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CHAPTER

5

Laboratory characteristics of cytokine storm syndrome in COVID-19 infection

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Cytokine storm or cytokine release syndrome: definition and features

Inflammation is a universal protective mechanism that activates in response to a pathogen invasion. In some pathologies, the immune system inability to eliminate pathogen can lead to its overstimulation and cytokine storm syndrome (CSS) development [1]. This severe condition is characterized by overwhelming systemic inflammation and hemodynamic instability, leading to a multiple organ dysfunction syndrome (MODS) and fatal outcomes in many cases. Common manifestations include sustained fever, splenomegaly, hepatomegaly with liver dysfunction, lymphadenopathy, coagulopathy, cytopenia, skin rash, and variable neurologic symptoms [2]. The documented conditions causing CSS are hemophagocytic lymphohistiocytosis (HLH), septic shock (SS), toxin-mediated shock syndrome (e.g., staph toxic shock), some acute viral infections, and graft versus host disease [3].

The main cytokine classes include interleukins, interferons, chemokines, mesenchymal growth factors, the tumor necrosis factor (TNF) family, and adipokines. Cytokines are secreted biologically active molecules that regulate cell growth and metabolism, as well as cellular interactions through their specific binding to certain receptors and subsequent induction of intracellular signaling. There exists cytokines that are involved in adaptive immunity (IL-2 and IL-4), proinflammatory cytokines and interleukins (interferon-I, -II and -III; IL-1, IL-6, and IL-17; and TNF-α) and antiinflammatory cytokines (e.g., IL-10). In response to

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stressful internal processes (such as cancer or microbial infection), host cells secrete cytokines that play a very important role in reprogramming cellular metabolism as a defense response [4]. Interferons, interleukins, chemokines, and TNF α are the main components involved in the development of the CSS.

Cytokines affect nearly every biological process, including embryonic development, disease pathogenesis, nonspecific and specific response to antigen, changes in cognitive functions and aging [5]. Under a pathogen invasion, cytokines serve as intercellular messengers in the immune system, integrating function of several cell types into a coherent immune response, targeted to eliminate infectious agent [6]. In the process of an innate immune response, the production and secretion of cytokines occurs due to the nonspecific stimulation of pattern-recognition receptors (PRRs), which detect distinct evolutionarily conserved structures on pathogens, termed pathogen-associated molecular patterns (PAMPs). These receptors are based in antigen-presenting cells (APCs), such as granulocytes, macrophages, and dendritic cells (DCs), where they are located on the cell surface, in endosomes or in cytoplasm according to the receptor subclass [7]. It is considered that key proinflammatory cytokines in different hyperinflammatory states are IFN-γ, TNF, IL-1β, IL-6, IL-10, and IL-18, and their metabolic effects explain most of manifestations, appearing during CSS development.

In March 2020, the World Health Organization announced a pandemic of new coronavirus disease 2019 (COVID-19), which in most severe cases was characterized by pulmonary infiltration, accompanied by CSS [8]. According to recent data, impaired clearance of SARS-CoV-2 due to its genetic characteristics and virulence factors, delayed, and weakened interferon response to SARS-CoV-2 in the early stages of the disease, increased nettosis and pyroptosis induced by the virus created background for a severe course of viral infection complicated by CSS syndrome [9–11]. A distinctive feature of CSS was an uncontrolled and dysfunctional immune response associated with constant activation of lymphocytes and macrophages, which produced large number of cytokines, causing CSS. Many clinical features of CS can be explained by the effects of proinflammatory cytokines such as interferon (INF)-gamma, TNF, interleukin (IL) -1, IL-6, and IL-18 [12–17].

The severity of COVID-19 is associated with robust systemic inflammatory response that can initiate a CSS including circulating cytokines and chemokines, such as IL-1α, IL-1β, IL-2, IL-7, IL-6, IL-8, IL-10, TNF, interferon (IFN)-γ, granulocyte colony-stimulating factor (G-CSF), IFN-inducible protein-10 (IP-10), CCL2 (monocyte chemotactic protein [MCP]-1), CCL3 (macrophage inflammatory protein one alpha [MIP1α]), CXC-chemokine ligand 10 (CXCL10), etc. [18–21]. Features of CSS and pathogenesis of COVID-19 have been proposed to be a consequence of immune hyperactivity and/or a failure to resolve the inflammatory response because of ongoing viral replication or immune dysregulation [22]. On the one hand, a correlation was found between viral load in the nasopharynx and levels of cytokines (e.g., IFN-α, IFN-γ and TNF). On the other hand, CSS associated with reduced cytotoxic CD4 + and CD8 + T cells, NK cells, memory T cells and regulatory T cells, B cells [23,24]. Probably CSS during COVID-19 is the result of a defective (or delayed) first line of defense, followed by persistent hypercytokinemia and dysfunctional T-cell response. This consequences in impaired clearance of apoptotic cells or infected/activated macrophages, increased viral replication, followed by IL-18/IFN-γ synthesis, activating macrophages, resulting in the release of multiple cytokines,

hemophagocytosis, coagulopathy, and ARDS. An unbalanced immune response in COVID-19 illustrates by a weak production of type I interferons (IFN-Is) [25]. Antagonism occurs at various phases in IFN signaling pathway, including by preventing pattern recognition by the viral RNA recognizing receptor (RRR), by preventing RRR signaling via TBK1/nuclear factor κB kinase-subunit-ε inhibitor (IKKε), TRAF3 and IRF3, preventing downstream interferon signaling through STAT1; and promoting host mRNA degradation and inhibiting host protein translation. Antagonism of the interferon response promotes viral replication, which leads to an increase in the release of pyroptosis products, which can further cause aberrant inflammatory reactions [26].

Clinical examples of cytokine storm syndrome

The term CSS was frequently used to describe the severe course of a number of diseases that led to an uncontrolled inflammatory response. Initially CSS was described as a part of excessive inflammatory response in graft versus host disease (GvHD) in 1993, and further the term was adopted for severe cases of viral and bacterial infections [27]. Pathological immune activation may occur under infectious, iatrogenic triggers or malignancies, and usually requires some latent or diagnosed preexisting conditions, such as genetic immune defects, prolonged immunosuppression, or chronic aberrant immune activity like in rheumatic diseases and persistent infections. Since the avian influenza epidemic in 2003–08 with high mortality rate, this term has become widespread [28]. Since the pandemic was recognized COVID-19-associated CSS development has become the major health problem [8].

