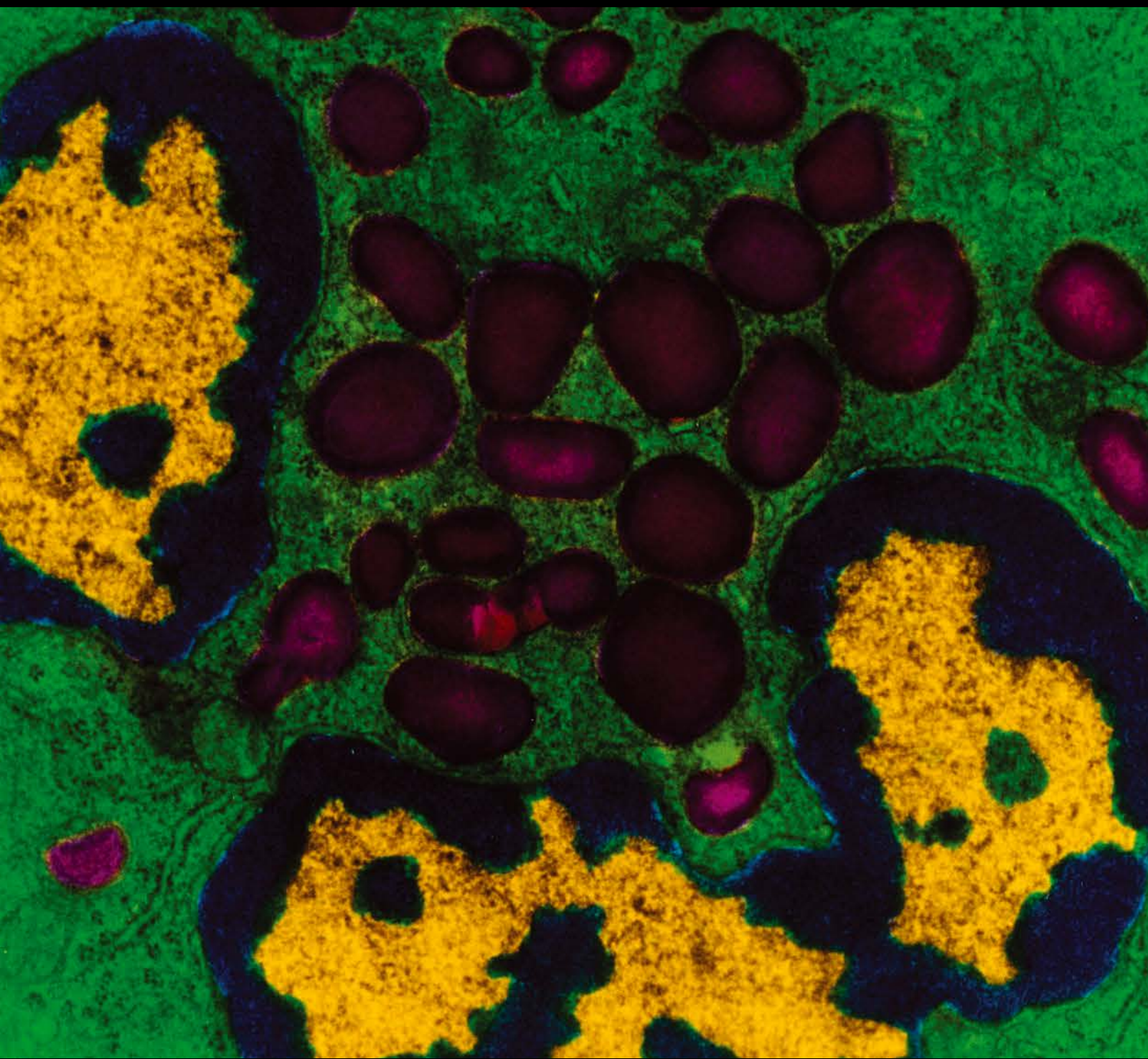
Mediators of Inflammation

Cardiovascular Involvement in Sepsis

Guest Editors: Emanuela Turillazzi, Vittorio Fineschi, Cristian Palmiere, and Consolato Sergi



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Editorial Board

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Editorial

Cardiovascular Involvement in Sepsis

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This special issue wants to contribute to a better understanding of cardiac involvement in sepsis. Sepsis is a complex syndrome that has recently been defined as "life-threatening organ dysfunction due to a dysregulated host response to infection" [1, 2]. It should be considered a major public health problem since it affects millions of people worldwide each year, and it accounts for most deaths in critically ill patients. The presence of myocardial dysfunction in sepsis is associated with higher mortality.

A great attention has been dedicated to improving our knowledge and understanding of the intricate mechanisms underlying sepsis. However, data from the literature suggest the need to implement strategies to reliably measure sepsis morbidity and mortality. In fact, methods based on analyses of insurance claim data using sepsis-specific codes or separate codes for infection and organ dysfunction are unreliable in informing or measuring the effects of policy changes [3, 4], and the postmortem diagnosis of sepsis is often elusive since postmortem investigations lack certain pathognomonic macroscopic and histopathological findings [5, 6]. From a morphological and diagnostic point of view, the term "septic disease" has been created to describe the cardiac involvement in the syndrome. However, this definition, rather than describing a morphological finding, was instead referred to the clinical setting. Although in recent years the concept of septic cardiomyopathy has evolved and it involves pathological alterations of myocardial cells in response to the multiplicity of acting mechanism of damage, the importance of structural changes during sepsis is often overlooked. In patients with sepsis, death is usually the result of a progressive multiorgan dysfunction, overlooking the primary infection through the hyperinflammation. The cardiac involvement as fundamental part of septic multiorgan dysfunction syndrome has been discussed for a long time.

C. Pomara et al. explored the state of current knowledge on the molecular and biohumoral mechanisms of sepsis and correlate them with our ability at postmortem diagnosis. The authors highlight that a complete methodological approach, integrating clinical data by means of autopsy and histological and laboratory findings aiming to identify and demonstrate the host response to infectious insult, is mandatory. Such an approach would be likely to produce an accurate objective surveillance of deaths due to sepsis and improve our knowledge of the clinical-pathological correlation in sepsis, thus contributing to the evaluation of the effectiveness of therapies.

Sepsis is characterized by symptoms and manifestations of organ dysfunction that may lead to fatal outcome. Myocardial dysfunction in sepsis is mediated by a complex interplay among several factors that still remains incompletely understood [7]. Circulatory compromise and microcirculatory alterations may, in part, explain cardiac impairment in sepsis. However, in recent years increasing attention has been paid to the study of other possible pathways of myocardial dysfunction in sepsis. The effects of the host's immune-inflammatory response with particular focus on depressant molecules,

complement molecules, cellular adhesion molecules, and altered intracellular energetic, dysregulated intracellular calcium fluxes have been called upon in the pathophysiology of myocardial depression in sepsis. Oxidative-nitrosative stress may contribute to cardiac dysfunction in sepsis, and mitochondria are one of the major sites for generation of reactive oxygen species and reactive nitrosative species as a detrimental side product of oxidative energy metabolism [8]. M. Neri et al. reviewed the evidence for a role of oxidative-nitrosative stress unbalance and mitochondria dysfunction in myocardial depression in sepsis. NADPH oxidase-derived reactive oxygen species, the role of mitochondria, and finally the involvement of nitric oxide and peroxynitrite as critical factors in mediating myocardial dysfunction in sepsis are discussed.

Measurement of biomarkers is a potential approach to early prediction of the risk of mortality in patients with sepsis. Over the years, a great amount of molecules has been proposed as potential biological markers [9]. However, only 20% of these biomarkers have been assessed specifically in appropriate studies for use in the diagnosis of sepsis [10]. To date an ideal clinical and postmortem marker of sepsis does not exist. Proadrenomedullin and copeptin are peptides cosynthesized together with adrenomedullin and vasopressin in endothelial cells and pituitary gland, respectively. These peptides are increased during sepsis. They have vasoactive, immune modulating, and metabolic properties. It was recently reported that adrenomedullin plays a central role in initiating the hyperdynamic response during the early stages of sepsis and was a useful predictor for development of severe sepsis and septic shock [11, 12]. W. Hu et al. discuss the prognostic value of adrenomedullin and atrial and brain natriuretic peptides in uroseptic patients induced by ureteroscopy. In their research article the authors suggest that the prognostic value of adrenomedullin is superior to atrial and brain natriuretic peptides and that all these molecules are robust independent predictors of in-hospital death in uroseptic patients. They conclude that adrenomedullin and atrial and brain natriuretic peptides may participate in initiating the hyperdynamic response during the early stages of sepsis in uroseptic patients and that these biomarkers could be considered strong predictors of adverse outcome in patients with urosepsis.

Finally, two articles of the special issue discuss the potential link existing between inflammatory status and cardiometabolic disorders. It has been well established that inflammation is also able to affect lipoprotein metabolism. However, the precise mechanisms pathophysiologically linking metabolic dysfunction and inflammation are still under investigation. The biological basis for these associations includes both systemic and local tissue effects.

Metabolic Syndrome (MetS) is a constellation of diseases that include obesity, diabetes, hypertension, dyslipidemia, hypertriglyceridemia, and hypercholesterolemia. These metabolic derangements trigger a persistent inflammatory cascade, whit recruitment of immune cells to the site of injury, and subsequent expression of cytokines and chemokines that amplify the inflammatory response [13]. A common link between inflammation and MetS has been suggested. In cardiometabolic disorders a high serum lipopolysaccharides activity has been demonstrated, thus suggesting a potential role of bacterial infections and immune response in their etiology [14]. One article discussed the serum adiponectin levels and the homeostasis model assessment-insulin resistance (HOMA-IR) that are reportedly associated with MetS. Y.-S. Ding et al. concluded that the adiponectin to HOMA-IR ratio (A/H) may be a better diagnostic marker for MetS than either HOMA-IR or adiponectin alone, and it may serve as an important biomarker to determine an increased risk for MetS in healthy middle-aged population. Y. Zhang et al. investigated the relationship between inflammatory markers and the atherogenic lipoprotein subfractions, and they hypothesized presence of heterogeneity in the relationship of systemic inflammatory markers with atherogenic lipoprotein subfractions, which would aid our understanding of their interplay in the pathogenesis of atherosclerotic disease.

This special issue is dedicated to the cardiovascular involvement in sepsis. The high mortality associated with sepsis makes a thorough knowledge of its underlying mechanisms and its pathophysiological connections with the cardiometabolic disorders important.

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Emanuela Turillazzi
Vittorio Fineschi
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Review Article

Oxidative-Nitrosative Stress and Myocardial Dysfunctions in Sepsis: Evidence from the Literature and Postmortem Observations

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Background. Myocardial depression in sepsis is common, and it is associated with higher mortality. In recent years, the hypothesis that the myocardial dysfunction during sepsis could be mediated by ischemia related to decreased coronary blood flow waned and a complex mechanism was invoked to explain cardiac dysfunction in sepsis. Oxidative stress unbalance is thought to play a critical role in the pathogenesis of cardiac impairment in septic patients. *Aim*. In this paper, we review the current literature regarding the pathophysiology of cardiac dysfunction in sepsis, focusing on the possible role of oxidative-nitrosative stress unbalance and mitochondria dysfunction. We discuss these mechanisms within the broad scenario of cardiac involvement in sepsis. *Conclusions*. Findings from the current literature broaden our understanding of the role of oxidative and nitrosative stress unbalance in the pathophysiology of cardiac dysfunction in sepsis, thus contributing to the establishment of a relationship between these settings and the occurrence of oxidative stress. The complex pathogenesis of septic cardiac failure may explain why, despite the therapeutic strategies, sepsis remains a big clinical challenge for effectively managing the disease to minimize mortality, leading to consideration of the potential therapeutic effects of antioxidant agents.

1. Introduction

Myocardial depression in the setting of sepsis and septic shock is common and has been recognized for a long time [1–5]. The presence of myocardial dysfunction in sepsis is associated with higher mortality. It has been shown that cardiovascular involvement increased mortality from 70% to 90%, compared to 20% in septic patients without myocardial impairment [2, 6]. Thus, cardiac dysfunction in sepsis is thought to have bad prognostic value as it coincides with its severity [6]. Septic cardiomyopathy is characterized by reversible biventricular dilatation, decreased ejection fraction, and impaired response to fluid resuscitation and catecholamine stimulation. However, septic myocardial impairment remains a clinical enigma [7, 8], and even its real incidence is uncertain due to the imprecision with which it is clinically described, the heterogeneity in patient selection in the published studies, and, finally, the lack of universally accepted definition of septic myocardial depression [9]. A reduced left ventricular ejection fraction (LVEF) is often used; on the other hand, septic cardiomyopathy can be defined as a global (systolic and diastolic) but reversible dysfunction of both the left and right sides of the heart [7, 10].

