COVID-19, what could sepsis, severe acute pancreatitis, gender differences and aging teach us?

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Abstract

Severe COVID-19 disease is characterised by an exaggerated inflammatory response, called cytokine storm, accompanied by a condition of immune depression. Even sepsis is characterised by an exaggerated inflammatory response, called SIRS (Systemic Inflammatory Response Syndrome), accompanied by a condition of immune depression called CARS (compensatory anti-inflammatory response syndrome). Clinical studies reveal that most sepsis patients who did not die during the hyper inflammatory response (SIRS) subsequently succumbed to the condition of immune depression (CARS). Severe acute pancreatitis begins with local inflammation that induces systemic inflammatory response syndrome (SIRS), accompanied and followed by a compensatory anti-inflammatory response (CARS). In COVID-19 disease, the male response to SARS CoV-2 virus is typically characterised by a robust inflammatory response. Instead, a cell-mediated immune response is dominant in women. This means that the male sex tends to have a more robust hyper inflammatory response than the female one. Furthermore, in women the condition of immune depression is less represented, therefore they are more protected. Sepsis, severe acute pancreatitis and COVID-19 disease evolve between two fundamental aspects: hyper inflammation and immunodepression. The experience gained over years of studies of sepsis and severe acute pancreatitis suggests that therapies should be differentiated according to the evolutionary stage of the disease. The goal is to save the lives of most patients with COVID-19 disease. The identification of critical points, suitable for designing the windows of therapeutic opportunity, may allow the use of therapeutic interventions, in the COVID-19 disease, which are effective (there are no approved drugs yet), safe (without significant side effects), targeted (based on the evolutionary phase of the disease) personalized, (based on sex, co-morbidities, age, etc.) and timely (based on signs, symptoms, laboratory parameters and instrumental investigations).

Sepsis

Sepsis is a serious clinical syndrome associated with an exaggerated systemic inflammatory response induced by an infection that can lead to multiple organ failure (MOF) and death. The main causes of sepsis are bacterial infections. A lower number of sepsis is due to viruses, parasites and fungi [12].

Sepsis is a complex process characterized by an early inflammatory response (systemic inflammatory response syndrome: SIRS), followed by a subsequent anti-inflammatory response (compensatory anti-inflammatory response syndrome: CARS). Overall, the immune response is appropriate when these two phases are balanced. An exaggerated early inflammatory response can lead to death in the initial stages. An immunosuppressive response can lead to multiple infections and multi-organ failure[9] in later stages. **Fig. 1.**

