The Cytokine Storm of COVID-19; *Focus on Prevention and Protection*

L. Pearce[1], SM. Davidson[1], DM. Yellon[1]

[1]The Hatter Cardiovascular Institute, University College London, United Kingdom

Corresponding Author: Professor Derek Yellon; d.yellon@ucl.ac.uk

(4,365)

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Abstract

The cytokine release syndrome (CRS) of COVID-19 is associated with the development of critical illness requiring multi-organ support. Further research is required to halt progression of multi-organ injury induced by hyper-inflammation. PubMed/MEDLINE^{TM} databases were accessed between May 9th-June 9th 2020, to review the latest perspectives on the treatment and pathogenesis of CRS. Over-activity of chemotaxis triggers a macrophage activation syndrome (MAS) resulting in the release of pro-inflammatory cytokines. IL-6 and TNF-*α* are at the forefront of hyper-inflammation.

The inflammatory cascade induces endothelial activation and capillary leak, leading to circulatory collapse and shock. As endothelial dysfunction persists, there is activation of the clotting cascade and microvascular obstruction. Continued endothelial activation results in multi-organ failure, regardless of pulmonary tissue damage. We propose that targeting the endothelium may interrupt this cycle.

Immuno-modulating therapies have been proposed, however further data is needed to confirm that they do not jeopardise adaptive immunity. Inhibition of IL-6 and the Janus Kinase, signal transducer and activator of transcription proteins pathway (JAK/STAT), are considered to be favourable targets. We propose that remote ischaemic conditioning (RIC) reduces the inflammation of sepsis in animal models, and should be considered as a low risk intervention, in combination with cardiovascular protection.

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1. Background

In December 2019, Hubei Province in China declared the emergence of a novel RNA coronavirus, which causes acute severe respiratory distress (ARDS) and rapid multi-organ failure [1]. Genomic sequencing demonstrated notable similarity (89% nucleotide identity) to the SARS coronavirus of 2003, responsible for significant respiratory disease outbreak [2]. The resultant COVID-19, has developed into an aggressive global pandemic, disabling the infrastructure of over 200 countries and infecting millions of people. Therapeutic challenges are mounting rapidly, due to the high virulence factor of the virus and the absence of pre-existing immunity. In the United Kingdom, current prognosis for patients admitted to critical care remains guarded, with mortality rates of approximately 50% reported [3].

COVID-19 causes a varied clinical picture of infection, ranging from asymptomatic, to critical illness with ARDS, requiring multi-organ support on the intensive care unit (ICU). The predictors of progression to critical disease remain unclear, however a trend towards deterioration at days 7-10 of clinical infection has been well documented [1]; [4]; [5]. As seen in the influenza pandemic of 1918, it has been suggested that a cytokine storm of hyper-inflammation is responsible for this detrimental disease course, leading to terminal outcomes in both those young and old, with and without pre-existing co-morbidities [1]; [4].

There is therefore a pressing need to develop or re-purpose therapies which target the COVID-19 induced cytokine storm. As this is not exclusive to COVID-19, we can learn much from known immuno-modulatory interventions, already established in the treatment of severe inflammation. In addition to seasonal influenza (which can also cause ARDS), research should also consider those mechanisms driving the hyper-inflammatory state of other novel coronavirus diseases, bacterial sepsis and haemorrhagic fevers. Considering that coagulopathy and disseminated intra-vascular coagulation (DIC) are features of advanced disease [4], the latter conditions may also be of increasing relevance.

Virus-induced cytokine storm is associated with Secondary haemophagocytic lymphohistocytosis (sHLH), a condition causing persistent pyrexia, haematological disturbance and ARDS in 50% of patients [5]. These clinical features are also observed following Chimeric Antigen Therapy (CAR-T), an autoimmune-modulating treatment used to target B-cells in haematological malignancy. CAR-T leads to a Cytokine Release Syndrome (CRS) in up to

70% of patients [6] and clinical scoring systems are used to pre-assess individuals who may be susceptible to developing hyper-inflammation. Likewise, it is paramount that we identify therapeutic measures to dampen CRS early in the COVID-19 clinical course, (days 1-7). Prompt recognition and intervention may halt progression to severe/critical disease, when the extent of host inflammation and cell injury is likely already, irreversible.

For the purpose of this perspectives opinion article, PubMed/MEDLINE™ databases were accessed at https://pubmed.ncbi.nlm.nih.gov/ between May 9th – June 9th 2020, to determine the latest relevant immunology and future treatment targets of COVID-19 associated CRS.

2. Defining Inflammatory Pathways

SARS-2-CoV is a single stranded RNA virus, which binds to ACE-2 receptors in the lung epithelium and enters the cell via endocytosis [4]. As part of viral replication and manufacture, damage associated molecular patterns (DAMPs) are released into the cytosol and are sensed by circulating macrophages via pattern recognition receptors (PRR) and RIG-I-like receptors (RLRs) [7]. Macrophage recognition of viral invasion initiates the process of chemotaxis and the recruitment of other immune cells via the secretion of acute phase response cytokines IL-6, TNF-α, IL-1β and type-1 interferons (innate immune system) [8]. Other, macrophage-bound receptors involved in the detection of foreign genetic material, include toll like receptors (TLRs), NOD like receptors (NLRs) and the interferon related STING/cGAS pathway [8]; [9]. The latter is involved in cellular cross-talk and transcription of anti-viral proteins in neighbouring cells.

NLRs also act to induce autophagy of pathogens and are involved in inflammasome formation (NLR). These molecules activate the enzyme Caspase-1, leading to production of IL-1β from its precursors [10]. They also facilitate the upregulation of tumour necrosis factor receptor-associated factor (TRAF) [8]. The Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT pathway) forms an integral part of innate immunity signalling and contributes to the differentiation of Natural Killer (NK) cells from lymphoid precursors. NK cells are activated by IL-12 and seek to destroy any host cell with altered or missing major histocompatibility complex (MHC) information [8].

