Sepsis-induced acute kidney injury revisited: pathophysiology, prevention and future therapies

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Purpose of review
Acute kidney injury (AKI) is a common complication in critically ill patients and is associated with increased morbidity and mortality. Sepsis is the most common cause of AKI. Considerable evidence now suggests that the pathogenic mechanisms of sepsis-induced AKI are different from those seen in other causes of AKI. This review focuses on the recent advances in this area and discusses possible therapeutic interventions that might derive from these new insights into the pathogenesis of sepsis-induced AKI.

Recent findings
The traditional paradigm that sepsis-induced AKI arises from ischemia has been challenged by recent evidence that total renal blood flow in is not universally impaired during sepsis, and AKI can develop in the presence of normal or even increased renal blood flow. Animal and human studies suggest that adaptive responses of tubular epithelial cells to injurious signals are responsible for renal dysfunction. Simultaneously occurring renal inflammation and microcirculatory dysfunction further amplify these mechanisms.

Summary
An understanding of the pathologic mechanisms of sepsis-induced AKI emphasizes the important role of maladaptive responses to the septic insult. Preventive and therapeutic measures should be based on counteracting these maladaptive responses of tubular epithelial cells, inflammation, and microvascular dysfunction.

Keywords
acute kidney injury, inflammation, microvascular dysfunction, sepsis, tubular epithelial cells

INTRODUCTION

Acute kidney injury (AKI) occurs in 1–35% of hospitalized patients and is associated with high mortality [1]. The incidence of AKI after general surgery has been reported to be about 1%, whereas the incidence among critically ill patients can be as high as 70%, with an in-hospital mortality of 50% when AKI is part of the multiple organ dysfunction syndrome [2,3]. AKI is an independent risk factor for death [4], and patients who survive have an increased risk to develop chronic kidney disease. AKI is a syndrome comprising multiple clinical conditions, and outcomes are influenced by underlying disease. The most common cause of AKI in critically ill patients is sepsis. Despite considerable research during the last decades, the pathophysiology of sepsis-induced AKI remains incompletely understood.

In the not-so-distant past, sepsis-induced AKI was considered a disease of the renal macrocirculation [5] resulting from global renal ischemia, cellular damage, and acute tubular necrosis. However, an increasing body of evidence suggests that AKI can occur in the absence of hypoperfusion [6▪,7]. In a human study, Prowle *et al.* [8] were able to demonstrate that decreased renal blood flow (RBF) was not a universal finding in patients with sepsis-induced AKI. In addition, Murugan *et al.* [9] demonstrated, in a prospective multicenter study of more than 1800 patients with community-acquired pneumonia,

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KEY POINTS

- The heterogeneous distribution of RBF induced by the microcirculatory dysfunction probably causes patchy tubular cell injury during sepsis-induced AKI, whereas hypoxia and hypoperfusion may amplify inflammation and contribute to an adaptive response of tubular epithelial cells.
- Proinflammatory cytokines released during sepsis are filtered in the glomerulus, enter the proximal tubulus and can directly activate tubular epithelial cells resulting in a change of the metabolic and functional state of these cells.
- Recent clinical evidence suggests that G1 cell cycle arrest of tubular epithelial cells is involved in AKI.
- As different mechanisms are involved in sepsis-induced AKI, it is unlikely that a single treatment may be able to prevent or treat sepsis-induced AKI.