The documented conditions, leading to CSS, also include genetically mediated immunological disorders, such as hemophagocytic lymphohistiocytosis (HLH) and its particular variant—macrophage activation syndrome (MAS), but also bacterial sepsis, toxin-mediated shock syndrome, some viral infections, Kawasaki disease, thrombocytopenic purpura [29]. Occasionally the term CSS is used to refer «cytokine release syndrome» (CRS), which occurs in result of targeted antitumor therapy (e.g., anti-CD19 CAR T-cell therapy), though this condition is considered to affect distinct immunological pathway and may be delayed until days or weeks after treatment [30]. Clinical examples of CSS presents in Table 5.1.

Although CSS and sepsis can cause deep endothelial activation and disseminated intravascular coagulation, some patients with primary immune-mediated vasculopathies develop syndromes similar to CSS (fever, disseminated intravascular coagulation, and organ dysfunction). This is perhaps best described in *Kawasaki disease* [31]. A significant proportion of patients with *thrombotic thrombocytopenic purpura* often develop characteristic signs of CSS such as prolonged fever, hemodynamic instability, central nervous system involvement, and coagulopathy.

Clinical signs of CSS include persistent fever, splenomegaly, hepatomegaly with liver dysfunction, lymphadenopathy, coagulopathy, cytopenia, skin rash, and various neurological symptoms. Symptoms of CSS are difficult to distinguish from those caused by underlying diseases [4,32–36]. Observation of patients with pathology associated with CSS is critical for the diagnosis of this condition. All signs are nonspecific in themselves, however, a combination of clinical symptoms and laboratory manifestations, their severity and changes over time

TABLE 5.1 Clinical examples of cytokine storm syndrome.

Disease/Condition	Damaging factor
Primary hemophagocytic lymphohistiocytosis	Mutations in genes PRF1, UNC13D or MUNC13-4, STX11 and STXBP2, UNC18B
Secondary HLH in the presence of malignant neoplasms, infections, autoimmune or autoinflammatory pathologies	Difficulty eliminating an infectious agent with a weakened T-cell response; cytokines produced by tumor cells, etc.
Macrophage activation syndrome	Disruption of perforin-mediated target cell lysis
Sepsis and septic shock	Lipopolysaccharide and other microbial components
Cytokine release syndrome (post-CAR-T cell therapy)	Hyperactivation of antigen-presenting cells upon infusion of antitumor T cells
Kawasaki disease and other primary vascular causes of CSS	Superantigen (viruses and bacteria)

underlie the diagnosis of CSS. Comparison of clinical and laboratory characteristics of different types of CSS is shown in Table 5.2.

Prognostic value of cytokine measurement in COVID-19

In a number of studies of cytokines in COVID-19, it was shown that in patients with severe COVID-19 and those who died from this infection, the levels of cytokines such as IL-1β, IL-2 and its soluble receptor, IL-6, IL-8, IL-17, IL-18, TNF-α, monocyte chemoattractant protein 1 (MCP1 or CCL2), macrophage inflammatory protein 1-alpha (MIP-1α or CCL3)), as well as the antiinflammatory cytokine IL-10, were significantly higher than in the group of patients with milder forms of SARS-CoV-2 [16,37].

During the first wave of coronavirus epidemic that developed in St. Petersburg, Russia, late spring of 2020 we collected serum samples, clinical data, and disease outcomes of 226 patients hospitalized in clinics of Pavlov State Medical University with viral pneumonia (CT severity score $\geq$2). In all COVID-19 patients, a SARS-CoV-2 nucleic acid sample was confirmed by PCR using samples from oropharyngeal swabs before hospitalization (Table 5.3). All serum samples were obtained at the time of hospitalization. This study was approved by the local ethics committee of Pavlov First Saint Petersburg State Medical University. The studied cohort of COVID-19 pneumonia included 138 (61%) men and 88 (39%) women, whose mean age was 56.82 $\pm$ 13.9 (from 23 to 87 years old). Any of the patients were vaccinated at that time. All examined patients had fever above 38°C, cough (158 [69.9%]), pain and chest pressure (31 [13,7%]). Diarrhea (11 [25%]) and anosmia (18.5 [42%]) were more common among patients with a favorable course of COVID-19.

In our cohort IL-1b, IL-2, IL-6, IL-8, IL-10, IL-18, TNF-a, IFNα, IFNy were measured with ELISA. The concentrations of IL-2, IL-1b, TNF-α, and IL-8 in patients with COVID-19-associated pneumonia were significantly higher than in healthy donors, but no differences

TABLE 5.2 Comparison of clinical and laboratory characteristics of CSS.

Characteristics	Primary HLH	Post CAR-T cell therapy	Sepsis	Macrophage activation syndrome	COVID-19
Clinical	Fever; Hepatosplenomegaly; Hepatobiliary dysfunction; Generalized lymphadenopathy; Coagulopathy; Neurologic symptoms; Headache; Cognitive changes; Focal neurologic deficits; Seizure	Fever; hypotension; Hypoxia; Malaise; Anorexia; Myalgias; Tachycardia; Widened pulse pressure; Capillary leak syndrome; Renal impairment; Hepatic failure; DIC; Multiorgan dysfunction;	Fever (more than 38.5°C) or hypothermia (less than 36°C); Hypotension; tachycardia; Tachypnea; Rigors; altered mental status; Mottled skin on inspection; petechiae; diaphoresis; Respiratory (cough, hemoptysis at el.); Gastrointestinal (abdominal distention on inspection, abdominal pain et al.); Genitourinary (costovertebral tenderness and suprapubic pain on palpatio, caginal discharge, or bleeding); Skin and soft tissue (erythema and/or edema, lymphadenopathy, wounds, ulcerations et al.); Meningismus; septic arthritis; endocarditis;	Fever hepatosplenome-galy, lymphadenopathy Hemorrhages (purpura, easy bruising, mucosal bleeding) Rash Central nervous system manifestations (lethargy, irritability, disorientation, headache, seizures, and coma)	Fever; Hypotension; Hypoxia; ARDS; Cardiomyopathy; Multi-organ dysfunction; Thrombosis;
Laboratory	Cytopenia (2 lineages in the peripheral blood); hypofibrinogenemia; ↑Ferritin; Hypertriglyceridemia; Hyperbilirubinemia; ↑ ALT, AST; ↑Cell count and/or protein content in CSF; ↑sCD25; ↑sCD163; Low or absent natural killer cell activity;	↑CRP; ↑↑Ferritin; ↑D dimer; ↑Fibrinogen; ↑Triglycerides;	↑↑↑WBC; ↓ Platelet; ↑Glucose; ↑Creatinine; ↑ ALT, AST; ↑Serum lactate; ↑Procalcitonin; blood, urine, stool, sputum, skin, cerebrospinal fluid cultures;	Pancytopenia; Hypofibrinogenemia; ↑ ALT, AST; Hypertriglyceridemia,; ↑↑↑ ferritin; Coagulopathy; Hyponatremia; Hyperfibrinogenemia (and low ESR)	Leukopenia/ lymphopenia/anemia; Thrombocytopenis (or Tr-cytosis); ↑CRP; Hyperferritinemia; Hypoalbminemia; Cytolysis (transaminases, direct bilirubin, and ammonia) ↑Alkaline phosphatase; ↑Triglycerides; CSF pleocytosis; ↑D-dimer; Hyperfibrinogenemia (and low ESR)

TABLE 5.3 Demographic and clinical characteristics of COVID-19 patients (n = 226).