The adaptive immune response is initiated when dendritic cells present antigen fragments to naïve CD4 T-cells. Interleukins 2,4,5 & 10 all have a role in the differentiation of naïve T-cells to T-helper cells, which in addition to IFN-γ, stimulate IgM antibody secretion from B cells (plasma cells) [11]. Memory B-cells are responsible for developing a 'cellular memory' of antigenic material, so that neutralising antibodies can be quickly manufactured, should viral invasion reoccur. A sub-group of T cells called T-regulator cells (Treg) are thought to have an important role in governing the amplitude of the humoral immune response, and it has been documented that reduction in Treg cell numbers is associated with increased susceptibility to auto-immune disorders [12]. Treg cell depletion leads to an increase in the levels of IL-,6, 17 and IFN-y, and reduces the clearance of neutrophils via the secretion of IL-8 [13]. This sequence of events contributes to the development of lung injury and ARDS [12].

3. Pathogenesis of the Cytokine Release Syndrome

The cytokine release syndrome (CRS) is a complex cascade of multiple chemokines released by the immune system in response to pathogenic material (Fig.1). The cytokines which are important in hyper-inflammation include IL-6, IL-8, TNF-α, IL1-β, MCP-1, GCS-F, IP-10, CCL1-3, IL-17 and IFNγ [4]; [5]; [14]; [8]; [11]; [15]; [16]. Delayed secretion of type-1 interferons (IFN-α/β) is also felt to accelerate the development of CRS [8]; [16]; [17]. Individual immune response may vary between different pathogens and the stage of the disease course [11]. Consequently, researchers are faced with the dilemma of quelling immune system over activity, whilst preserving mechanisms of viral clearance and anti-body production. It is challenging to identify the cytokines which are most damaging in COVID-19, without compromising this delicate balance. The risk of impairing a beneficial adaptive immune response, from cytokine inhibition, has recently been defined [11].

The acute-phase response cytokines, IL-6 and TNF-α, are considered by many to be the major 'culprits' in the pathogenesis of COVID-19 hyper-inflammation [4]; [8]; [15]. It has been suggested that IL-6 promotes a macrophage activation syndrome (MAS) [17], triggering mass production of pro-inflammatory cytokines and inducing migration of neutrophils and fibroblasts into the pulmonary epithelium. This results in increased deposition of collagen and fibrin, leading to damage to underlining lung tissue [8]; [15]; [17]; [18]. In humans

with severe COVID-19, SARS and Ebola haemorrhagic fever (EHF), there is positive correlation between elevated IL-6 levels, disease severity and mortality [4]; [19]; [20]; [21]. In a study of patients receiving CAR-T therapy, increased IL-6 was associated with grade-4 CRS and peaked between days 2-5 of treatment [6]. IL-6 additionally causes detrimental endothelial activation, and nitrous oxide (NO) dependent increase in vascular permeability, which will be discussed below.

It is possible to consider IL-6 as both 'friend and foe'; given its ability to 'class-switch' into either a pro- or anti-inflammatory form, as mediated by the protease TACE (TNF-α-converting enzyme). When IL-6 is bound to its soluble receptor (sIL-6R), a cascade of hyper-inflammation is induced, whereas the IL-6-membrane bound IL-6 complex, downregulates this response [20]. IL-6 intra-cellular signalling is complex, involving the gp130 receptor, JAK/STAT, RAS/RAF and AKT/P13K pathways [19]. The IL-6 'classical' pathway involves the IL-6 receptor (IL-6R) which binds directly to gp130. The 'trans' signalling pathway uses soluble sIL-6 receptors to aid gp130 binding and signal transduction in cells which do not express membrane bound IL-6R [19]. In an animal model of influenza, IL-6 knock-out mice were found to have an increased mortality, reduced phagocytic function and increased fibroblast proliferation [22]. Therefore, the ability of IL-6 to initiate chemotaxis and immune system polarisation, may make its inhibition unfavourable in the early disease course.

TNF-α has a similar role to IL-6, as an acute phase response cytokine, with pro-inflammatory actions in COVID-19. Increased serum levels of TNF-α are associated with increased CRS severity [6]; [16]; [17]. In addition, TNF-α has a role in tissue regeneration, by activating nuclear kappa factor B (NF-kB) signalling pathways, to recruit circulating progenitor cells [16]. Murine models of SARS virus (using recombinant S 'spike' protein) have demonstrated that TNF-α and IL-6 may be stimulated directly by the virus via the NF-kB pathway [23]. This would explain the supra-normal levels of the acute phase response cytokines observed in response to infection by coronaviruses.

In addition to stimulating the production of pro-inflammatory cytokines, COVID-19 may induce TNF-α-mediated lymphocyte apoptosis [16]. TNF-α induces caspase-mediated cell death in other disease states such as rheumatoid arthritis, by triggering the release of mitochondrial cytochrome c into the cytosol [11]. Under normal circumstances, this is a carefully regulated homeostatic mechanism and TNF- α /NF-kB activation can also induce cytoprotective proteins [10]. Cell death leads to reduced CD4/8 count (lymphopenia); a commonly reported

clinical finding of severe COVID-19 [4]; [8]; [15]. Moreover, the reduction in T lymphocytes appears too great to be explained by lymphocyte sequestration in lymphoid tissue. Both the protective and detrimental actions of IL-6 and TNF- α, mean that their inhibition must be considered carefully in COVID-19 [24]; [25]; [26].

IL-6, in combination with IL-23, activates the JAK/STAT pathway, leading to differentiation of Th_{17} cells; a sub-type of T-helper cells. TH_{17} secrete IL-17, which has been associated with the development of ARDS in severe influenza [13]. IL-17 may be an important driver of COVID-19 CRS and its inhibition is considered safe when this is not combined with other cytokine suppressants [11]. Another important contributor to CRS is the delayed or insufficient secretion of type-1 interferon, early in the immune response [15]. Although the exact mechanism remains unclear, lower levels of Type-1 interferon have been associated with increased MAS and worsening cytokine storm [8]. Moreover, when type I IFNs have been introduced to SARS infected animals, lower levels of pro-inflammatory cytokines have been reported [17]. Some viruses such as EHF, are able to actively down-regulate the type-I interferon response via disruption of RIG pathways [21]. It is possible that COVID-19 is also capable of disrupting type I IFN defence mechanisms.