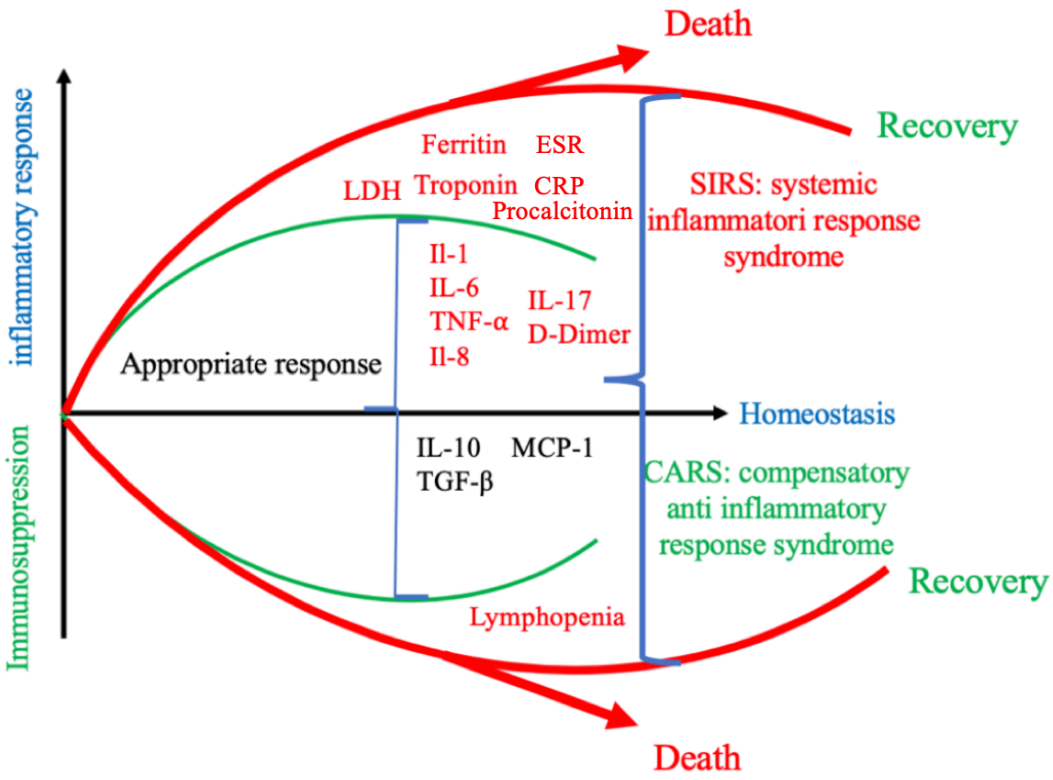

Fig. 1. **SIRS and CARS in sepsis**. Activation of both pro- and anti-inflammatory immune responses occurs promptly after sepsis onset.

Sepsis is a serious syndrome associated with the host's response to infection. An inappropriate sequence of early pro-inflammation (SIRS) and subsequently anti-inflammation (CARS) is responsible for the fatal outcomes in sepsis. A self-amplifying cytokine production, termed cytokine storm is the main pathogenetic mechanism by which infections can cause sepsis. In the early phase of immune response patient death is caused by SIRS. In the later phase patient death is associated with secondary infections and other complications from immunosuppression (CARS). Immune system cells and their associated molecules, inflammation mediators and coagulation factors are involved in the pathogenesis of sepsis[11]. During the process of sepsis the two pro and anti inflammatory phases are almost superimposed. Following recognition of the pathogen, the acute phase of inflammation is triggered. This is characterized by the release of some cytokines (IL-1β, TNF-α, IL-6, IL-8), mediators of inflammation, activation of coagulation factors and complement pathways. Pro-inflammatory cytokines, within 6 hours, induce the production of C-reactive protein (CRP) by the liver. CRP, in turn, promotes bacterial opsonization and activates complement. However, CRP is an aspecific biomarker of acute conditions and it does not allow to distinguish between infectious and non-infectious diseases. Instead, procalcitonin begins to increase already 2-4 hours after the start of the immune cascade and peaks after 6-24 hours[13]. Procalcitonin increases and reaches very high values in the course of bacterial infections. It allows to distinguish between infectious and non-infectious inflammatory diseases and between bacterial and viral pathologies[13,14]. Only a few cytokines are constitutively expressed under non pathological conditions. On the other hand, most of these mediators are produced and secreted following the activation of several intra-cell

cascades paths. The expression of genes codyfing the cytokines is strongly regulated in terms of transcription and translation. The transcription factor NF kB, for example, promotes the expression of IL-1 and IL-6 [15].

The goal of the inflammatory response is to fight the pathogen and restore homeostasis. Adaptive immunity is also activated immediately and anti-inflammatory cytokines are produced to reduce hyperinflammation. The body responds to the phase of hyperinflammation with an immunosuppression phase[6]. The main pro inflammatory interleukins are: TNF-α, IL-1β, IL-6, IL-8. These interleukins are not specifically produced during sepsis, but have a well-known prognostic value. The main cytokine of the immunosuppression phase is IL-10, IL1ra, TGF-β[16]. IL-10 has the function of reducing hyperinflammation[4,5,7,8]. **Fig. 2.**

