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Review

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New Frontiers in Sepsis-Induced Acute Kidney Injury and Blood Purification Therapies: The Role of Polymethylmethacrylate Membrane Hemofilter

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Abstract
Acute kidney injury (AKI) is a common consequence of sepsis with a mortality rate of up to 40%. The pathogenesis of septic AKI is complex and involves several mechanisms leading to exacerbated inflammatory response associated with renal injury. A large body of evidence suggests that inflammation is tightly linked to AKI through bidirectional interaction between renal and immune cells. Preclinical data from our and other laboratories have identified in complement system activation a crucial mediator of AKI. Partial recovery following AKI could lead to long-term consequences that predispose to chronic dysfunction and may also accelerate the progression of preexisting chronic kidney disease. Recent findings have revealed striking morphological and functional changes in renal parenchymal cells induced by mitochondrial dysfunction, cell cycle arrest via the activation of signaling pathways involved in aging process, microvascular rarefaction, and early fibrosis. Although major advances have been made in our understanding of the pathophysiology of AKI, there are no available preventive and therapeutic strategies in this field. The identification of ideal clinical biomarkers for AKI enables prompt and effective therapeutic strategy that could prevent the progression of renal injury and promote repair process. Therefore, the use of novel biomarkers associated with clinical and functional criteria could provide early interventions and better outcome. Several new drugs for AKI are currently being investigated; however, the complexity of this disease might explain the failure of pharmacological intervention targeting just one of the many systems involved. The hypothesis that blood purification could improve the outcome of septic AKI has attracted much attention. New relevant findings on the role of polymethylmethacrylate-based continuous veno-venous hemofiltration in septic AKI have been reported. Herein, we provide a comprehensive literature review on advances in the pathophysiology of septic AKI and potential therapeutic approaches in this field.

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Introduction

Sepsis-induced acute kidney injury (SI-AKI) is a common clinical complication of the critically ill patients and is associated with unacceptable high risk for mortality, and its impact extends into long-term outcomes, predisposing to the development of chronic kidney disease (CKD). SI-AKI has a complex and unique pathophysiology principally characterized by an exacerbated immune response associated with systemic endothelial dysfunction and alterations of renal resident cells that may promote progression of kidney disease. The host immune response is enhanced by several mediators including damage- and pathogen-associated molecular patterns (DAMPs/PAMPs) that bind the pattern recognition receptors (PRR), such as toll-like receptors (TLR), expressed on the surface of immune cells [1–3], inducing their subsequent activation. Additionally, renal resident cells are able to directly interact with these factors, through TLR-2 and TLR-4, and actively participate in amplifying renal damage [1].

A crucial mediator of innate immune response involved in sepsis is the complement system. The kidney is particularly susceptible to complement cascade, activated both by the pathogen itself and by damaged tissue. Additionally, the end product of complement activation, the C5b-9 complex, is associated to the development of multiple organ failure (MOF) as well as other complement fragments with AKI severity after cardiac surgery [4]. Several preclinical and clinical studies have underlined the involvement of complement factors in the pathogenesis of AKI [5]. Unresolved recovery of renal function is associated with a great risk of physiological and structural changes that lead to the progression of chronic renal failure [6]. A broad range of potential pathophysiological mechanisms have been proposed to be involved in AKI-to-CKD transition, including hypoxia and microvascular rarefaction, persistence of chronic inflammation and cell cycle arrest, development of interstitial fibrosis, cell and tissue senescence, and mitochondrial dysfunction [5, 7]. Although considerable advances have been made in our understanding of the pathophysiology of AKI and AKI-to-CKD process, there are no effective and standardized therapeutic strategies in this field.

The introduction of highly sensitive and diagnostic-specific biomarkers might enable the prompt detection and effective treatment of SI-AKI. This approach is critical for the development of new therapies that could take place in the earliest stages, before kidney damage occurs.

Several molecules have been tested in preclinical and clinical studies, to recover mitochondrial dysfunction, inflammation, and oxidative stress. Most of these interventions have been proven ineffective since sepsis is a complex disease that involves several mediators. Clinical studies showed that the use of adsorptive membrane hemofilter reduced systemic inflammation, improving blood pressure and urine output in critically ill patients [8]. In line with these findings, our group reported relevant results on the role of polymethylmethacrylate (PMMA)-based continuous veno-venous hemofiltration (CVVH) in an animal model of SI-AKI. PMMA treatment induced significant modulation of hemodynamic parameters, with preservation of renal function and avoidance of structural changes in the renal parenchyma of endotoxemic animals. This review deeply analyzes the potential mechanisms involved in the pathogenesis of septic AKI and the new advance in diagnosis and therapeutic strategies.

Pathophysiology of SI-AKI

As it is now largely recognized, SI-AKI pathophysiology is complex and multifactorial. Over and above intrarenal hemodynamic changes, a key role has emerged for several elements such as inflammation, vascular dysfunction, bioenergetics, and tubular cell adaptation to injury [1, 9, 10]. Consistently, Gomez et al. [11] proposed a "unified theory" of SI-AKI, consolidating the various mechanisms into a coherent framework of synergic interactions.

