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Systematic Review of Hematological Finding in Critical Covid-19 patients

Sumiah Abdullah M Shati*

King Khalid University, Saudi Arabia

ABSTRACT

Severe COVID-19 infection results in bilateral interstitial pneumonia, often leading to acute respiratory distress syndrome (ARDS) and pulmonary fibrosis in survivors. Most patients with severe COVID-19 infections who died had developed ARDS. Currently, ARDS is treated with supportive measures, but regenerative medicine approaches including extracellular vesicle (EV)-based therapies have shown promise. Herein, we aimed to analyze whether EV-based therapies could be effective in treating severe pulmonary conditions that affect COVID-19 patients and to understand their relevance for an eventual therapeutic application to human patients. Using a defined search strategy, we conducted a systematic review of the literature and found 39 articles (2014–2020) that reported effects of EVs, mainly derived from stem cells, in lung injury models (one large animal study, none in human). EV treatment resulted in: (1) Attenuation of inflammation (reduction of pro-inflammatory cytokines and neutrophil infiltration, M2 macrophage polarization); (2) Regeneration of alveolar epithelium (decreased apoptosis and stimulation of surfactant production); (3) Repair of microvascular permeability (increased endothelial cell junction proteins); (4) Prevention of fibrosis (reduced fibrin production). These effects were mediated by the release of EV cargo and identified factors including mi Rs-126, 30b-3p, 145, 27a-3p, syndecan-1, hepatocyte growth factor and angiopoietin-1. This review indicates that EV-based therapies hold great potential for COVID-19 related lung injuries as they target multiple pathways and enhance tissue regeneration. However, before translating EV therapies into human clinical trials, efforts should be directed at developing good manufacturing practice solutions for EVs and testing optimal dosage and administration route in large animal models.

Key words: COVID-19, Inflammation, Pneumonia, Alveolar epithelium

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Corresponding author: Sumiah Abdullah M Shati
e-mail ✉ :sama.shati23@gmail.com
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INTRODUCTION

Three epidemics connected to emerging coronaviruses have occurred in the last two decades: severe acute respiratory syndrome (SARS) in 2002, Middle East respiratory syndrome (MERS) in 2012, and the continuing pandemic of coronavirus disease in 2019. (COVID-19) [1].

In December 2019, the first reports of COVID-19, a disease linked to SARS-CoV-2, were reported in China. The virus has continued to spread since then, and the World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020 [2]. More than 34 million cases have been reported worldwide as of October 3, 2020, with over one million deaths. COVID-19 causes fever and respiratory symptoms that are similar to pneumonia in the majority of patients. GI problems, dermatological signs, cardiovascular events, and neurological manifestations are among the less common symptoms recorded [1].

The novel viral was identified as an enveloped, single-stranded, positive-strand RNA virus that can cause respiratory infections in people. These zoonotic viruses could transmit to humans from various animal species, similar to (SARS-Covid) in 2002 and (MERS-Covid) in 2012 and suggested that additional emergence events are likely [3,4].

SARS-CoV-2 had an incubation period of 5.2 days (95 percent confidence interval: 4.1–7.0), with a range of 2 to 14 days [5,6]. The most common modes of transmission are respiratory droplets or close contact [7]. The role of aerosolized particles in transmission is still debatable. [8]. Men are consistently overrepresented in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and coronavirus disease 2019 (COVID-19) severe outcomes, including higher fatality rates. These differences are likely due to gender-specific behaviours, genetic and hormonal factors, and sex differences in biological pathways related to SARS-CoV-2 infection. Several social, behavioural, and comorbid factors are implicated in the generally worse outcomes in men compared with women. Underlying biological sex differences and their effects on COVID-19 outcomes, however, have received less attention. The present review summarizes the available literature regarding proposed molecular and cellular markers of COVID-19 infection,

their associations with health outcomes, and any reported modification by sex. Biological sex differences characterized by such biomarkers exist within healthy populations and also differ with age- and sex-specific conditions, such as pregnancy and menopause. In the context of COVID-19, descriptive biomarker levels are often reported by sex, but data pertaining to the effect of patient sex on the relationship between biomarkers and COVID-19 disease severity/outcomes are scarce. Such biomarkers may offer plausible explanations for the worse COVID-19 outcomes seen in men. There is the need for larger studies with sex-specific reporting and robust analyses to elucidate how sex modifies cellular and molecular pathways associated with SARS-CoV-2. This will improve interpretation of biomarkers and clinical management of COVID-19 patients by facilitating a personalized medical approach to risk stratification, prevention, and treatment.

The relationship between biological sex and the risk of infection and illness severity is complicated. The immune response is a good example: While females have a higher immune response overall, males are more prone to produce the cytokine storm linked to poor COVID-19 outcomes [9].

Sex variations in immune responses to SARS-CoV-2 have been described [9], as well as the fact that only a few people with blood type O are infected [10]. 10–15 percent of individuals having severe disease and over 105,952 deaths recorded [11]. Most of patients have a mild sickness (80%), 15% are very unwell (requiring oxygen), and 5% will need to be admitted to an intensive care unit (ICU). The Clinical Blood Sciences Laboratory (CBSL) is an important part of the COVID-19 disease monitoring and management process [12].

Inflammation is the body's first line of defenses against infection, triggering both innate and adaptive immune responses in response to threats [12].

During bacterial infections, procalcitonin (PCT) is produced into the blood stream and maintained by the interleukins IL1-, IL-6, and tumor necrosis factor alpha (TNF-). Interferon gamma (IFN-), the primary activator of macrophages as well as a stimulator of natural killer cells and neutrophils, inhibits PCT. As a result, with uncomplicated COVID-19 disease, PCT levels should remain within the reference interval. In patients who acquire severe manifestations of the condition, markedly aberrant PCT findings are consistent with bacterial coinfection [6,10,12-14].

When infection is suspected or confirmed, lactate is less than 2 mmol/L, and vasopressors are required to maintain a mean arterial pressure (MAP) of 60–65 mmHg in the absence of hypovolemia, septic shock is diagnosed [12,15].