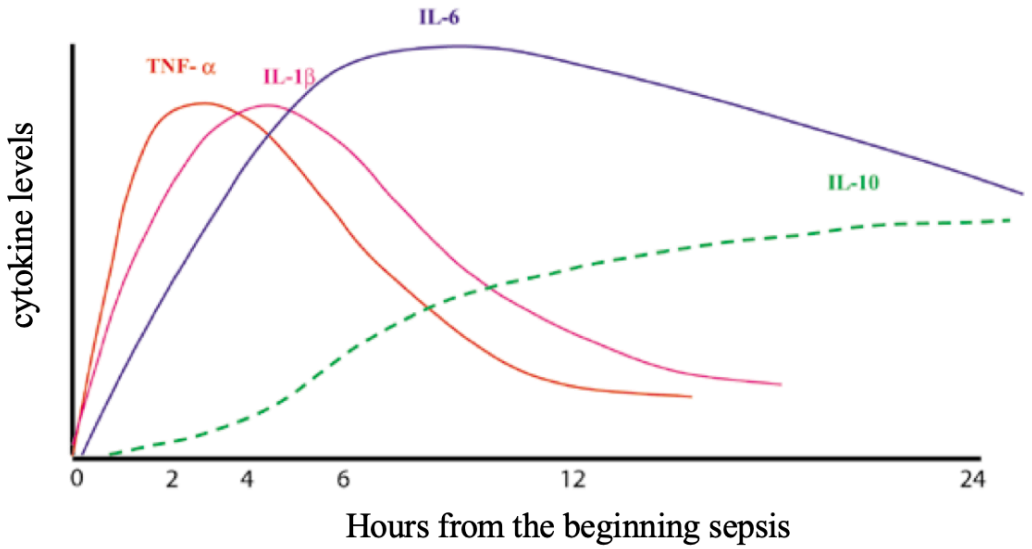

Fig. 2. Chronobiology of the inflammatory and anti-inflammatory process in sepsis (Boontham et al., 2003). IL-1 and TNF-α represent the starters in the chronobiology of inflammation. IL-6 and IL-8 peak after IL-1β and TNF-α. IL-10 increases significantly and has the role of blocking the inflammation by modulating inflammatory cytokines.

The inflammatory response involves the release of inflammatory mediators that can trigger a self-amplifying cascade of events responsible for the "cytokine storm". Cytokine storm represents the main cause of infection severity. The local inflammatory response triggered by the infection can extend throughout the body, through the cells of the immune system and soluble mediators released into the circulation [10]. In the first phases of this process, the cytokines are mainly produced from macrophages, within 30-90 minutes of interaction with the pathogens [16] and induces the release of other cytokines, eicosanoids and further inflammatory mediators, reactive oxygen species (ROS), adhesion molecules. IL-1 and TNF-α represent the starters in the chronobiology of inflammation. **Fig. 2.** Interleukin 1, produced by the stimulated monocytes, comprises two forms: IL-1α and IL-1β. Both molecules share the same receptor and, consequently, have the same biological effects. They possess a lot of common biological actions, therefore, they promote the development of inflammation with a synergistic action by binding to specific receptors and stimulate the gene expression of other inflammatory cytokines (IL-6, IL-8)[18]. IL-6 and IL-8 peak after IL-1β and TNF α[17] (Fig.2). IL-6 induces the synthesis of the acute phase proteins, stimulates B and T lymphocytes and works as a pyrogen [18]. IL-8 works by attracting neutrophils to the inflammation site [19] and it has been found in the bronchoalveolar lavage fluids (BALF) of patients with respiratory acute distress syndrome (ARDS). In addition, it's involved in multiple organ dysfunction syndrome (MOF)[20]. In the chronobiology of the inflammatory process, IL-10 increases significantly and has the role of blocking the inflammation by modulating inflammatory cytokines (IL-6)[18]. IL-1Ra prevents the effects of IL-

1 by competitive binding to the IL-1R receptor [21]. The cytokine profile in the septic patient has a prognostic value, providing useful information to establish the stage of the pathology and can help personalize the therapies.

