivity 71.6% Specificity 89.1%	Unsure

Continued



**Table 8** Continued

Author	Design	Statistical model	Population	Score components	Diagnostic performances	RoB
Ji <i>et al</i> <sup>74</sup>	Retrospective	Multivariate logistic regression	Training: 86 Validation: 62	Comorbidity, dyspnoea on admission, lactate dehydrogenase, lymphocyte count	Disease progression Training: AUC=0.856 Validation: AUC=0.819 Sensitivity 94% Specificity 63.1%	High
Li <i>et al</i> <sup>73</sup>	Retrospective	Multivariate logistic regression	Training: 322 Validation: 317	(Age×LDH)/CD4 T-cell count	Disease progression Training: AUC=0.92 Validation: AUC=0.92 Sensitivity 81% Specificity 93%	High
Xu <i>et al</i> <sup>72</sup>	Retrospective	Multivariate logistic regression	Training: 315 Validation N°1: 69 Validation N°2: 123	Age, comorbid diseases, neutrophil-to-lymphocyte ratio, d-dimer, C-reactive protein, and platelet count	Disease progression to critical illness Training: AUC=0.923 Validation N°1: AUC=0.882 Validation N°2: AUC=0.906	High
Xiao <i>et al</i> <sup>77</sup>	Retrospective	Multivariate logistic regression	Training: 231 Validation No 1: 101 Validation No 2: 110	HNC-LL (hypertension, neutrophil count, C reactive protein, lymphocyte count, lactate dehydrogenase)	Disease severity Training: AUC=0.861, p<0.001 Validation: AUC=0.871, p<0.001 V Validation No 2: AUC=0.826, p<0.001	Unsure
Zhang <i>et al</i> <sup>78</sup>	Retrospective	Multivariate logistic regression	Training: 80 Validation: 22	Age, white cell count, neutrophil, glomerular filtration rate and myoglobin	Disease severity Training: AUC=0.906 Validation: AUC=0.958 Sensitivity 70.8% Specificity 89.3%	High
Laing <i>et al</i> <sup>79</sup>	Retrospective	LASSO and multivariate logistic regression	Training: 1590 Validation: 710	Chest radiographic abnormality, age, hemoptysis, dyspnoea, unconsciousness, number of comorbidities, cancer history, neutrophil-to-lymphocyte ratio, lactate dehydrogenase and direct bilirubin	Disease progression to critical illness Training: AUC=0.9 Validation: AUC=0.813	Unsure

AUC, area under the curve; RoB, risk of bias.

opportunistic pathogens. Of interest, these abnormalities persisted even after recovery from COVID-19.<sup>83–86</sup> In addition, when comparing the gut microbiome of patients with COVID-19 with that of patients with H1N1 influenza, differing overall compositions were observed. Opportunistic pathogens were reported in with more pronounced abundance in COVID-19.<sup>84</sup> Interestingly, a specific set of bacterial species allowed to discriminate the two patient groups.<sup>86</sup> One study identified increased levels of biomarkers of gut leakage and gut homing, while no difference in biomarkers of enterocyte damage were observed in patients with COVID-19 compared with normal subjects.<sup>87</sup>

### Histological lesions related to SARS-CoV-2 infections

Most histological studies have assessed lung tissue damage linked to COVID-19 in deceased individuals

(online supplemental table S4). Two studies reported viral inclusions assessed by electronic microscopy immunohistochemistry with or without in situ hybridisation.<sup>4 40</sup> Viral inclusions were observed mainly in airways and tracheal epithelium and pneumocytes. Histological studies of autopsy specimens from patients with identified cause of death being various among which ARDS, consistently reported the following tissular lesions: exudative, proliferative, mixed, organising or fibrosing diffuse alveolar damage (DAD); associated with microvascular and macrovascular thrombi.<sup>4 88 89</sup> Of interest in the study from Li *et al*, patients with fibrosing DAD were younger (p=0.034) and had a longer duration of illness (p=0.033), hospitalisation (p=0.037) and mechanical ventilation (p=0.014) compared with those with acute DAD. Similarly, patients displaying organising DAD

features had a longer duration of illness ( $p=0.032$ ) and hospitalisation ( $p=0.023$ ) compared with those with acute DAD.<sup>89</sup> De Michele *et al*<sup>90</sup> have identified different histological patterns associated with COVID-19 severity. In their autopsy series, 29 (73%) of 40 patients presented with acute lung injury (ALI) defined by the presence of hyaline membranes, DAD—with or without—an organising (proliferative) phase. This pattern was associated with longer hospitalisation ( $p=0.02$ ), longer ventilation ( $p=0.003$ ) and more radiographic alveolar infiltrates ( $p=0.01$ ).

### Comorbidities and immune response to SARS-CoV-2 infection

Although many studies assessed the impact of comorbidities on clinical outcomes of COVID-19,<sup>91</sup> only one study explored the effect of comorbidities, namely, type 2 diabetes (T2D) on immune response in patients with COVID-19 (online supplemental table S5). By means of unsupervised analyses of cytometry data and principal component analysis) including lymphocyte and monocyte subpopulations, the authors identified three distinct clusters of patients corresponding to COVID-19 without T2D, COVID-19+T2D and T2D without COVID-19. In more detail, an increase of CD14+ monocytes, increased phenotypically switched monocytes and decreased classical monocytes were observed in patients with COVID-19+T2D compared with those with COVID-19 without T2D.<sup>92</sup>

### Immunosenescence and SARS-CoV-2 infection

Three studies assessed the impact of immunosenescence on immune response to SARS-CoV-2 infection using different age cut-offs.<sup>30 93 94</sup> Among other, CD4+ and CD8+ T cells were reduced compared with younger patients, and CD8+ T cell cytotoxicity was reduced as demonstrated by a decrease in granzyme and perforin expression. Two studies reported that CD8+ T cells displayed an exhausted phenotype as shown by higher PD-1 expression.<sup>93 94</sup> However, it is worth noting that PD-1 is known to be increased in older patients regardless of their SARS-CoV-2 infection status.<sup>95</sup> Other cell population are reported in only one study, and the results are detailed in online supplemental table S6.