Hyperinflammation is a pivotal hallmark in the pathophysiology of SI-AKI, characterized by a humoral and a cellular mediator which exacerbate the renal injury. However, as the new sepsis definition implies, hyperinflammation leads to neutrophil and macrophage/lymphocyte infiltration followed by MOF and poor outcomes [12]. Mounting evidence has shown that in AKI-induced sepsis, inflammatory mediators including DAMPs/PAMPs are released in the intravascular compartment. DAMP/PAMP interaction with tubular injured cells via PRRs enhances the inflammatory damage by stimulating the production of cytokines (i.e., TNF-α, IL-6, and IL-1β), chemokines (i.e., MCP-1), and adhesion molecules (i.e., VCAMs and ICAMs) (Fig. 1) [13]. Notably, among the PRR family, TLRs are the most extensively studied, following DAMP/PAMP interaction, leading to the recruitment of innate immune system cells [14]. On the other hand, DAMP/PAMP interaction with tubular epithelial cells (TECs) stimulates the production of reactive oxygen

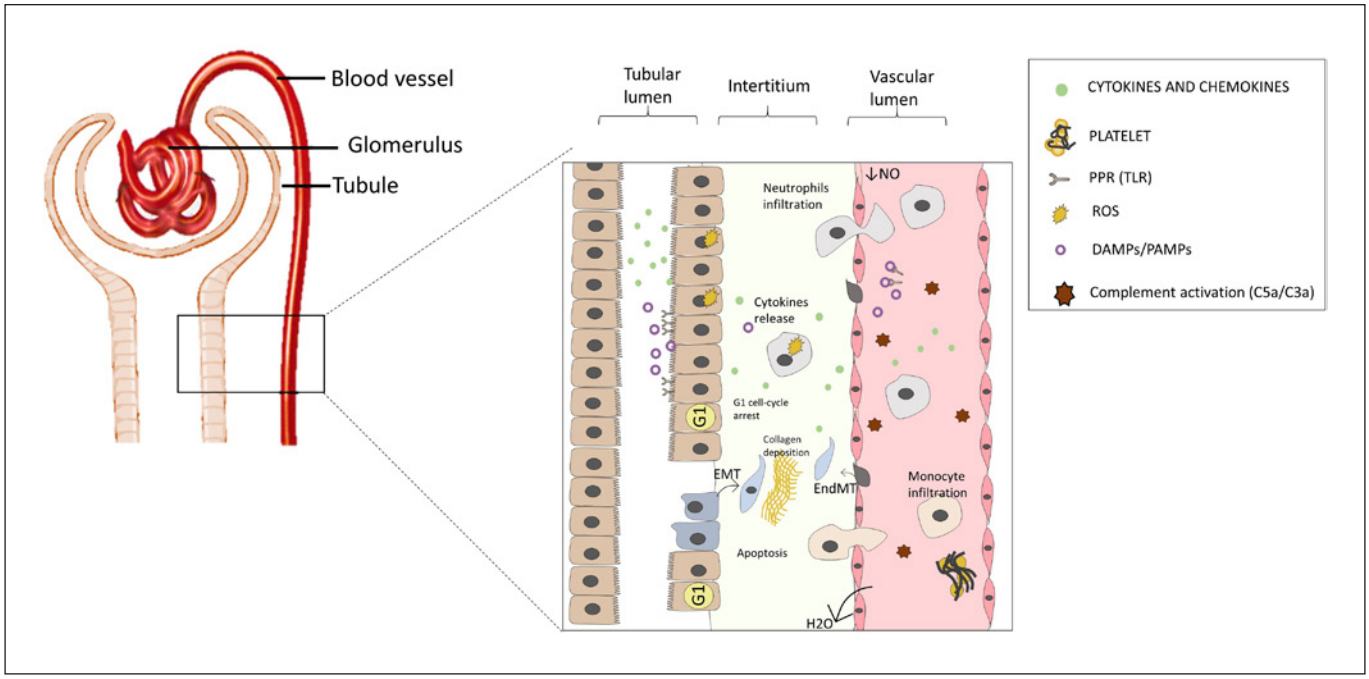

Fig. 1. Pathophysiology of SI-AKI. SI-AKI is recognized by a complex mechanism characterized by the interplay between resident renal cells and immune system. Hyperinflammation is a physiological stimulus triggered by sepsis injury and is characterized by a humoral and a cellular mediator which exacerbate the renal injury. Hyperinflammation leads to neutrophil and macrophage/lymphocyte infiltration followed by organ failure. PAMPs/DAMPs interaction with tubular injured cells via PRRs enhances the inflammatory damage by stimulating the production of cytokines and adhesion molecules. On the other hand, DAMP/PAMP interaction with proximal TECs results in ROS production, apoptosis, and cell cycle arrest.

species (ROS) resulting in exacerbating oxidative stress, mitochondrial injury, and apoptosis [15]. Thus, the pro-inflammatory renal microenvironment milieu induced by sepsis plays a central role in causing tubular dysfunction. In this scenario, renal resident cells actively participate in amplifying this "cytokine storm" and perpetuating the renal damage [16].

The most relevant morphological change observed in the proximal and distal tubule is cellular shedding, characterized by cellular desquamation with increased permeability, resulting in the leakage of glomerular filtrate from the tubular lumen to the interstitium [17, 18]. According to these findings, in a recent study, a swine model of sepsis showed substantial histological changes compared to healthy animals. The observed differences included morphological changes such as vacuolization, epithelial flattening, and necrosis associated with the glomerular capillary rarefaction with monocyte infiltration [19].

Besides the pro-inflammatory microenvironment, the vascular rarefaction after SI-AKI leads to hemodynamic derangement and tissue hypoxia. Regardless of hemodynamic circulation, several clinical studies have observed a wide heterogeneity in blood flow distribution within tissues [20]. The same microcirculatory derangement has been described in different models of SI-AKI [21].