Measuring C-reactive protein (CRP), interleukin 6 (IL-6), ferritin, and procalcitonin can help doctors identify people who need these medicines. CRP is driven by IL-6, which is an early indicator of a patient's inflammatory condition. Elevated IL-6 concentrations, together with the rate at which the result is increasing, indicate that clinical status is about to deteriorate [12]. Conclusions Baseline IL-6 greater than 30 pg/mL predicts IMV requirement in patients with COVID-19 and contributes to establish an adequate indication for TCZ administration.

Abbreviations used: ARDS, Acute respiratory distress syndrome; CAR, Chimeric antigen receptor; COPD, Chronic obstructive pulmonary disease; COVID-19, Coronavirus disease 2019; CRP, C-reactive protein; FiO2, Fraction of inspired oxygen; IL-6R, IL-6 receptor; IMV, Invasive mechanical ventilation; IQR, Interquartile range; LDH, Lactate dehydrogenase; PaO2, Arterial oxygen tension; ROC, Receiver-operating characteristic; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; TCZ, Tocilizumab.

Severity of the respiratory sickness produced by SARS-CoV-2 is thought to be attributable in part to an increased immunological response to the virus [16,17]. This reaction has been documented in patients treated with chimeric antigen receptor (CAR) T-cell treatment and in prior respiratory virus outbreaks [16,18]. Proinflammatory cytokines like IL-1 and IL-6 play an important role in this process [17-19].

The only medicine currently approved for the treatment of the cytokine release syndrome associated with CAR T-cell therapy is Tocilizumab (TCZ), an anti–IL-6 receptor (IL-6R) antibody [19].

Several hospitalized severe and critical COVID-19 patients have high levels of cytokines and chemokine's such as IL-2, IL-6, IL-7, IL-10, G-CSF, CCL2, CCL3, CXCL10, and TNF, as seen in SARS and MERS severe cases, implying a significant role for these Innate immune cells are the earliest responders following infection, identifying virus pathogen-associated molecular patterns that often elicits a type I IFN signature (PAMPs) [20].

It is widely known that endosomal RNA receptors (TLR3 and TLR7) or cytosolic RNA sensors such as RIG-I/MDA5 [21,22]. Identify genomic RNA or intermediary molecules created during viral replication such as dsRNA. Because SARS-Covid and MERS-Covid use different signalling pathways-blocking strategies, the innate response mediated by type I IFN is occasionally repressed, particularly in severe illness cases [23].

T-cell responses to the SARS-CoV-2 spike protein have recently been described and found to correlate with either IgG or IgA antibody titters in COVID-19 patient cohorts, both of which are important aspects for vaccine development and long-term (memory) immunological responses [24-26].

B cells have been linked to harmful roles in autoimmune disorders, transplantation, cancer, and even infections, exerting alternative antibody-independent responses that frequently rely on cytokine release with pro- or anti-inflammatory qualities. Immature recent bone marrow emigrants (transitional B cells), (nave B cells), and (memory B cells and antibody-secreting plasma blasts/ plasma cell) are all examples of circulating human B cells.

We became interested in mapping these cells in COVID-19 patients because many of them have been implicated in the pathogenesis of acute and chronic viral infections [20,27].

Severe COVID-19 is frequently exacerbated by coagulopathy, according to certain research, and increased levels of D-dimer and fibrin/fibrinogen degradation products (FDP) are linked to a poor prognosis in severe COVID-19 [28-30].

Increased d-dimer is the most documented coagulation/ fibrinolysis anomaly in COVID-19, and its association with prognosis has been explored. However, the utility of d-dimer assessment alone has significant limitations [31].

Many aspects of COVID-19's pathogenesis are yet unknown. The pathogenesis is still being investigated from the standpoint of laboratory haematology. Although the d-dimer is a good marker, it has limitations when it comes to determining the etiology of the coagulation abnormalities seen in COVID-19 [31].

Ten severe COVID-19 patients received one dose of 200 mL convalescent plasma with neutralizing antibody titers over 1:640. Within three days of receiving convalescent plasma therapy, clinical symptoms improved dramatically, associated with an increase in oxyhemoglobin saturation [31].

Children have a lower incidence than adults; according to the latest Morbidity and Mortality Weekly Report (MMWR) from the US Centres for Disease Control and Prevention (CDC), children accounted for 5% of the cases, 2 while the Chinese CDC stated that 2% of the cases were under the age of 19 [31].

Lymphopenia is common in adults with severe illness, and leukocytosis with neutrophilia is regarded an undesirable characteristic. Because of their immature immune system and ACE2 expression, leukocyte alterations, particularly lymphopenia, were less common in children with COVD-19. When hematological abnormalities were discovered in children with COVID-19, the most common finding was leukopenia. Lymphopenia was mostly detected in older hospitalized children. The most prevalent hematological abnormality in COVID-19 new-borns and babies was lymphocytosis [31].

In patients with End Stage Kidney Disease (ESKD), Yoon et al. discovered that naive T cell lymphocytes and memory T cells are more susceptible to apoptosis [32]. T cell, Th cell, killer T cell, and NK cell numbers are all lower in dialysis patients, regardless of SARS-CoV-2 infection. As a result, absolute cut-off values for lymphopenia may not be useful in identifying SARS-CoV-2 infection in haemodialysis patients. However, lymphopenia that is lower than the prior baseline levels may still provide insight into the underlying COVID-19, as lymphopenia is frequently reported in haemodialysis patients [33].

In haemodialysis patients, the kinetics of the antibody response to SARS-CoV-2 infection is still unknown. The first study of possible antibody responses in six haemodialysis patients was published by De Vriese et al. They discovered that the SARS-CoV-2 nucleocapsid (N) protein elicited an IgG response in the second week following symptom onset. Although it is unclear if those findings apply to the entire haemodialysis population [34], the use of antibody-detecting fast diagnostic tests is not advised. It could be administered with caution in low-resource facilities 10–14 days following the onset of symptoms [33].