that AKI was a common condition, even in patients without severe disease. Higher cytokine levels [e.g., interleukin 6 (IL-6)] were associated with severity and worsening of AKI [9]. Moreover, most of the patients with sepsis-induced AKI were never admitted to the ICU nor suffered from hemodynamic instability [9]. Complementary to the insights from clinical studies, in-vitro experiments have revealed that incubating human epithelial cells with plasma from septic patients resulted in decreased cell function and shortened the survival of tubular cells and podocytes, suggesting that the plasma from septic patients can induce renal cell injury and dysfunction absent any vasculature or circulating immune effector cells. Recent post-mortem studies attempted to more closely describe the pathological changes in septic kidneys [10▪▪,11]. Despite representing the latest stages of the disease, these kidneys were characterized by a strikingly bland histology with focal areas of tubular injury, which was also entirely discordant with the profound functional impairment seen *premortem*. Interestingly, all these changes can occur in the presence of a normal RBF, and define the clinical phenotype characterized by a reduced glomerular filtration rate and tubular dysfunction. Although RBF does not universally decrease during sepsis, some data exist suggesting that the blood pressure can directly influence the perfusion of the kidney and glomerular filtration rate under some pathological conditions [12] and that a higher blood pressure in patients with previous hypertension can prevent AKI during sepsis [13]. These data support the hypothesis that mechanisms other than tissue hypoperfusion are involved in the pathogenesis of sepsis-induced AKI. A consistent finding in septic humans, independent of the severity of AKI, is the presence of the following three pathologic findings: microcirculatory dysfunction, inflammation, and bioenergetic adaptive response to injury. The aim of this review is to discuss the role of these mechanisms in the genesis of sepsis-induced AKI and the potential therapeutic implications.

PATHOPHYSIOLOGY OF SEPSIS-INDUCED ACUTE KIDNEY INJURY

Although the functional consequences during sepsis-induced AKI are dramatic, the histological changes are moderate and do not entirely explain the clinical phenotype. Recent evidence suggests that instead of a single mechanism being responsible for its cause, sepsis is associated with an entire orchestra of cellular mechanisms, adaptive and maladaptive, which potentiate each other and ultimately give rise to clinical AKI. The microcirculation is perhaps the more important physiological compartment in which these mechanisms come together and exert their integrated and deleterious action. These mechanisms include endothelial dysfunction, inflammation, coagulation disturbance, and adaptive cell responses to injury (Fig. 1) [7]. Therefore, we hypothesize that a key event in the early dysfunction of the kidney during sepsis is a bioenergetic stress of the tubular epithelial cells, in response to the amplified inflammatory signal that peritubular microvascular dysfunction generates.

RENAL MICROCIRCULATION DURING SEPSIS-INDUCED ACUTE KIDNEY INJURY

Sepsis causes a profound alteration of the macrocirculation and microcirculation and is characterized by a decreased peripheral vascular resistance, maldistribution of tissue blood flow, and derangement of microcirculatory perfusion. These alterations cause a significant decrease in functional capillary density [14,15] and an increment in the heterogeneity of regional blood flow distribution [16].

During the initial hyperdynamic stage of sepsis, when AKI develops, cardiac output is usually increased. RBF was markedly increased in a sheep model of sepsis [17], and yet AKI developed despite increased RBF [18]. Similarly, post-mortem studies on septic patients have shown the heterogeneous distribution of tubular cellular injury with apical vacuolization, but without extensive apoptosis or necrosis [10▪▪]. Alterations in the microcirculation in the renal cortex or renal medulla can occur despite normal or even increased global RBF [19]. Increased renal vascular resistance may represent an