Characteristics	All patients (n = 226)	Recovery (n = 190)	Death (n = 36)	*P* value
Demographic				
Age 0–45 years, % (n)	17.8 (41)	18.9 (36)	8.3 (3)	ns
Age 45–65 years,% (n)	50.4 (114)	52.6 (100)	30.5 (11)	0,01
Age 65–85 years,% (n)	31.4 (71)	26.8 (51)	58.3 (21)	< 0,0005
Men, %,n)	61.0 (138)	60.5 (115)	63.8 (23)	ns
BMI (kg/m^2), median (25–75th)	29.41 (8–33)	29.7 (2–34)	27.9 (24.9–31.96)	ns
Clinical symptoms				
Fever (°C), median (25–75th)	38.9	39.0	38.8	ns
Cough, % (n)	69.9 (158)	70.5 (134)	66.6 (24)	< 0,0001
Pain/tightness in the chest, % (n)	13,731)	13,1 (25)	16,6(6)	NS
Diarrhea, % (n)	11 (25)	12.1 (23)	5.55 (2)	Nns
Anosmia, % (n)	18.58 (42)	21.57 (41)	2.7 (1)	0,01
CT severity score (Estimation of lung parenchymal involvement)				
CT-2, moderate (25-50%)	42.47 (96)	44.2 (84)	33.3 (12)	0,02
CT-3, severe (50-75%)	39.8 (90)	41.5 (79)	30.5 (11)	0,02
CT-4, critical (>75%)	6.19 (14)	14.2 (27)	33.3 (12)	0,01
SpO2,%,median (25–75th)	89.5 (84–94)	91 (86–94)	81(70–87.5)	< 0,0001
NEWS2 >4, median (25–75th)	6 (3–7)	5 (3–7)	7 (6–8)	< 0,0001

were found between the deceased and surviving patients. In our study, the patients who died were more likely to have initially elevated concentrations of IL-6, IL-10, IL-18, and PCT than recovered patients (Fig. 5.1).

Clinical significance of cytokine concentration was confirmed by a number of studies. It was known that IL-6 is synthesized by T-lymphocytes, fibroblasts, endothelial cells, monocytes and was an important mediator during the acute phase response in sepsis and other infections [38]. It was found that this cytokine is elevated in both severe and mild COVID-19, while it has a direct correlation with the volume of the affected lung tissue in patients with ARDS. E. Giofoni et al. (2020) showed that PZ IL-6 25 pg/mL in blood is an independent risk factor for the progression of severe COVID-19 [39]. In another study, IL-6 levels> 80 pg/mL were associated with the need for mechanical ventilation [40].

Several studies have shown that the concentration of IL-18 in the blood has a significant correlation with the severity of COVID-19 and damage to vital organs [41]. Noteworthy is the fact that the increase in blood levels of IL-18, due to the activation of NLRP3/

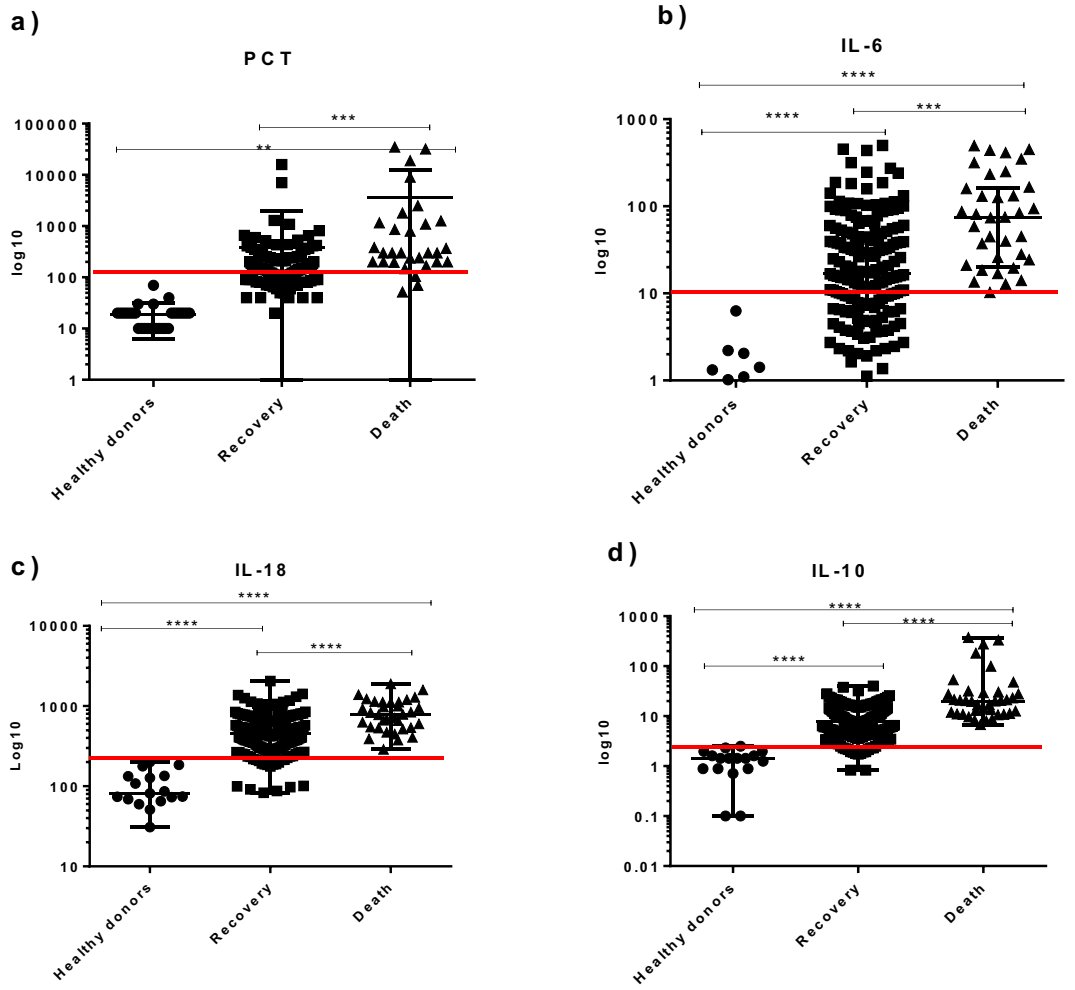

FIGURE 5.1 Concentration of procalcitonin (a, PCT), interleukin 6 (b,IL-6), interleukin 18 (c,IL18), interleukin 10 (d,IL10) in healthy individuals, recovered and deceased patients with COVID-19-associated pneumonia. Cytokine concentrations are presented in ng/mL on vertical axis as log10. Notes: IL-6—interleukin 6, IL-18—interleukin 18, IL-10—interleukin 10, PCT—procalcitonin.