Other molecules that circulate at increased levels in severe COVID-19, include monocyte chemo-attractant [8]; [11]; [15]; [26] protein-1 (MCP-1), and the small, inducible chemokines CCL 1,2 & 3 . MCP-1 is a key molecule in assisting macrophage recruitment and migration, and so could contribute to an uncontrolled macrophage activation syndrome (MAS). Elevated levels of MCP-1 have also been implicated in the CRS associated with CAR-T cancer therapy [6]. Although not exclusively limited to haematological conditions, CAR-T is frequently used in the treatment of B-cell malignancy following lymphodepletion chemotherapy. The CRS of CAR-T therapy may be highly relevant to COVID-19, as this also features circulatory collapse and coagulopathy. In a study of 133 haematology patients receiving CAR-T, levels of both IL-6 and MCP-1 peaked between 2-5 days [6]. This illustrates the short amount of time to administer preventative therapy, before key cytokines begin to rise.

The closely linked CCL chemokines, are implicated in many inflammatory disease processes such as allergic asthma, and insulin resistance syndromes and can also induce the prothrombotic, tissue factor (TF) [27]. Tissue factor is expressed on macrophages during endothelial activation and triggers the clotting cascade, to initiate thrombosis. This is homeostatic, when there is localised haemorrhage, but can be detrimental when this is systemic during sepsis [8]. Although pulmonary ARDS is linked to poor prognosis, COVID-19 enters a critical

phase when multi-organ injury and circulatory collapse develop. Viraemia and hyper-cytokinaemia, may result in widespread vascular damage, which continues to drive the production of pro-inflammatory cytokines [8].

4. Endothelial activation – the eye of the Cytokine Storm?

Evidence is emerging that the multi-organ injury observed in COVID-19 is a consequence of cytokine-induced endothelial dysfunction (endothelitis) [28]. IL-6 causes endothelial activation and neutrophil infiltration, which results in NO (nitric oxide)-mediated changes to vascular permeability and loss of vascular tone [8]. This is reflected clinically by increased neutrophil to T-cell lymphocyte ratio, and the development of septic shock [4]. Endothelial activation occurs initially within the microcirculation, in order to prevent pathogenic material from translocating to larger vessels. Unfortunately, this increases microvascular complications including micro-thrombi and capillary haemorrhage; pathologies which have been observed following the post-mortems of both COVID-19 and SARS patients [28]; [29]. Clinical markers such as d-dimer, which are predictive of poor outcome in COVID-19 [4] are products of fibrin degradation and reflect underlying endothelial-mediated activation of the clotting cascade [8].

Multiple cases of ST-elevation Myocardial Infarction (STEMI), in the absence of major epicardial coronary obstruction, have been reported in COVID-19 [30]; [31]. In a cohort of 28 patients from the Italian district of Lombardy, 40% of COVID-19 patients presenting with typical STEMI had no flow limiting lesion [31]. Moreover, biopsies from a STEMI patient with unobstructed coronaries in COVID-19, did not demonstrate myocarditis [28]. Myocardial infarction (MI) in COVID-19 may be triggered by cytokine-induced microvascular dysfunction [28]. Troponin remains a gold standard biomarker in the context of infarction, but may also be significantly elevated in microvascular obstruction (MVO). Marked elevations are also seen in septic shock and are prognostic [31]. Thrombolysis is not deemed appropriate treatment in COVID-19 STEMI, when type-2 myocardial infarction predominates [30]; [31].

The role of ACE inhibitors has proved controversial in COVID-19, however some suggest that they do not cause harm, and may be beneficial [32]; [33]. ACE inhibitors are proven to be cardioprotective and help to reduce long-term endothelial activation, by controlling hypertension and encouraging LV remodelling [32]. It is possible that

this cohort of patients may be better faced to withstand CRS-induced inflammation, given that angiotensin-II is linked to vascular NO pathways [8]. In animal models, therapeutic heparin has improved survival following challenge with LPS [34]. Heparin, however, did not reduce inflammatory cytokine levels, and the development of subsequent coagulopathy made the activated partial thromboplastin time (APTT) difficult to interpret.

In addition to microvascular events, COVID-19 is associated with an increase in arterial thrombosis. A recent case study of five COVID-19 positive patients under 50 years, reported ischaemic stroke as a cerebrovascular manifestation of the disease [35]. An occlusive thrombus was identified in all cases and treatment consisted of anti-coagulation with clot retrieval. These phenomena may not be surprising considering that endothelial activation in non-COVID disorders such as atherosclerosis, affects the cardiovascular and neurovascular systems in a similar manner [36]. Moreover, inflammatory molecules and leukocytes are known to traverse the blood-brain barrier causing central nervous system (CNS) inflammation; a mechanism which may be further augmented when endothelial integrity is compromised [37]. The CNS is able to synthesise its own cytokines via type-1 astrocytes and microglia, resulting in other neurological manifestations of coronavirus such as encephalitis [38]; [39].

It is now well appreciated that COVID-19 increases the incidence of venous thrombo-embolic events (VTEs) such as pulmonary emboli; often in absence of traditional risk factors [4]; [40]; [41]. A French study of 107 critical care patients found a significantly higher proportion of VTEs in a COVID-19 cohort, compared to a controls with severe influenza [42]. The relationship between systemic inflammation and coagulation cascade activation is well recognised in many diseases, and in septic shock, the pro-inflammatory cytokines such as TNF and IL-1 are thought to induce pro-coagulant thrombin factor (TF), triggering important 'immuno-thrombotic' pathways [43]. A new novel mechanism in immune mediated thrombosis has been proposed, which identifies the depletion of anti-coagulant Protein S (PROS1) by clot consumption, as significant in the development of COVID-19 pro-thrombotic state [44]

Clinicians are currently in the early stages of identifying a new paediatric illness, which resembles a large/medium vessel vasculitis such as Kawasaki's disease [45]; [47]. This is felt to be a late sequela of COVID-19 infection in children, and is perhaps a consequence of the paediatric cytokine storm causing immune mediated vascular damage. Further research must focus on endothelial protection to prevent multi-organ injury, cardiac/cerebrovascular complications, and multi-system vasculitis/endothelitis. This may be particularly

important in individuals with conditions such as type-2 diabetes who have a chronic, persistent level of endothelial dysfunction and a poor prognosis in COVID-19 [4]. The endothelial system as an organ must be considered as a pivotal mediator of COVID-19 induced hyper-inflammation and thrombosis.