In this regard, we should consider two important pathological processes. First, local hypoxia generated in hyperperfused areas worsens the inflammation and triggers an adaptive metabolic downregulation of the TECs. Of note, hypoxia per se exerts a central role in the pathogenesis of both vascular dysfunction and renal injury [22]. Second, the production of the pro-inflammatory cytokines by endothelial cells increases the expression of adhesion molecules, resulting in leukocyte adhesion, microthrombi formation, and endothelial damage exacerbation. In addition, endothelial decline is associated with nitric oxide (NO) reduction. Consequently, the loss of NO-mediated vasodilation further cooperates to worsen a preexisting hyperperfused environment [23, 24]. Finally, the patchy pattern of NO distribution in renal parenchyma involves a heterogeneous distribution of renal blood flow caused by microcirculatory dysfunction in SI-AKI [18, 25].

Alongside hypoxia, an increasing number of studies suggest that oxidative stress is an important hallmark of sepsis-induced tubular injury, as it seems spatially associated with renal areas of sluggish flow [26]. Interestingly, in vitro experiments revealed increased production of ROS in TECs and podocytes treated with bacteria-derived toxins or plasma from septic patients [27, 28]. Nevertheless, postmortem studies on septic patients have shown the heterogeneous distribution of tubular cellular injury with apical vacuolization but without extensive apoptosis or necrosis [22]. Accordingly, recent experimental studies provide essential insights into the central interplay between SI-AKI and apoptosis. The paucity of TECs apoptosis may be explained by the metabolic adaptations to a harmful renal environment favoring cell survival to the detriment of organ function.

In this scenario, mitochondrial dysfunction together with the oxidative outburst orchestrates the metabolic adaptation of TEC resulting in energy optimization, reprogramming metabolism, and counteraction of proapoptotic triggers [25, 29, 30]. Therefore, inflammation implies an optimization and rearrangement of energy consumption, supporting vital functions. Interestingly, the downregulation of tubular transporters (i.e., ion channel and solute carriers) associated with apoptosis inhibition suggests an adaptive mechanism for survival [31]. Although it is still unclear how metabolic reprogramming occurs, experimental studies in septic AKI indicate that the energy requirement may induce a switch from aerobic glycolysis to anaerobic glycolysis [32].

Hence, in SI-AKI physiopathology, the interplay of inflammation and microvascular dysfunction characterizes and amplifies renal injury. In addition, mitochondrial dysfunction may orchestrate a complete metabolic rearrangement, favoring cell survival processes (such as mitophagy, autophagy, and cell cycle arrest), with significant reduction in kidney function (i.e., tubular absorption and secretion of solutes).

Role of Immune Response and Complement System in SI-AKI

Recent evidence has observed that hyperinflammation in the renal environment after SI-AKI is associated with elevated innate and adaptive immune responses [33]. Additionally, a broad range of mediators such as cytokines and chemokines together with complement system have been identified as pivotal factors in sepsis-related tissue injury. Notably, the complement system has been demonstrated to have a significant function since its activation affects organ damage resulting in poor prognosis for septic patients [34].

In this point of view, Singbartl et al. [33] proposed a bidirectional interplay between the immune system and SI-AKI. Following the early host-microbial interactions, a widespread activation of the innate immune system coordinates a defensive response followed by macrophage/lymphocyte infiltration engaged by pro-inflammatory mediators released by injured cells.

First, both DAMPs and PAMPs participate in the development of hyperinflammation since they activate macrophage via TLR [35]. The binding between DAMPs/PAMPs and TLRs in both immune and non-immune cells triggers the assembly of inflammasome which mediates the maturation and secretion of pro-inflammatory factors such as pro-IL-18 and pro-IL-1β [36]. As previously underlined, a wave of inflammatory cells including monocyte/macrophages and T- and B-cells infiltrate the renal interstitium. Many findings showed the importance of the TLRs in the development of sepsis, as the expression of TLR-2 and TLR-4 in monocytes of sepsis patients was upregulated when compared with healthy individuals [37].

Furthermore, a mechanistic role of lymphocytes in the pathogenesis of SI-AKI has been described. Especially an inflammatory subset of CD4+ T cells, the Th17, stimulates neutrophil infiltration by IL-17 production. Remarkably, hyperactivated CD4+Th17 cells were associated with poor outcomes in patients with septic shock [38]. IL-17 knockout mice exhibit a reduced neutrophil infiltration correlated with a reduced TECs apoptosis in SI-AKI. Additionally, IL-17 expression was associated with renal fibrosis in AKI-to-CKD transition [39].

Besides immune cell activation, cytokines and complement systems mediate a tight crosstalk between inflammatory and renal resident cells [40–42]. Cytokines such as are IL-6, IL-17, IL-8, and TNF-α have a wide range of action in endothelial dysfunction, immune cell activation, and exert an antiapoptotic and profibrotic activity [33].