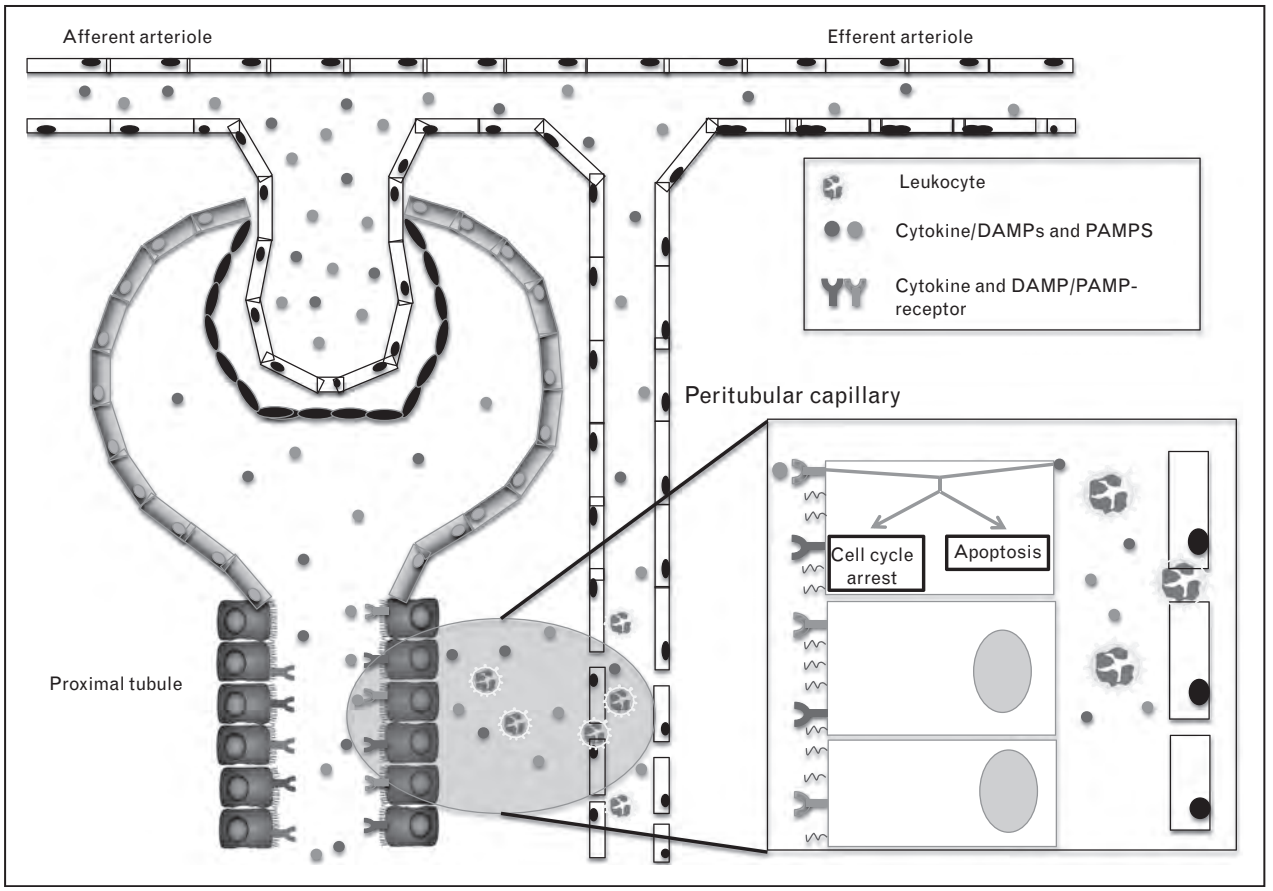

FIGURE 1. During sepsis, DAMPs, PAMPs, and cytokines may potentially injure tubular cells from the tubular and interstitial side. Inflammatory mediators derived from bacteria or immune cells are filtered in the glomerulus, enter the tubular space and can subsquently injure tubular cells by binding to their respective receptors. In addition, cytokines, DAMPs, and PAMPs are released from extravasated leukocytes and can also activate tubular cells from the interstitial side. The activation of cytokine or DAMP/PAMP receptors may induce apoptosis or cell cycle arrest. DAMPs, damage-associated molecular pattern molecules; PAMPs, pathogen-associated molecular patterns.

important hemodynamic factor that is involved in the development of sepsis-induced AKI.

Of course, decreased RBF can cause injury to kidney and when sepsis-induced renal microvascular dysfunction is combined with an increase in intra-abdominal pressure, increased renal vascular resistance results. Measurement of renal vascular resistance using renal Doppler at the bedside has been proposed by Deruddre *et al.* [20] as a tool to titrate norepinephrine in septic shock patients based on the renal arterial resistance to determine the optimal mean arterial pressure. However, whether improvement of regional blood flow, even in this subpopulation of patients, will prevent tubular damage remains to be substantiated.

Platelets, fibrin, stiff red blood cells, and leukocytes together with endothelial cell swelling are responsible for capillary occlusion [21]. Increased vascular permeability is a common feature in sepsis and leads to interstitial edema and fluid retention (Fig. 1) [22,23]. In addition to its association with the severity of sepsis, fluid overload and interstitial edema increase the diffusion distance for oxygen to target cells [24]. Similar findings can be observed in the renal microcirculation [25]. Furthermore, as the kidney is an encapsulated organ, fluid accumulation and tissue edema contribute to the observed deterioration of renal microcirculatory perfusion by altering transmural pressures and by aggravating venous congestion [26▪,27].