5. New frontiers in Cytokine Suppression

As the detrimental effects of COVID-19 associated CRS are recognised, there has been a rush to trial pre-existing immune-modulating therapies, used in the treatment of chronic inflammatory conditions. Anti-viral agents which predominantly target the host cell (modulating endocytosis, viral replication and manufacture) such as Remdesivir, Hydroxychloroquine/chloroquine and arbidol have been investigated widely [45]; [46] and are the subject of many clinical studies [47]; [48]. Although these agents have shown some early promise, they may be considered less relevant as direct mediators of the CRS of COVID-19, hence this article will focus on new frontiers in targeting cytokine release.

The IL-6 receptor blocker, Tocilizumab (TCZ) has generated much interest in this context [5]; [8]; [11]; [19]. Acting directly on the IL-6R, TCZ can inhibit both classical and trans-signalling pathways and is licensed for treatment of CRS associated with CAR-T therapy [19]. Traditionally, TCZ is used in the treatment of rheumatoid arthritis (RA) and sHLH caused by Still's Disease (Juvenile onset arthritis) [5]; [11]; [15]; [25]. It is generally well tolerated in patients with chronic inflammatory conditions, however in a review of phase III RCT's of RA, TCZ was found to cause upper respiratory tract infection in approximately 7% of patients and hypertension in 6% of patients [49]. A retrospective study from Wuhan, China analysed the outcomes of 15 critical care patients who received TCZ for COVID-19. TCZ was associated with mitigation of IL-6 response, however the study lacked controls, was retrospective and patients received multi immune-modulating therapies [50]. The results of randomised control trials (RCTs) are currently awaited.

An important relationship may be emerging between IL-6 and androgen regulation [54]. It has been proposed that upregulation of androgens in males leads to increased expression of cellular transmembrane serine protease 2 receptors (TMPRSS2) via IL-6 signalling [54]. Animal models have demonstrated that TMPRSS2, alongside ACE-2 receptors, govern entry of COVID-19 into the host cell. Moreover, inhibition of TMPRSS2 has

been found to kill the SARS-CoV-2 virus [51]. Attention has turned to established anti-androgen therapies such as spironolactone and bicalutamide as potential modulators of pro-inflammatory cytokines [52]. Similarly, inhibitors of IL-1β (Anakinra™) and TNF- α (Etanercept™) have been proposed as anti-inflammatory agents in already-established severe CRS [8]. Whilst they have shown promise in animal models with LPS-induced infection, [24] data in humans is limited.

The JAK/STAT pathway is considered an attractive target for cytokine suppression [5]; [8]; [11]; [16]; [17]. Of note, inhibition of this pathway would disrupt the signalling of multiple cytokines and maximise anti-inflammatory effect. Caution must be exercised however, as there is a theoretical danger in blocking cytokines associated with viral clearance via (JAK 1&3) [11]. Immunosuppressants can impede viral clearance via interference with type I IFN signalling, which upregulates NK cells and the adaptive immune response. In addition, JAK inhibitors can increase the risk of venous thrombosis, which is a significant pathology in COVID-19 [16]. The tyrosine-kinase inhibitor Ponatinib, has been found to inhibit the release of multiple cytokines in animal models of severe influenza [53]. In vivo, Ponatinib significantly down-regulated three key cytokines (IP-10, IL-8 and MCP-1), and reduced overall mortality.

Other pharmacological agents proposed in influenza associated cytokine storms include peroxisome proliferator-activated receptor agonists (PPARs), sphingosine-1-phosphate receptor modulator agonists (S1P1) and COX inhibitors [13]. Corticosteroids remain controversial [54], as they have previously increased mortality in critical care patients suffering from severe influenza and ARDS [55]. Administration of IVIG and convalescent plasma of survivors has shown early promise, however these are therapies suited to critical care and treatment of well-established grade 4 CRS [56]. A new novel target of the rapamycin (mTOR) pathway has recently been proposed [57]. This is based on the principle that tissue damage in CRS is related to antibody-dependent enhancement (ADE) and this class of drugs has been utilised previously in the prevention of CRS in transplant and H1N1 [58].

Circulatory support methods such as Extra Corporeal Membrane Oxygenation (ECMO) used on critical care, are an effective means of cytokine removal and therefore reduce multi-organ involvement [59]. It is important clinically, when considering pharmaceutical options to also consider the haemodynamic status of the patient and the consequences of shock physiology. Critical care teams have observed the importance of ensuring that the severely unwell COVID-19 patient has optimal fluid balance, so as to avoid development of ARDS, fluid

overload and congestive renal injury with renal hypo-perfusion [60]. Similarly, cardio-renal syndrome related to COVID-19 can occur by this mechanism of excessive volume expansion and third space fluid accumulation, caused by IL-6 induced changes to vascular permeability.

In animal models of sepsis, Remote Ischaemic Conditioning (RIC) has shown significant promise in reducing mortality [61]. RIC is a widely reported cardio-protective phenomenon, evident when periods of sequential ischaemia and reperfusion at a remote site to the target organ, confer protection of cardiomyocytes from reperfusion injury (RPI) [61]; [62]. RIC is performed in humans by gradual inflations and deflations of a blood pressure cuff situated on the upper limb [63].

In mice challenged with LPS, RIC significantly reduced the serum concentration of pro-inflammatory cytokines (IFN-γ, IL-1β, TNF-α) and improved survival. The exact mechanism by which this occurs remains unclear, however RIC may modulate adenosine receptors and downregulate the NF-kB pathway of cytokine release [62]. In humans, RIC uses vagally-mediated pathways to reduce the inflammation from reperfusion injury, which can occur in both sepsis and acute myocardial infarction [62]; [64]. RIC could therefore have a role in suppressing the inflammatory storm of COVID-19 and is both an accessible and simple intervention.