Together with any other immune mediator, the complement system plays a crucial function in SI-AKI [34]. Complement involves numerous factors which exert a broad number of physiological functions. Mainly, the complement system exerts a first-line defense against bacterial infection and mediates the cross-link between innate and adaptive immunity. Recent clinical and experimental findings suggest that complement activation is associated with MOF and detrimental outcomes during

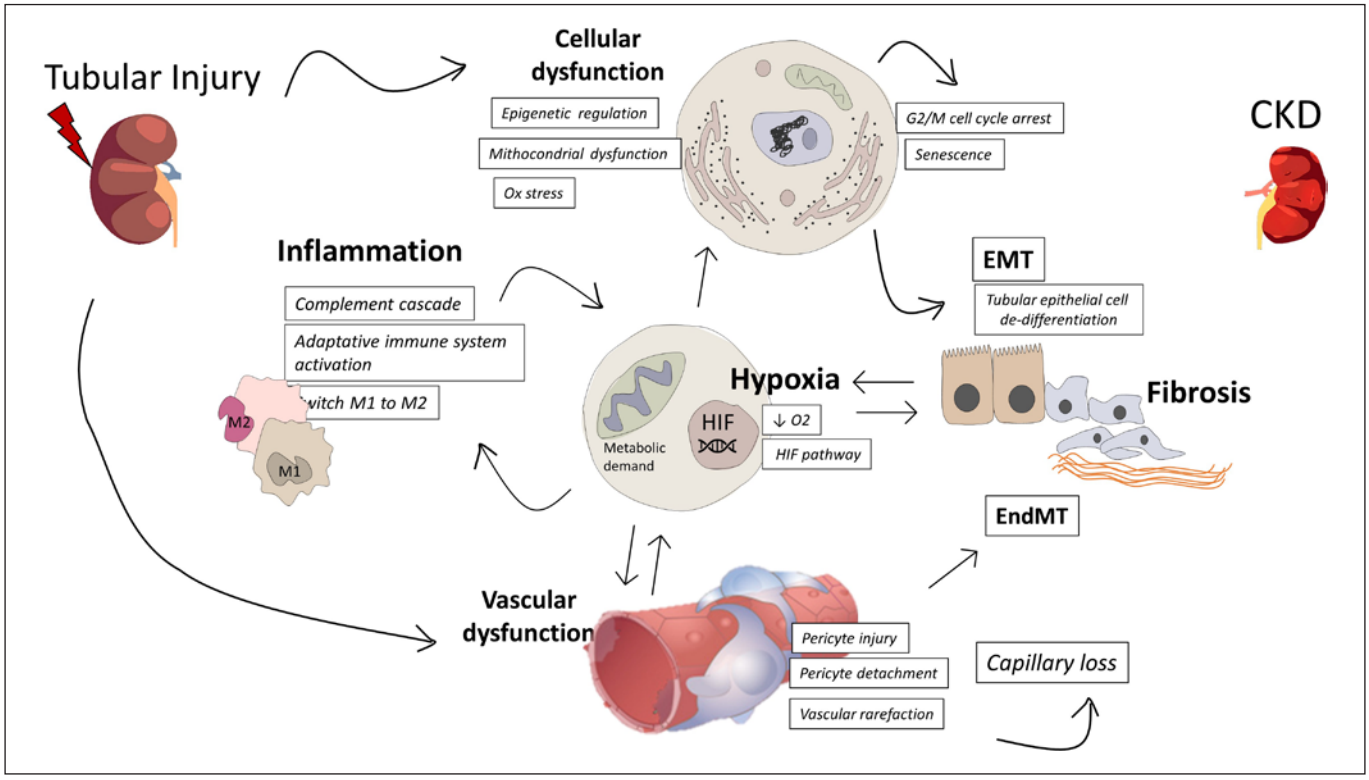

Fig. 2. Pathophysiological mechanism involved in the AKI-to-CKD transition. Several mechanisms contribute to maladaptive repair to AKI leading to CKD progression. Main mechanism includes inflammation, hypoxia, vascular rarefaction, and cellular dysfunction which ultimately lead to kidney fibrosis. HIF, hypoxia-inducible factor; EMT, epithelial/mesenchymal transition; EndMT, endothelial/mesenchymal transition.

septic shock [43]. Additionally, anaphylatoxins C3a and C5a are mainly involved in vascular permeability, kidney fibrosis, and leukocyte extravasation [44, 45]. Similarly, anaphylatoxins C3a triggers a local inflammation and chemotaxis by binding with receptors on peripheral blood mononuclear cells (i.e., C3aR, C5aR1, and C5aR2) [5]. By this observation, severe septic patients showed elevated levels of C5a strongly associated with MOF and reduced survival rates [46].

Notably, a further player recently detected in the complement activation is PTX3 [47]. Following SI-AKI, PTX3 protein stimulates the classical pathway activation via C1q, resulting in the worsening of injury [48]. In our recent study in a swine model of LPS-induced AKI, PTX3 and C5b-9 deposits significantly increased in peritubular and glomerular capillaries after 24 h of LPS infusion [19]. Besides, increased activation of complement pathways was observed. These findings corroborate the essential role of PTX3 in complement activation and severity of sepsis disease. Finally, further evidence indicates a deep association between serum PTX3 levels and injury severity in several inflammatory and cardiovascular diseases [47].

Additionally, a tight association was found between C5b-9 and MOF development in SI-AKI [49]. C5b-9 is believed to play an important role in the pathogenesis of various kidney diseases by causing cellular injury together with tissue fibrosis and inflammation [50]. Animal models of SI-AKI show a significant activation of C5b-9, especially in the tubulointerstitial compartment. Several in vitro experiments have suggested that C5b-9 exerts a profibrotic activity associated with the progression of renal injury. In addition, human glomerular epithelial cells and TECs treated with C5b-9 significantly increased collagen synthesis and cytokine production [51, 52]. Similar results were obtained in endothelial cells [53]. Collectively, these findings support the crucial role of the immune response and complement in the pathophysiology of SI-AKI and suggest a critical role of inflammation in the AKI-to-CKD transition.