In recent years, the hypothesis that the myocardial dysfunction during sepsis could be mediated by ischemia related to decreased coronary blood flow waned and a complex mechanism was invoked to explain cardiac dysfunction in sepsis [11, 12].

In fact, septic shock is characterized by circulatory compromise, microcirculatory alterations, and mitochondrial damage, which all reduce cellular energy production. In order to reduce the risk of major cell death and a diminished likelihood of recovery, adaptive changes appear to be activated in

sepsis. As a result, cells and organs may survive in a nonfunctioning hibernation-like condition. Sepsis-induced cardiac dysfunction may represent an example of such functional shutdown [13]. Furthermore, the effects of the host's immune-inflammatory response with particular focus on depressant molecules (i.e., TNF-alfa, IL-1), complement molecules, cellular adhesion molecules, and altered intracellular energetic and dysregulated intracellular calcium fluxes have been called upon in the pathophysiology of myocardial depression in sepsis [14–19]. A role of unbalance in oxidative status leading to high production of reactive oxygen species (ROS) has been hypothesized as playing a pivotal role in myocardial depression in sepsis. Nitric oxide (NO), a mediator involved in sepsis, is known to have a strong multifaceted influence on cardiac function since it affects the systemic and cardiac vascular tone and has direct effects on cardiomyocytes [20]. Finally, in the recent years, mitochondrial dysfunction has been considered as a crucial mechanism of heart impairment in sepsis [21]. Conclusively, a very complex pathogenesis involving a combination of hemodynamic, molecular, genetic, and metabolic cardiac alterations underlies cardiac involvement in sepsis.

In this paper, we review the current literature regarding the pathophysiology of cardiac dysfunction in sepsis focusing on the possible role of oxidative-nitrosative stress unbalance and mitochondria dysfunction. We discuss these mechanisms within the broad scenario of cardiac involvement in sepsis, presenting also our related data obtained on *postmortem* cardiac samples of septic patients.

2. NADPH Oxidase-Derived ROS

Oxidative stress (OS) arises as a result of an imbalance between free radical production and antioxidant defense. When antioxidant strategies are overwhelmed, OS results and excessive ROS and reactive nitrosative species (RNS) are produced. ROS can cause oxidation damage to all cellular components, including lipids, proteins, and DNA. The latter is the most detrimental, since replication of damaged DNA can lead to genetic mutations or apoptosis [22–26].

Some of these species interfere with signaling cascades, while others provoke deleterious effects on various biological molecules and structures. It is clear that the increased production of signaling species and strong oxidants act in synergy with collapse in energy metabolism to provoke cell dysfunction, which may result in organ failure and death.

OS in patients with sepsis has been widely described over the last years [27–29], and it is now widely accepted that oxidative stress is central to the etiology of cell and organ dysfunction and tissue damage in sepsis [30–33].

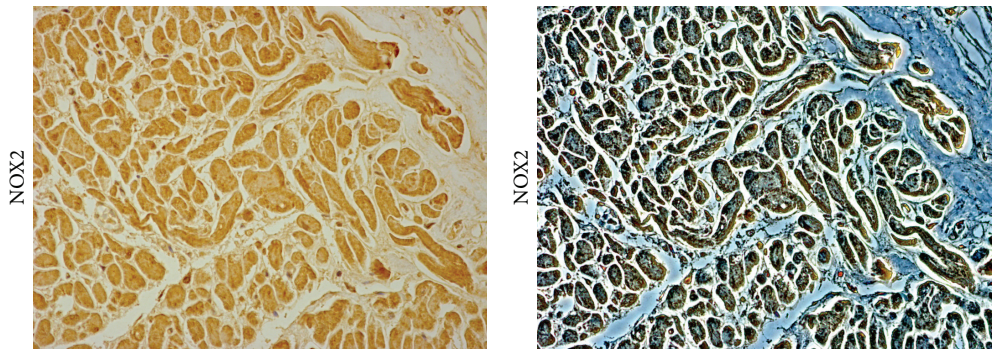
Although several sources of ROS may be involved, a family of the NADPH oxidases appears to be especially important for redox signaling; during sepsis, a major source of ROS is the NADPH oxidases that are present in a variety of cells, especially the professional phagocytes and endothelial cells, and that are central to the genesis of the inflammatory response [34] (Figure 1). ROS production after LPS stimulation in leukocytes is primarily mediated by NADPH oxidase activation [35].

NADPH oxidase is a superoxide-generating enzyme comprising a membrane-bound catalytic subunit (NOX) and several cytosolic regulatory subunits. NOX2 is the catalytic subunit of phagocyte NADPH oxidase [35, 36]. On activation, the cytosolic components translocate to the transmembrane catalytic protein $gp91^{phox}$, which results in the formation of functional NADPH oxidase complex. NADPH oxidase expression has been demonstrated in cardiomyocytes [37, 38]. Conclusively, NADPH oxidase is a pivotal source of ROS that subsequently triggers the release of ROS by other enzymes [39], thus playing a pivotal determinant role of the redox state of the myocardium [37, 38, 40, 41], and it has also been implicated in the TNF-α production induced by LPS [42].

Experimental data derived from animal models showed a strong increase in NADPH oxidase activity and O_2^- in the heart in response to LPS (lipopolysaccharides) [43, 44]. However, the role of this enzyme complex in myocardial septic depression is not completely clarified, partially due to the fact that the membrane subunit of the NADPH oxidase $gp91^{phox}$ (NOX2) has at least 3 other homologs, NOX1, NOX3, and NOX4 [45], which are expressed in a cell- and tissue-specific fashion, are subject to independent activation and regulation, and may subserve distinct functions [46]. Both $gp91^{phox}$ and NOX4 are expressed in cardiomyocytes [47].

NOX2-derived ROS were likely to mediate hyperinflammatory responses and sepsis-induced mortality in mice [48]. In particular, Peng et al. [42] demonstrated that the subunit $gp91^{phox}$ of NADPH oxidase plays a critical role in myocardial depression induced by endotoxemia and that $gp91^{phox}$-containing NADPH oxidase signaling contributes to LPS-stimulated TNF-α expression in cardiomyocytes. Also the role of NOX1/NADPH oxidase in septic myocardial dysfunction has been investigated, and recently Matsuno et al. [49] investigated the involvement of NOX1-derived ROS in endotoxemia-induced cardiac dysfunction. Using an animal model, the authors demonstrated a correlation between the increase of NOX1 mRNA and the production of ROS in cardiac tissue of septic mice and that the increase in cardiomyocytes apoptosis and activation of caspase-3 induced by LPS were attenuated in mice deficient in NOX1. In particular, ROS derived from NADPH oxidase are known to induce cardiomyocytes apoptosis [50], and it has been suggested that NOX1-induced ROS might cause apoptosis by reducing Akt signaling in the heart [49]. Apoptosis may be directly involved in cardiac dysfunction in sepsis as demonstrated in studies on animal models [51], showing that endotoxin may induce a TNF-alpha-dependent apoptotic cascade in the myocardium [52].

Finally, it is noteworthy that complex interactions among different ROS sources exist and that NADPH oxidase-produced ROS may induce ROS production by other sources, thus increasing the total level of ROS [46]. Mitochondrial ROS production can be increased by ROS produced by different sources [53] and it was demonstrated that mitochondrial ROS production may, in turn, stimulate NADPH oxidase ROS production in endothelial cells [54].



(a) (b)

Figure 1: NOX2 expression in the cardiac tissue of a patient died following sepsis. Bright field (a) and contrast phase (b) microscopy image of human cardiac sample, demonstrating a moderate immunopositivity to anti-NOX 2 antibody (Santa Cruz, CA, USA). Personal observation of a 45-year-old septic patient admitted to an emergency unit due to acute abdominal pain and constipation. Abdominal X-ray revealed large amount of fecal content in the colon and dilated small bowel suggestive of bowel obstruction from fecal impaction. The patient was started on broad spectrum antibiotics after pan cultures were obtained and intravenous fluids were administered, and surgical evaluation was requested for possible surgical intervention of bowel obstruction. However, he had a rapid decline in clinical status with worsening hypotension. An echocardiogram revealed severe left ventricular dysfunction (EF 37%) and dilatation of the right ventricle with medium-apical akinesis. Despite a vigorous fluid resuscitation, dopamine, dobutamine, and norepinephrine infusion, the patient died.

3. The Role of Mitochondria

Mitochondria, which occupy 30–50% of the cardiomyocyte cytoplasmic volume, are critical in cardiac energy balance since energy supply for cardiomyocytes is mostly derived from mitochondrial oxidative phosphorylation (OXPHOS). On the other hand, they are a favored site of intracellular damage [55–57]. Mitochondrial dysfunction, reflected in the structure, function, and number of mitochondria within the cardiomyocyte, leads to diminished energy production, loss of myocyte contractility, altered electrical properties, and eventual cardiomyocyte cell death [58].

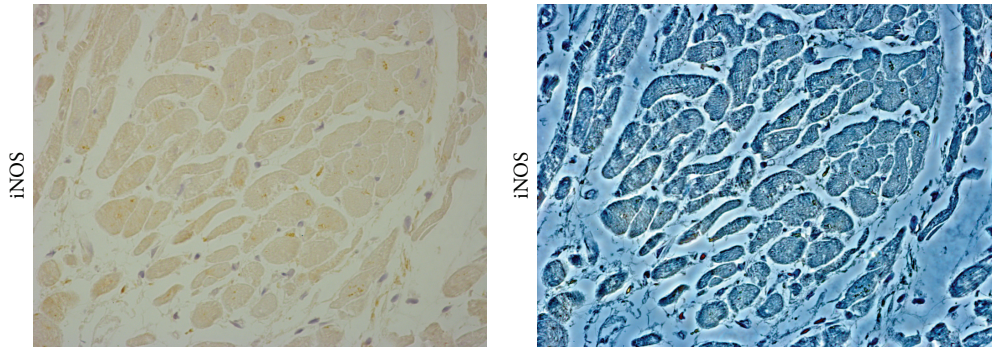
Furthermore, since 1966 when Jensen was among the first investigators to demonstrate that mitochondria produce ROS [59], a solid evidence exists regarding ROS production in mitochondria [60–64]. Mitochondrial dysfunction and its consequence, oxidative stress, have long been considered contributory factors in cardiac tissue damage [65–68].

In sepsis, mitochondrial dysfunction exists [69–72] and mitochondrial damage is thought to play a pivotal role in cardiac dysfunction during sepsis [73, 74]. Several animal models of sepsis have demonstrated cardiac mitochondrial dysfunction during sepsis [75–82]. Evidence exists that mitochondrial dysfunction is a key feature in endotoxemia and the associated multiorgan failure syndrome including heart failure [83]. Soriano et al. [84] studied twenty-five patients presenting with severe sepsis or septic shock and histologically demonstrated on heart sections derangements of mitochondrial cristae in patients who died. Also Takasu et al. found mitochondrial abnormalities in patients who died from sepsis: hydropic change (edema of the mitochondrial matrix), cystic alterations of the cristae, and collapse into small myelin-like clusters were described in septic patients who died in surgical and medical intensive care units [85].

Conclusively, a large body of evidence supports the hypothesis that mitochondrial dysfunction and mitochondria-induced ROS are key factors in cardiac impairment in sepsis [21].