Therefore, early mortality can be attributed to the hyperinflammatory phase. Instead, late deaths are related to the immunosuppressive phase and secondary infections[6]. Accordingly, the cytokine profile in the septic patient could provide information about the stage of the disease and the patient's prognosis, contributing to a better management. Indeed, studies have reported that IL-6 is a very promising biomarker. However, IL-1β, TNF-α and IL-6 are not altered only in sepsis. Nevertheless, several studies have shown the importance of IL-6 in the prognosis of sepsis as it presents strong correlations with patient mortality[2,3,4,5]. There are similarities and differences between severe COVID-19 disease and Sepsis. In fact, many COVID-19 patients have multiple organ and system involvement: lung, liver, immune system, kidneys, brain, digestive system, heart, vessels, thromboembolism[27,42]. In addition, they develop typical clinical manifestations of septic shock: severe metabolic acidosis, cold extremities and weak peripheral pulses, regardless of evident hypotension. Therefore, according to Li's observations and hypotheses[22], and on the basis of the pathogenesis of a known and already widely studied disease, such as sepsis, it is possible to draw lessons for the treatment of a new and still poorly known pathology, like COVID-19.

IL-6 is one of the main actors of the cytokine storm, contributing significantly to the increase in vascular permeability and to the impairment of multi-organ functionality[26]. In fact, it is increased both in sepsis and in serious COVID-19 disease. It constitutes a useful biomarker and prognostic factor in both diseases. Even though IL-6 levels are significantly elevated in patients requiring ventilation, they are relatively low (hundreds of picograms/mL) compared to levels detectable in patients with septic shock (hundreds to thousands of picograms/mL)[23,28]. IL-6 levels in COVID-19 patients increase over time with disease severity and deterioration of lung function[29]. The kinetics of IL-6 clearly distinguishes COVID-19 patient response from septic patients[28]. In COVID-19 patients, moderately elevated IL-6 levels are a prognostic factor associated with respiratory failure and need for mechanical ventilation (cutoff: ≥80pg / ml). 92% of patients with IL-6 values ≥80pg/ml require mechanical ventilation within a median time of 1.5 days (range 0-4 days)[24]. IL-6 concentration ≥100 pg/mL were exclusively observed in critically ill patients and extremely high IL-6 level was closely correlated with viral load and mortality[25].

A useful approach for the treatment of patients with septic shock and with COVID 19 includes both in the counteracting the pathogen, but also in the suppressing the molecular response known as cytokine storm. In fact, an exaggerated cytokine response has been recognized as a possible cause of organ damage. Therefore, it was speculated that suppressing the inflammatory response by targeting IL-6, TNF-alpha, Il-1, could have a beneficial effect.

The observation of the clinical and laboratory features emerging in most studies in patients with COVID-19 underlines some analogies with the evolution of sepsis. In sepsis, the majority of patients who survive the hyperinflammatory response syndrome (SIRS) die later during the immunosuppression phase (compensatory anti-inflammatory response syndrome: CARS. In COVID-19 patients, as in septic patients, there is a condition of deep immune depression. IL-10, a cytokine with immune suppressive action, is elevated. There is a profound lymphopenia, with loss of T lymphocytes (CD4+ and CD8+), B lymphocytes, NK cells[39,40,41]. The loss of effector cells and the lack of antibody production correlates with the development of secondary infections and mortality[30,31]. Recent studies have highlighted the importance of cell-mediated immune response, independently from antibody production, in the defense against the SARS CoV-2 virus[32,33,34,35].

The autopsy results revealed significant damage in the spleen and peripheral lymphatic organs in deceased patients[36,37,43]. Over 50% of hospitalized patients experience complications, including hospital-related infections associated with the compromission of immune system[30].