AKI-to-CKD Transition

An adaptive response to AKI may lead to the complete recovery of the damage with the total repair of pathological changes [54]. On the other hand, depending on the severity and frequency of the lesion, a maladaptive repair may affect the renal tissue leading to the so-called "AKI-to-CKD transition" which exacerbates the risk of developing CKD and end-stage renal disease. Regardless of the causes of AKI, CKD progression ensue a well-defined pathway leading to the detrimental effect of renal fibrosis and chronic damage [55]. So far, several pathophysiological occurrences and actors have been investigated in the maladaptive response to AKI (Fig. 2).

Tubulointerstitial Fibrosis

Regardless of the different origins of the acute damage, tubulointerstitial fibrosis is one of the main driving forces of AKI-to-CKD progression. Tubulointerstitial fibrosis occurs as a consequence of extracellular matrix (ECM) deposition via mesenchymal cells (fibroblasts or pericytes) or both tubular and endothelial cells by tubular epithelial/mesenchymal transition (EMT) or endothelial/mesenchymal transition, respectively. Of note, EMT arises when TECs reach a mesenchymal phenotype and lose the ability to re-differentiate since they arrest the cell cycle in the G2/M phase [56]. Interestingly, a causal association between cell cycle arrest and fibrosis was supposed since, following AKI, the expression of both epithelial and mesenchymal markers (i.e., N-cadherin, vimentin, and αSMA) in tubular cells promote a partial EMT associated with a senescence-related secretory phenotype [57]. The consequent profibrotic factors production such as TGF-β1, PDGF-β, and CTGF/CCN2 together with stress-induced factors expression (i.e., JNK and MCP-1) enhances an interstitial profibrotic milieu [58]. Other profibrotic growth factors produced by injured TEC are listed in Table 1. Additionally, the production of PAMPs and DAMPs triggers a systemic inflammatory response marked by both humoral (cytokines and chemokines) and cellular (dendritic cells, macrophages, NK, and neutrophils) components boosting the renal inflammation [59]. At once, the Wnt/β-catenin signaling pathway seems to play a pivotal role in this process by supporting both the inflammatory response and ECM deposition [60, 61].

Increasing studies corroborate the role of capillary rarefaction in the development of renal fibrosis and chronic failure after AKI [62]. First, AKI-induced vascular injury leads to long-term implication since renal vascular endothelial cells exhibit a poor regenerative capacity. Besides, experimental evidence in an animal model of endotoxemia-induced AKI suggests that endothelial/mesenchymal transition is one of the most important mechanisms in augmenting capillary rarefaction and chronic interstitial fibrosis [63–65]. In addition, the detachment of the pericytes from the capillaries represents another mechanism involved in AKI-induced CKD. Evidence demonstrated that pericyte migration was followed by pericyte-to-myofibroblast trans-differentiation during renal ischemia/reperfusion injury and endotoxemia, resulting in the loss of endothelial integrity and vascular rarefaction on one side and advancing collagen deposition on the other [59, 66–68]. Hence, the interplay between vascular dysfunction, inflammation, and ECM neogenesis are the three mainly detrimental outcomes causing the AKI-to-CKD breakthrough.

Hypoxia and Mithocondrial Dysfunction

Hypoxia is one of the most pivotal triggers for the maladaptive repair of acute damage. The counteracting player of hypoxia is the hypoxia-inducible factor (HIF) activated during the hypoxic state which upregulates plentiful target of downstream genes controlling hematopoiesis, angiogenesis, and metabolism [69]. Furthermore, plenty of evidence highlighted an HIF-dependent regulation addressed for several pro-fibrogenic genes including Col-I, PAI-1, ET-1, CTGF, MMP-2, and TIMP1 [70]. Additionally, HIF activation can stimulate both the proliferation and recruitment of inflammatory cells to the injury site [69]. Consistent with these findings, the genetic ablation of HIF-1α in TECs improved the development of long-term tubulointerstitial fibrosis and inflammation in a mouse model of AKI [71].

Mitochondrial dysfunction affects cellular function, leading to the loss of kidney function during acute injury. In this scenario, an abnormal mitophagy is associated with a failure of renal recovery after AKI by increasing the susceptibility to extended injury in tubular cells [7]. Several studies on ischemia/reperfusion and sepsis models showed a weakened renal repair due to the persistent disruption of mitochondrial homeostasis resulting in severe tubular damage [72, 73]. Key elements in mitophagy, including the PINK1-PARK2 pathway and BNIP3-mediated mitophagy pathway, seem to exert a protective role in preserving the renal tubular integrity and the normal renal function following injury [74]. Interestingly, the PINK1-PARK2 downregulation in a model of LPS-induced sepsis, correlated with TGF-β1 activation, mitochondrial ROS production, and inhibition of mitophagy [75].