4. Nitric Oxide and Peroxynitrite

NO is a free gaseous radical with function of messenger and effector molecule, synthesized by a family of enzymes (nitric oxide synthase (NOS)) [86]. NO synthesis is activated by one of the three isoforms of NOS that are obligated homodimers that catalyze NADPH-dependent oxidation of l-arginine to NO and l-citrulline: NOS1 (neuronal or nNOS), NOS2 (inducible or iNOS), and NOS3 (endothelial or eNOS) [87]. NO is an important bioactive substance which plays an important role in the regulation of normal body function and disease occurrence, and it is recognized to be a ubiquitous signaling molecule with a multitude of biological actions and targets. Signaling may involve direct reactions between NO and a molecular target or can occur through indirect reactions of secondary ROS [88]. In fact, actions of NO are multifaceted, and its interactions with oxygen or oxygen-related reactive intermediates (e.g., superoxide) yield numerous reactive nitrogen as well as oxygen species. These account for most of the so-called indirect effects attributed to NO through oxidation, nitrosation, and nitrate reactions referred to as oxidative, nitrosative, and nitrative stress, respectively. However, much about NO biological actions remains contradictory, especially with regard to pathophysiologic disturbances in NO signaling. There is an ongoing debate about the levels of NO involved, whether there is a clearly defined threshold at which NO crosses from being beneficial to being destructive. Some authors hypothesized that the biological function of NO depends mostly on concentration and time course of exposure to NO, supposing that cytotoxic events, such as arrest of the cell cycle, cell senescence, or apoptosis, can occur at high NO



(a) (b)

Figure 2: Inducible nitric oxide synthase (iNOS) expression in the cardiac tissue of patient died following sepsis. Bright field (a) and contrast phase (b) microscopy image of cardiac sample, showing a moderate immunopositivity to anti-iNOS antibody (Santa Cruz, CA, USA). Personal observation of a 38-year-old woman with no history of cardiac disease who was referred to an intensive care unit from the obstetrics department 3 days after a cesarean delivery. She was febrile, and laboratory findings included a white blood cell count of 12,800/mm^3. A postpartum sepsis was diagnosed. Echocardiography showed severe LV systolic function and hypokinesia. Coronary angiography on the same day revealed no significant coronary stenoses. Because of the patient's hemodynamic instability, medical treatment that included inotropic agents and antibiotics was started. She did not respond to the treatment and three days later died.

concentrations [89]. However, other authors suggested that the chemical and biological reactivity of NO that has been studied using very high NO concentrations is of doubtful physiological relevance [90].

Vascular bioavailability of NO is a critical factor in regulation of many physiological processes including blood pressure [91], vascular tone [92–95], vascular permeability [96], adhesion and aggregation of platelets [97], and smooth muscle cell proliferation [98]. NO influences on several aspects of cardiomyocytes functioning. The physiological production of NO in the heart maintains coronary vasodilator tone and inhibits platelet aggregation and neutrophil and platelet adhesion, so performing an active role in cardioprotection. It is now determined that NO protects the heart against ischemia-reperfusion injury [99, 100]; however, excessive NO formation is thought to contribute to contractile dysfunction [101, 102].

The inducible NOS (iNOS/NOS2) is generally believed to be the high-capacity NO-producing enzyme in sepsis since endotoxin and cytokines and various mediators are demonstrated to overstimulate the iNOS which is inactive under physiological conditions. Unlike the other two NOS isoforms, iNOS is not constitutively expressed in cells, and its production is elicited by several stimuli like bacterial lipopolysaccharide and cytokines. Primarly identified in macrophages, this enzyme may be expressed in virtually any cell or tissue, such as in the myocardium [103], and once expressed, iNOS is constantly active [87].

Although some studies demonstrated that high levels of NO in sepsis could be beneficial due to a bactericidal effect [104], excessive production of NO is an important player during hypotension and catecholamine-resistant septic shock [105] and contributes to myocardial dysfunction [106–109] (Figure 2).

In the inflammatory condition that is central in endotoxemia, NO and peroxynitrite ($ONOO^-$) have a central role in the development of mitochondrial dysfunction. Several experimental studies performed on animals subjected to endotoxemia demonstrated that NO production, production of $O_2^{\bullet -}$ and H_2O_2, global protein nitration, nitrotyrosine content, protein carbonylation, and lipid peroxidation are increased in cardiac mitochondria [76, 80, 81, 110, 111]. Moreover, the antioxidant systems seem to be inhibited as shown by decreased activity of Mn-superoxide dismutase and glutathione peroxidase and depletion of glutathione [112, 113]. In their elegant experiment, Escames et al. [114] demonstrated that increased oxidative stress, impairment in OXPHOS function, and a decrease in ATP production were restored by genetic deletion of iNOS ($iNOS^{-/-}$ mice). This argument is further supported by the fact that treatment with melatonin, an inhibitor of iNOS, prevented the impairment of mitochondrial homeostasis after sepsis, restored ATP production, and improved survival [114]. Other experimental studies conducted in septic animal models demonstrated an improvement of cardiac function by pharmacological inhibition or genetic deletion of NOS [115].

Finally, the possible role of eNOS in cardiac involvement during sepsis has to be discussed. NOS 3 is expressed in endothelial cells and in cardiac myocytes; NOS3-derived NO physiologically has a positive inotropic and lusitropic effect, thus contributing to optimal cardiac performance and filling [116, 117]. Studies demonstrated that during sepsis an increase in iNOS is induced by endotoxins and cytokines while a decrease in eNOS activity would be likely to happen [118]. Functioning of NOS3 in sepsis is not yet completely clarified and controversial results are reported with studies showing that NOS3 has no proinflammatory or anti-inflammatory effects in sepsis [119]. For example, Yamashita et al. examined the effect of chronic eNOS overexpression and the role of eNOS-derived NO in LPS-induced septic shock using eNOS transgenic mice and demonstrated that chronic eNOS overexpression in the endothelium of mice resulted

in resistance to LPS-induced hypotension, lung injury, and death. These effects are associated with the reduced vascular reactivity to NO and the reduced anti-inflammatory effects of NO [120]. Other studies postulated a proinflammatory role for eNOS and that eNOS-derived NO is critical for maximal iNOS expression in the vasculature [121, 122]. More recently, Bougaki et al. examined the impact of NOS3 deficiency on systemic inflammation and myocardial dysfunction in vivo and in cardiomyocytes isolated from mice subjected to peritonitis-induced polymicrobial sepsis and reported that NOS3 protects against systemic inflammation and myocardial dysfunction during polymicrobial sepsis [119]. The detrimental effects of NOS3 deficiency on myocardial function appeared to be caused by impaired Ca2+ handling of isolated cardiomyocytes obtained from mice subjected to colon ascendens stent peritonitis. Depressed Ca2+ handling of cardiomyocytes of NOS3KO mice were associated with impairment of mitochondrial integrity and marked depression of the ability of mitochondria to produced ATP, a determinant of the function of the SR Ca2+-ATPase pump [119]. Moreover, Ichinose et al. demonstrated that cardiomyocyte-specific overexpression of eNOS prevented cardiac impairment and death after sepsis induction in mice, thus underscoring an important protective role of myocardial NOS3 against endotoxin-induced myocardial dysfunction and death [123]. Finally, a recent study by van de Sandt et al. demonstrated that endothelial NOS plays a key role in the development of sepsis. In their experiment on male $NOS3^{-/-}$ and C57BL/6 wildtype mice rendered septic by cecum ligation and puncture, the authors found that NOS3 promoted a drop in mean arterial blood pressure and systemic vascular resistance, a hyperdinamc state despite impaired left ventricular function, a rapid deterioration of cardiac output, and limited coronary flow reserve, thus leading to short survival times. These findings were not observed in septic $NOS3^{-/-}$ mice which showed that survival times extended more than twofold [110].

Although a two-step oxidation of l-arginine to l-citrulline, with concomitant production of NO, represents the reaction assumed to be catalyzed by NOS, these enzymes are also capable of catalyzing the production of additional products, notably superoxide anion ($O_2^{\bullet-}$), depending on the conditions [124]. During the reaction of molecular oxygen with the amino acid substrate l-arginine to produce l-citrulline and NO, electrons donated by NADPH at the carboxy-terminal reductase domain of NOS are passed to the heme catalytic center of the oxidase domain, where activation of molecular oxygen is "coupled" to NO synthesis by two successive monooxygenations of l-arginine [125]. The cofactor 6R-5,6,7,8-tetrahydrobiopterin (BH4) is required for these reactions; in its absence, electron flow to molecular oxygen becomes "uncoupled" from l-arginine oxidation, resulting in production of O_2^- instead of NO [126, 127]. Furthermore, low concentrations or absence of l-arginine and accumulation of endogenous methylarginines are supposed to cause the uncoupled reduction of molecular oxygen [87]. Both eNOS and iNOS are thought to be involved in the uncoupled reduction of oxygen, leading to the production of $O_2^{\bullet-}$ and H_2O_2 [124]. These two products can react together extremely rapidly to form the potent oxidant peroxynitrite. Superoxide derived from uncoupled NOS or other mechanisms is an important source of cellular oxidative stress, including BH4 oxidation which may occur both directly by superoxide [128] and through the oxidization to BH2 by $ONOO^-$ [129, 130].

Conclusively, NOS uncoupling leads to reduced de novo NO production; sequestration of bioactive NO by superoxide anions via peroxynitrite formation; and peroxynitrite-mediated oxidation of BH4 to BH2, resulting in further propagation of NOS uncoupling [126].

The production of $ONOO^-$ is a crucial pathophysiological event which occurs during sepsis since it represents a critical cytotoxic factor in oxidative stress-mediated tissue damage, supposed to be the NO toxicity mediator [131–134], which, in turn, exhibits multiple inhibitory actions in the mitochondrial respiratory chain [135].

$ONOO^-$ and its derivatives are able to enter the cell membrane and consequently oxidize multiple target molecules, either directly or through the generation of highly reactive radicals, resulting in structural modification and dysfunctions in lipids, proteins, and nucleic acids with significant cytotoxic consequences [136–139]. $ONOO^-$ can also react with carbon dioxide (in equilibrium with physiological levels of bicarbonate anion) leading to the formation of carbonate ($CO_3^{\bullet-}$) and nitrogen dioxide ($\bullet NO_2$) radicals, which are one-electron oxidants. $\bullet NO_2$ can undergo diffusion-controlled radical-radical termination reactions, resulting in nitrated species, such as nitrotyrosine (Figure 3).

Alternatively, it can undergo homolytic fission to generate one-electron oxidants hydroxyl ($\bullet OH$) and $\bullet NO_2$ radicals. The proton-catalyzed decomposition to form $\bullet OH$ and $\bullet NO_2$ radicals may become relevant in hydrophobic phases resulting in the initiation of lipid peroxidation processes [140, 141]. Another important interaction of $ONOO^-$ occurs with nucleic acids, with the production of 8-hydroxydeoxyguanosine [142] or 8-nitroguanine [143] (Figure 4).

$ONOO^-$ can disrupt DNA integrity, impair the activity of ion channel, break down mitochondrial respiratory chain, and induce cell death. It mediates nitration of tyrosine and cysteine residues in proteins which is one of the crucial pathways contributing to its cytotoxicity [144]. The involvement of $ONOO^-$ in sepsis has been demonstrated in different animal models. Several studies suggested that $ONOO^-$ is responsible, at least in part, for the development of endotoxin-induced hypotension, endothelial injury, multiple organ dysfunction, and subsequent death [145, 146]. In their nice experiment in sepsis model mice induced by LPS, Okazaki et al. [147] found that the LPS-treated mice were under oxidative stress and that species, such as superoxide and peroxynitrite, were mainly involved in the oxidative stress. In support of these results, superoxide, nitric oxide, and peroxynitrite cardiac formation has been demonstrated in septic hearts, which has been implicated in the pathogenesis of the myocardial depression and cell death in sepsis [148, 149]. Finally, data from the literature showed that $ONOO^-$ could mimic many of the cardiovascular alterations associated with shock (endothelial dysfunction, vascular hyporeactivity, myocardial impairment, and cellular energetic failure [150]) and that

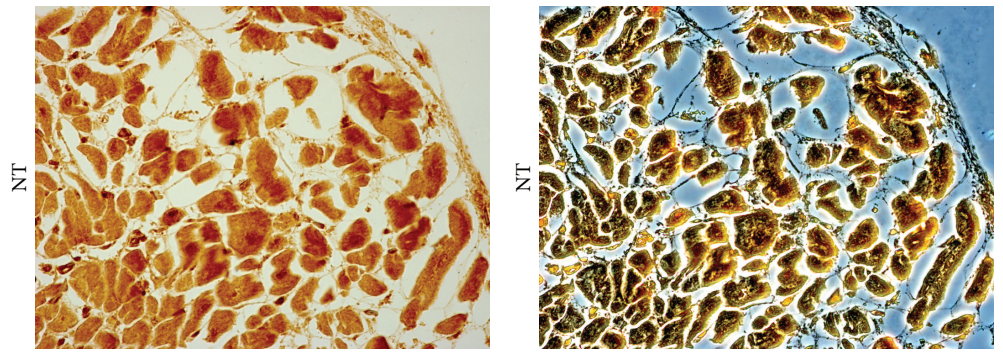


Figure 3: Nitrotyrosine (NT) expression in the cardiac tissue of patient died following sepsis. Bright field (a) and contrast phase (b) microscopy image of cardiac sample of a septic patient, showing a strong immunopositivity to anti-nitrotyrosine antibody (Abcam, Cambridge, UK). Personal observation of a 60-year-old man diagnosed with acute suppurative cholangitis and treated with antibiotic therapy and endoscopic drainage. Jaundice slightly improved; however, on the third day after admission, an echocardiography showed biventricular wall dysfunction. The patient died on third day despite massive fluid and vasopressor support.