Therefore, the distinctive features of COVID-19 disease, against sepsis, are a) an exaggerated but more modest inflammatory response b) a deeper and progressive immunosuppression of adaptive immunity. **Fig. 3.**

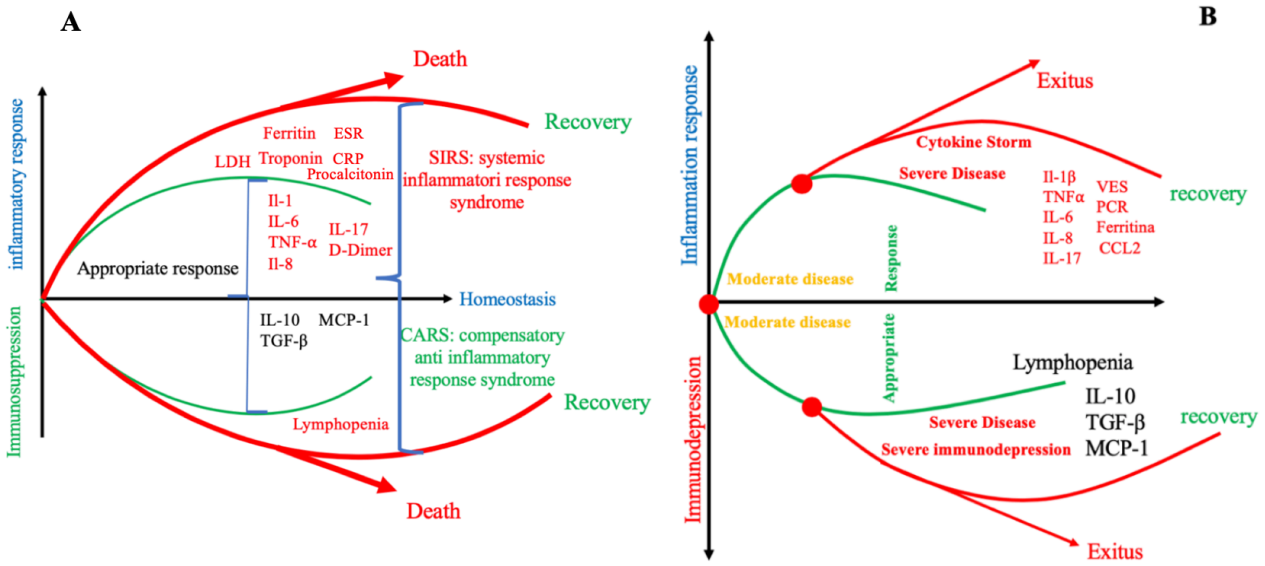

Fig. 3. Sepsis and COVID-19 in the mirror. In both pathologies the immune response includes two phases: an early pro-inflammatory condition associated with an anti-inflammatory condition. When both of these phases are balanced, an appropriate response occurs. A) An excessive early response in sepsis leads to early shock and mortality. On the other hand, an exaggerated anti-inflammatory response leads to immunosuppression or immunoparalysis with multiple infectious complications and multiorgan failure[9]. B) The immunological characteristics of COVID-19 are similar to the features detectable in sepsis, with some important differences: in sepsis the cytokine storm is more robust, while in COVID-19 there is a deeper impairment of the adaptive immune response.

Severe Acute Pancreatitis

Severe acute pancreatitis is usually fatal disease associated with a systemic inflammatory response, dominated by the production and release of pro-inflammatory cytokines and chemokines[46,50]. It may result in ALI/ARDS (Acute Lung Injury/Acute Respiratory Distress Syndrome), MOF (Multiple Organ Failure) and culminate in patient death. Acute lung injury is the leading cause of early death in patients with severe acute pancreatitis[47,48]. Acute pancreatitis begins with local damage at the level of the acini, due to the abnormal and early activation of pancreatic proteases[45]. In response to local damage, an inflammatory reaction develops (sterile inflammation) which, thanks to self-amplification mechanisms in which the NLRP3 inflammasome is involved, causes severe disease[49]. It was proposed to divide the picture into an initial hyper inflammatory phase called SIRS (systemic inflammatory response syndrome), followed by a compensatory anti-inflammatory response syndrome (CARS) phase, dominated by immunodepression and anti-inflammatory components[49]. In this second phase, secondary infections mainly develop.