Endothelial cells are important determinants of vascular tone, leukocyte recruitment and function, and alter the responsiveness of smooth muscles [28]. Injured endothelial cells produce less vasodilators (e.g., nitric oxide), resulting in a more pronounced response to vasoconstrictors with a redistribution of blood flow. The imbalance between vasoconstrictors, vasodilators, and oxidative stress at the endothelial level is receiving considerable attention as a major contributor to the development of AKI.

Augmented vasoconstriction, small vessel occlusion due to the interaction of leukocytes with activated endothelial cells, and activation of the coagulation system results in local compromise of the microcirculation and regional ischemia [25,29]. The patchy tubular cell injury [10▪▪] probably reflects the heterogeneous distribution of RBF caused by microcirculatory dysfunction.

Nitric oxide plays a pivotal and multifaceted role in the complex pathophysiology of sepsis [30] and sepsis-induced AKI [31]. During sepsis, global nitric oxide production increases, whereas the producing enzyme, inducible nitric oxide synthase (iNOS), has a heterogeneous expression pattern, resulting in different regional concentrations of nitric oxide [30]. The uneven distribution of nitric oxide production may contribute to the heterogeneous perfusion pattern. However, elevated nitric oxide also influences renal hemodynamics and causes peroxynitrite-related tubular injury through the local generation of reactive nitrogen species during sepsis [32]. Evidence suggests that this may play an important role as upregulation of iNOS has been associated with proximal tubular injury during systemic inflammation, and its selective inhibition, with amelioration of the functional impairment caused by cecal ligation and puncture [33]. Therefore, the selective inhibition of renal iNOS might have an implication for the treatment of sepsis-induced AKI.

INFLAMMATION PROPAGATES RENAL DAMAGE DURING SEPSIS

There is a strong association between cytokine levels (IL-6, IL-10, and macrophage migration inhibitory factor) and the development of sepsis-induced AKI [9,34], suggesting an important role of systemic inflammatory mediators in this process. During sepsis, infection triggers a host response, in which inflammatory mechanisms contribute to clearance of infection and tissue recovery on the one hand, and organ injury on the other [35]. Pathogens activate a variety of cells, including renal epithelial and dendritic cells through an interaction with pattern-recognition receptors including toll-like receptors (TLR), C-type lectin receptors, retinoic acid inducible gene 1-like receptors, and nucleotide-binding oligomerization domain-like receptors [36]. The engagement of these receptors results in the upregulation of inflammatory gene transcription and initiation of innate immunity. The same receptors can also detect endogenous molecules released from injured cells, so-called damage-associated molecular patterns, such as DNA, RNA, histones, HMGPB1, and S100 proteins [37].

The cytokine storm during the initial phase of severe sepsis activates leukocytes, endothelial cells, and epithelial cells leading to leukocyte and platelet activation, microvascular dysfunction, hypoxia, and tissue damage [35]. Proinflammatory mediators activate endothelial cells and increase vascular permeability (Fig. 1). Activated endothelial cells upregulate the expression of adhesion molecules and release additional proinflammatory mediators. E-selectin, specifically induced on the endothelium upon inflammatory stimulation, has been demonstrated to play a major role in leukocyte recruitment into the kidney during the late stages of sepsis-induced AKI [38▪]. Experimental data highlight the importance of leukocyte recruitment into the kidney [38▪], especially in later stages of AKI. Although not seen in all models of sepsis-induced AKI [39], elimination of neutrophils or blocking adhesion molecules that are required for neutrophil recruitment into the kidney completely abolished sepsis-induced AKI in a cecal ligation and puncture-induced sepsis model [38▪]. This observation can be explained by the fact that adherent and transmigrated neutrophils release reactive oxygen species (ROS), proteases, elastases, myeloperoxidase, and other enzymes that damage the tissue. These substances, together with leukotriene B_4 and platelet-activating factor, can both increase vascular permeability and upregulate the expression of adhesion molecules that promote further inflammation [6▪,40,41]. Leukocytes leaving peritubular capillaries have a close proximity to tubular epithelial cells and can directly activate tubular epithelial cells by releasing proinflammatory mediators and damage-associated molecular pattern molecules (DAMPs) (Fig. 1). On the basis of the special location of tubular epithelial cells, these cells can also be activated from the tubular side [42]. DAMPs, pathogen-associated molecular patterns (PAMPs), and proinflammatory cytokines are filtered in the glomerulus, enter the proximal tubulus and can directly activate tubular epithelial cells resulting in a change of the metabolic and functional state of these cells (Fig. 1). It has been recently shown that these molecules can bind to and activate tubular cells by binding to TLR2 and TLR4 [43,44,45▪,46]. Although animal studies have linked TLR4 signaling to kidney injury, the relevance of TLR activation in human kidney was unknown until recently. In a very elegant human study, Krüger *et al.* [43] demonstrate that TLR4 is constitutively expressed in kidneys and that tubules in damaged kidneys also stain positively for HMGB1, a known endogenous TLR4 ligand. In-vitro stimulation of human tubular epithelial cells with HMGB1 confirmed that HMGB1 can stimulate proinflammatory responses through TLR4 [43].