METHODS

A systematic review of the literature was carried out in order to find all studies that mentioned Hematological Findings in Critical Covid-19 patients. The procedures stated in the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [34] was used to conduct this review. The systematic review was carried out utilizing an electronic database search technique (PubMed/MEDLINE, Scopus, and Cochrane).

Eligibility criteria

Terms used were “blood changes in critical covid patients or critical coronavirus disease or critical covid patients” and “hematological finding in covid OR hematological finding in critical covid patients OR laboratory changes in critical covid patients”. Studies published from 2020 until September 10th, 2021, were included. Reference lists were searched to identify relevant cross references. Books, documents, clinical trials, randomized clinical trials, review and systematic reviews were included. Meta-analysis reports and studies on animals were excluded. All studies that reported at least one outcome of interest (Covid-19, critical Covid patients, hematological finding, laboratory changes) were included in the analysis. After the selection of potential eligible papers using the title and the abstract, three reviewers independently retrieved the full-text articles to assess the final eligibility.

Any disagreement over the eligibility of a specific study was resolved through the discussion with a fourth author.

RESULTS

Original studies describing cases meeting the definition of Critical covid patients by World Health Organization (WHO), or Centres for Disease Control and Prevention (CDC) [35], Primary outcome analysis focused on hematological parameters in critical covid patients.

A search strategy was designed with keywords combining the blood changes in critical covid patients, critical coronavirus disease, critical covid patients, hematological finding in critical covid patients, laboratory changes in critical covid patients and severe coronavirus disease, including articles published from August 13, 2020, to August 13, 2021. Electronic databases were searched (PubMed), including COVID-19-specific repositories (Cochrane COVID-19 Study Register

and WHO COVID-19 Global Research Database). The reference lists of included studies were considered additional sources.

Search strategy criteria

Inclusion criteria

- Study population: blood changes in critical covid patients, critical coronavirus disease, critical covid patients, hematological finding in critical covid patients, laboratory changes in critical covid patients and severe coronavirus disease.
- Types of study designs: RCT, observational studies, case-control studies, cross-sectional studies, case reports, and case series.

Exclusion criteria

- Studies with incomplete or lacking necessary data.
- Duplicate studies.
- Studies without accessible full-text versions.
- Studies not in English language.
- Studies on animals.
- Studies with therapeutic Agent.

Study characteristics

The search strategy yielded 67 records. After removing duplicates, 61 unique publications were screened on title and abstract of which 29 were excluded. 32 full-text articles were assessed for eligibility. Finally, 32 studies were included (Figure 1).

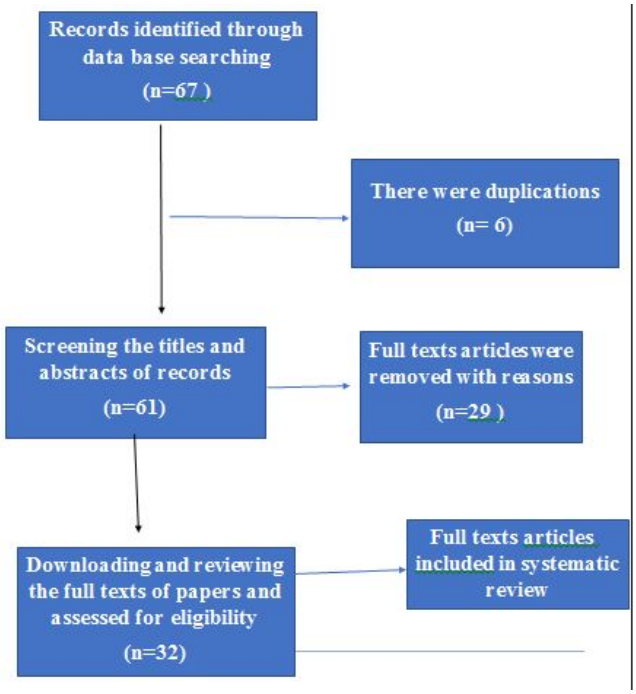

Figure 1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram.

Study of B cell subsets as severity-associated signatures in critical covid-19 patients is being carried out by researchers at the University of California, San Francisco (UCSF) on 100 critical COVID-19 patients

Routine laboratory tests were taken, including a complete blood count, glucose, blood urea nitrogen (BUN), Creatinine (Cr), liver function tests, C-reactive protein (CRP), lactate dehydrogenase (LDH), Creatinine kinase (CPK), fibrinogen, D-dimer, coagulation tests and ferritin. The following disease-severity scores were also calculated for these patients: qSOFA (quick Sepsis-related Organ Failure Assessment: identifies patients outside the ICU with a suspected infection that are at high risk for in-hospital mortality), NEWS (National Early Warning Score: determines the degree of illness and prompts critical care intervention), PSI-PORT (Pneumonia Severity Index/Pneumonia-patient Outcomes Research Team: calculates the probability of morbidity/mortality among patients with community-acquired pneumonia) [15,36].

The CD19+B cell fraction of peripheral blood mononuclear cells (PBMCs) in COVID-19 patients appears to be slightly increased at severe and then critical cases. This is because of a shift in this compartment's composition with a more evident expansion in the Tr CD24hi CD21/lo fraction compared to its correspondent in healthy controls. There were no differences in the gross naïve B compartment, but a significant increase in CD11c+ activated naïve (actN) lymphocytes was observed in severe cases compared to mild/moderate patients [36] (Figures 2 and 3).