Sex difference and aging in COVID-19

The prevalence of COVID-19 disease is superimposable in both sexes. Male sex, however, is a risk factor for severe disease and death, regardless of age[53,54]. Age is also an independent risk factor for severe COVID-19 disease. The immune system has different characteristics between two sexes and evolves with age. The differences are significant and affect the outcomes of COVID-19 disease. Human monocytes represent a heterogeneous population of cells of the innate immune system. At least three monocyte subpopulations have been identified, based on the expression of the CD14 and CD16 membrane receptors:
$CD14^{++}CD16^{-}$ o cMonocytes (classic monocytes) (normally the prevailing subpopulation: 80%);
$CD14^{+}CD16^{+}$ o IntMonocytes (intermediate monocytes)

$CD14^{+}CD16^{++}$ o ncmonocytes (non-classic monocytes).

	Men	Women
IntMonocytes	+/-	++/-
ncMonocytes	+++	+/-
Inflammatory Cytokines	+++	+/-
cell-mediated immunity	+/-	+++

Table 1. IntMonocytes: intermediate monocytes; Red: Non-classical monocytes are pathogenetic. Red: inflammatory cytokines are pathogenetic. Green: cell-mediated immunity is protective.

The transition from the classic to the intermediate and non-classic phenotype is associated with the progressive loss of the CD14 receptor and the gain of the CD16 receptor. In vitro studies have shown that non-classical monocytes are the most inflammatory in response to TLR[56]. Senescence, NFkB and IL-1β promote the phenotypic switch towards non-classic monocytes. The increase in the proportion of ncMonocytes is associated with the increase in the production of TNFα, IL-1β and IL-8[52,56,57,58,59,60,61,62].

A recent study highlights an exaggerated switch towards the ncMonociti phenotype in patients with type 2 diabetes affected by COVID-19. As is known, diabetes is one of the major risk factors for severe COVID-19 disease[63,64]. A recent study examines the different characteristics of the basic immune response to SARS Cov-2 virus in both sexes, during the early stages of the disease[65]. In male subjects, a higher percentage of ncMonocytes is found which translates into high levels of cytokines and inflammatory chemokines such as IL-8 and CCL5 (Rantes).

The CCL5 chemokine (Rantes) has several effects on innate and adaptive immunity (promotes macrophage phagocytosis in the inflamed lung, contributes to the migration of dendritic cells from the lung to the draining lymph nodes, recruits the effector T lymphocytes, promoting the immune response adaptive in the lung). IL-8 is a chemokine that recruits neutrophils at the infection site level. Neutrophils are associated with a more severe prognosis for COVID-19 disease[96,97].

nc-Monocytes contribute to endothelial inflammation and can help to promote micro thrombosis and thromboembolism[69,70]. Male subjects have a robust inflammatory response, with an increased production of inflammatory cytokines of the innate immune system. In female subjects there is a robust cell-mediated response, with a prevalence of CD8 T lymphocytes. **Tab. 1.**

Consistent with the effects of these peculiar immune responses, male subjects with poorer Cell-mediated response have a worse prognosis. Instead, the prognosis is worse in female subjects with a higher level of cytokines from the innate immune system. Finally, the impairment of the cell-mediated response correlates with age in male subjects but not in female subjects. **Fig. 4.**