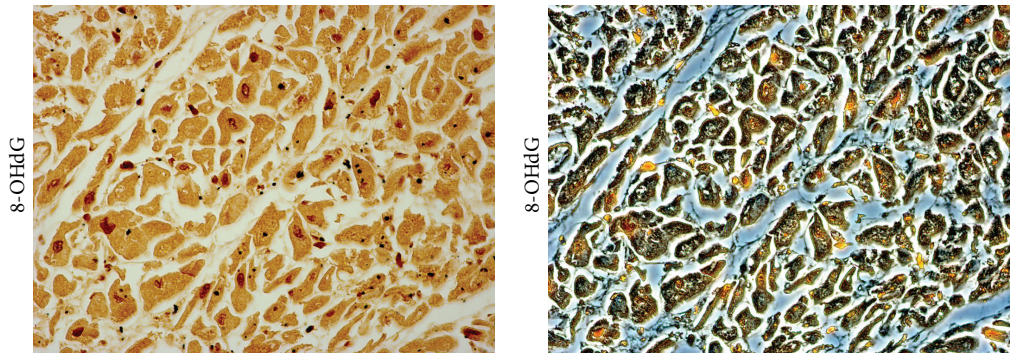


Figure 4: 8-Hydroxy-2′-deoxyguanosine (8-OHdG) expression in the cardiac tissue of patient died following sepsis. Bright field (a) and contrast phase (b) microscopy image of cardiac sample of a septic patient, showing a high positive rate of 8-OHdG expression in myocardial nuclei using an anti-8-hydroxy-2′-deoxyguanosine (8-OHdG) antibody (JaICA, Japan). Personal observation of a 31-year-old woman with severe burn wounds of the right thigh. After two days of antibiotic therapy, her clinical condition worsened with the onset of cardiac failure symptoms. Immediate transthoracic echocardiogram revealed profound diffuse hypokinesis and severely depressed systolic function of the left ventricle with ejection fraction of 21%, right ventricular dilatation, and no valvular abnormalities. Fluid and vasopressor support were started; however, several hours later, LV function started to deteriorate with severe hemodynamic instability and progression to death.

peroxynitrite neutralizers reduced peroxynitrite accumulation and improved myocardial contractile dysfunction and inflammation in septic animal models [136, 151]. Peroxynitrite contributes to the cardiovascular collapse of septic shock, promoting vascular contractile failure and endothelial and myocardial dysfunction, and is also implicated in the occurrence of multiple organ dysfunction in this setting [152–154].

5. Conclusion

Collectively, these findings broaden our understanding of the role of oxidative and nitrosative stress unbalance in the pathophysiology of cardiac dysfunction in sepsis, thus contributing to establishment of a relationship between these settings and the occurrence of oxidative stress. Cardiac impairment is common in severe sepsis and septic shock and it is demonstrated that it strongly contributes to the high rate of mortality in these subjects. Oxidative-nitrosative stress may contribute to cardiac dysfunction in sepsis, and mitochondria are one of the major sites for generation of ROS/RNS as a detrimental side product of oxidative energy metabolism. In turn, damaged mitochondria may increase the production of ROS/RNS.

To improve mortality and morbidity in the septic patient, the most important focus are a prompt and specific management of the infectious finalized to eradicate the causative pathogen as well as supportive therapy to maintain and restore organ function. This strategy is also recognized as the standard therapy for sepsis-induced cardiomyopathy [155]; however, attempts to reduce high mortality rates of patients with sepsis, severe sepsis, and septic shock by manipulating these functional alterations have provided limited success [156]. The complex pathogenesis of septic cardiac failure,

involving a combination of interconnected hemodynamic, structural, metabolic, molecular, and genetic alterations, may explain why despite all these therapeutic strategies, sepsis remains a big clinical challenge for effectively managing the disease to minimize mortality [157]. Numerous basic research and clinical trials have been undertaken to evaluate the possible modulation of the uncontrolled response in sepsis [158]. The knowledge that unbalanced oxidative stress could be critical in the pathophysiology of cardiac impairment in sepsis has naturally led to consideration of the potential therapeutic effects of antioxidant agents [159]. Over the years, treatment of the impaired myocardial energetics rather than cardiac inflammation has been postulated to curb the lethal tools of sepsis with drugs that target the oxidative stress unbalance and the deep mechanisms within mitochondria [21, 160–167].

Competing Interests

The authors declare that they have no competing interests.

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Review Article

A Pathophysiological Insight into Sepsis and Its Correlation with Postmortem Diagnosis

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Background. Sepsis is among the leading causes of death worldwide and is the focus of a great deal of attention from policymakers and caregivers. However, sepsis poses significant challenges from a clinical point of view regarding its early detection and the best organization of sepsis care. Furthermore, we do not yet have reliable tools for measuring the incidence of sepsis. Methods based on analyses of insurance claims are unreliable, and postmortem diagnosis is still challenging since autopsy findings are often nonspecific. *Aim*. The objective of this review is to assess the state of our knowledge of the molecular and biohumoral mechanisms of sepsis and to correlate them with our postmortem diagnosis ability. *Conclusion*. The diagnosis of sepsis-related deaths is an illustrative example of the reciprocal value of autopsy both for clinicians and for pathologists. A complete methodological approach, integrating clinical data by means of autopsy and histological and laboratory findings aiming to identify and demonstrate the host response to infectious insults, is mandatory to illuminate the exact cause of death. This would help clinicians to compare pre- and postmortem findings and to reliably measure the incidence of sepsis.

1. Introduction

Sepsis can be defined as a syndrome of dysregulated inflammation caused by the failure of infection control and containment mechanisms. It should be considered a major public health problem since it affects millions of people worldwide each year, with an incidence which is dramatically increasing [1]. It accounts for most deaths in critically ill patients [2–4]. The hospital mortality of patients with sepsis ranges from 28.3 to 41.1% in North America and Europe [5, 6].

Consequently, considerable attention is dedicated to sepsis by policymakers and caregivers. However, these efforts are tempered by several limitations. First of all, difficulty in defining sepsis still exists due to the emerging biological insights and reported variation in epidemiology. Recently the Third International Consensus Definitions Task Force defined sepsis as "life-threatening organ dysfunction due to a dysregulated host response to infection" [7, 8]; however the performance of clinical criteria for this sepsis definition is unknown [7]. Furthermore, sepsis poses heavy challenges from a clinical point of view regarding its early detection and the best organization of sepsis care [6, 9]. Finally, we do not yet have reliable tools for measuring the incidence of sepsis. Methods based on analyses of insurance claim data using sepsis-specific codes or separate codes for infection and organ dysfunction are unreliable in informing or measuring the effects of policy changes [1], and the postmortem diagnosis of sepsis is still challenging as the results of postmortem investigations often show a relative paucity of significant macroscopic and histopathological findings [10, 11]. Apart from the possibility of demonstrating infected sites in the internal organs (i.e., septicopyaemic abscess), the inflammatory organ changes observed at autopsy are mediated by the endogenous inflammatory mediators that are neither specific nor sensitive with regard to sepsis [11]. These can also be demonstrated in different clinical conditions going along with systemic inflammatory response syndrome (SIRS) or prolonged ischemia [11]. Furthermore, the presence of a pathogen in the blood or tissues does not necessarily indicate that the complex syndrome of sepsis has occurred [12]. These issues are further demonstrated by the discrepancies existing between clinical and postmortem diagnosis of sepsis [13–15].

Despite the fact that the latter is still a diagnosis of exclusion, sepsis is an intriguing field of interest in autopsy practice, and the diagnosis of sepsis-related deaths is an illustrative example of the reciprocal value of autopsy both for clinicians and for pathologists. Upon studying these deaths, the pathologist needs to approach the complex pathophysiological mechanisms underlying sepsis. This may lead in turn to an increased understanding of the pathogenic hypotheses and to the accuracy of existing diagnostic tools to be checked [16]. Autopsies should still serve as a very important part of quality control in clinical diagnosis and treatment of sepsis [17, 18]. Last but not least, the issue of the diagnostic reliability of fatal sepsis is even more pivotal. One important area which has seen a rising number of clinical negligence claims comprises healthcare-associated infections and sepsis [19].

The objective of this review is to assess the state of our knowledge of the molecular and biohumoral mechanisms of sepsis and to correlate them with our postmortem diagnosis ability.

2. The Inflammatory Response

The septic response is an extremely complex chain of events involving inflammatory and anti-inflammatory processes, humoral and cellular reactions, and circulatory changes. When an infectious insult occurs, it initiates a series of events, resulting in the release of inflammation mediators which involves both a local reaction and a systemic response and which, finally, could impact on organs function [20].

Sepsis triggers the production of a diverse array of cytokines that are proinflammatory and anti-inflammatory [21, 22]. Cytokines are low molecular weight compounds which are considered potent positive and negative regulators of inflammation. Some cytokines possess proinflammatory effects such as tumour necrosis factor- (TNF-) alpha, interleukin- (IL-) 1, and interleukin-8, while others have anti-inflammatory effects including IL-10 and IL-1 receptor antagonist, and some are supposed to act as pro- and anti-inflammatory IL-6 as an example [23].

Proinflammatory cytokines trigger a beneficial response, such as increased local coagulation, limited tissue damage, and elimination of the pathogen. Overwhelming production of these proinflammatory cytokines, however, can be very dangerous in that excessive cytokines destroy the normal regulation of the immune response and induce pathological inflammatory disorders, such as capillary leakage, tissue injury, and organ failure. Similarly, the anti-inflammatory cytokines play a critical role in regulating overall immune response, in establishing homeostasis, and in checking the effects of the proinflammatory ones in order to solve inflammation and heal tissue. Therefore, their dysregulation can also trigger pathogenesis [24], since it has been shown that an intense anti-inflammatory response may induce a state of immunosuppression in patients with sepsis [25]. When a state of anti-inflammatory predominance occurs, monocytes are deactivated. This results in reduced antigen presentation and decreased production of proinflammatory cytokines, leading to pathogen persistence and further fuelling the inflammatory process [21, 22, 25]. A tightly regulated balance in the cytokine network, which comprises pro- and anti-inflammatory cytokines, is crucial for eliminating invading pathogens on the one hand and restricting excessive, tissue-damaging inflammation on the other [26].

In brief, when an infectious insult occurs, pattern recognition receptors (PRRs), which are expressed on epithelial barriers as well as on resident immune cells such as dendritic cells and macrophages, detect invading microorganisms. A specific family of PRRs named toll-like receptors (TLRs) recognizes conserved macromolecular motifs from microorganisms, called pathogen-associated molecular patterns (PAMPs). The stimulation of TLRs or the nucleotide oligomerization domain NOD-like receptor (NLR) family of intracellular PRRs results in the triggering of downstream signalling cascades. Depending on the particular receptor engaged, this process leads to the activation of a transcriptional response programme that includes nuclear factor κB (NF-κB), followed by the production and secretion of cytokines, chemokines, and nitric oxide [21, 22]. Cytokines usually bind their specific receptors, induce signalling pathways, and thus regulate immune responses and other cell functions [24].

Therefore, different types of cells, tissues/organs, or protein/other molecules may function as effectors, modulating the immune response through various pro- or anti-inflammatory mediators. Resident macrophages and polymorphonuclear cells (PMCs) initiate the primary host response to the invading microorganisms. They are responsible for the primary phagocytosis and subsequent activation and recruitment of granulocytes and monocytes. Leukostasis of neutrophils (the so-called leucocyte sticking) in the liver sinusoids, in the pulmonary vessels, and so forth is the common histological counterpart of this phenomenon [11]. Proliferation of astrocytes and microglial cells is a common postmortem histological finding in septic patients. However, it is highly nonspecific as it may reflect several kinds of insults, including ischemia [11].