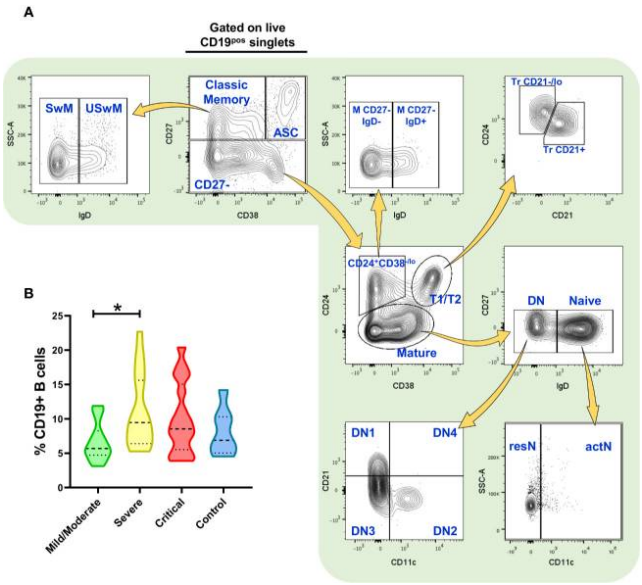

Figure 2: Flow cytometry analysis of B cell subsets and frequency of total (CD19+) B cells. (A) Gating strategy for the identification of the indicated B cell subsets in peripheral blood mononuclear cells (PBMCs) (depicting representative results from a healthy control) previously selected from singlets gate (SSC-A vs. SSC-H) and live Zombie Green- cells. (B) Frequency of total CD19+B cells in PBMCs from patients infected with SARS-CoV-2 (n=52) and healthy controls (n=7, negative PCR for SARS-CoV-2).

Frequency values are displayed as mean (dashed line) plus lower and upper quartiles (dotted lines). The data were analyzed by a Kruskal-Wallis test followed by a Dunn's post-hoc test. *p<0.01. This figure was made using arrows from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

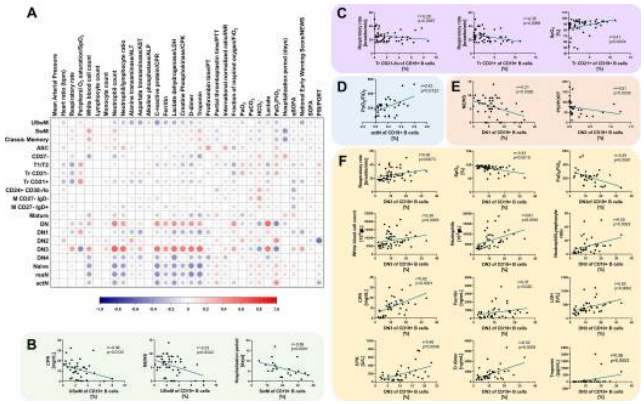

Figure 3: B cell subsets correlate with severity scores and different clinical parameters in Coronavirus Disease 2019 (COVID-19) patients. (A) Correlation matrix showing a graphical representation of calculated Spearman's coefficient calculations between the B cell subset frequencies and indicated severity indexes/clinical and laboratory variables. The underlying color scale indicates Spearman's coefficient values. The size of each dot also denotes the correlation strength. (B) Statistically significant correlations between memory B cell subsets and C-reactive protein (CRP), NEWS score and hospitalization length. (C) Statistically significant correlations between Tr B cell subsets and respiratory rate and SpO2. (D) The statistically significant correlation between actN subset and PaO2/FiO2. (E) Statistically significant correlations between DN1 B cell subset and NEWS score and DN2 subset with PSI/PORT score. (F) Statistically significant correlations between DN3 cell subset and respiratory rate, SpO2, PaO2/FiO2, white blood cell count, neutrophils, neutrophil/lymphocyte ratio, CPR, ferritin, lactate dehydrogenase (LDH), Creatinine kinase (CPK), D-dimer, and troponin. In all graphs, calculated Spearman's correlation (r) and significant p values (at least p<0.01) are shown.

Patient clusters of M CD27-IgD+, actN, and DN2 cell frequencies in each of the COVID-19 presentations were mapped. Clade "C" shows a pattern where DN3, ASC, and Swum subsets seem to be increased in critical cases [20].

COVID-19 in haemodialysis patient reported by the Royal College of Anaesthetists (RCAN)

Patient was not suspected of having co-infection with bacterial pneumonia, as he denied any epidemiological exposure. A SARS-CoV-2 antibody test was performed, but the result was negative. The patient was discharged from hospital on the seventh day of his hospitalization. Laboratory tests showed anemia (haemoglobin level 9.2 g/dL), a white blood cell count of 15,452 cells/μL, with a low absolute lymphocyte count of 920 cells/μL, and a platelet count of 153,000 cells/μL [33].

Suspicion of viral pneumonia was raised, and a SARS-CoV-2 antibody test was performed, but the result was negative. The patient was discharged from hospital on the seventh day of his hospitalization. Ten days later, he presented with a progressive shortness of breath and dry cough, without fever. His physical examination revealed tachypnea, peripheral oxygen saturation was 88% in room air, and he was afebrile. Co-infection with bacterial pneumonia was suspected. [33].

Laboratory tests showed anemia (haemoglobin level 9.2 g/dL), a white blood cell count of 15,452 cells/μL, with a low absolute lymphocyte count of 920 cells/μL, and a platelet count of 153,000 cells/μL [33].

ACE2 and TMPRSS2: Sex differences in expression and regulation

The risk of SARS-CoV-2 infection and COVID-19 severity may be related to the gene expression of ACE2 and TMPRSS2, which lie on the X chromosome and escape X-chromosome inactivation. COPD, smoking and COPD are more prevalent among males than females [9].

Immunological and inflammatory biomarkers

Males with severe COVID-19 reportedly have a higher CRP concentration compared with females, independent of age and co-morbidities. Early elevation in C-reactive protein (CRP) greater than 15 mg/L provides a marker of disease severity [9].

Adaptive immune response

Lymphocytes are among the first responders to viral agents, including SARS-CoV-2, and are associated with COVID-19 severity. A single-centre Wuhan study showed that in ill patients, concentrations of SARS-CoV-2 immunoglobulin G were significantly higher in females compared with males and remained so until 4 weeks from hospital admission.

A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: a possible reason underlying different outcome between sexes [37].