Table 1. Profibrotic growth factors involved in AKI-to-CKD transition

Factors	Repored effect	Study	Authors
NGAL and KIM-1	Profibrotic and pro-inflammatory	In vivo	Ko et al. 2010 [66]
SerpinA3	Profibrotic and pro-inflammatory	In vivo	Navarro et al. 2019 [67]
CSF-1	M2 macrophage activation and polarization	In vivo	Wang et al. 2015 [68]
CTGF	Profibrotic, fibroblast proliferation, and cytokine production	In vivo and in vitro	Geng et al. 2012 [69]
Notch pathway	Profibrotic and pro-inflammatory	In vivo and in vitro	Kobayashi et al. 2008 [70]
WNT/β catenin pathway	Fibroblast to myofibroblast differentiation and regulation of Klotho	In vivo and in vitro	Maarouf et al. 2016 [71] Kuang et al. 2021 [72] Xiao et al. 2016 [73]
VEGF	Macrophage recruitment; VEGF promoter gene hypermethylation at HIF-1α binding site promote fibrosis	In vivo	Leonard et al. 2008 [74]
HIF	HIF-1α promotes fibrogenesis via EMT and profibrotic gene activation	In vivo and in vitro	Rosenberger et al. 2002 [75] Higgins et al. 2007 [76]
YAP	YAP activation promote renal fibrosis via KLF4 and MCP-1	In vivo	Xu et al. 2021 [77] Zheng et al. 2021 [78]
NFAT	Increased expression of NFAT2 contributes to renal fibrosis	In vivo and in vitro	Xie et al. 2021 [79]
SIK1	Profibrotic effect via EMT process	In vivo and in vitro	Hu et al. 2021 [80]
Ang II	Profibrotic, vascular rarefaction, and stimulation of cytokine production	In vitro and in vivo	Chou et al. 2018 [81]
Snail1	Induction of partial EMT	In vivo and in vitro	Grande et al. 2015 [82]

Cellular Senescence

Hypoxia, mitochondrial dysfunction, and epigenetic change affect cell cycle arrest and cellular senescence[76]. Cellular senescence is involved in the detrimental consequences of long-term AKI damage and, consequently, in accelerating the maladaptive repair linking AKI to CKD. One of the well-known hallmarks of renal senescence is the downregulation of the antiaging molecule Klotho since the renal environment plays a central role in its regulation and homeostasis [77]. Cellular and animal models show an increase in downregulation of Klotho expression following AKI, outlining a thigh regulation by a few numbers of factors including HIF. For instance, inflammatory components together with the complement mediators such as C1 and C5a can reduce Klotho expression [60, 78]. Finally, it has been suggested that several triggers, including the Notch signaling pathway, play an effective function in activating others pro-senescent molecules such as p21 [79].

Several epigenetic mechanisms orchestrate structural and functional changes in AKI, leading to extension of the tubular and vascular injury. During an acute lesion, the so-called "hypoxia memory" mediates important epigenetic changes in renal cellular chromatin thanks to histone acetylation and histone methylation processes [80–84]. Several endogenous mediators such as complement factors can affect the histone modification by various mechanisms. For instance, the complement C5a factor induces fibroblast-like phenotype and ECM deposition, via epigenetic modification in TECs [60].

Renal Replacement Therapy in SI-AKI

In course of sepsis, current therapeutic strategies are based on hemodynamic stability, support therapy, and early appropriate antibiotic administration to counteract infection. However, the use of inappropriate antibiotics is associated to overall increased risk of mortality [85] whereas every hour of delay in the administration increases mortality by 8% [86]. Moreover, delayed and/or inappropriate antibiotic administration seems responsible for the developing of multidrug resistance gram-negative sepsis [87]. Indeed, gram-negative sepsis (Enterobacteriaceae, Pseudomonas aeruginosa, and Klebsiella pneumonia) has the highest incidence of multidrug resistance sepsis [88]. The most common drugs associated with AKI are aminoglycosides, vancomycin, radiocontrast media, cisplatin, amphotericin B, foscarnet, and osmotically active agents [89]. Thus, they should be used with caution to avoid renal damage, according to the KDIGO AKI guidelines.

Another important consideration is that there is no specific treatment to prevent or recover renal injury in septic patients, and the support by renal replacement therapy (RRT) becomes necessary when renal function is

compromised [90]. In most critically ill patients, the indication for RRT is unquestionable, and the procedure should be initiated without delay. Indeed, the 2012 KDIGO AKI guidelines [91] suggested early initiating RRT in patients with urgent indications such as severe acidosis, severe hyperkalemia, and acute lung edema.

However, the initiation of RRT might find several obstacles related to unstable hemodynamic phase of septic patients. In addition, delays in RRT could compromise acid-base, electrolyte, and fluid balance, causing more severe complications of AKI. Since 2012, several studies have attempted to provide an answer to this issue. Recent randomized clinical trials have evaluated the optimal timing to start RRT in critically ill AKI patients. A meta-analysis, mostly derived from observational studies, suggested a reduction in 28-day mortality in favor of earlier starts [92]. In contrast, both in the Artificial Kidney Initiation in Kidney Injury (AKIKI) multicenter trial [93] and Dialysis Early versus Late in the Intensive Care Unit (IDEAL-ICU) study [94], the benefit of an early RRT start was not provided. Therefore, these controversial findings contribute to lack of a strong recommendation for the use of early RRT in the KDIGO guidelines. In addition, the Surviving Sepsis Campaign Guidelines contain weak recommendations for the choice of intermittent hemodialysis or continuous RRT (CRRT) [95].

In the late 1970s, CRRT was introduced in the intensive care unit [96] in order to manage critically ill patients needing renal support therapy due to AKI and sepsis [97]. CRRT includes four basic techniques as follows: CVVH, continuous veno-venous hemodialysis, and continuous veno-venous hemodiafiltration. CRRT is being increasingly performed in ICU because it offers certain practical advantages such as the cardiovascular tolerance, the control of electrolyte, and acid-base homeostasis [98]. Bellomo et al. [99] suggested that CRRT should be the therapy of choice for critically ill patients requiring RRT, especially for those with hemodynamic instability. CRRT has been criticized for its lack of specificity because it removes both useful molecules and inflammatory mediators [100]. However, the absence of specificity could be seen as an advantage considering the complexity of the septic process.