Endothelial cells (ECs) activation occurs during sepsis. The interaction between ECs and leukocytes, a hallmark of the inflammatory process, comprises adhesive and migratory molecular events including low-affinity transient and reversible rolling adhesions, integrin-dependent firm adhesive interactions, and migratory events of the leukocytes through the endothelium and, finally, in the interstitial space [27, 28]. A variety of chemical mediators secreted from inflamed tissue and the entire process of leukocyte-endothelial cell adhesion are regulated by the sequential activation of different families of adhesion molecules that are expressed on the surface of leukocytes and ECs. Lectin-like adhesion glycoproteins, selectins, mediate leukocyte rolling. The firm adhesion and subsequent transendothelial migration of leukocytes are mediated by the interaction of integrins expressed by leukocytes with immunoglobulin-like adhesion molecules on ECs, for example, intercellular cell adhesion molecules (ICAM) 1–5, vascular cell adhesion molecule-1 (VCAM-1), and the junctional adhesion molecules (JAMs), which are expressed on endothelial and other cells [29, 30]. Recently, EC-active molecules (i.e., the angiopoietin pathway, Ang-2, soluble fms-like tyrosine kinase-1) have been

proposed to correlate significantly with organ dysfunction and mortality in patients with sepsis [31, 32].

Sepsis is also associated with robust activation of the complement system, as demonstrated by the presence of complement activation products (C3a, C5a, and C5b-9) in the plasma [33]. C3a and C5a anaphylatoxins are small cleavage products of C3 and C5 and possess proinflammatory activities. C5a especially reacts with its receptors on phagocytes (neutrophils, macrophages) and on a variety of organs to trigger numerous biological effects. These include increasing vascular permeability and inducing smooth muscle contraction, inducing chemotaxis of PMCs, monocytes, and other cell types [34, 35]. In experimental studies on animal models (rats or mice with cecal ligation and puncture, CLP), both rabbit polyclonal neutralizing antibodies and mouse monoclonal antibodies that neutralized C5a were highly effective in attenuating the parameters of sepsis (clinical symptoms, evidence of multiorgan failure, MOF, consumptive coagulopathy, innate immune functions, apoptosis, etc.), resulting in greatly improved survival [36, 37]. The effects of C5a contribute to immunoparalysis, MOF, the apoptosis of thymocytes and adrenal medullary cells, and imbalances in the coagulation system [38]. In addition, C5a is involved in the development of septic cardiomyopathy and severe left ventricular dysfunction [39]. At autopsy, the left ventricle is often dilated and the ventricular walls have a flaccid appearance [11].

Other products derived from activation of the complement system play an important role in sepsis, such as C3b (from C3), which is a key opsonisation factor that reacts with phagocytes receptors to favour internalization of bacteria and their subsequent elimination. The membrane attack complex (C5b-9) causes lysis of Gram-negative bacteria [38].

Finally, sepsis also affects other biological systems, such as the coagulation system and the autonomic nervous system [39]. In the clinical setting of sepsis, dysregulation of the coagulation cascade results in major complications. The extent of activation of the coagulation cascade during sepsis can range from an insignificant level to the occurrence of disseminated intravascular coagulation (DIC). When DIC occurs, at autopsy various degree of haemorrhage can be observed on the skin, on mucocutaneous surfaces and serous membranes, and, finally, in parenchymal organs [10, 11]. Increasing evidence points to an extensive cross talk between inflammation and coagulation, in which the protease activated cell receptors play an important role (Figure 1).

3. The Concept of "Compartmentalization" of the Inflammatory Response in Sepsis

A fundamental step in the comprehension of sepsis pathophysiology is to appreciate that the inflammatory response varies from one compartment to another, and not all compartments behave similarly [40]. Chinnaiyan et al. supported this concept by studying gene expression in different tissues in CLP model of sepsis in rats. They showed that the sepsis response elicited gene expression profiles that were either organ specific, common to more than one organ, or distinctly opposite in some organs [41].

Each organ has a distinctive molecular response to systemic inflammation. Several experimental pieces of data confirm this observation. For example, neutrophil sequestration in lung and liver resulted differently regulated by chemokines in a murine experimental model of peritonitis [42]. After injection of LPS (lipopolysaccharide) in a mice model, NF-κB activation in liver was mediated through TNF and IL-1 receptor-dependent pathways, but, in the lung, LPS induced NF-κB activation was largely independent of these receptors [43]. Although data are still missing in humans, mouse alveolar macrophages do not produce IL-10 [44], do not express TLR9, and are thus insensitive to bacterial DNA [45] and fail to produce IFN-β in response to TLR4 and TLR3 agonists [46], thus demonstrating that, in this animal model, alveolar macrophages behave differently to other types of macrophages.

Through the bloodstream a strict cross talk exists between the different organs and compartments. Both pro- and anti-inflammatory mediators are present concomitantly in the bloodstream, evoking different responses in the various organs. The latter, in turn, respond through the local production of different mediators and the activation of different cellular types [40]. Most tissues contribute to the release of inflammatory mediators and there is local activation of intracellular signalling pathways [40].

This concept is of paramount clinical importance as the various organs may respond differently to therapeutic strategies. On the other hand, upon approaching sepsis-related deaths, pathologists must keep in mind that tissue injury can be initiated remotely from an insult in a faraway site and that all organs and compartments may be involved (Figure 2).

Different organs and systems are interconnected via humoral and biochemical interactions and are clustered into functional modules sharing many common pathophysiological mechanisms. Diagnostic postmortem strategies based on the measurement of compartmentalized mediators may prove useful as a diagnostic strategy [47, 48].

4. Microcirculation and Microvesicles

Sepsis is a disease of microcirculation [49]. Nuclear vacuolization, cytoplasmic swelling and protrusion, cytoplasmic fragmentation, and various degrees of endothelial detachment from its basement membrane have been demonstrated during sepsis [50, 51]. Endothelial physical disruption leads to an extravascular leak of protein-rich oedema and polymorphonuclear cells (PMNs) influx into organs. Furthermore, endothelial damage may induce leukocyte and platelet aggregation, as well as aggravation of coagulopathy, thus favouring impaired perfusion, tissue hypoxia, and subsequent organ dysfunction [51, 52].

Deleterious effects on the vascular function are mediated by increased synthesis of inflammatory cytokines and chemokines and increased expression of endothelial adhesion molecules [49–51]. Microvascular endothelial cells (MVECs) that are critical modulators of blood flow and microvascular function are principal targets of the systemic inflammation of sepsis [52].

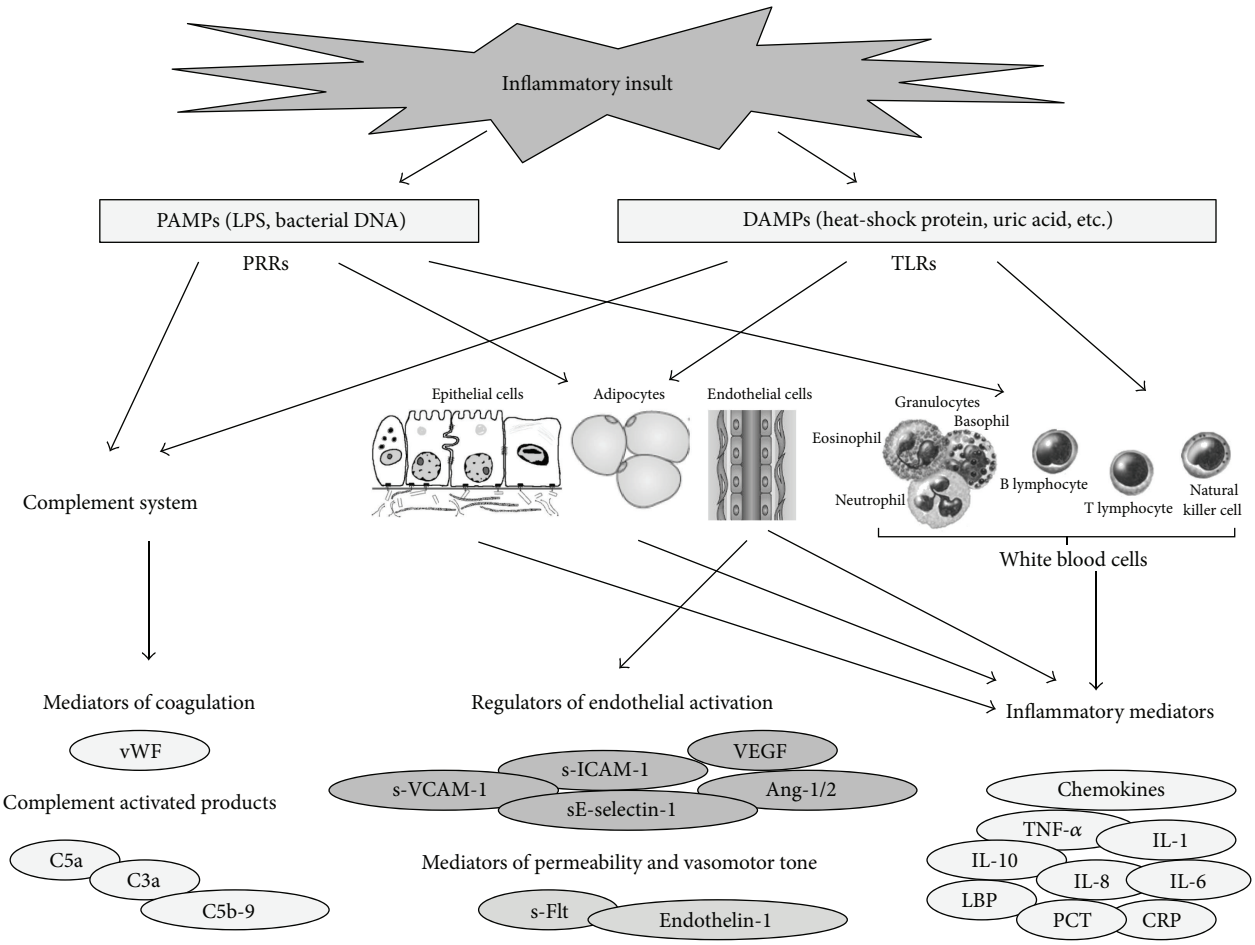

Figure 1: Schematic of events associated with major mediators of cytokine cascade on initiation of sepsis. The release of a large amount of pathogen and damage associated molecular patterns (PAMPs and DAMPs) from invading microorganisms and/or damaged host tissue, respectively, results in the overstimulation of pattern-recognition receptors (PRRs) of immune cells. This process activates an inflammatory cascade in which large amounts of cytokines are released into the body. Macrophages and endothelial cells are then hyperactivated by the unusually large quantity of circulating cytokines. The activation of macrophages and endothelial cells results in the release of more cytokines, exacerbating the inflammatory response. The profound proinflammatory response is counteracted by certain anti-inflammatory cytokines, including IL-10, transforming growth factor-β (TGF-β), and IL-4, which attempt to restore immunological equilibrium.

Microvasculature dysfunction in sepsis is almost ubiquitous. Pulmonary MVECs injury and barrier dysfunction result in the leak of protein-rich fluid and circulating neutrophils into the pulmonary interstitium and alveolar spaces [52–54]. Recently it has been demonstrated that septic pulmonary microvascular barrier dysfunction is associated with significant pulmonary MVECs death, which is largely apoptotic [52]. Experimental observations provide proof that septic acute kidney injury (AKI) can occur in the setting of renal hyperaemia and that ischemia is not necessarily present. Nonhemodynamic mechanisms of cell injury are likely to be at work, due to a combination of immunologic, toxic, and inflammatory factors that may affect the microvasculature and the tubular cells [55–58]. There is evidence that adhesion molecule activation, both on the renal endothelium and on epithelial cells, leads to enhanced leukocyte adhesion, followed by the influx of activated leukocytes into the renal interstitium [57]. Finally, renal mitochondrial dysfunction has been demonstrated in CLP murine model of sepsis leading to a decrease in renal *complexes I* and *II/III* respiration, MnSOD (manganese superoxide dismutase) activity, and ATP levels. This was associated with increased mitochondrial superoxide levels, impaired renal microcirculation, and impaired renal function [59]. Oxidant generation by the renal tubules and renal microvascular failure are early events, which lead to AKI [60–62]. Cerebral microcirculatory dysfunction has been demonstrated in various experimental models of sepsis [63], and it is thought to be one of the main pathophysiological mechanisms leading to brain damage also in humans [64]. Both endotoxin, or more accurately termed bacterial lipopolysaccharide (LPS), and proinflammatory cytokines induce the expression of CD40, VCAM-1 or ICAM-1, and E-selectin on human brain microvessel endothelial cells [65–69].