Innate immune system

Males have higher baseline numbers of total leukocytes, monocytes, neutrophils, eosinophil's, and basophils compared with females. Toll-like receptors, which Upregulated type 1 interferon (IFN), may be up to 10-fold higher in females than males

Sex hormones, menopause, and hormone replacement therapy

Sex steroids, including testosterone, estrogen, and progesterone, regulate immune and inflammatory responses. Estrogen during pre-menopause has anti-

inflammatory effects, attended by lower levels of IL-6, IL-8, and TNF-alpha. Low levels of testosterone in elderly men have been associated with up regulation of inflammatory markers [38].

Markers of calcium homeostasis and COVID-19

Procalcitonin

Procalcitonin levels are higher in severe cases of COVID-19. Risk of severe infection may be five-fold higher in patients with elevated levels of PCT. PCT level of greater than 0.5 ng/mL is typically considered a sign of bacterial but not viral infection [39].

Vitamin D

Vitamin D deficiency increases the risk of acute viral respiratory infection and community-acquired pneumonia, according to meta-analyses [39].

Vitamin D has been shown to reduce IL-6 production in monocytes. This evidence provides a rationale for supplementing COVID-19-positive patients with vitamin D [39].

Organ-specific biomarkers

Cardiac biomarkers

Sex-specific troponin and NT-pro BNP thresholds do not improve the predictive value of myocardial infarction or death in patients with COVID-19 infections, according to a study published in The Lancet Infectious Diseases (2013) by researchers at the University of Bristol [40].

Liver function

SARS-CoV-2 causes liver damage through various mechanisms, from direct cellular toxicity to the effects of immune-related inflammation. One study of 168 patients critically ill with COVID-19 reported significantly higher levels of ALT and aspartate aminotransferase in males than females [40].

Renal function

A large prospective cohort study of 701 patients in Wuhan, China, noted that acute kidney injury (AKI) occurred in 5% of patients. Higher elevations in these renal biomarkers, along with proteinuria, and hematuria, occur in critically ill patients compared with patients with mild or moderate infection. 126, 127 and females have been previously shown to be at lower risk of AKI compared with males.

Muscle

Muscle breakdown as evidenced by raised levels of creatine phosphokinase (CPK) and myoglobin has been reported to occur in patients with COVID-19 disease [13].

Dynamic fluctuations in coagulation and fibrinolysis

Study of 184 severe COVID-19 cases in the ICU found 75 cases of thrombosis. 60% of ICU inpatients had VTE, compared to 10% of general ward patients [35,41].

Coagulation and fibrinolysis pathology of COVID-19 patients was investigated in a seminal paper published by the US Department of Health, Welfare and Human Services (HHS) in 1974. Figure 4 shows the results of that study, which is cited in almost every study discussing the coagulation pathology of this disease [28].

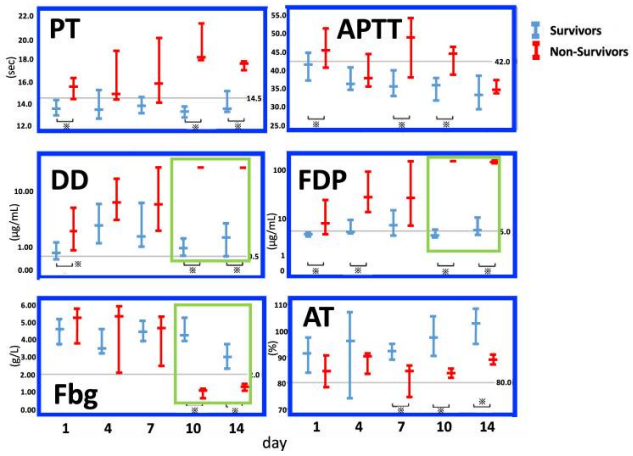

Figure 4: Time-dependent changes in coagulation and fibrinolytic markers in COVID-19 (modified from Reference [6]. PT prothrombin time, APTT activated partial thromboplastin time, DD d di mer, FDP fibrin/ fibrinogen degradationproducts, Fbg fibrinogen, AT a ntithrombin.

Prediction of prognosis by d-dimer level on admission

Many studies have suggested that the prognosis of COVID-19 can be estimated by levels of d-dimer on admission [42-45], but the paper by Tang et al. was a pioneering work [28]. However, accurate assessment of prognosis cannot be achieved with admission data alone.

Large fluctuations in coagulation and fibrinolysis

In fatal cases, fibrinogen was as high as 400 mg/dL on Day 7 but dropped sharply to about 100 mg/dl on Day 10. FDP and d-dimer levels increased sharply in just 3 days.

Schmidt et al. reviewed the outcomes of patients under Extracorporeal membrane oxygenation (ECMO), reporting a mortality rate of 31%, major bleeding in 42%, and haemorrhagic infarction in 5%. Complications of enhanced-fibrinolytic-type DIC: described above; Vascular endotheliosis, which is considered to cause severe vascular endothelial injury and vascular fragility; Acquired von Willebrand factor multimer structural analysis is required for definitive diagnosis [46].

Decreased platelet and lymphocyte counts

When the platelet count fell below 10 × 104/μL during the course of treatment, the prognosis became particularly severe. Mortality rates were reported as 92.1%, 61.2%, 17.5% and 4.7%. The cause of decreasing platelet counts was considered to be DIC in that paper.

ICU COVID+ patients show more pronounced CD8+ T lymphopenia and higher B/T8 cell ratios. CD4+ T-lymphocyte count (359 vs. 533 cells/μL), platelets (239 vs. 182 platelets/nL, p=0.01), respectively, was observed in both groups of patients with acute lymphoprolithiasis patients. An exploratory analysis of the B cell to CD8+ T cell ratio showed a significant difference between ICU COVI's and COVID's patients (1.80 vs. 0.98).

Intermediate and activated monocytes are consumed in the ICU COVID+ cohort

Comparing percentages of monocytes, the ICU COVID+ cohort showed significantly lower rates of intermediate monocytes compared to the ICU COVID–group (5.6 vs. 13%, p<0.01), which was also true for activated HLA-DR/CD14 positive monocytes (17 vs. 34 %, p<0.01).