High-volume hemofiltration (HVHF) is defined as a CRRT with a convective dose >35 mL/kg per h. The benefits of HVHF were investigated in sepsis-like syndromes such as resuscitated cardiac arrest patients [101] and patients with severe acute pancreatitis [102]. A multicenter study (Hemofiltration Study: High Volume in Intensive Care [IVOIRE]) investigated the 28-, 60-, or 90-day mortality after HVHF (70 mL/kg per h) or standard hemofiltration (35 mL/kg per h) for 96 h. No differences were found in terms of survival rate, renal function, hemodynamics, or organ failure between two treatment modalities. However, the results were underpowered since only 30% of the estimated sampling size was effectively recruited [103]. In accordance, another clinical study that compared the HVHF treatment with two different convective doses (85 mL/kg and 50 mL/kg per h) failed to demonstrate improved survival and renal outcome [104].

Some clinical studies demonstrated that HVHF induced an improvement in hemodynamics and organ perfusion and a decrease in circulating inflammatory cytokines. However, these improvements did not ameliorate survival and clinical outcome [95]. Otherwise, recent clinical trial with septic children and children with septic AKI assessed the efficacy of HVHF (convective dose of 50–100 mL/kg/h) in decreasing the plasma concentration of inflammatory mediators and improving hemodynamics and survival rate [105]. The discordance between these findings is probably due to the use of conventional hemofilters in HVHF [106], such as membranes without adsorbing properties [107]. Combining HVHF and adsorptive membranes may optimize the technique, providing a significant clinical outcome and mortality benefit in septic patients.

Experimental Therapies for SI-AKI: Blood Purification Approaches

The complexity of septic disease characterized by a dysfunctional immune response with a cytokine storm, poor clinical outcomes, and low survival rates led to optimize available extracorporeal blood purification techniques [96, 106]. Systemic immune imbalance is the common denominator between renal failure and sepsis. Indeed, early renal dysfunction has been well documented in experimental septic models with systemic inflammation [19, 63, 67, 68, 108] and also in critically ill patients, according to SOFA scores [8, 109]. Ronco et al. [110] proposed the "cytokine peak hypothesis," affirming that the early removal of both pro- and anti-inflammatory mediators from the bloodstream may effectively prevent the toxic tolerance, reducing local and systemic injuries. In addition, the removal of cytokines might influence the level of local inflammatory mediators, preserving organ function [110–112]. The hypothesis that blood purification improves the outcome of SI-AKI remains to be established.

In the last decades, new adsorptive membranes have been developed to offer both renal support and amelioration of hemodynamic stability. These membranes have the advantage to enhance the clearance of middle-to-high molecular weight mediators. Polymyxin B is a resin membrane with demonstrated capacity to bind endotoxin, decreasing circulating LPS levels in septic patients [113]. Multiple clinical trials have been conducted to determine the efficacy of PMX-HP and have shown conflicting results [114]. A multicenter pilot trial enrolled 36 surgical patients with intra-abdominal sepsis and demonstrated that the treatment with 2-h PMX-HP improved left ventricular function and decreased RRT requirement [114]. Accordingly, another clinical trial, Early Use of Polymyxin B Hemoperfusion in Abdominal Sepsis (EUPHAS), reported significant improvements in terms of hemodynamic stability, renal function, and 28-day survival in patients with severe intra-abdominal sepsis, treated with PMX-HP [115]. A multicenter trial, Safety and Efficacy of Polymyxin B Hemoperfusion for Septic Shock (EUPHRATES) analyzed the effects of 2 PMX-HP sessions versus hemoperfusion plus standard therapy in 450 patients with septic shock. The authors did not find significant improvement in survival rate and renal function [116]; however, a post hoc analysis of the trial focusing on patients with endotoxin activity assay between 0.6 and 0.9 reported a significant improvement in mortality as well as in hemodynamic and respiratory endpoints [117]. Interestingly, Srisawat et al. [118] demonstrated for the first time that PMX-HP improved mHLA-DR expression in severe sepsis patients, providing beneficial effects in immune response.

CytoSorb membrane is used as hemoperfusion cartridge to absorb and remove high cytokines. Several studies showed the capacity of this membrane to remove cytokines, complement mediators, PAMPs, and DAMPs [119–121]. However, these studies did not report improvements in terms of hemodynamics and renal function. CytoSorb has been used to reduce inflammatory response in course of severe pancreatitis or cardio-pulmonary bypass [122]. The first results of the clinical trial, NCT02312024, related to the use of CytoSorb adsorber in real-life critically ill patients, reported significantly decrease of IL-6 levels and no declines in SOFA scores [123].

Oxiris is AN69-based membrane designed specifically for cytokine and LPS removal, through its surface treated with polyethyleneimine and heparin [95]; it is capable of removing inflammatory mediators. Moreover, a randomized double-blind crossover study of septic shock-related acute renal failure showed beneficial hemodynamic effects and reduced levels of LPS and pro-inflammatory mediators compared to standard hemofilter [124]. However, evidence supporting its favorable outcomes on renal function has to be demonstrated.