Finally, in recent years a growing body of evidence has been established regarding the role of microvesicles (MVs)

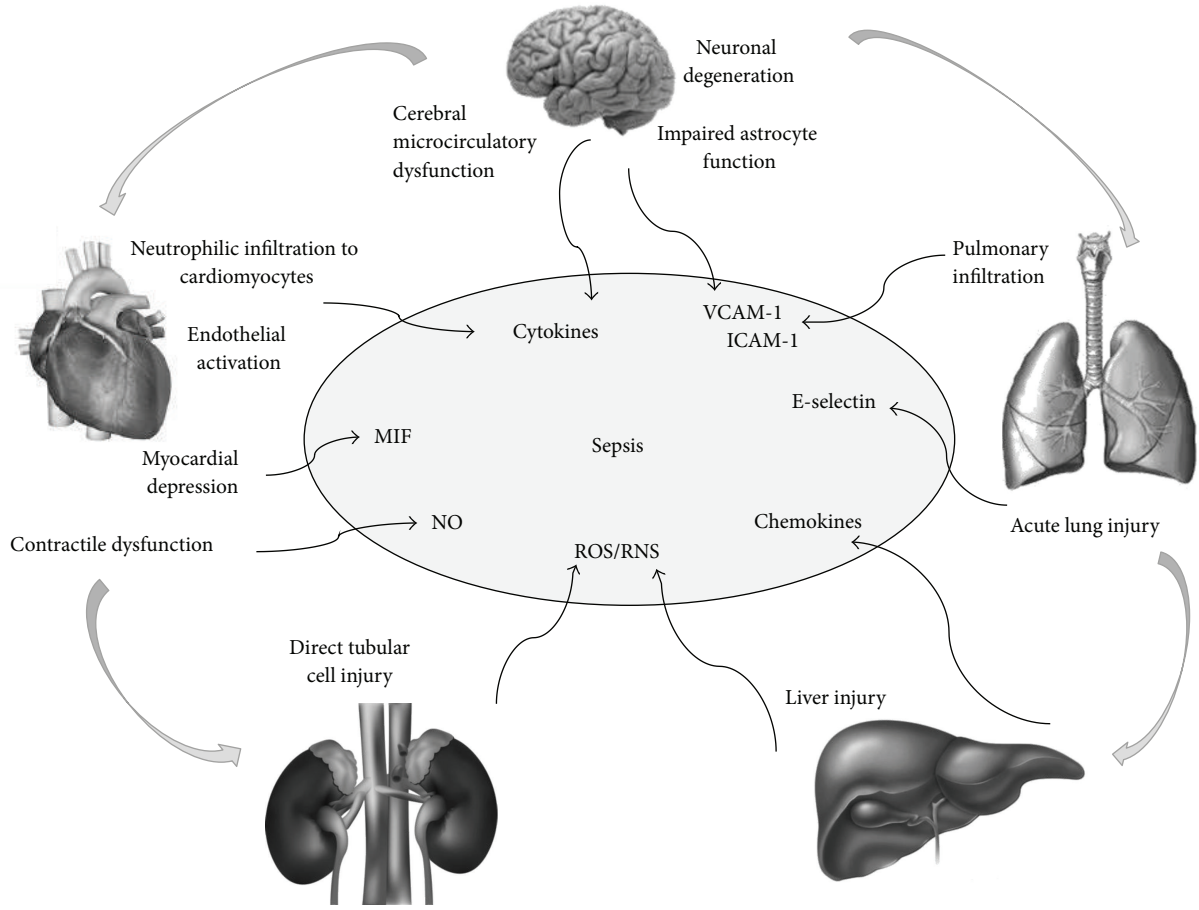

Figure 2: A schematic representing the involvement of different organs in sepsis. During sepsis large amounts of inflammatory mediators are found within the bloodstream. They can act on different organs and induce tissue injury that in turn will favour further production of inflammatory mediators. Cross talk between the different organs and tissues is further mediated by the local delivery of mediators that can amplify or limit the inflammatory response (MIF, macrophage migration inhibitory factor; NO, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; VCAM-1, vascular adhesion molecule-1; and ICAM-1, intercellular adhesion molecule-1).

in sepsis [70]. These extracellular vesicles are released in the extracellular environment through a membrane reorganization and blebbing process following cell activation or apoptosis. They constitute a storage pool of bioactive effectors with varied cellular origins and are able to act as intercellular messengers or effectors through multiple amplification and regulatory loops affecting vascular cells functions [71]. Thus, MVs contribute to the spread of inflammatory and prothrombotic vascular status. They may also affect smooth muscle tissue through adhesion molecules, activation of NF-κB, and the expression of inducible nitric oxide synthase and cyclooxygenase-2, with an increase in nitric oxide and vasodilator prostanoids, leading to arterial hyporeactivity [72–74].

In recent years, the analysis of circulating-cell-derived MVs has become more defined and clinically more useful, and several groups suggest that it may enter mainstream clinical testing [75, 76]. Endothelial-derived microvesicles are considered relevant biomarkers of septic shock-induced DIC and have been proposed as a significant diagnostic tool to evaluate early vascular injury [74]. An increase of platelet derived microvesicles has been demonstrated in the development of sepsis-related renal impairment [77] and in severe fungal sepsis [78]. Finally, MVs released from peripheral blood PMNs have a similar size and orientation but differ in protein composition and functional properties. These affect endothelial cells, platelets, monocytes, and macrophages. They also show antibacterial properties since they are capable of a significant reduction in bacterial growth (Figure 3) [79–81].

5. The Blood Compartment

Blood compartment plays a key role in the inflammatory response. Waves of mediators with both anti- and proinflammatory properties are detectable in the plasma of septic patients. Many of these are considered reliable markers for *in vita* diagnosis of sepsis and some have been proposed also as biological markers of the severity of sepsis, but none alone is entirely specific for infection because they can be also detected in the absence of infection [82]. The future direction of research is most likely to focus on the use of

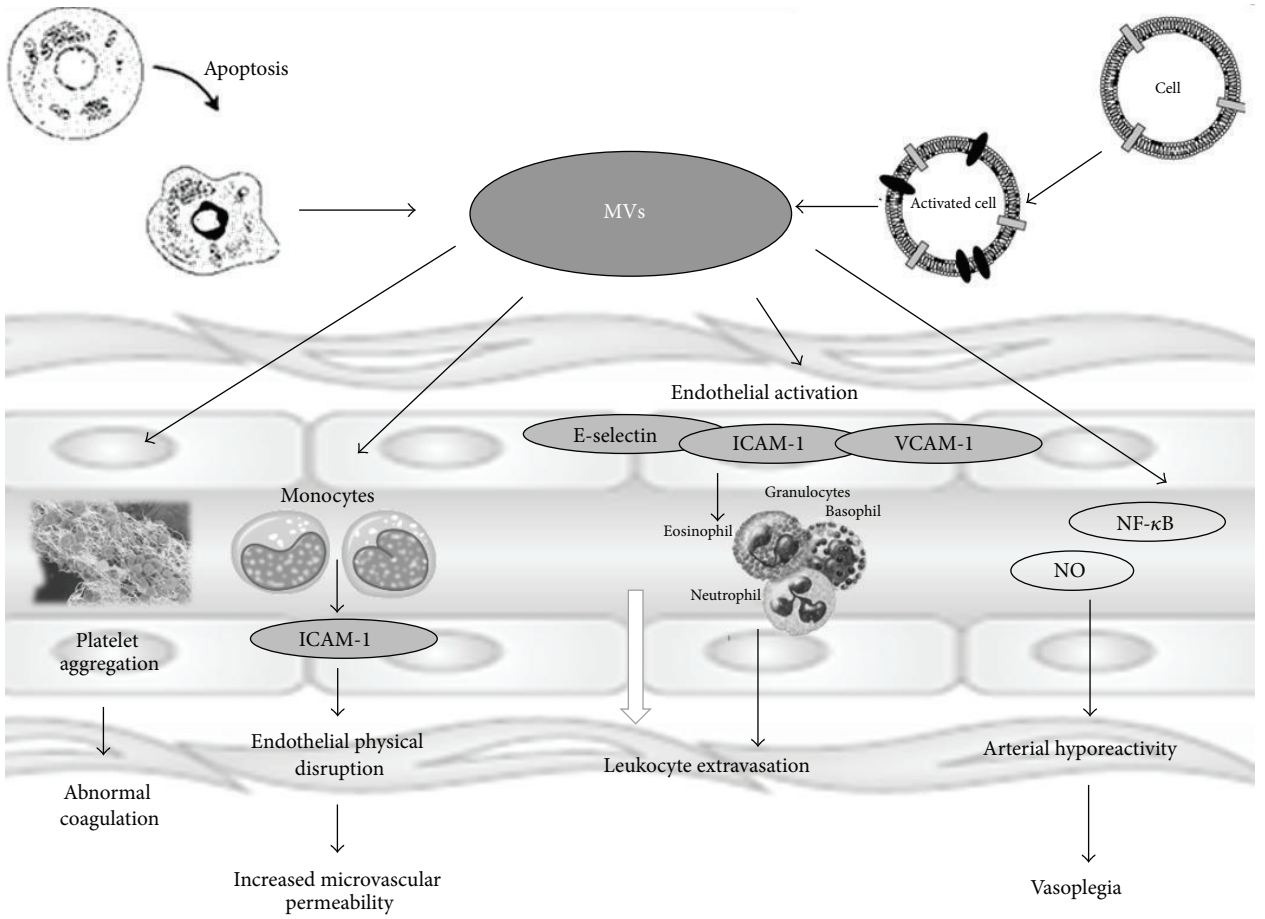

Figure 3: A schematic representing microvesicles (MVs) functions. MVs are released in the extracellular environment through a membrane reorganization and blebbing process following cell activation or apoptosis. They contribute to the spread of inflammatory and prothrombotic vascular status and they may affect smooth muscle tissue through adhesion molecules (E-selectin, ICAM, and VCAM) activation of nuclear factor κB and the expression of inducible nitric oxide synthase and cyclooxygenase-2, with an increase in nitric oxide (NO) and vasodilator prostanoids, leading to arterial hyporeactivity.

panels or combinations of markers with clinical signs. Some biomarkers may also be useful for prognosis and guiding therapy [83–86]. However, nowadays the ideal biomarker, with high sensitivity and specificity and cost effectiveness and with definite cut-off ranges and time of blood sampling, is yet to be found [87].

As molecular and humoral mechanisms are thought to play a key part in the pathophysiology of sepsis, postmortem diagnostic strategies based on the concentration of such mediators have been investigated.

Many authors propose the detection of procalcitonin (PCT) as a useful and reliable marker of sepsis in routine autopsy investigations [88–91]. PCT, a prohormone composed of 116 amino acids, is the precursor of the calcium homoeostasis hormone calcitonin, which is found in thyroid C cells and pulmonary endocrine cells. Clinically relevant levels of PCT influence the immunologic responses that contribute to systemic inflammatory responses and septic shock. Many studies have indicated that PCT is an excellent marker of bacterial infection in patients with sepsis and its related conditions. Bode-Jänisch et al. outline that, at PCT levels <2 ng/mL, bacterial sepsis or septic shock can almost certainly be excluded as cause of death [89]. PCT levels ≥10 ng/mL can be detected occasionally in conditions other than sepsis. A final assessment should therefore take into account the PCT levels, autopsy results, and the histopathological and microbiological findings [91]. Other authors indicate high diagnostic accuracy for both lipopolysaccharide-binding protein (LBP) and PCT, considered individually and combined, in detecting sepsis-related outcomes in postmortem [92].

However, due to the detectability of high levels of PCT also in aseptic inflammation (i.e., chronic inflammatory and autoimmune conditions, myocardial infarction, etc.) [93–103], a pressing need to identify additional biomarkers is evident [91].

As demonstrated by the studies mentioned above, much attention has been focused on cytokines and other mediators as diagnostic tools in many diseases and they certainly hold promise also for the discovery of reliable postmortem biomarkers of sepsis.

Soluble interleukin-2 receptor (sIL-2R) and LBP seem to represent appropriate diagnostic tools for the postmortem diagnosis of sepsis [104]. Postmortem IL-6 and C-reactive protein (CRP) serum levels were investigated by Tsokos et al. in sepsis and nonseptic fatalities and both IL-6 and CRP serum concentrations seem to be suitable biochemical markers of sepsis [105]. However, since pathological conditions other than sepsis (trauma, burn injury, etc.) may be associated with elevated IL-6 and/or CRP levels, the authors themselves warn about the need to rule out such conditions upon interpreting postmortem values of IL-6 and CRP [105].

Other biomarkers of potential relevance to sepsis/septic shock diagnostics have been proposed more recently.