inflammasome, is characteristic of both COVID-19 and autoinflammatory diseases. In the cohort we studied, in those who died the concentration of IL-18 was significantly higher than in those patients who survived. It was noted that the levels of IL-18 in our study had correlations with the severity of respiratory failure, the degree of lung damage according to CT data, as well as the NEWS and SOFA scores.

A unique feature of COVID-19 is an increase in the level of IL-10 in patients with severe disease [14,42,43]. It should be noted that IL-10 is also one of the key cytokines in sepsis and systemic inflammatory processes. On the one hand, the induction of IL-10 synthesis at

the initial stage of COVID-19 inhibits cellular immunity. However, as the production of endogenous IL-10 increases, as in endotoxemia and sepsis, IL-10 can enhance the hyperinflammatory response [44]. The results of our study indicate that IL-10, according to the ROC analysis, is a more informative indicator of a poor prognosis in patients with COVID-19-associated pneumonia compared to other biomarkers.

Many studies have shown that an increased PCT level is significantly associated with the severity of COVID-19 [13,45,46]. It is assumed that the cascade of inflammatory reactions triggered by the coronavirus through the release of proinflammatory cytokines such as IL-1b and IL-6 can induce the release of PCT in patients even without bacterial coinfection. A meta-analysis showed that the severe form of COVID-19 can be distinguished from the moderate one by a slightly higher PCT (by 0.2 ng/mL). In our cohort, the procalcitonin level of 0.32 ng/mL or higher was recorded in almost half of the patients who died, which confirms its high predictive value. An increase in both CRP and PCT may be associated not only with a huge inflammatory process, but also with a higher incidence of bacterial superinfections among critically ill patients with COVID-19 (up to 50% among deceased patients).

Immunological patterns of secondary hemophagocytic lymphohistiocytosis, bacterial sepsis, and COVID-19

Secondary HLH (sHLS) and sepsis are both typical hyperinflammatory diseases associated with CSS. Some common features were found in CSS caused by COVID-19 and sHLH due to the severity of their clinical course, frequent lungs involvement [6], and similar immunological profile including elevated IL-1β, IL-2, IL-8, IL-10, IL-17, TNF-α, IFN-γ et al. [7]. Furthermore, both COVID-19 and HLH also has common immunological and clinical characteristics with septic shock (SS), caused by bacterial infection [8,9]. Although an elevation of many inflammatory parameters is essential for all mentioned conditions, some of them could be combined and serve as patterns, reflecting immunopathological characteristics of underlying diseases.

There were also available retrospective collections of 49 samples from patients with secondary hemophagocytic syndrome and 52 samples from patient with septic shock collected a year before. Serum samples and clinical data of 39 sHLH and 47 SS consecutive fatal cases that had been collected within 2018−2019 years were analyzed retrospectively and compared to fatal COVID-19 group. The median number of days from admission and taking blood until the end point was 6.0 days in sHLH and 1.0 day in SS. 7-day and 28-day CFRs consisted 50.0% and 75.0% in sHLH, and 95.4% and 100.0% in SS, respectively. The sHLH diagnosis was established with the use of HLH-2004 criteria [12] and H-Score [13]. The median number of points was 5.0 (4.0−6.0) by HLH-2004 criteria and 227 (178.5−256.0) by H-Score. The diagnosis of sepsis was established according to ACCP/SCCM sepsis criteria and confirmed based on the positive blood culture result in 57.45% (27/47) cases [14]. The median value on the Sequential Organ Failure Assessment (SOFA) Score in SS patients consisted 12.0 points. Assessing the risk of developing a severe course of COVID-19 has evaluated using previously developed National Early Warning Score 2 (NEWS2). The median value on National Early Warning Score 2 (NEWS2) Score in fatal COVID-19 patients consisted seven points.

Serum samples, demographic, and clinical data were collected from 37 lethal cases of COVID-19, 39 retrospective fatal cases sHLH and 47 retrospective fatal SS (Table 5.4). Secondary HPS with hyperferrininemia was related to hemato-oncology diseases, and in over half of septic patients the microbial agent was known. The samples came from patients during last two weeks of the disease before death.

Serum cytokine levels in patients with fatal COVID-19, SS, and sHLH are presented in a Table 5.5:

Hypercytokinemia is essential component of systemic inflammation and by itself is not specific for any definite pathology; however, the proportions of certain parameters may vary depending on the pathogen type, underlying disease, and patient's individual features. It was noticed, that WBC rate [47], CRP [48], and PCT values [49] are typically higher, while NPT and ferritin are lower in bacterial infections compared to viral [50]. The novel concept divides infectious pathogens into intracellular and extracellular due to their ability to affect different PPRs and induce different inflammatory pathways [7]. In particular, Slaats J et al. have proposed IL-1/IL-6/CRP pattern for extracellular pathogen-induced response and IL-18/ferritin pattern for intracellular pathogen-induced response [51]. In this view, CSS may be considered as a condition prevalently induced by extracellular parasites, though the cases of intracellular sepsis were also reported [52]. Meanwhile, hyperinflammation in sHLH is

TABLE 5.4 Demographic parameters of patients with fatal COVID-19, SS, and sHLH outcomes.