6. Expert Opinion: Consider RISK and Multi-organ Protection in CRS

COVID-19 is no longer just an infection confined to the pulmonary epithelium, but a multi-system inflammatory disorder causing end-organ failure [28]. Although anti-viral agents such as Remdesivir have reduced disease severity in case studies [46], there is still no definitive cure for COVID-19. We must therefore, turn our attention to other modalities of preventing end-organ destruction, whilst perfecting pharmacological options. As is evident from critical care reports, multi-organ ischaemia in COVID-19 occurs as a result of cardio-renal syndrome, cytokine release and global hypoperfusion due to loss of vascular integrity [60]. It may be possible to apply the principles of organ protection, utilised in other ischaemic conditions to limit cell injury and apoptosis. The aforementioned observation that RIC can influence cytokine release in animal models, provides an excellent foundation for further research in COVID-19 [61].

Cardioprotection is a well-established concept, developed to minimise damage to cardiac myocytes following myocardial infarction and re-perfusion injury (RPI) [65]; [66]; [67]. As the heart is central to circulatory function and multi-organ perfusion, it follows that cardiac protection may confer multi-system benefit in COVID-19. Following ST elevation myocardial infarction (STEMI), there is a significant immune response which leads to systemic inflammation [68]. Similarly, there is upregulation of pro-inflammatory cytokines, inflammasomes and immune mediated thrombosis following plaque rupture [68]. The discovery of the Reperfusion Injury Salvage Kinase (RISK) pathway was a major breakthrough in cardioprotective research [69]. RISK describes a group of pro-survival protein kinases which act to minimise cell death by reducing mitochondrial transition pore opening (MTP), when activated early in ischaemia [65].

Organ protection from tissue hypoxia is most optimal when a 'multi-hit' approach is adopted [70]. The authors feel this is best achieved by the fusion of proven cardioprotective medications, and physiological interventions which minimise reperfusion injury and cytokine release, such as RIC [62]. Moreover, within cardioprotective pharmacology there is the opportunity to target multiple pathways which reduce cell death and inflammation (additive effect) [70]. Such pro-survival pathways consist of RISK, Survivor Activating Factor Enhancement (SAFE) and Nitrous Oxide/Protein Kinase (NO/PKG) [65]. This is relevant when considering that hyper-inflammation can trigger apoptosis via NF-*k*B and TNF-*a* [16]. RIC has been more difficult to translate to humans, despite the clear benefits demonstrated in animal models [61]; [63]; [67]. Importantly, COVID-19 provides an optimal opportunity to apply this intervention at the onset of mild disease, before the cytokine storm develops.

Preserving cardiac function during severe sepsis and CRS provides global benefit by improving contractility, increasing oxygen utilisation and maintaining perfusion pressure [71]. Improving cardiac function therefore, has the potential to protect renal function and neurological status. The heart is especially susceptible to injury during infection by circulating active substances known as myocardial depressant factors (MDFs). These substances include the cytokines IL-1*B*, IL-6, TNF-*a*, complements and high levels of NO. MDFs may act via NF-*k*B pathways of inflammation when binding to toll-like receptors such as TLR-4 [72]. Therapies which have been investigated in the septic heart of animals include caspase inhibitors, hydrogen gas (H_2), melatonin and independent growth factor I (GFI-I) [74]; [73]. Cytokine suppression has been attempted in this context; however, these treatments have thus far failed to show significant benefit [74].

In other cardioprotective developments, research is ongoing to identify the exact mechanisms by which sodium-glucose cotransporter 2 (SGLT2) inhibitors are beneficial in the diabetic heart [74]. Treatments targeting ischaemia induced microvascular dysfunction may equally be of interest in COVID-19. In addition to $P2Y_{12}$ inhibitors and statins, Rho Kinase inhibitors (ROCKi) have demonstrated many novel actions in cardiovascular protection, including modulation of vascular tone, angiogenesis and apoptosis [70]; [75]; [76]. ROCK inhibition can additionally mediate endothelial barrier function and reduce leukocyte migration [78]; [77]. These protective effects have been seen in the CNS in addition to the cardiovascular system [78], which makes this class of drug a versatile and exciting prospect for further research.

7. Conclusion

The cytokine storm of COVID-19 is an important instigator of severe disease and multi-organ injury. We describe the immune-modulating therapies which are currently under review in the COVID-19 pandemic, and consider the benefits of remote ischaemic conditioning (RIC) in mediating harmful cytokine release. The endothelial system as an organ is a crucial mediator of the cytokine storm and its persistent activation drives a 'septic swirl'. This opinion considers the importance of cardiovascular protection in whole body ischaemia during sepsis, and provides insights into implications for further research. Only time will tell whether a fusion of novel immune-modulating drugs and cardiovascular protection can influence the dire outcomes of severe COVID-19.

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Highlights

- IL-6 and TNF-*α* are key mediators of the cytokine release syndrome in COVID-19
- Macrophage activation syndrome and endothelial activation are cornerstone in persistent inflammation
- Endothelial involvement is associated with microvascular thrombi and pro-thrombotic state
- Risk to the adaptive immune response should be considered when developing cytokine suppression therapy
- Remote Ischaemic Conditioning and Cardiovascular Protection should be considered in the prevention and protection of COVID-19 CRS