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently discovered member of the immunoglobulin superfamily of receptors that is specifically expressed on the surfaces of neutrophils and monocytes and upregulated in bacterial sepsis. This is associated with a marked plasma elevation in the soluble form of this molecule (sTREM-1). Studies have indicated that sTREM-1 could be a valuable diagnostic biomarker for sepsis [106]. Palmiere et al. investigated sTREM-1 concentration in the serum of patients who died from sepsis and found that when individually considered, it did not provide better sensitivity and specificity than PCT in detecting sepsis. However, simultaneous assessment of PCT and sTREM-1 in postmortem serum could improve diagnostic accuracy [87]. Results from septic patients in intensive care units found that sTREM-1, PCT, and CRP levels indicate infection, while sTREM-1 and PCT levels predict prognosis. Moreover, sTREM-1 appears to be the best indicator for the diagnosis of sepsis and assessment of prognosis of blood culture-positive bacteremia [107].

Presepsin (sCD14-ST) is a soluble N-terminal fragment of protein CD14 which is released into circulation during monocyte activation on the recognition of LPS from infectious agents [108]; it shows promise for diagnostic and prognostic purposes in septic patients [109]. It has been investigated in sepsis-related death. The results show that even though increases in both PCT and sCD14-ST concentrations were observed in the control cases, coherent PCT and sCD14-ST results in cases with suspected sepsis allowed the diagnosis to be confirmed. Conversely, no relevant correlation was identified between postmortem serum and pericardial fluid sCD14-ST levels in either the septic or control groups [110].

Endocan (endothelial cell-specific molecule-1), a 50-kDa dermatan sulphate proteoglycan, expressed by endothelial cells in lung and kidney, can be detected at low levels in the serum of healthy subjects. Increased concentrations were described in patients with sepsis, severe sepsis, and septic shock compared to healthy individuals, with serum concentrations related to the severity of illness [111]. Palmiere and Augsburger found that postmortem serum endocan concentrations were significantly higher in sepsis fatal cases, with values ranging from 0.519 ng/mL to 6.756 ng/mL, while, in most patients of the control group, endocan was undetectable. The authors argue that endocan could be considered a suitable biological parameter for the detection of sepsis-related deaths in forensic pathology routine [112].

Neopterin (D-erytro-1′,2′,3′-trihydroxypropylterin), a biochemical product of guanosine triphosphate pathway, has been proposed to aid in the diagnosis of bacterial [113] and viral infections [114]. Also in the forensic literature neopterin has been proposed as a marker of inflammatory diseases [115–117]. Postmortem serum neopterin levels over 500 nmol/L were observed in bacterial and viral infection cases as well as in delayed deaths due to trauma [118]. For this reason, the specificity of neopterin as a clinical marker of bacterial sepsis is limited [119].

Conclusively, there is a strong body of evidence that postmortem concentration of serum cytokines and other mediators of inflammation may be an area of great interest with exciting diagnostic possibilities for sepsis/septic shock-related deaths [118, 119]. However, at present, an ideal clinical and postmortem marker of sepsis does not exist [118].

In a previous review, Pierrakos and Vincent identified nearly 180 distinct molecules that have been proposed as potential biological markers of sepsis [83]. However, only 20% of these biomarkers have been assessed specifically in appropriate studies for use in the diagnosis of sepsis [84].

6. Conclusion

The difficulties of the postmortem diagnosis of death due to sepsis are well known. The major limitation is the poor specificity of macroscopic and routine histological findings encountered in such cases. Due to the complex molecular and cellular mechanisms underlying sepsis, proof of the presence of germs alone cannot be of evidential value in establishing a causal relationship between infection and outcome. Thus, the old saying by William Osler (1849 to 1919) "except on few occasions, the patient appears to die from the body's response to infection rather than from the infection" is still true [84].

A complete methodological approach, integrating clinical data by means of autopsy and histological and laboratory findings aiming to identify and demonstrate the host response to infectious insult, is mandatory. Such an approach would be likely to produce an accurate objective surveillance of deaths due to sepsis and improve our knowledge of the clinical-pathological correlation in sepsis, thus contributing to the evaluation of the effectiveness of therapies. Finally, autopsy is a critical tool for protection from false liability claims and settling valid claims quickly and fairly.

Disclosure

Readers can refer to *Sepulchretum Sive Anatomia Practica ex Cadaveribus Morbo Denatis (Burial vault/cemetery or anatomical studies on bodies affected by disease)* Bonet T (1679), Leonardus Chouet, Geneva.

Competing Interests

The authors declare that they have no competing interests.

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Research Article

Prognostic Value of Adrenomedullin and Natriuretic Peptides in Uroseptic Patients Induced by Ureteroscopy

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The aim of this paper is to investigate whether urosepsis is related to irrigation pressure of ureteroscopy (URS) and evaluate the prognostic value of adrenomedullin (ADM) and atrial and brain natriuretic peptides (ANP and BNP) in URS-induced uroseptic patients. From July 2008 to October 2013, we enrolled 332 patients with untreated unilateral ureteral obstruction (UUO). The UUO group included three subgroups of, respectively, 118, 132, and 82 patients who underwent URS under intermittent stable irrigation pressure of, respectively, 80, 120, and 160 mmHg. The plasma concentrations of ADM, ANP, and BNP were measured in all subjects. URS was performed for all UUO patients; the values of the three peptides were measured again after URS. Irrigation pressure and stone size were independent risk factors of urosepsis. After URS, the plasma concentrations of ADM, ANP, and BNP were significantly higher in uroseptic patients. Moreover, the concentrations were significantly higher depending on the disease severity. Plasma concentrations of the three peptides were correlated with plasma ET concentration in the uroseptic patients. The areas under receiver operating characteristic (ROC) curve of ADM, ANP, and BNP for predicting urosepsis were 0.811, 0.728, and 0.764, respectively. In conclusion, ADM, along with ANP and BNP, is valuable for prognosis in urosepsis secondary to URS which is associated with irrigation pressure.

1. Introduction

Urosepsis is defined as sepsis caused by infection of the urogenital tract and is a systemic response to infection [1]. Frequent causes for urosepsis are obstructive diseases of the urinary tract, such as ureteral stones, anomalies, stenosis, or tumor [2]. It can also occur after interventions in the urogenital tract, such as transrectal prostate biopsy, percutaneous nephrolithotomy (PCNL), or ureteroscopy (URS) [3–5]. Early diagnosis and timely intervention have great importance for urosepsis. Effective treatment in the early periods of urosepsis can prevent irreversible organ damage and reduce mortality. Therefore, finding useful biomarkers plays a crucial role in early diagnosis of urosepsis.

Adrenomedullin (ADM) was first isolated from human pheochromocytoma [6]. It has been detected in the plasma and other fluids of normal individuals [7]. Adrenomedullin possesses anti-inflammatory, bactericidal, positive inotropic, and, perhaps most importantly, vasodilatory activities [8]. Several clinical studies have demonstrated that ADM increases significantly in septic patients and is correlated with disease severity, which is valuable for prognosis in septic patients [9, 10].

Atrial and brain natriuretic peptides (ANP and BNP, resp.) are polypeptide hormones comprising the cardiac-derived natriuretic peptide system which are involved in the long-term regulation of sodium and water balance, blood volume, and arterial pressure [11–13]. Increased plasma ANP and BNP have been identified as predictors of cardiac dysfunction in sepsis and prognosis in patients with congestive heart failure or ischemic heart disease [14, 15]. Many reports indicate that ANP or BNP levels are elevated in septic patients and they can provide useful diagnostic and prognostic information in septic patients [14, 16, 17].

To measure the plasma concentrations of ADM, ANP, and BNP and evaluate their prognostic value in uroseptic patients induced by URS, we measured the values of the three peptides in untreated patients with unilateral ureteral obstruction

(UUO) secondary to ureteral stones and compared the results with those of healthy control subjects. Additionally, we measured the values of the three peptides after URS for UUO patients.

2. Materials and Methods

2.1. Study Subjects. This retrospective, case-control study was conducted in Renmin Hospital of Wuhan University between July 2008 and October 2013 in accordance with our institutional standards and under the appropriate license of the Ethics Committee of Renmin Hospital, as well as in adherence to national regulations. The study groups consisted of 90 healthy control subjects (50 men and 40 women, mean age 41.3 ± 12.6 years, range 18 to 66 years) and 332 patients with untreated UUO (185 men and 147 women, mean age 43.3 ± 12.0 years, range 18 to 68 years). In order to investigate whether urosepsis is related to irrigation pressure of ureteroscopy (URS), all UUO patients underwent URS with intermittent stable irrigation pressure of 80 mmHg (group I, 66 men and 52 women, mean age 43.2 ± 12.1 years; range 18 to 68 years), 120 mmHg (group II, 73 men and 59 women, mean age 43.3 ± 11.6 years; range 19 to 66 years), and 160 mmHg (group III, 46 men and 36 women, mean age 43.6 ± 12.8 years; range 19 to 67 years), respectively. All patients agreed to participate in this study and provided written informed consent. The operation was performed by only one experienced surgeon. The irrigation pressure was randomly selected and the patients and surgeon were blind to it according to the double blind method. All patient information was anonymized and deidentified prior to analysis. Routine laboratory studies and image examinations were conducted before URS. The inclusion criteria for UUO subjects were as follows: simple unilateral ureteral stone, without urinary tract infections by urinalysis (the presence of 10 urine leukocytes/HPF and no microorganisms), without any symptoms of urogenital tract infections, without antimicrobial prophylaxis before the procedure. Ureteroscopy was performed using a Wolf rigid ureteroscope. UUO was definitely diagnosed on the basis of intravenous pyelonephrography or computed tomography. All patients undergoing laser lithotripsy had a double-J stent and a Foley catheter placed at the end of the procedure. The indwelling Foley catheter was drawn within 72 hours. All uroseptic patients were symptomatic or signs of potential sepsis were present within 24 hours after URS. They fulfilled the systemic inflammatory response syndrome (SIRS) criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine [18], regardless of the procedure duration and residual stones. Patients who developed sepsis were treated with vasopressors such as phenylephrine and norepinephrine to sustain blood pressure when necessary. None of these UUO patients had clinical evidence of active infection, malignant cancer of any type, acquired immunodeficiency syndrome (AIDS), end-stage renal or liver disease, diabetes, pulmonary disease, valvular heart disease, congenital heart disease, acute myocarditis, angina pectoris, myocardial infarction, essential hypertension, or other diseases. Control subjects were age- and gender-matched healthy subjects who had been hospitalized for a health checkup.

2.2. Assay Procedures. Venous blood samples were drawn from an antecubital vein within 24 hours for nonseptic subjects or septic patients before vasopressor treatment and were transferred to ice-chilled tubes containing Trasylol (500 KIU/mL) and ethylenediaminetetraacetic acid (EDTA, 1 g/L). They were then centrifuged at 3,000 rpm for 15 min at 4°C and the plasma was immediately frozen and stored in polypropylene tubes at −80°C until radioimmunoassay (RIA).

Baseline clinical data were recorded as follows: endotoxin (ET) using the Pyrochrome test kit (Pyroquant Diagnostik GmbH, Mörfelden, Germany), white blood cell counts (WBC) using a Sysmex SE-9000 analyzer (Toa Medical Instruments, Kobe, Japan), C-reactive protein (CRP) using an immunoturbidimetric assay (Modular Analytics P, Roche Diagnostics, Mannheim, Germany), lactate (LAC) using enzymatic method (Modular Analytics P, Roche Diagnostics, Mannheim, Germany), and procalcitonin (PCT) using an immunoassay analyzer (Block Scientific, Bohemia, NY).

The plasma ADM levels were measured with specific RIA for human ADM (ADM RIA SHIONOGI, Shionogi Pharmaceutical, Co., Ltd., Osaka, Japan). The intra- and interassay coefficients of variation were 3.8% to 7.9% and 4.5% to 8.8%, respectively. All assays were performed in duplicate. ADM concentrations were expressed as ng/L.

The plasma ANP concentrations were measured with a specific immunoradiometric assay for human ANP (Shiono RIA ANP kit, Shionogi and Co., Osaka, Japan). The intra- and interassay coefficients of variation were 4.7% to 9.8% and 5.9% to 11.6%, respectively. The plasma BNP concentrations were measured by a method similar to that for ANP, developed by the same company (Shiono RIA BNP kit). The intra- and interassay coefficients of variation were 5.8% to 10.7% and 6.5% to 12.5%, respectively. All assays were performed in duplicate. The concentrations of ANP and BNP were expressed as ng/L.