	Secondary hemophagocytic syndrome (N = 39)	**Bacterial sepsis and septic shock (N = 47)**	**Pneumonia COVID-19 (N = 36)**
Study	**Retrospective, 2018–19**	**Retrospective, 2018–19**	**Prospective, 2020**
Age, median	58.0	65.0	69.0
Gender (m/f)	19/20	30/17	24/13
Etiology	• Hemato-oncology: 71.8% • Infections(Leishmania spp., HIV, Epstein–Barr virus): 15.4% • Idiopathic: 12.8%	• Klebsiella pheumoniae: 21.3% • Staphylococcus spp: 19.2% • Acinetobacter baumannii: 10.6% • Pseudomonas aeruginosa: 6.4% • Streptococci spp.: 6.4% • Others—6.3%	SARS-CoV-2: 100.0%
Cause of death	• Poly-organic failure: 61.6% • Oncology progression: 33.3% • Stroke: 5.13%	• Polyorganic failure: 100.0%	• ARDS: 73.0% • Poly-organ failure 21.6% • Heart attack 5.4%
Therapy	Chemotherapy (etoposide), IVIG, cyclosporin A, steroids	Antibiotics	Anticytokine treatment, Jak inhibitors, steroids,

Note: AMI, Acute Myocardial Infarction; *ARDS*, Acute Respiratory Distress Syndrome; *COVID-19*, coronavirus disease 2019; *MODS*, Multiple Organ Dysfunction Syndrome; *SARS-CoV-2*, Severe Acute Respiratory Syndrome Coronavirus 2; *sHLH*, secondary Hemophagocytic Lymphohistiocytosis; *SS*, Septic Shock (caused by bacteria).

TABLE 5.5 Cytokine levels in patients with fatal COVID-19, SS, and sHLH outcomes.

Parameter	Donor values	sHLH	SS	COVID-19	sHLH/ SS	sHLH/ COVID-19	SS/ COVID-19
IL-1β, pg/mL,median (25 −75th)	0.20 (0.10−0.87)	0.13 (0.1−1.47)	0.36 (0.11−19.95)	0.88 (0.15−1.67)	ns	ns	ns
IL-2, pg/mL,median (25 −75th)	0.15 (0.10−0.20)	1.46 (0.32−43.07)	0.01 (0.01−0.14)	0.07 (0.01−0.18)	*	ns	ns
IL-6, pg/mL,median (25 −75th)	0.63 (0.25−1.37)	24.23 (7.91−122.90)	99.80 (16.94−512.3)	75.27 (24.30−166.1)	**	**	ns
IL-8, pg/mL,median (25 −75th)	2.90 (2.21−3.98)	141.3 (74.31−343.5)	498.1 (80.47−643.2)	23.78 (14.86−32.28)	ns	****	****
IL-10, pg/mL,median (25 −75th)	1.43 (0.89−1.79)	64.70 (17.90−236.8)	42.33 (15.87−500.0)	20.00 (11.16−29.80)	ns	*	*
IL-17A, pg/mL,median (25 −75th)	4.85 (0.19−21.57)	51.32 (6.89−101.5)	8.11 (0.21−30.53)	31.95 (30.29−33.66)	*	ns	ns
IL-18, pg/mL,median (25 −75th)	81.40 (67.06−133.80)	2690.0 (2042.0−2741.0)	1575.0 (942.4−2368.0)	783.9 (537.4−1117.0)	*	****	****
IFN-γ, pg/mL,median (25 −75th)	0.20 (0.10−0.52)	35.24 (21.00−55.62)	19.35 (11.02−36.61)	4.55 (0.21−10.19)	*	****	*
TNF-α, pg/mL,median (25 −75th)	0.71 (0.30−1.04)	36.90 (22.13−60.73)	19.33 (9.58−37.62)	3.77 (2.17−6.11)	ns	**	*

Note: P values for each pair are presented as ns ($P > 0.04$, not significant), *($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and **** ($P < 0.0001$). *COVID-19*, coronavirus disease 2019; *sHLH*, secondary Hemophagocytic Lymphohistiocytosis; *SS*, Septic Shock (caused by bacteria). The groups were compared by Kruskal−Wallis one-way analysis of variance and Dunn's pairwise posttest.

triggered by either intracellular infectious agents (viral, bacterial, or protozoan) or malignancies [53] and inflated in result of genetic defect of CD8+T-cells or natural killers [54]. Both viruses and malignancies are known to activate IFN-γ mediated cell immunity [55]. The distributions of inflammatory biomarkers in deceased patients with COVID-19, sHLH, and SS and in different COVID-19 outcomes (fatal v/s nonfatal) are presented in Figs. 5.2–5.3 .

Considering the chart in a Fig. 5.2, it was noted that each hyperinflammatory condition had distinct increased and decreased parameters, suggesting that this imbalance may be a better differentiating marker compared to a set of the most increased parameters. Figs. 5.2 and 5.3 illustrated that despite deceased COVID-19 patients had significantly higher IL-6, IL-8, IL-10, IL-18, PCT, CRP, and NPT levels compared to survived, some of these parameters were lower compared to SS and sHLH. In particular, patients with COVID-19 had the lowest IFN-γ median concentration compared to other fatal conditions, what can be explained by the fact that SARS-CoV-2 is able to suppress both interferon types I and II expression in monocytes and epithelial cells [27,28].

Although both COVID-19 and sHLH were provoked by intracellular pathogens, the pattern of COVID-19 was more similar to SS. In particular, deceased patients with COVID-19 and SS had comparable IL-6, ferritin, %GF, and NLR values. Recently NLR was identified as an

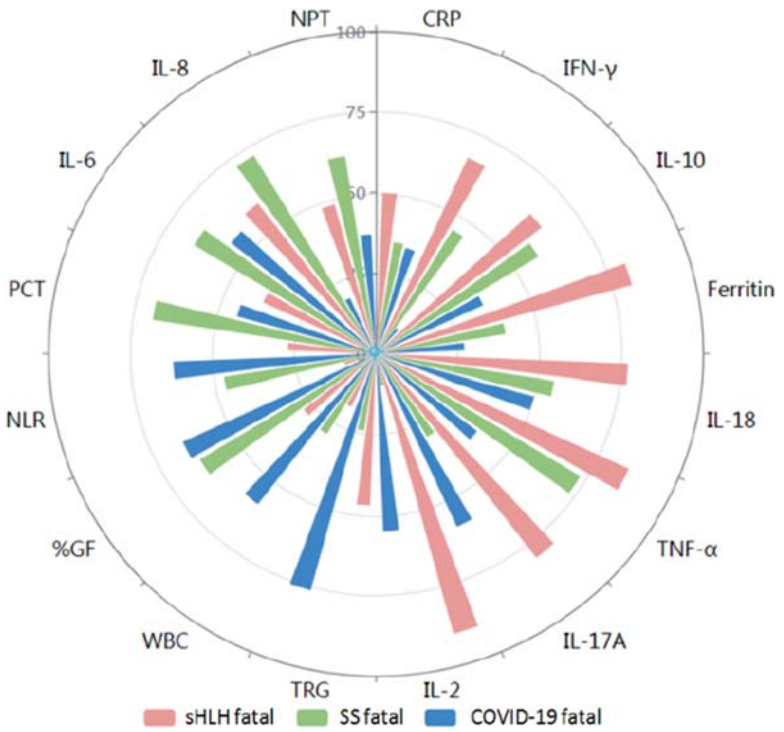

FIGURE 5.2 Rose Plote describing inflammatory parameters in patients with fatal COVID-19, sHLH, and SS outcomes. Median values of laboratory parameters are transformed into percentile values of these parameters in total group of patients with fatal conditions.