Complex immune changes are observed in ICU COVID+ but not in NCU COVID+ patients

ICU COVID+ patients treated in the ICU of the NCU showed significant differences in leukocyte counts, proportion of lymphocytes (7 vs. 20%) and CRP (180 vs. 37 mg/L, p<0.01) as well as CRP and haemoglobin levels (10 vs. 13 g/dL, p=0.07) between the two groups.

Patients with dismal outcome presented with more pronounced immune changes compared to survivors

A larger proportion of patients in the positive outcome group reached normalization of lymphocytes and its subpopulations than in the group of patients with negative outcome. In the ICU COVID+ group with favourable outcome, it took 12 days for lymphocytes, 2.9 days for B cells, 7.3 days for CD4 T cells, 19 days to recover (Figure 5).

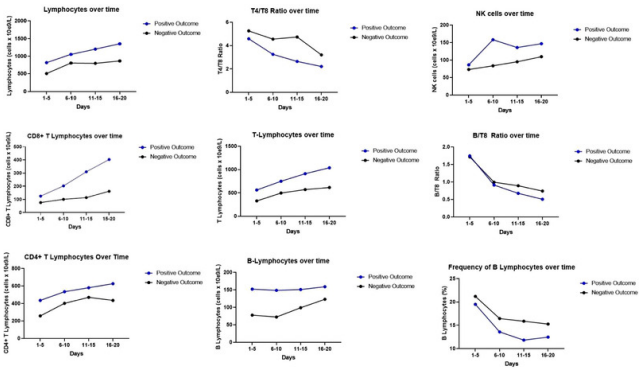

Figure 5: Comparisons of parameters per outcome over time until day 20. Graphs show cell counts or cell frequencies in four different time points for two subgroups of the ICU COVID+ cohort. Blue dots are means of the subgroup with positive outcome (survival) (N=16) and black dots are means of the subgroup with negative outcome (death) (N=8). Comparisons made using mixed effect model showed no significant differences between curves. A statistically significant interaction of cell count and outcome was verified for lymphocytes, T lymphocytes, and CD8+ lymphocytes.

We found lower concentrations of IL 6 (624 vs. 1560 pg./mL), ferritin (1253 vs. 2295 μg/L), and CRP (125.6 vs. 197 g/dL) in the positive outcome group as well as higher platelet counts (262.9 vs. 178.4/nL) in patients with negative outcomes. Fig. shows cell counts, cell ratios, and cell frequencies according to outcome in specific time intervals until day 20 (Figure 5).

NK cells and B cells exhibit increased proliferation in COVID-19 patients

Analysis of the lymphoid compartment in COVID-19 patients revealed a low frequency of CD56bright NK cells and enhanced expression of the proliferation marker Ki. 67 compared to healthy individuals. Analysis of the B cell compartment also demonstrated increased proliferation in severely diseased patients (Figure 6).

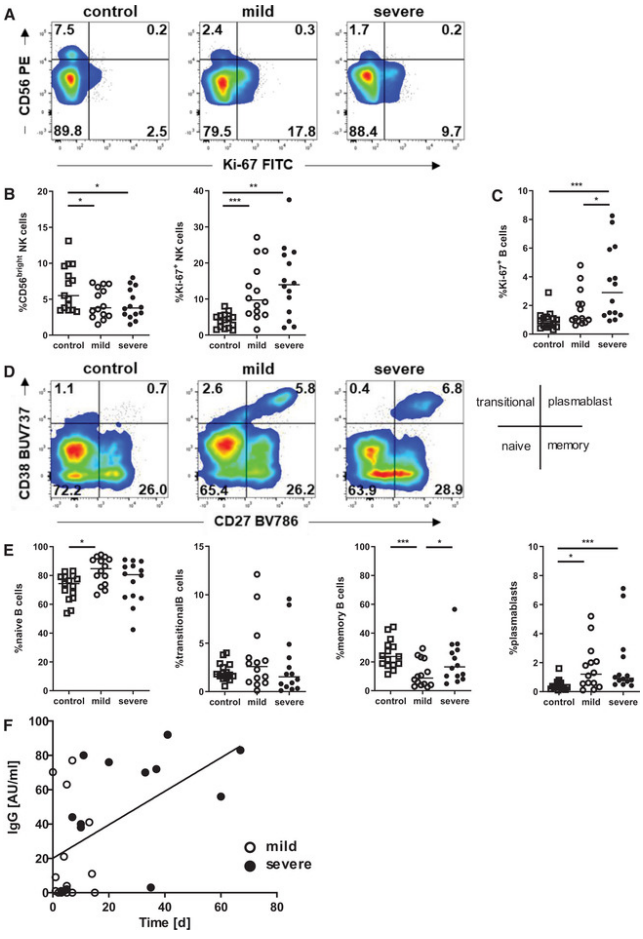

Figure 6: NK cells and B cells exhibit increased proliferation in COVID-19 patients. (A, B) Samples from healthy controls and patients with mild and severe COVID-19 infection were gated for NK cells and analyzed for CD56 and Ki-67 expression by flow cytometry. (A) Density plots show a representative experiment out of 5 experiments with 6-10 donors

per experiment, (B) graphs exhibit cumulative data of all COVID-19 patients (mild: open circle, n=14; severe: black circle, n=14) and healthy controls (open squares, n=15). (C) Proliferation of B cells was analyzed by Ki-67 staining, each symbol represents an individual subject (healthy controls: n=15; mild: n=14; severe: n=14). (D) Expression of CD27 and CD38 were determined in B cells, density plots show a representative experiment out of 5 experiments with 6-10 donors per experiment. Diagram defines B-cell subsets and data were measured by flow cytometry. (E) Frequencies of naïve B cells (CD27−/CD38−), transitional B cells (CD27−/CD38+), memory B cells (CD27+/CD38−), and plasma blasts (CD27high/CD38high) among all live B cells are shown. Healthy controls (n=15) and patients with mild (n=14) and severe COVID-19 infection (n=14) are compared. (B, C, E) Bars represent median, significance within these cohorts is calculated using Mann–Whitney-U test with *<0.05, **<0.01, and *<0.001. (F) SARS-CoV-2-specific IgG titers in patients with mild (white dots) and severe (black dots) COVID-19 are depicted as measured on the indicated day after onset of symptoms by chemiluminescent Immunoassay (CLIA). Data from 2 experiments with 10-20 donors per experiment r=0.5272, **p < 0.01 (Spearman's test).**

COVID-19 patients with severe disease show enhanced T-cell activation and differentiation

There was a clear increase in the activation of both T-cell subsets in patients with COVID 19 disease and patients with more severe forms of the disease. Activation of CD4 Th cells and cytotoxic CD8 T cells by HLA-DR and CD38 expression increased in patients suffering from COVID 19 disease.