The released proinflammatory mediators can act in an autocrine and paracrine fashion and may contribute to further tubular cell damage. In agreement with these findings, kidneys with a TLR4 loss-of-function allele contained less TNF-α, MCP-1, and more heme oxygenase 1 [43]. During sepsis, endotoxin in the tubule binds to TLR4 on S1 proximal tubule cells, which subsequently causes oxidative stress in cells of the neighboring S2 segment [44], suggesting that targeting TLR4 signaling may have value in preventing or treating AKI.

ADAPTIVE RESPONSES OF TUBULAR CELLS TO CHANGES IN THE LOCAL ENVIRONMENT

Tubular cells exposed to inflammation and the consequences of microcirculatory dysfunction act as primary targets and respond by adaptation to the altered tubular environment. They may also spread this signal and shutdown other tubular cells in a paracrine fashion [43]. Microvascular dysfunction occurs in heterogeneous regions of the kidney and, therefore, may explain the heterogeneous histopathologic changes of tubular epithelial cells.

Oxidative stress is a hallmark of sepsis-induced AKI. Post-mortem studies in humans with sepsis-induced AKI show apical epithelial tubular cell vacuolization, which has been linked to oxidative stress [47]. Cultured tubular cells and podocytes treated with components of bacteria or plasma from patients with severe burns and sepsis-associated AKI produce ROS [48] or undergo apoptosis [48,49]. Oxidative stress is also linked to tubular dysfunction [50].

Recent studies demonstrate that apoptosis of tubular cells is rare during sepsis-induced AKI [10▪▪], suggesting that tubular epithelial cells exposed to hypoxia and inflammation limit processes that can result in apoptosis or necrosis (Fig. 1). This can be achieved by an adaptive response of tubular cells characterized by downregulating metabolism and undergoing cell cycle arrest (Fig. 1) [51–53]. This response may be orchestrated by mitochondria and it limits further damage and provides cells with the opportunity to recover function. Swollen and injured mitochondria, which can be found in humans with sepsis-induced AKI, cause a reduced tubular cell function by prioritizing the existing energy to functions that are required for cell survival. Another important feature is mitophagy. This is a process that removes damaged mitochondria through autophagy and can be induced in the kidney by several factors including inflammation and oxidative stress [54]. Decreased mitophagy is associated with a proximal tubular dysfunction, cell and organ dysfunction, and worse outcome in critically ill patients [54]. Furthermore, abnormal mitophagy has also been linked to progressive renal injury [55]. However, mitophagy was significantly upregulated in septic kidneys [10▪▪]. As most of the patients had an already established AKI, this observation let us speculate that increased mitophagy contributes to renal recovery.

Mitochondria are also involved in cell cycle arrest, which is a quality control process of cell division. Recent clinical studies have independently demonstrated that two markers involved in G1 cell cycle arrest, insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2), predict AKI in critically ill patients and in patients undergoing cardiac surgery [56▪,57,58▪▪], suggesting that cell cycle arrest of tubular epithelial cells is involved in AKI. The reduction in ATP production triggers cell cycle arrest [59]. Therefore, a reduced ATP level may induce cell cycle arrest in these cells and prevent the cell from undertaking a process that could end in cell death. Interestingly, these cell cycle arrest markers can also predict renal recovery [56▪,60].