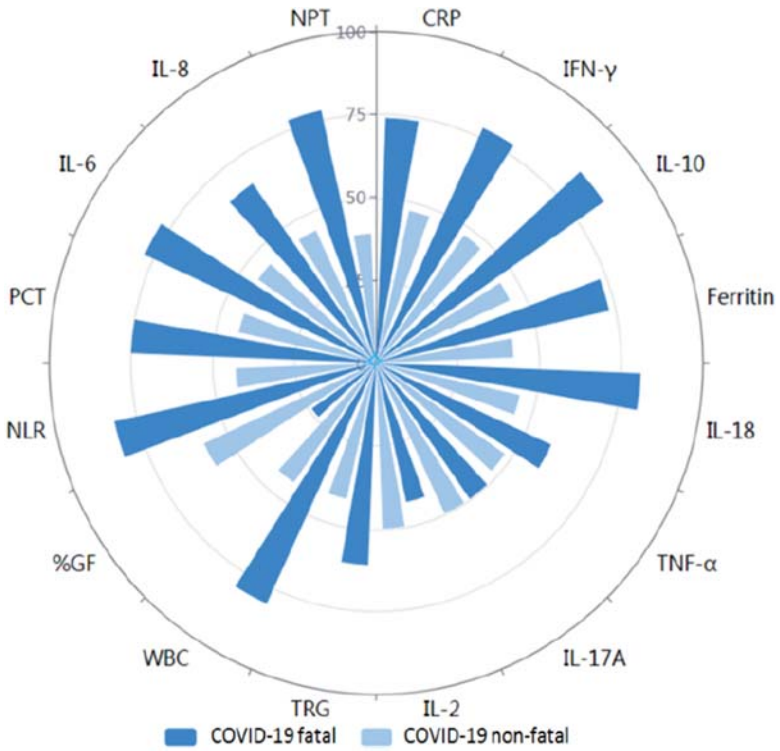

FIGURE 5.3 Rose Plot describing inflammatory parameters in patients with fatal and nonfatal COVID-19 outcomes. Median values of laboratory parameters are transformed into percentile values of these parameters in total COVID-19 group. *Note: %GF*, the percent of Glycosylated Ferritin fraction; *COVID-19*, coronavirus disease 2019; *CRP*, C-reactive protein; *NLR*, Neutrophil to Lymphocyte ratio; *NPT*, neopterin; *PCT*, procalcitonin; *sHLH*, secondary Hemophagocytic Lymphohistiocytosis; *SS*, Septic Shock (caused by bacteria); *TRG*, triglycerides; *WBC*, White Blood Cells.

independent risk factor for critical illness in patients with COVID-19 infection (with a threshold value of 3.13 in early stage) [29], and in the current study deceased COVID-19 patients demonstrated its highest level. Revealed disproportions in COVID-19 included high IL-6, IL-17A, WBC, NLR, and low IFN-γ, TNF-α, IL-8, IL-2 concentrations that seemed to be the sign of its unfavorable course. Previously Bert NL and al. reported an imbalanced response in symptomatic COVID-19 patients with elevated IL-6, TNF-α, IL-1β, and IL-10, but decreased IL-2 and IFN-γ production, whereas asymptomatic patients had proportionally high IL-2 and IFN-γ levels [30]. Nevertheless, both decreased [31] and increased [32] IFN-γ levels in severe COVID-19 compared to its mild form were reported. Low IL-2 and IFN-γ levels most likely reflected cytotoxic immunity suppression, while an activation of IL-6/IL-17 pathway with subsequent neutrophil recruitment may play the role of an alternative mechanism of antiviral defense. Recent studies revealed a range of effects of these cells, aimed at eliminating intracellular pathogens via binding their extracellular forms by neutrophil extracellular traps [33], shuttling to the lymph nodes and presenting to lymphocytes [34], expression chemokines for macrophages and T-lymphocytes [4], etc.

As it was said above, hyperinflammation is a result of immune system inability to eliminate pathogen that also manifests as imbalanced response. Each fatal condition has demonstrated some immunological pattern: high IL-6/NLR and low IFN-γ/TNF-α in COVID-19; high IL-8/IL-6 and low IL-17A/IL-2/sTfR in SS; high IL-18/ferritin/IL-17A and low IL-6/PCT/NLR%GF in sHLH.

Cytokine storm syndrome score for disease prognosis

Predicting the course of COVID-19 infection is of fundamental importance for the timely and adequate distribution of treatment efforts in the face of limited time and material resources caused by the massive admission of patients. A significant number of new clinical algorithms and models have been proposed to solve this problem. A number of studies have evaluated the use of previously developed clinical scales for assessing the risk of developing a severe course, including the pneumonia severity index (PSI), the CURB-65 and CRB-65 pneumonia severity scores, A-DROP and SMART-COP, the severity rating score patient NEWS2, sequential assessment of organ failure qSOFA, and criteria for systemic inflammatory response syndrome [56]. Thus, the NEWS2 scale was superior to qSOFA and other scales in terms of predicting the critical course in hospitalized patients [57]. New scales for assessing the severity of COVID-19 were also developed, based on demographic data, the presence of concomitant diseases, instrumental studies, saturation, and laboratory parameters [56]. A large-scale study of the informativeness of this approach was carried out in China, where the area under the ROC curve of the clinical risk scale was 0.88 (95% CI, 0.85−0.91), and when validated it was also 0.88 (95% CI, 0.84−0.93). The American 10-point scale for assessing the severity of COVID-19, including age, oxygen saturation, blood pressure, blood urea, CRP, and international normalized ratio, showed similar prognostic indicators [58]. Despite the decisive role of cytokines and the development of CSS, they are not included in risk stratification algorithms due to the inaccessibility of their routine measurement in most clinical laboratories.

Since IL-6, IL-10, IL-18, and PCT were associated with disease severity and death, these indicators were integrated into a 12-point scale called the CSS score. The f ranges of IL-6, IL-18, IL-10, PCT, and the corresponding score are presented in Table 5.4. Cut-off of these ranges was established on the basis of ROC analysis. The cut-off between low and medium levels were determined on the basis of the concentrations of the studied laboratory parameters and were characterized by a sensitivity of 60% and a specificity of 75%, and values between the medium and high levels—a sensitivity of 40% and a specificity of 90%. The CSS score represents the sum of IL-6, IL-10, IL-18, and PCT scores (Table 5.6), from 0 to 12. Patients who score 6 points or more have a high risk of an unfavorable outcome of the disease. According to the ROC analysis, the area under the curve for the CSS score was superior to for each of the four markers separately (AUC 0.90 (95% CI 0.84−0.95), $P < 0.001$).