SARS patients with severe disease showed an expansion of central memory (CM) CD4 T cells pointing to an active immune response. There was a significant correlation of CD8 T-cell activation and differentiation in patients with SARS-CoV2 infection as well as high activation state associated with increased effector and decreased terminal differentiation.

SARS-CoV-2 infection modulates T-cell functionality

Cytotoxic molecules perforin and granzyme B were strongly Upregulated in CD8 T cells in COVID 19 patients. Expression of cytokines IFN-γ, TNF, IL-4, and CD40L in CD4 T cells was comparable in healthy individuals and COVID-19 patients.

Altered chemokine receptor expression in severe COVID-19 attracts T cells to the lungs CD8 T cells from patients with severe disease showed enhanced attracting to sites of inflammation and limited homing to secondary lymphoid organs. CD4 T cells showed higher CCR4 expression, which was restricted again to severe cases. In contrast, CCR6 expression was downregulated in both mild and severe disease [47].

Study of COVID-19 patients who were admitted in Huoshenshan hospital and Taikang Tongji hospital

A total of 159 patients were diagnosed with severe COVID-19. The median age of these patients was 69 years (interquartile ranges: 63.00, 78.00). One hundred four (71.72%) patients had comorbidities including hypertension (46.21%), diabetes (18.62%), coronary heart disease (13.10%) and cerebrovascular disease (8.28%). In the entire cohort, 57 (39.31%) patients died.

Predicted efficiency of the combination scores

Almost all the combined scores could predict in-hospital death in COVID-19 with a relatively high accuracy, but the LDH-lymphocyte ratio (LLR) had the highest accuracy compared with other scores. These scoring systems were calculated by the up-regulated parameters divided by the most common down-regulated parameter, which was the lymphocyte count.

Elevated LLR correlated significantly with the prognosis of severe COVID-19

We found that higher levels of LLR were significantly associated with the incidence of common complications on admission including liver injury, renal injury and higher DIC scores.

3.5. LLR was an independent prognostic factor for severe COVID-19 in both training and validation cohorts

LLR >345 was still an independent risk factor for prognosis in patients with severe COVID-19, according to multivariate logistic regression analysis. The AUC of LLR in the validation cohort was 0.857 (95% CI: 0.718–0.997) [48].

Hematological finding in COVID-19 children

Abnormalities in the white blood cell count in children with COVID-19

Original studies published up to July 27, 2020 providing data on white blood cell (WBC) abnormalities in children with COVID-19 [49-60].

The association of lymphopenia with COVID-19 severity was documented in two studies from China, of 171 and 36children, respectively. In a systematic review of 486 hospitalized children, the most common abnormalities were lymphocytosis (22%) and leukopenia (21%) [54, 58].

2.2. Abnormalities in the red blood cell count in children with COVID-19

Data on children with COVID-19 have, to date, shown no abnormalities in red blood cell (RBC) count or level of hemoglobin (Hb) [50,53-55] Hb levels were normal in asymptomatic children but also in severe disease, and did not differ between children admitted to the ICU or to a medical unit. Anemia was a common feature in the

children with a Kawasaki-like disease associated with SARS-CoV-2 infection, called multisystem inflammatory syndrome(MIS). One case report described a 17-year-old male with a history of refractory chronic immune thrombocytopenia that manifested as autoimmune hemolytic anemia during infection with SARS-CoV-2 [61].

More than 900 articles on pediatric diseases associated with SARS-CoV-2 were searched. Risk of bias was low, despite short follow-up in all studies and publication length of 1,000 sesquipedias. After removing duplicates, 567 unique publications were screened for inclusion.

Increased inflammatory markers, including C-reactive protein (CRP), ferritin (910 µg/l [457–1521), and interleukin-6 (244.5 pg/ml (107–379) were frequently documented.

Coagulation markers such as d-dimers (3750 ng/ml [1946–6896) and fibrinogen (640 mg/dl [504–800) were Upregulated. Myocardial injury marker troponins (188 ng/l [60–61) and brain natriuretic peptide (BNP) (424–3325) were often elevated [62-66].

Two-thirds of patients were IgG-positive (362/569; 63.6%). Of single cases, 115/138 (83.3%) had a microbiologically confirmed SARS-CoV-2 infection (PCR and/or serology-positive). Only 338/901 (37.5%) had positive respiratory RT-PCR.

DISCUSSION AND CONCLUSION

Severe and critical groups of patients in COVID-19 showed a decrease in USwM B cells while only severe patients exhibit a significant decrease in SwM, both cases compared to healthy donors. This memory decline could be the result of the activation of pre-existing memory cells (specific for a coronavirus different from SARS-CoV-2) changing into "atypical" memory (CD27-) and/or ASC.

Most critical COVID-19 patients aberrantly display high proportions of DN2, atypical CD27-switched memory, and SwM B cells in a similar trend to severe patients. DN1 cells decreased in all three patient groups, while DN2/DN3 seems to be increased in the severe and/or critical groups. The DN1:DN3 ratio was reversed among these clinical categories.

B cells specific to the viral "spike" glycoprotein S are highly correlated with the clinical phenotype in COVID-19. The correlation between different B cell subsets and ventilatory parameters could be related to the hypoxic conditions during infection. Our study has several limitations, including the limited sample size for elderly healthy donors [67].

PT is one of the most important parameters used to assess coagulation function in the clinic. COVID-19 patients had significantly higher levels of IL-2R, IL-6, and TNF-α in addition to abnormal PT and INR.