Fig.1

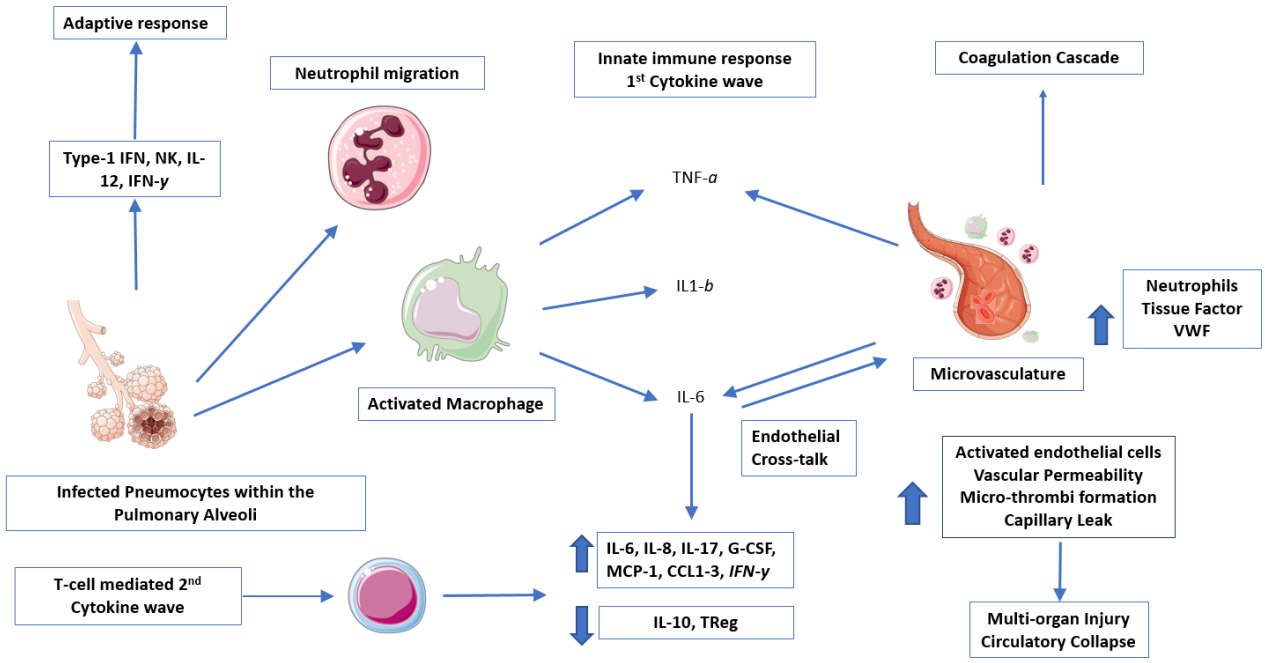

Fig.1 illustrates the key pathways in the development of cytokine release syndrome (CRS), and microvascular involvement. The SARS-CoV-2 virus infects type II pneumocytes within the pulmonary system. Local macrophages respond to foreign genetic material. The acute phase response cytokines (TNF-a, IL1-b and IL-6) trigger a first cytokine wave, whilst type I IFNs initiate the differentiation of Natural Killer cells (NK) and the adaptive immune response. The cytokine IL-6 induces endothelial activation, and inflammatory cell migration. There is an increase in Tissue Factor secretion and upregulation of the coagulation cascade leading to immune mediated thrombosis. Endothelial activation stimulates inflammatory cross-talk contributing to a second cytokine wave. Figure adapted from [9,12,17]. Abbreviations: IFN – Interferon, NK – natural killer cells, VWF – Von Willebrand Factor, G-CSF – Granulocyte colony-stimulating factor, MCP-1 – monocyte chemoattractant protein 1, CCL – chemokine ligand

References

[1] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727–733.

[2] Chan JF-W, Kok K-H, Zhu Z, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect [Internet]. 2020 [cited 2020 May 16];9:221–236. Available from: https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1719902.

[3] Source: ICNARC Case Mix Programme Database. 2020.

[4] Zhou F, Yu T, Du R, et al. Articles Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet [Internet]. 2020 [cited 2020 May 16];395:1054–1062. Available from: https://doi.org/10.1016/. **An important cohort of COVID-19 patients from the first wave of infection in Wuhan, China*

[5] Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. Lancet Publishing Group; 2020. p. 1033–1034.

[6] Hay KA, La¨ıla-A¨ıcha L, Hanafi A, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy Key Points. 2017 [cited 2020 May 12]; Available from: https://ashpublications.org/blood/article-pdf/130/21/2295/1403588/blood793141.pdf.

[7] Kim YK, Shin JS, Nahm MH. NOD-like receptors in infection, immunity, and diseases. Yonsei Med. J. Yonsei University College of Medicine; 2016. p. 5–14.

[8] Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages [Internet]. Nat. Rev. Immunol. [cited 2020 May 17]. Available from: www.nature.com/nri.**An extensive review proposing the macrophage activation sydrome*

[9] Ni G, Ma Z, Damania B. cGAS and STING: At the intersection of DNA and RNA virus-sensing networks.

Evans MJ, editor. PLOS Pathog [Internet]. 2018 [cited 2020 May 16];14:e1007148. Available from: https://dx.plos.org/10.1371/journal.ppat.1007148.

[10] Bezbradica JS, Joyce S. NKT Cells Join the Two Step for Inflammasome-Independent IL-1β Release. Cell Rep. 2020;31:107481.

[11] Schett G, Sticherling M, Neurath MF. COVID-19: risk for cytokine targeting in chronic inflammatory diseases? Nat. Rev. Immunol. Nature Research; 2020. p. 271–272.**An important review evaluating clinical risk of individual cytokine suppression*

[12] Kumar P, Saini S, Khan S, et al. Restoring self-tolerance in autoimmune diseases by enhancing regulatory T-cells. Cell Immunol. 2019;339:41–49.

[13] Lin S, Wu H, Wang C, et al. Regulatory T cells and acute lung injury: Cytokines, uncontrolled inflammation, and therapeutic implications. Front. Immunol. Frontiers Media S.A.; 2018. p. 1545.

[14] Sun X, Wang T, Cai D, et al. Cytokine storm intervention in the early stages of COVID-19 pneumonia. Cytokine Growth Factor Rev. 2020;

[15] Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'Cytokine Storm'' in COVID-19.' J. Infect. W.B. Saunders Ltd; 2020.

[16] Jamilloux Y, Henry T, Belot A, et al. Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. Autoimmun Rev [Internet]. 2020 [cited 2020 May 17];102567. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32376392.

[17] Mcgonagle D, Sharif K, O'regan A, et al. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. 2020 [cited 2020 May 17]; Available from: www.elsevier.com/locate/autrev.