To assess the prognosis of the outcome of the disease, we carried out an analysis of laboratory data, which revealed significant differences between patients who died from COVID-19 and those who survived (Table 5.3). Thus, among patients with a poor outcome, the following were significantly more frequent: leukocytosis (26 patients [72%] versus 55 [28.9%]; $P < 0.001$), as well as lymphopenia (25 [69.4%] patients versus 69 [36%]; $P < 0.001$). The average number of leukocytes and neutrophils in deceased patients was

TABLE 5.6 Cytokine storm syndrome score.

	Score = 0	Score = 1	Score = 2	Score = 3
Biomarkers	**Normal range**	**Borderline increase**	**Moderately increase**	**Ultimately high**
IL-6, pg/mL	0–10	10–40	40–100	>100
IL-18, pg/mL	0–300	300–650	650–1000	>1000
IL-10,pg/mL	0–5	5–10	10–30	>30
PCT, pg/mL	0–0.25	0.25–0.99	1.0–2.0	>2.0

Notes: IL-10, interleukin 10; *IL-18*, interleukin 18; *IL-6*, interleukin 6; *PCT*, procalcitonin; The score of cytokine storm is a 12-point score that includes different levels of concentrations of IL-6, IL-18, IL-10, PCT. Scores from one to three correspond to normal, borderline, medium and high level of these biomarkers. A value of **six and more points** is associated with a high risk of unfavorable outcome.

significantly higher, and the average number of lymphocytes and platelets was significantly lower than in recovered patients (Table 5.2). There was also a significant difference in the levels of a number of biochemical and coagulation parameters. It should be noted that 16 (44%) of 36 deceased patients and 53 (27%) of 190 recovered patients had a D-dimer concentration above 1000 ng/mL. The levels of C-reactive protein and ferritin in the blood in deceased patients was significantly higher than in the group of recovered individuals (60 vs 144 mg/L and 605 vs 1243 μg/L, $P < 0.0005$, respectively).

To compare the predictive value of the CSS score and other pro-inflammatory and other biomarkers, ROC curves of the levels of D-dimer, neutrophils, C-reactive protein, ferritin, and LDH were constructed (Fig. 5.4). The area under the curve was the largest for CSS score and amounted to 0.90 (0,85- 0,96) with sensitivity of 83.33% (62.62-95.26) and specificity 84.48% (78.23–89.52). For the diagnosis of critical COVID-19, the area under the curve for

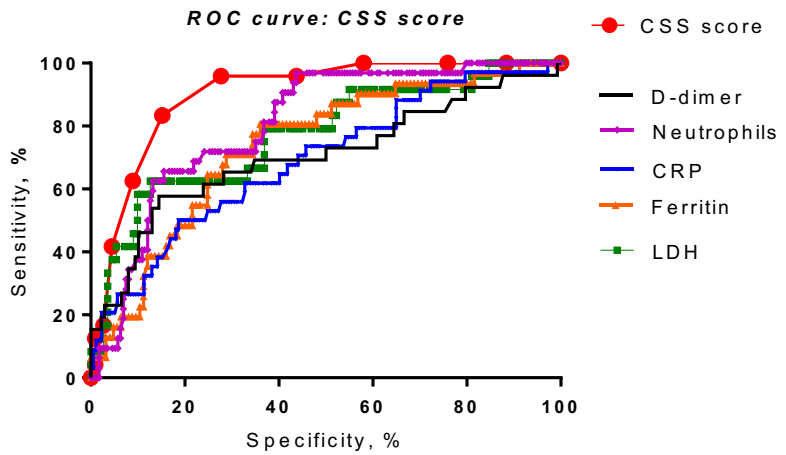

FIGURE 5.4 ROC curves of tCSS score, C-reactive protein, lactate hydrogenase, ferritin, D-dimer, neutrophils for predicting the critical course of COVID-19. Notes: *CRP*, C-reactive protein; *CSS*, cytokine storm syndrome; *LDH*, lactatdehydrogenase.

neutrophils was 0.80 (0.73-0.88), for LDH - 0.77 (0.66−0.88), for D-dimer—0.70 (0.57−0.82). Proinflammatory markers such as CRP and ferritin showed an area under the ROC curve of 0.69 (0.59−0.78) and 0.74 (0.64−0.83), respectively.

Validation of cytokine storm syndrome score and its clinical relationship

To validate the CSS score and to prove its prognostic significance, we have also tested samples of 121 patient collected during second wave of the epidemic that happened in St. Petersburg, Russia, during the winter of 2020. There were 48 hospitalized patients with COVID-19 pneumonia not required ICU and 80 hospitalized patients with COVID-19 severe and critical pneumonia.

To identify patients at risk of death in the validation cohort, the integration of markers into the CSS score showed a relatively high sensitivity (78.79%, 95% CI% 0.61−0.91) and significantly increased the specificity (81.12%, 95% CI 71.24−88.80). Clinical sensitivity of CSS score (≥6 points) for fatal outcomes was 78.79% and specificity of 81.12% (Table 5.7). The CSS score positively and statistically significantly correlates with the NEWS2 (0.56, $P < 0.05$), the duration of oxygen therapy ($R = 4.49$, $P < 0.05$) and the degree of lung damage based on CT results (3.48, $P < 0.05$).

Thus, we have confirmed the predictive value of the CSS score in relation to the risk of an unfavorable prognosis of the course of COVID-19. The combined prognostic capabilities of IL-6, IL-18, IL-10, and PCT, integrated into CSS score, can help identify patients at high risk of death in COVID-19-associated pneumonia. Important clinical issue of COVID-19 pneumonia is the relationship between severe disease and older age and the number of comorbidities.

TABLE 5.7 Diagnostic test evaluation of IL-6, IL-10, IL-18, PCT, cytokine storm syndrome score.