### Consequences of immunomodulatory drugs on viral load and host antiviral immune response

Only a few studies assessed the effects of immunomodulatory drugs on viral load and host antiviral immune response (online supplemental table S7). Several cytokines levels, including ↓ IL-6, MCP-3 and IFN- $\gamma$  were reduced after baricitinib treatment in four patients.<sup>44</sup> After tocilizumab treatment, two studies reported an increase in lymphocyte counts in small groups of five patients.<sup>29 96</sup> This is in line with data from clinical trials.<sup>97</sup> In addition, the administration of Tocilizumab restored of NK cell cytotoxicity<sup>29</sup> and rescued HLA-DR expression in conventional monocytes.<sup>96</sup>

## DISCUSSION

This SLR summarises current evidence on SARS-CoV-2 infection pathogenesis as viewed from the Rheumatology perspective. We gathered a large amount of publications showing how SARS-CoV-2 infection affects immune and non-immune cells. While some features were consistently reported across studies for both the lymphoid and the myeloid compartment, the heterogeneity of results prevented any firm conclusion being drawn in many publications. We did not retrieve data on mast cell and eosinophils since studies remain scarce.<sup>98</sup> From a cytokines' point of view, IL-6, TNF $\alpha$  and IL-1 $\beta$  production and release were associated with COVID-19 severity.<sup>99</sup> It is noteworthy that the elevations in cytokine levels including IL-6 were reported to be mild or modest in general compared with sepsis, or oncoimmunotherapy-related cytokine storm and MAS.<sup>6</sup>

In addition, genetic predisposition, especially linked to the type I IFN pathway, was shown to be responsible for more severe phenotypes in different cohorts, highlighting molecules and pathways deemed essential for a functional anti-SARS-CoV-2 response. Despite the clear genetic evidence incriminating loss-of-function mutations in the IFN pathway and the strong history of IFN link to viral immunity, a clear beneficial role of type I IFN cannot be determined so far and may vary depending on the timing and the stage of the disease. In fact, type I IFN response appeared to be variably described across studies, probably because of variable methods (transcriptomic data vs protein measurements) and timing of analysis. It is very likely that, while IFN response is initiated in all patients with COVID-19, the magnitude of IFN production and its duration to clear the virus may differ according to disease severity, and it probably fails to remain high in severe and critical patients, therefore contributing to impaired viral response and worse outcome. Longitudinal studies measuring IFN response across time and disease severity are warranted to confirm this hypothesis. In addition, IFN protein measurement in blood may not reflect disease in tissue.

Humoral response to SARS-CoV-2 tended to be variable among individuals and the presence of IgM was inconsistently reported, suggesting that some individuals do not develop an IgM response. Non-immune cells such as platelets and endothelial cells exhibit an activated phenotype favouring clotting along with a hypercoagulability state. Of interest, children and young adults were displaying different features compared with infected adults, presenting with extremely common mild forms of the disease and more rarely severe disease termed multi-system inflammatory syndrome in children. All these findings taken together address partly the knowledge gap in understanding SARS-CoV-2 infection mechanisms.

While conducting this SLR, we were faced with several limitations that prevented from drawing conclusions in several aspects of disease pathogenesis. First, limitations related to study design itself or methods used since most studies were assessing only a few randomly selected

cytokines or cell subsets. Such approaches are biased and could potentially lead to inaccurate or non-generalisable results. In this SLR, we included only studies using unsupervised clustering approaches through single cell RNA seq or similar techniques; or at least multiparametric flow cytometry for cell assessment. Similarly, only mass spectrometry or multiplexed cytokine assays that could simultaneously assess several cytokines (eg, Luminex, Muliplex) were included and analysed. Through this strict approach, we aimed at reducing the risk of biased results. The second type of limitations pertained to the heterogeneity of inclusion criteria and treatments received by individuals included in the studies. In fact, the definition of disease severity was extremely variable across studies, since the WHO scale was not always used, and also two WHO scales exist, classifying patient of moderate severity differently.<sup>100</sup>

In addition, in several studies, patients who were enrolled could receive several treatments including immunomodulators such as steroids or IL-6 receptor antagonists; and the results were presented without clustering or subgroup analysis, hereby leading to high risk of results' misinterpretation. Another aspect pertains to multiparametric algorithm studies, where in addition of the very common retrospective design, most of the algorithms published were not validated in other cohorts, while those who did, were in fact validated in temporally different cohorts but in the same population. Although the current context of the pandemic and associated rush in delivering useful science to better understand and treat the disease might explain some of these issues, the interpretation of the results needs to be guarded.

In conclusion, the results of the present SLR highlight the aberrant immune and non-immune cellular response to SARS-CoV-2 infection. Despite the massive amount of literature published, the knowledge gap in SARS-CoV-2 disease mechanisms as viewed from the Rheumatology perspective on how immunity is contributing to severe outcomes remains incompletely understood. Future studies should endeavour to address pending questions such as a better description of host-virus interactions across disease spectrum, and differences in immune response between mild and severe forms. Another important aspect to be further explored is the identification of new therapeutic targets and the study of humoral immune response to vaccination compared with infected individuals. This SLR informs the EULAR PtCs on COVID-19 pathophysiology and immunomodulatory therapies.

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