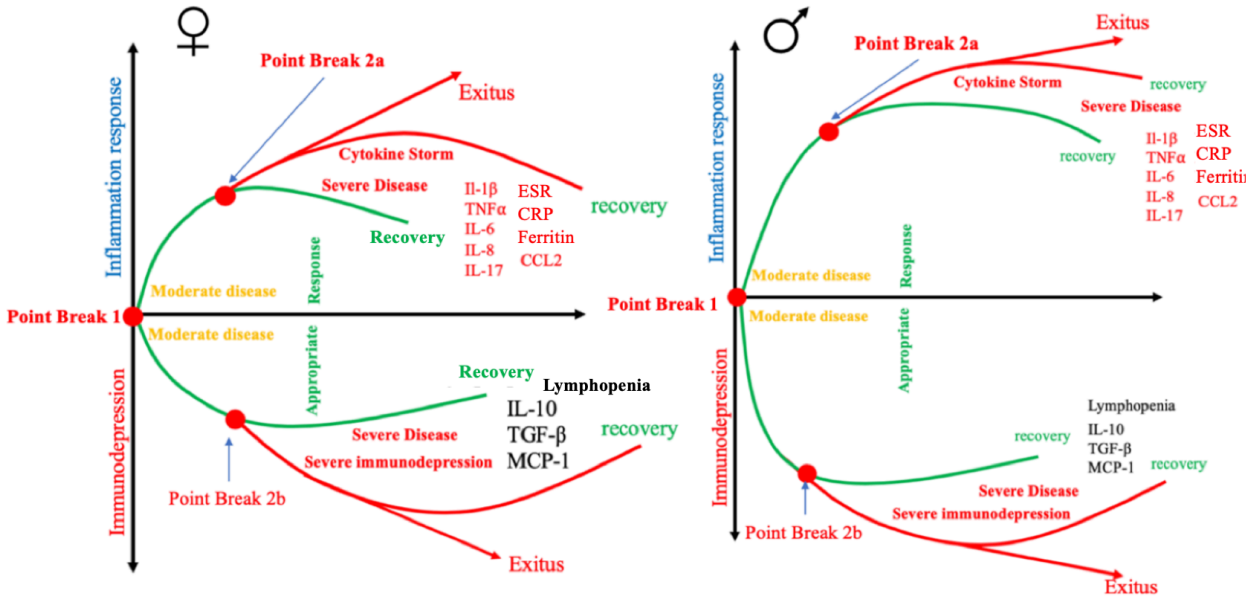

Fig. 4. comparison between the immune response to SARS CoV-2 virus of women ♀ and men ♂. Under basic conditions, the immune response to the SARS CoV-2 virus in men is more inflammatory and less immunocompetent than in women.

Conclusive remarks

Exaggerated inflammation, accompanied by immunodepression, characterises sepsis, severe acute pancreatitis and severe COVID-19 disease. Both conditions are basic for some important risk factors for severe COVID-19 disease: advanced age, male gender, type 2 diabetes. These two different conditions require a different therapeutic approach. In COVID-19 disease, in addition to modulating the exaggerated innate inflammatory response, it is essential to include a proper support for immune defenses as therapeutic strategies. The correct balance between cytokine storm contrast and adaptive immune function support must be adapted to the stage of the disease.
The windows of therapeutic opportunities should be designed to promptly identify precise reference points (symptoms, signs, laboratory parameters, instrumental investigations). Therapies should be optomised by diversifying them according to the evolutionary phase. The results of the vast amount of work in progress will provide, in the near future, a plethora of data. The correct examination of the hypotheses formulated should allow us to refine the therapies, sewn on like a tailored suit.

List of abbreviations:

ALI: Acute Lung Injury
BALF: Bronchoalveolar Lavage Fluid
ARDS: Acute Respiratory Distress Syndrome
CARS: compensatory anti-inflammatory response syndrome
CCL5: Chemokine (C-C motif) ligand 5
cMonocytes: classical Monocytes
CRP: C-reactive Protein
ESR: erythrocyte sedimentation rate
IL-1β: Interleukin 1β
IL-6: Interleukin 6
IL-8: Interleukin 8
IL-10: Interleukin 10
IL-17: Interleukin 17
IL-1R: IL-1 Receptor
IL-1RA: Interleukin-1 Receptor Antagonist
intMonocytes: Intermediate Monocytes
LDH: Lactate Dehydrogenase
MCP-1: Monocyte Chemoattractant Protein-1
MOF: Multiple organ failure
ncMonocytes: non classical Monocytes
Rantes: regulated on activation, normal T cell expressed and secreted
SIRS: Systemic Inflammatory Response Syndrome
TGF-β: transforming growth factor β
TLRs: Toll-Like Receptors
TNFα: Tumor Necrosis Factor α

Declaration of interests:

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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