POTENTIAL FOR DIAGNOSTIC AND THERAPEUTIC TARGETS

To date, no therapeutic measures are available to prevent or treat sepsis-induced AKI. A potential reason for this may be that often therapy is started too late in the disease process. The development of new biomarkers, which also provide insights in the pathophysiology of the disease, makes it possible to detect kidneys at risk for injury and thus enable earlier initiation of interventions [56▪,57,58▪▪].

The knowledge that inflammation, microvascular dysfunction, and adaptive responses of tubular cells are involved in the development of sepsis-induced AKI provides new diagnostic and therapeutic avenues. As these mechanisms are closely interlinked with each other, modulating one of these components simultaneously alters other components. The recognition of inflammation has triggered the investigation of therapeutic strategies to dampen inflammation to prevent/treat AKI. As increased levels of proinflammatory mediators (e.g., IL-6) are associated with the development of AKI [34], it is tempting to speculate that eliminating these mediators or endotoxin can prevent sepsis-induced AKI. Indeed, elimination of cytokines and endotoxin is feasible by hemoadsorption [61,62] and experimentally it has been shown that hemoadsorption completely protects against AKI in a cecal ligation and puncture model of sepsis [63]. A clinical study demonstrated that reducing endotoxin by

polymyxin-B hemoperfusion reduced RIFLE scores and urine tubular enzymes [62]. Another option to interfere with cytokines and endotoxin is the application of exogenous alkaline phosphatase. Alkaline phosphatase is an endogenous enzyme that exerts detoxifying effects through dephosphorylation of endotoxins and proinflammatory extracellular ATP and is reduced during systemic inflammation. Heemskerk *et al.* [64] demonstrated that alkaline phosphatase application was associated with a decreased expression of iNOS synthase in proximal tubule cells isolated from urine related to an attenuated urinary excretion of a proximal tubule injury marker. In a small, randomized trial, Pickkers *et al.* [65] showed that the administration of exogenous alkaline phosphatase in septic patients improved endogenous creatinine clearance and reduced the requirement and duration of renal replacement therapy. Modulating TNF-α signaling might be another therapy option because a polymorphism in the promoter region of the *TNFA* gene is associated with markers of kidney disease severity and distant organ dysfunction [66]. However, it is important to keep in mind that these proinflammatory mediators are required for the host response and bacterial clearance during sepsis and that they can later provide necessary signals for the resolution of injury.

Microcirculatory dysfunction during AKI initiates hypoxia and inflammation. To improve the microcirculatory perfusion, vasodilators in the setting of sepsis are currently under investigation including nitroglycerin [14,67], nitric oxide administration, and modulation of nitric oxide production [30,32]. Furthermore, drugs with pleiotropic effects on the vasculature, such as statins [68] and erythropoietin [69], have the potential to prevent kidney injury by enhancing endothelial nitric oxide synthase expression and decreasing vascular permeability. However, on the basis of the different mechanisms involved in sepsis-induced AKI and the interrelationship among these mechanisms, it is unlikely that a single treatment modality may emerge as a magic bullet in the prevention and/or treatment of sepsis-induced AKI.

CONCLUSION

In conclusion, the old paradigm that sepsis-induced AKI is initiated by renal ischemia as a result of macrovascular dysfunction has been called into question because AKI can also develop in the presence of normal or increased RBF. Furthermore, in contrast to renal ischemia reperfusion injury, which is characterized by apoptosis or necrosis of tubular epithelial cells, sepsis-induced AKI is characterized by healthy or reversibly injured renal tubular epithelial cells.

New evidence suggests that the inflammatory response during sepsis causes an adaptive response of the tubular epithelial cells. These alterations induce a downregulation of the cell function in order to minimize energy demand and to ensure cell survival. The result is reduced kidney function. The simultaneous occurrence of renal inflammation and microvascular dysfunction exacerbates the adaptive response of tubular epithelial cells to injurious signals. In addition, the endothelial cell injury is also of importance in the initiation and development of sepsis-induced AKI through the nitric oxide pathway, leukocyte adhesion, ROS, and inflammation. Targeting tubular epithelial cells and components of the microcirculation may be an effective strategy in preventing and/or treating sepsis-induced AKI.

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Conflicts of interest

The authors have no conflicts of interest.

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