[18] Tanaka T, Narazaki M, Kishimoto T. Cite this article as. Cold Spring Harb Perspect Biol [Internet]. 2014 [cited 2020 May 17];6:16295–16296. Available from: www.cshperspectives.org.

[19] Zhang C, Wu Z, Li JW, et al. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. Int J Antimicrob

Agents. 2020;

[20] Mahmud-Al-Rafat A, Majumder A, Rahman KMT, et al. Decoding the enigma of antiviral crisis: Does one target molecule regulate all? 2018 [cited 2020 May 17]; Available from: www.elsevier.com/locate/cytokine.

[21] Falasca L, Agrati C, Petrosillo N, et al. Molecular mechanisms of Ebola virus pathogenesis: Focus on cell death. Cell Death Differ. Nature Publishing Group; 2015. p. 1250–1259.

[22] Yang ML, Wang CT, Yang SJ, et al. IL-6 ameliorates acute lung injury in influenza virus infection. Sci Rep. 2017;7.

[23] Wang W, Ye L, Ye L, et al. Up-regulation of IL-6 and TNF-α induced by SARS-coronavirus spike protein in murine macrophages via NF-κB pathway. Virus Res. 2007;128:1–8.

[24] Lai WY, Wang JW, Huang BT, et al. A novel TNF-α-targeting aptamer for TNF-α-mediated acute lung injury and acute liver failure. Theranostics. 2019;9:1741–1751.

[25] Perricone C, Triggianese P, Bartoloni E, et al. The anti-viral facet of anti-rheumatic drugs: Lessons from COVID-19. 2020 [cited 2020 May 18]; Available from: www.elsevier.com/locate/jautimm.

[26] Pedersen SF, Ho YC. SARS-CoV-2: A Storm is Raging. J Clin Invest. 2020;130:2202–2205.

[27] Deshmane SL, Kremlev S, Amini S, et al. Monocyte chemoattractant protein-1 (MCP-1): An overview. J. Interf. Cytokine Res. Mary Ann Liebert, Inc.; 2009. p. 313–325.

[28] Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. 2020 [cited 2020 May 17]; Available from: https://doi.org/.**Key opinion in the endothelial involvement of COVID-19*

[29] Hwang DM, Chamberlain DW, Poutanen SM, et al. Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mod Pathol. 2005;18:1–10.

[30] Stefanini GG, Montorfano M, Trabattoni D, et al. ST-Elevation Myocardial Infarction in Patients with COVID-19: Clinical and Angiographic Outcomes. Circulation [Internet]. 2020 [cited 2020 May 18];CIRCULATIONAHA.120.047525. Available from:

https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.120.047525.

[31] Chieffo A, Stefanini GG, Price S, et al. EAPCI Position Statement on Invasive Management of Acute Coronary Syndromes during the COVID-19 pandemic Interventional cardiology Abbreviations ACS = acute coronary syndrome ED = emergency department HCWs = healthcare workers NSTEMI = non-ST-elevation myocardial infarction PCI = percutaneous coronary intervention PPE = personal protective equipment STEMI = ST-elevation myocardial infarction. Eur Heart J [Internet]. 2020 [cited 2020 May 18];41:1839–1851. Available from: https://academic.oup.com/eurheartj/article-abstract/41/19/1839/5836093.***Position paper on the management of COVID-19 associated MI*

[32] Guzik TJ, Mohiddin SA, Dimarco A, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. [cited 2020 May 18]; Available from: https://www.arcgis.com/apps/opsdash.

[33] Bean DM, Kraljevic Z, Searle T, et al. ACE-inhibitors and Angiotensin-2 Receptor Blockers are not associated with severe SARS- COVID19 infection in a multi-site UK acute Hospital Trust. [cited 2020 May 18]; Available from: https://doi.org/10.1101/2020.04.07.20056788.

[34] Li Y, Sun JF, Cui X, et al. The effect of heparin administration in animal models of sepsis: A prospective study in Escherichia coli-challenged mice and a systematic review and metaregression analysis of published studies. Crit Care Med. Lippincott Williams and Wilkins; 2011. p. 1104–1112.

[35] Oxley TJ, Mocco J, Majidi S, et al. Large-Vessel Stroke as a Presenting Feature of Covid-19 in the Young. N. Engl. J. Med. NLM (Medline); 2020. p. e60.

[36] Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat. Immunol. Nat Immunol; 2011. p. 204–212.

[37] Wu F, Liu L, Zhou H. Endothelial cell activation in central nervous system inflammation. J Leukoc Biol. 2017;101:1119–1132.

[38] Lavi E, Cong L. Type I astrocytes and microglia induce a cytokine response in an encephalitic murine coronavirus infection. Exp Mol Pathol [Internet]. 2020 [cited 2020 Jun 6];115:104474. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32454103.

[39] Li Y, Fu L, Gonzales DM, et al. Coronavirus Neurovirulence Correlates with the Ability of the Virus To Induce Proinflammatory Cytokine Signals from Astrocytes and Microglia. J Virol. 2004;78:3398–3406.

[40] Danzi GB, Loffi M, Galeazzi G, et al. Acute pulmonary embolism and COVID-19 pneumonia: a random association?

[41] Bertoletti L, Couturaud F, Montani D, et al. Venous thromboembolism and Covid-19. Respir Med Res. 2020;78:100759.

[42] Poissy J, Goutay J, Caplan M, et al. Pulmonary Embolism in COVID-19 Patients: Awareness of an Increased Prevalence. Circulation. 2020;

[43] Allen KS. Anticoagulant modulation of inflammation in severe sepsis. World J Crit Care Med. 2015;4:105.

[44] Lemke G, Silverman GJ. Blood clots and TAM receptor signalling in COVID-19 pathogenesis. Nat Rev Immunol [Internet]. 2020 [cited 2020 Jun 9];1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32488201.

[45] Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Preliminary Report. N Engl J Med [Internet]. 2020; Available from: http://www.ncbi.nlm.nih.gov/pubmed/32445440.

[46] Sanders JM, Monogue ML, Jodlowski TZ, et al. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. JAMA - J. Am. Med. Assoc. American Medical Association; 2020. p. 1824–1836.

[47] Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 2020;395:1569–1578.