PMMA Membrane Hemofilter: Recent Advances

Another membrane with adsorptive properties is the PMMA characterized by larger and longer pores and an overall high-specific surface area dedicated, almost exclusively, to trap substances with high molecular weights [95]. PMMA is principally composed of two methylmethacrylate polymer elements that, when mixed together, generated a synthetic polymeric membrane [95]. This filter was developed for dialysis treatment in chronic field; moreover, PMMA showed excellent capacity to remove β2-MG by adsorption, decreasing toxic effects of this pathogenic molecule, involved in dialysis-related amyloidosis [125]. In the last forty years, the potential of this adsorptive membrane has been well recognized. Indeed, several clinical studies revealed significant effects in critically ill patients that cannot be explained as those of RRT alone [95]. In particular, there was a rapid improvement in several clinical symptoms and also clinical parameters such as recovery of urine output and mean arterial pressure [109, 126, 127]. Of course, these results could be related to the capacity of PMMA to remove pathogenic mediators.

Matsuda et al. [126] investigated the effectiveness of PMMA-CHDF treatment in 43 consecutive septic shock patients with AKI, comparing it to a standard hemofilter made of polyacrylonitrile. Following 24 h of treatment, the authors found an increase of urine output and amelioration of hemodynamic stability compared with standard hemofilter. Accordingly, Sakamoto et al. [127] showed a great efficacy of PMMA for removal of several cytokines in septic patients. Then, these clinical findings showed that PMMA membrane hemofilter in CHDF modality reduced systemic inflammation and improved hemodynamic stability and renal function in critically ill patients [8].

Different publications show in vitro data on the effect of PMMA high adsorptive properties in removing IL-6, IL-8, IL1-β, TNF-α, and HMGB-1 [95, 128]. Recently, a new version of PMMA membrane hemofilter for continuous RRT obtained the CE mark and is available for the clinical use in Europe. This membrane, HEMOFEEL CH-1.8 W, is a non-ionic PMMA membrane with an effective surface area of 1.8 m^2, an internal hollow fiber diameter of 240 μm, and a wall thickness of 30 μm. A declared cut-off of 38 kD with an ultrafiltration rate of 30 mL/h/mm

Hg, measured in standard experimental setting condition with a Qb = 200 mL/min.

Considering the principal clinical signs for AKI, such as the significant increase in serum creatinine, decrease in urine output, and the sepsis-induced hypotension, our group recently demonstrated the effectiveness of PMMA to recover renal function and hemodynamic status compared to polysulphone (PS) treatment in a swine model of LPS-induced AKI [19]. Moreover, histological analysis and, in particular, Masson trichrome staining underlined the impact of PMMA in reducing renal damage and early fibrosis with respect to PS [19]. Interestingly, at systemic level, we found that PMMA and not PS reduced LBP, serum complement activation, and significantly reduced circulating sCD40 and sCD40L [19].

LBP is an acute-phase protein, synthesized by hepatocytes that enhances and amplifies cellular response to endotoxin, and it is crucial in the development of early renal fibrosis [63, 67, 129–131]. Interestingly, PMMA significantly removed LBP from blood circulation, suggesting its efficacy in preventing renal fibrosis and the subsequent progression to CKD. In addition, it was able to modulate the sCD40L/sCD40 axis, through the removal of both mediators [19]. Then, the ability of PMMA is to remove those mediators that are present in large number, preserving homeostatic balance, assuring better immune competence in septic patients, and avoiding immunodepression phase and secondary infections.

Several studies reported that the gene expression profiles of circulating leukocytes correlate with renal diseases [132, 133] and might be a potentially useful tool for discovery-oriented studies of the pathogenesis of sepsis and severe infection [134, 135]. Indeed, such studies are based on the assumption that molecular profiling of circulating blood cells might reflect physiological and pathological events occurring in different tissues of the body [135]. Interestingly, we analyzed gene expression profile of circulating leukocytes, and we provided molecular explanation of the PMMA effectiveness by modulation of PBMC transcriptome [19]. In particular, our analysis demonstrated an increased expression of several genes involved in inflammatory response and complement system activation with significant downregulation in PMMA-treated peripheral blood mononuclear cells that was also associated to better renal recovery [19].

In addition, hemadsorption with this new membrane-modulated local and systemic complement activation was contributing to the balance between pro- and anti-inflammatory processes. Therefore, the use of the new PMMA membrane hemofilter might prevent an exacerbated inflammatory response on one hand and the paralysis of cell-mediated immunity on the other, resulting in early recovery of renal function. Considering these findings, we believe that PMMA-CVVH treatment might represent a promising therapeutic strategy to modulate cytokine storm and to assure immune competence with a significant impact on short- and long-term outcomes for patients with systemic inflammatory syndrome.

Conclusion

The early application of blood purification therapies to remove circulating inflammatory mediators and bacterial toxins might improve immune homeostasis, preventing the subsequent molecular mechanisms involved in SI-AKI and CKD progression. Despite initially promising results in preclinical studies, the application of these novel techniques in clinical studies did not provide sustainable survival benefits. Large-scale randomized controlled trials could measure the effectiveness of this intervention in septic-AKI field. Then, the hypothesis that blood purification improves the outcome of septic AKI remains to be established. Therefore, technological advancements in blood purification approaches and well-designed, prospective randomized controlled trials are the way to obtain concrete evidence in terms of clinical outcome.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Alessandra Stasi contributed to conceptualization, design, writing, and editing of the work. Rossana Franzin and Gianvito Caggiano contributed to the conceptualization, design, and drafting of the work; Gianvito Caggiano conceived figure and table; Rosa Losapio, Marco Fiorentino, and Carlo Alfieri contributed to the draft; Loreto Gesualdo and Giovanni Stallone critically revised the manuscript; Giuseppe Castellano contributed substantially to the work reported by critical revisions and draft editing. All authors have read and agreed to the published version of the manuscript.

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