Marker	Cut-off	P	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Odds ratio (95% CI)	Relative risk (95% CI)	PPV (95% CI)	NPV (95% CI)
IL-6 (pg/mL)	16.53	< 0.0001	94.23 (79.77−99.26)	52.0 (42.96−64.00)	18.28 (4.11−81.33)	10.63 (2.66−42.34)	0.44 (0.32 −0.56)	0.95 (0.85 −0.99)
IL-18 (pg/mL)	535.9	0.0235	75.76 (0.57−0.88)	47.06 (36.13−58.13)	2.77 (1.12−6.85)	2.14 (1.05−4.34)	0.35 (0.24 −0.48)	0.83 (0.69 −0.92)
IL-10 (pg/mL)	17.45	< 0.0001	78.79 (0.61−0.91)	74.12 (0.63−0.83)	10.64 (4.04−27.94)	5.41 (2.56−11.46)	0.54 (0.39 −0.68)	0.90 (0.80 −0.95)
PCT (pg/mL)	0.56	< 0.0001	45.71 (28.83−63.35)	87.06 (78.02 93.36)	5.66 (2.26−14.20)	2.90 (1.74−4.82)	0.59 (0.38 −0.77)	0.79 (0.69 −0.87)
CSS score	>6	< 0.0001	78.79 (61.09−0.91)	81.1 (71.2−88.84)	16.02 (5.90−43.38) 5.914 to 43.38	6.71 (3.19−14.16)	0.62 (0.45 −0.76)	0.88 (0.82 −0.96)

Note: NPV, negative predictive value; *PPV*, positive predictive value; *CSS*, cytokine storm syndrome.

We have studied the prevalence of comorbidity, that was found in 70% among hospitalized patients, while hypertension (HD) was observed in 57.8% (n = 130), coronary heart disease (IHD)—in 27% (n = 61), diabetes mellitus (DM)—in 16.2% (n = 36), chronic heart failure (CHF)—in 8.6% (n = 19). About 9.6% (n = 21) of patients had oncological diseases in the active stage, and 3.7% (n = 8) had chronic kidney disease. It should be noted that a high incidence of comorbidities was observed in critically ill patients, as well as in the group of patients with fatal outcome (Fig. 5.5). Chronic kidney disease, coronary heart disease, and cancer were directly correlated with number of deaths.

The number of deaths of patients under 45 years old was 3 (7.31%), from 45 to 65 years old—12 (10.5%), while the largest number of deaths was observed in the group of patients aged 65–85 years (21 [58, 3%]). Body mass index (BMI) in 42% of cases exceeded 30 kg/m^2. BMI in women was 33.0 ± 1.4 kg/m^2 and was significantly ($P < 0.01$) higher than the average BMI in men (29.3 ± 0.7 kg/m^2). We have found the association between CSS score and age group and the presence of life threating comorbidities (Fig. 5.6).

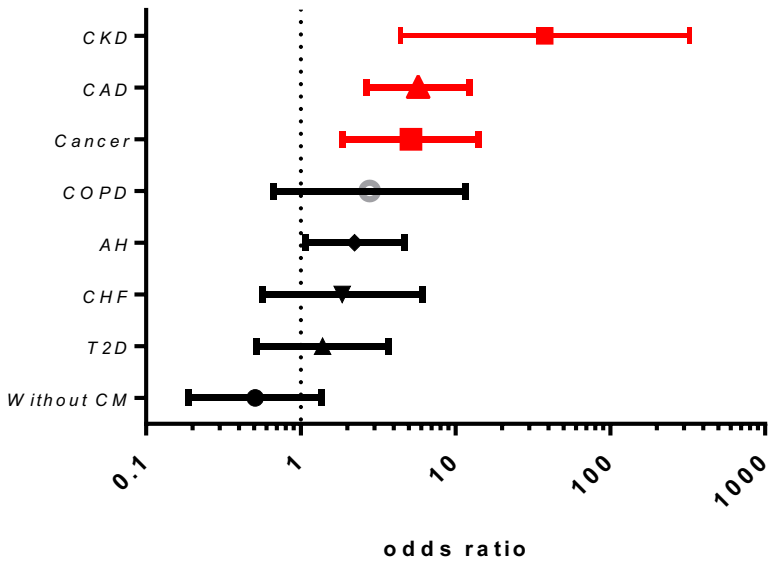

FIGURE 5.5 Forest graph showing the relationship of various comorbidities and the risk of death. On the ordinate axis comorbidities represent, on logarithmic axis—odds ratio of the risks of death. Groups of comorbidity with the highest odds ratios are highlighted in red. Note: *AH*, arterial hypertension; *CAD*, coronary artery disease; *CHF*, congestive heart failure; *CKD*, chronic kidney disease; *CM*, comorbidity; *COPD*, chronic obstructive pulmonary disease; *T2D*, type 2 diabetes.

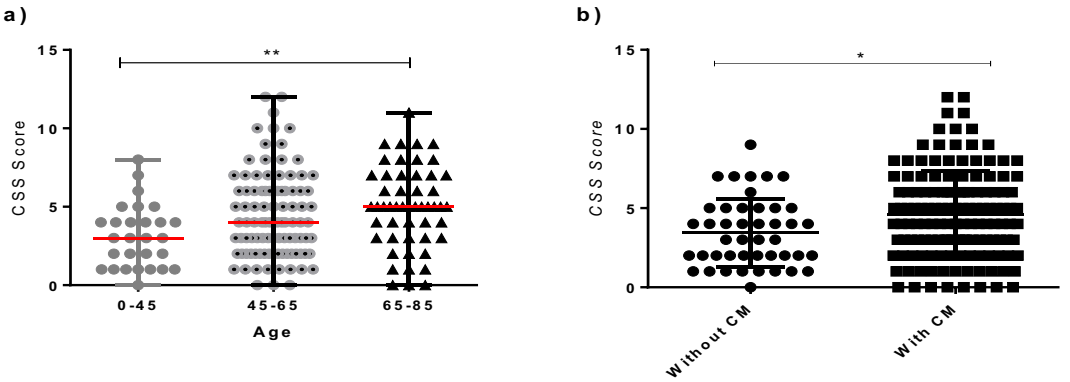

FIGURE 5.6 Relationship between SCC score points and age (A) and presence of life threating comorbidities (B)
Notes: CM, comorbidities; *CSS*, cytokine storm syndrome.

Conclusion remarks

- COVID19 in characterized by unique pattern of proinflammatory cytokine synthesis including IL-18/ferritin and IL-17A; IL-6/CRP and IL-8/TNFa/procalcitonin; IL-10/lymphopenia;
- Profiles of cytokines and laboratory biomarkers resemble both sepsis and hemophagocytic (MAS) syndrome;
- Levels of proinflammatory monocytic cytokines are higher in patients further transferred to ICU for pulmonary support and are associated with high mortality;
- Cytokine storm parameters are higher in patients belonging to risk groups (higher age and with higher number of comorbidities);
- Integrated Cytokine Storm Score can help to predict most severe cases of COVID-19 disease.

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