[48] Rosenberg ES, Dufort EM, Udo T, et al. Association of Treatment with Hydroxychloroquine or Azithromycin with In-Hospital Mortality in Patients with COVID-19 in New York State. JAMA - J Am Med Assoc. 2020;

[49] Scott LJ. Tocilizumab: A Review in Rheumatoid Arthritis. Drugs. 2017;77:1865–1879.

[50] Luo P, Liu Y, Qiu L, et al. Tocilizumab treatment in COVID-19: A single center experience. J Med Virol

[Internet]. 2020 [cited 2020 May 20]; Available from: http://doi.wiley.com/10.1002/jmv.25801.

[51] Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181:271-280.e8.

[52] Liaudet L, Szabo C. Blocking mineralocorticoid receptor with spironolactone may have a wide range of therapeutic actions in severe COVID-19 disease. Crit Care [Internet]. 2020 [cited 2020 Jun 9];24:318. Available from: https://ccforum.biomedcentral.com/articles/10.1186/s13054-020-03055-6.

[53] Feng CG, Karupiah G, Levy DE, et al. Ponatinib Protects Mice From Lethal Influenza Infection by Suppressing Cytokine Storm. Front Immunol | www.frontiersin.org [Internet]. 2019 [cited 2020 May 14];1:1393. Available from: www.frontiersin.org.

[54] Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet. Lancet Publishing Group; 2020. p. 473–475.

[55] Brun-Buisson C, Richard JCM, Mercat A, et al. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. Am J Respir Crit Care Med. 2011;183:1200–1206.

[56] Chen L, Xiong J, Bao L, et al. Convalescent plasma as a potential therapy for COVID-19. Lancet Infect. Dis. Lancet Publishing Group; 2020. p. 398–400.

[57] Zheng Y, Li R, Liu S. Immunoregulation with mTOR inhibitors to prevent COVID-19 severity: A novel intervention strategy beyond vaccines and specific antiviral medicines. J Med Virol [Internet]. 2020 [cited 2020 May 18];jmv.26009. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.26009.

[58] Wang C-H, Chung F-T, Lin S-M, et al. Adjuvant Treatment With a Mammalian Target of Rapamycin Inhibitor, Sirolimus, and Steroids Improves Outcomes in Patients With Severe H1N1 Pneumonia and Acute Respiratory Failure. Crit Care Med [Internet]. 2014 [cited 2020 May 18];42:313–321. Available from: http://journals.lww.com/00003246-201402000-00010.

[59] Ronco C, Navalesi P, Vincent JL. Coronavirus epidemic: preparing for extracorporeal organ support in intensive care. Lancet Respir Med. 2020;8:240–241.

[60] Kazory A, Ronco C, McCullough PA. SARS-CoV-2 (COVID-19) and intravascular volume management strategies in the critically ill. Baylor Univ. Med. Cent. Proc. Taylor and Francis Inc.; 2020.

[61] Joseph B, Khalil M, Hashmi A, et al. Survival benefits of remote ischemic conditioning in sepsis. J Surg Res. 2017;213:131–137.**Key evidence of the benefits for RIC in Sepsis*

[62] Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. Nat. Rev. Cardiol. Nature Publishing Group; 2016. p. 193–209.

[63] Hausenloy DJ, Kharbanda RK, Møller UK, et al. Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. Lancet. 2019;394:1415–1424.

[64] Pickard JMJ, Davidson SM, Hausenloy DJ, et al. Co-dependence of the neural and humoral pathways in the mechanism of remote ischemic conditioning. Basic Res Cardiol. 2016;111.

[65] Rossello X, Yellon DM. The RISK pathway and beyond. Basic Res. Cardiol. Dr. Dietrich Steinkopff Verlag GmbH and Co. KG; 2018.**An important description of cardioprotection via RISK*

[66] Bromage DI, Pickard JMJ, Rossello X, et al. Remote ischaemic conditioning reduces infarct size in animal in vivo models of ischaemia-reperfusion injury : a systematic review and meta-analysis. 2017;288–297.

[67] Heusch G. Critical Issues for the Translation of Cardioprotection. Circ. Res. Lippincott Williams and Wilkins; 2017. p. 1477–1486.

[68] Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. 2002;53:31–47.

[69] Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: Taking a RISK for cardioprotection. Heart Fail Rev. 2007;12:217–234.

[70] Davidson SM, Ferdinandy P, Andreadou I, et al. Multitarget Strategies to Reduce Myocardial Ischemia / Reperfusion Injury JACC Review Topic of the Week. 2019;73.**Position paper in multi-targets for cardioprotection*

[71] Ronco C, Reis T. Kidney involvement in COVID-19 and rationale for extracorporeal therapies. Nat. Rev. Nephrol. Nature Research; 2020.

[72] Lin H, Wang W, Lee M, et al. Current Status of Septic Cardiomyopathy: Basic Science and Clinical Progress. Front. Pharmacol. Frontiers Media S.A.; 2020.

[73] Zhong J, Tan Y, Lu J, et al. Therapeutic contribution of melatonin to the treatment of septic cardiomyopathy: A novel mechanism linking Ripk3-modified mitochondrial performance and endoplasmic reticulum function. Redox Biol. 2019;26.

[74] Mcmurray JJ V, Solomon SD, Inzucchi SE, et al. Dapagli fl ozin in Patients with Heart Failure and Reduced Ejection Fraction. 2019;

[75] Narumiya S, Thumkeo D. Rho signaling research: history, current status and future directions. FEBS Lett. 2018;592:1763–1776.

[76] Shimokawa H, Rashid M. Development of Rho-kinase inhibitors for cardiovascular medicine. Trends Pharmacol Sci. 2007;28:296–302.

[77] Galvão I, Athayde RM, Perez DA, et al. ROCK Inhibition Drives Resolution of Acute Inflammation by Enhancing Neutrophil Apoptosis. Cells. 2019;8:964.

[78] Batchelor TJP, Sadaba JR, Ishola A, et al. Rho-kinase inhibitors prevent agonist-induced vasospasm in human internal mammary artery. Br J Pharmacol. 2001;132:302–308.