PT-act, D-dimer, and FDP are significant predictors of mortality in patients with COVID-19. The accuracy of coagulation parameters for predicting in-hospital mortality has been evaluated by researchers at the University of Texas Medical Branch.

There are several limitations to the study, which was limited by factors that are inherent to retrospective analysis.

Coagulation tests required for COVID-19

APTT testing in patients with COVID-19 also provides useful for screening for lupus anticoagulant as well as monitoring heparin. Fibrinogen can also be measured regularly to check for changes in the type of DIC.

Macro thrombosis

In COVID-19, both arterial and venous thrombosis has been reported. In general, DVT and PTE may occur at the same time, collectively referred to as venous thromboembolism (VTE). [45] (Figure 6).

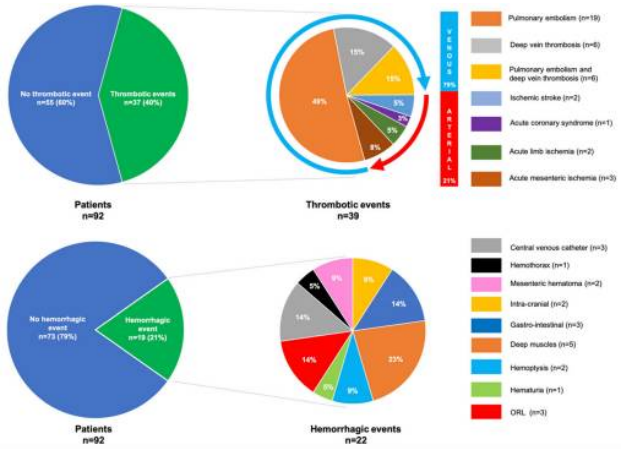

Figure 6: Sites of thrombosis and bleeding in COVID-19.

Micro thrombosis

Macro thrombosis such as VTE can be diagnosed by imaging modalities such as contrast-enhanced CT. Pathophysiology of enhanced-fibrinolytic-type DIC may have been present in those fatal COVID-19 patients [67].

Covid in haemodialysis patients

ESKD patients on haemodialysis are considered to be at high risk for SARS-CoV-2 infection. Symptoms can mimic uraemia conditions or fluid overload from inadequate dialysis. The prevalence of COVID-19 and its severity among ICDs varies worldwide.

The kinetics of the antibody response to SARS-CoV-2 infection in hemodialysis patients remains to be determined. A majority of patients develop an antibody response in the second week after symptom onset of symptoms as a result of infection.

Sex differences

Sex differences may affect the risk of infection and the severity of the disease, its outcomes, and its biomarkers. Several comorbidities, which disproportionally occur in men, likely contribute to worse COVID-19 outcomes. The

role of biological sex and risk for infection and disease severity is complex.

Septic shock

Septic shock in adults is recognized when infection is suspected or confirmed, lactate is ≥ 2 mmol/L and vasopressors are needed to maintain a mean arterial pressure (MAP) of 60–65 mmHg in the absence of hypovolemia [15].

Cytokine storm syndrome

Procalcitonin (PCT) is released into the circulation during bacterial infections and sustained by interleukins IL1-β, IL-6 and tumor necrosis factor alpha (TNF-α). PCT levels should remain within the reference interval in uncomplicated COVID-19 disease.

Liver: hepatocyte injury and disseminated intravascular coagulopathy. Elevated liver chemistries (alanine transaminase (ALT) aspartate aminotransferase (AST) in COVID-19 disease are indicative of hepatocyte injury. Prothrombin test (PT) measures the time it takes blood to clot.

Heart: myocardial injury, high-sensitivity cardiac troponin and B-type natriuretic peptide. Patients with heart failure are prone to hemodynamic decompensation and an unfavorable course when infected with SARS-CoV-2. This raises the possibility of myocarditis occurring in some COVID-19 patients irrespective of existing or pre-existing CVD.

Problems of previous studies using coagulation tests

D-Dimer is certainly a useful marker in assessing the thrombotic pathology of COVID-19. However, previous reports discussing coagulation and fibrinolysis in COVID-19 have shown that Prothrombin time (PT) and d-dimer (dimer) are often measured, but fibrinogen, activated partial thromboplastin time (APTT) and other factors are often left measured.

Immune dysfunction in COVID-19 patients

The mechanisms involving immune dysfunction, especially of T lymphocytes, seem to be multifactorial and associated with a complex context of overstimulation, excessive migration, cell exhaustion and simultaneous inhibition. Patients with severe disease seem to have higher levels of SARS-CoV2-specific CD4+ Lymphopenia. ICU patients in the ICU COVID+ cohort had higher levels of IgG3 and IgG1 compared to controls. This was found despite the use of hydroxychloroquine, an anti-diuretic drug used to treat COVID (Crohn's and Idiopathic Vertigo).

T cells in patients with severe SARS-CoV-2 infection were not phenotypically exhaustive, but produced high amounts of effector molecules perforin, granzyme B, and IFN-y. This raises the assumption that even in clinically severe cases functionality of T-effector cells seems to be maintained.

T lymphocytes and NK cells play a role in the control of COVID-19. Patients with decreased B cells at diagnosis were reported to shed the virus for a longer period. Increased B-cell activation leads to a lupus-like phenotype in critically ill SARS patients.

LDH is a sensitive parameter for evaluating organ injury in COVID-19. Elevation of LDH was associated with liver injury, renal injury, DIC score ≥ 2, and poor outcome. However, liver injury or renal injury was not independent risk factors for prognosis.

Children with COVID-19

Most children with severe manifestations of COVID-19 recover completely. In a case series, obesity and asthma were prevalent in the cohort but not significantly associated with pediatric intensive care units (PICU) admission.

In children with COVID-19, lymphopenia was the most common hematological abnormality. Anemia and thrombocytopenia were rarely found in children with the disease. SARS-CoV-2 infection is a rare complication in children. Thus, in children, not only the clinical severity but also the age may have an impact on the WBC.

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