2.3. Statistical Analysis. All continuous data were expressed as mean ± SD and analyzed with SPSS software, version 19.0 (SPSS Inc., Chicago, IL). Comparisons between two variables were performed with unpaired t-test or Mann-Whitney U test. Multiple comparisons were evaluated with analysis of variance followed by Student-Newman-Keuls or Kruskal-Wallis method. The significance of differences between paired variables was determined by paired t-test or Wilcoxon test. Categorical variables were assessed by the chi-square test or Fisher's exact test. Stepwise multiple linear regression analysis was used to evaluate the most important factor for ADM, ANP, and BNP. The correlation between two variables was done by linear regression analysis and further confirmed by Spearman's rank test. Receiver operating characteristic (ROC) curves were used to predict urosepsis and determine the cutoff values. Kaplan-Meier curves were plotted according to the identified cutoff values of ADM, ANP, and BNP and further confirmed by log-rank test. A 2-sided p value less than 0.05 was considered to indicate statistical significance.

Table 1: Baseline characteristics of study subjects.

Characteristics	Control (n = 90)	UUO (n = 332)			p value
		Group I (n = 118)	Group II (n = 132)	Group III (n = 82)	
Age (years)	41.3 ± 12.6	43.2 ± 12.1	43.3 ± 11.6	43.6 ± 12.8	>0.05
Gender					
Men	50 (55.6%)	66 (55.9%)	73 (55.3%)	46 (56.1%)	>0.05
Women	40 (44.4%)	52 (44.1%)	59 (44.7%)	36 (43.9%)	
Side					
Left	—	60 (50.8%)	67 (50.8%)	42 (51.2%)	>0.05
Right	—	58 (49.2%)	65 (49.2%)	40 (48.8%)	
Stone site					
Proximal ureter	—	37 (31.4%)	40 (30.3%)	25 (30.5%)	
Mid ureter	—	41 (34.7%)	45 (34.1%)	28 (34.1%)	>0.05
Distal ureter		40 (33.9%)	47 (35.6%)	29 (35.4%)	
Stone size (mm)	—	9.2 ± 4.1	9.6 ± 4.5	10.3 ± 5.2	>0.05
WBC ($\times 10^9$/L)	7.1 ± 1.5	7.2 ± 1.6	6.8 ± 1.5	7.0 ± 1.7	>0.05
ET (ng/L)	3.6 ± 1.4	3.5 ± 1.2	3.6 ± 1.6	3.5 ± 1.7	>0.05
CRP (mg/L)	6.0 ± 2.2	5.8 ± 2.1	5.9 ± 2.1	6.2 ± 2.1	>0.05
LAC (mmol/L)	1.1 ± 0.5	1.2 ± 0.6	1.3 ± 0.6	1.2 ± 0.5	>0.05
PCT (ng/mL)	0.24 ± 0.13	0.23 ± 0.12	0.25 ± 0.13	0.22 ± 0.10	>0.05
Scr (μmol/L)	75 ± 12	77 ± 14	76 ± 13	74 ± 11	>0.05
Ccr (mL/min)	100 ± 10	101 ± 12	99 ± 11	102 ± 14	>0.05

UUO: unilateral ureteral obstruction; WBC: white blood cell count; ET: endotoxin; CRP: C-reactive protein; LAC: lactate; PCT: procalcitonin; Scr: serum creatinine; Ccr: creatinine clearance.
The normal values are as follows: WBC, 4.0~10.0 $\times 10^9$/L; ET, 0~10 ng/L; CRP, 0~10 mg/L; LAC, 0~2.4 mmol/L; PCT, 0~0.5 ng/mL; Scr, 53~106 μmol/L (male), 44~97 μmol/L (female); Ccr, 80~120 mL/min.

3. Results

Table 1 shows the baseline characteristics of the study groups. There were no significant differences in age, sex distribution, WBC, and plasma concentrations of ET, CRP, LAC, PCT, Scr, and Ccr among the four groups. No significant differences were observed in stone side distribution, stone site, and stone size among the three UUO subgroups.

After URS, the uroseptic rates of the three UUO subgroups were 8.5% (10/118), 18.2% (24/132), and 30.5% (25/82), respectively, which were significantly higher and higher in proportion to the irrigation pressure ($p < 0.05$). However, there were no significant differences in age, sex distribution, Scr, and Ccr among the three uroseptic groups (data not shown).

We analyzed the risk factors of urosepsis by stepwise multiple logistic regression analysis which revealed that stone size ($B = 0.695$, OR = 2.004, $p = 0.024$) and irrigation pressure ($B = 0.750$, OR = 2.118, $p = 0.000$) were the most important independent factors of urosepsis, when age, sex, stone side, stone site, stone size, and irrigation pressure were taken into account.

Table 2 shows the clinical parameters at diagnosis and after URS in uroseptic patients. As expected, WBC and plasma concentrations of ET, CRP, LAC, and PCT were significantly higher after URS than at diagnosis ($p < 0.05$). However, Scr and Ccr remained unchanged after URS.

The plasma concentrations of ADM, ANP, and BNP in controls and uroseptic patients before and after URS are depicted in Figures 1(a)–1(c). The mean value of ADM was significantly higher in uroseptic group I after URS (50.19 ± 20.67 ng/L) than before URS (19.08 ± 7.36 ng/L) and in controls (18.50 ± 6.46 ng/L) ($p < 0.05$). There was no significant difference in mean ADM value between uroseptic group I before URS and controls. The mean values of ANP in controls and uroseptic group I before and after URS were 23.63 ± 8.98, 22.56 ± 8.70, and 82.91 ± 30.43 ng/L, respectively, while the mean values of BNP were 12.72 ± 5.52, 13.24 ± 4.11, and 137.97 ± 57.79 ng/L, respectively. Similar changes were observed in mean values of ANP and BNP in uroseptic group I. Similar changes were found in mean values of the three peptides in uroseptic groups II and III.

Table 3 shows the clinical parameters of uroseptic patients depending on the disease severity. There were no significant differences in age, sex distribution, WBC, Scr, and Ccr among the three groups. No significant differences were detected in plasma concentrations of CRP, LAC, and PCT between sepsis and severe sepsis, while a significant difference was observed in plasma ET ($p < 0.05$). However, plasma concentrations of ET, CRP, LAC, and PCT were significantly higher in septic shock than in sepsis ($p < 0.05$).

As shown in Table 4, ET was the most important factor associated with ADM, ANP, and BNP in the uroseptic patients. Stepwise multiple regression analysis of independent parameters (WBC, ET, CRP, LAC, and PCT) related to the values of plasma ADM, ANP, and BNP was also conducted.

Table 2: Parameters of uroseptic patients before and after URS.

Parameters	Uroseptic group I ($n = 10$)		Uroseptic group II ($n = 24$)		Uroseptic group III ($n = 25$)	
	At diagnosis	After URS	At diagnosis	After URS	At diagnosis	After URS
WBC ($\times 10^9$/L)	7.5 ± 1.8	13.4 ± 5.8*	6.9 ± 1.6	15.6 ± 6.6*	7.2 ± 1.9	17.2 ± 9.4*
ET (ng/L)	3.8 ± 1.9	22.1 ± 10.2*	3.4 ± 1.8	29.5 ± 14.4*	3.7 ± 1.7	36.4 ± 15.6*
CRP (mg/L)	5.0 ± 2.4	59.2 ± 19.0*	5.2 ± 2.0	109.7 ± 48.9*	6.0 ± 2.2	145.9 ± 59.3*
LAC (mmol/L)	1.4 ± 0.6	2.4 ± 0.9*	1.1 ± 0.6	2.3 ± 0.8*	1.1 ± 0.5	2.5 ± 0.9*
PCT (ng/mL)	0.20 ± 0.13	0.59 ± 0.23*	0.25 ± 0.14	0.58 ± 0.24*	0.24 ± 0.11	0.60 ± 0.24*
Scr (μmol/L)	73 ± 8	74 ± 10	75 ± 10	77 ± 13	74 ± 9	75 ± 12
Ccr (mL/min)	101 ± 8	100 ± 11	100 ± 9	99 ± 12	99 ± 10	97 ± 14

URS: ureteroscopy; WBC: white blood cell count; ET: endotoxin; CRP: C-reactive protein; LAC: lactate; PCT: procalcitonin; Scr: serum creatinine; Ccr: creatinine clearance.
* $p < 0.05$, compared with subjects at diagnosis.

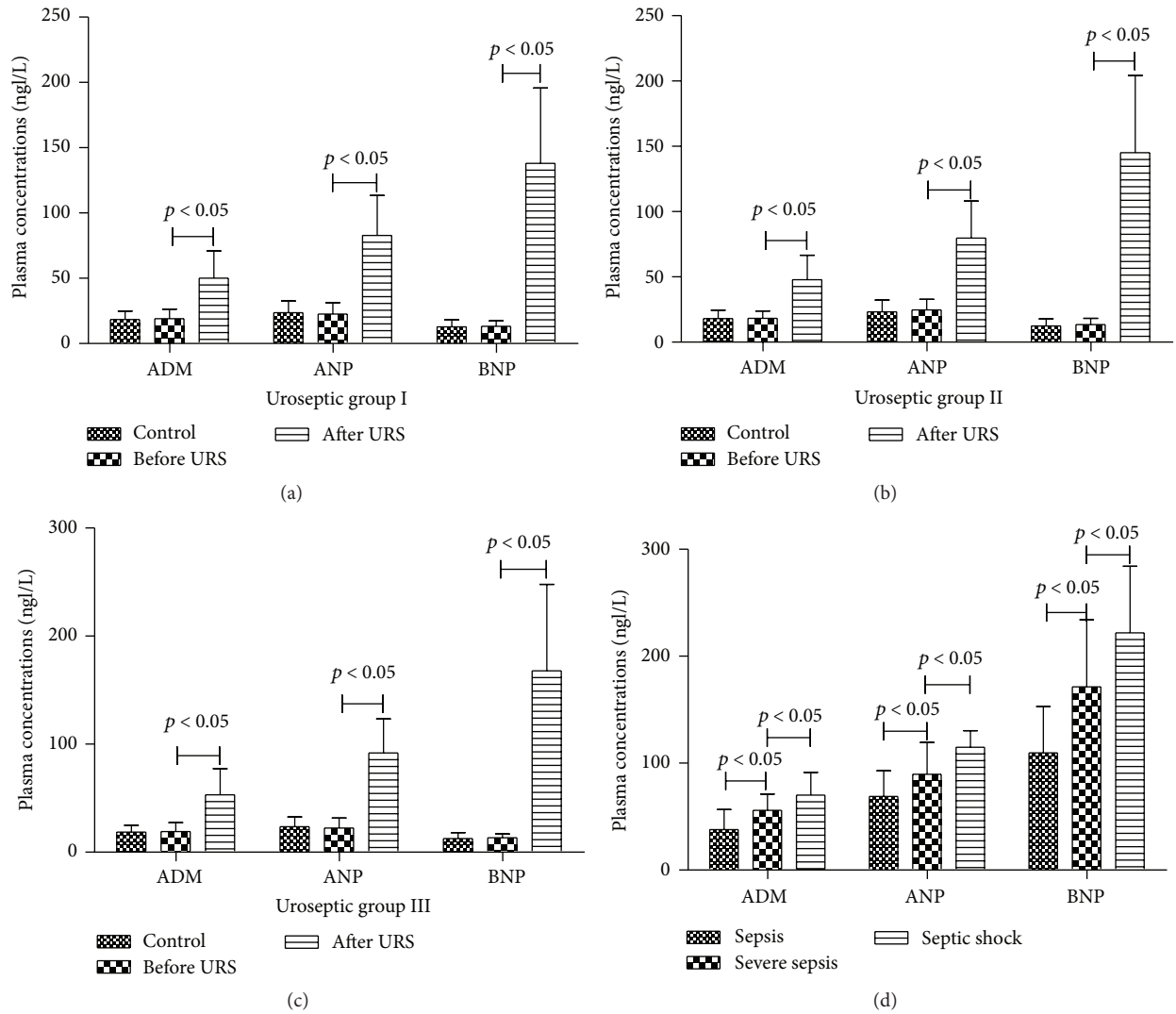

Figure 1: (a) Plasma concentrations of ADM, ANP, and BNP in controls and uroseptic group I before and after URS under 80 mmHg. (b) Plasma concentrations of ADM, ANP, and BNP in controls and uroseptic group II before and after URS under 120 mmHg. (c) Plasma concentrations of ADM, ANP, and BNP in controls and uroseptic group III before and after URS under 160 mmHg. (d) Plasma concentrations of ADM, ANP, and BNP in URS-induced sepsis, severe sepsis, and septic shock.

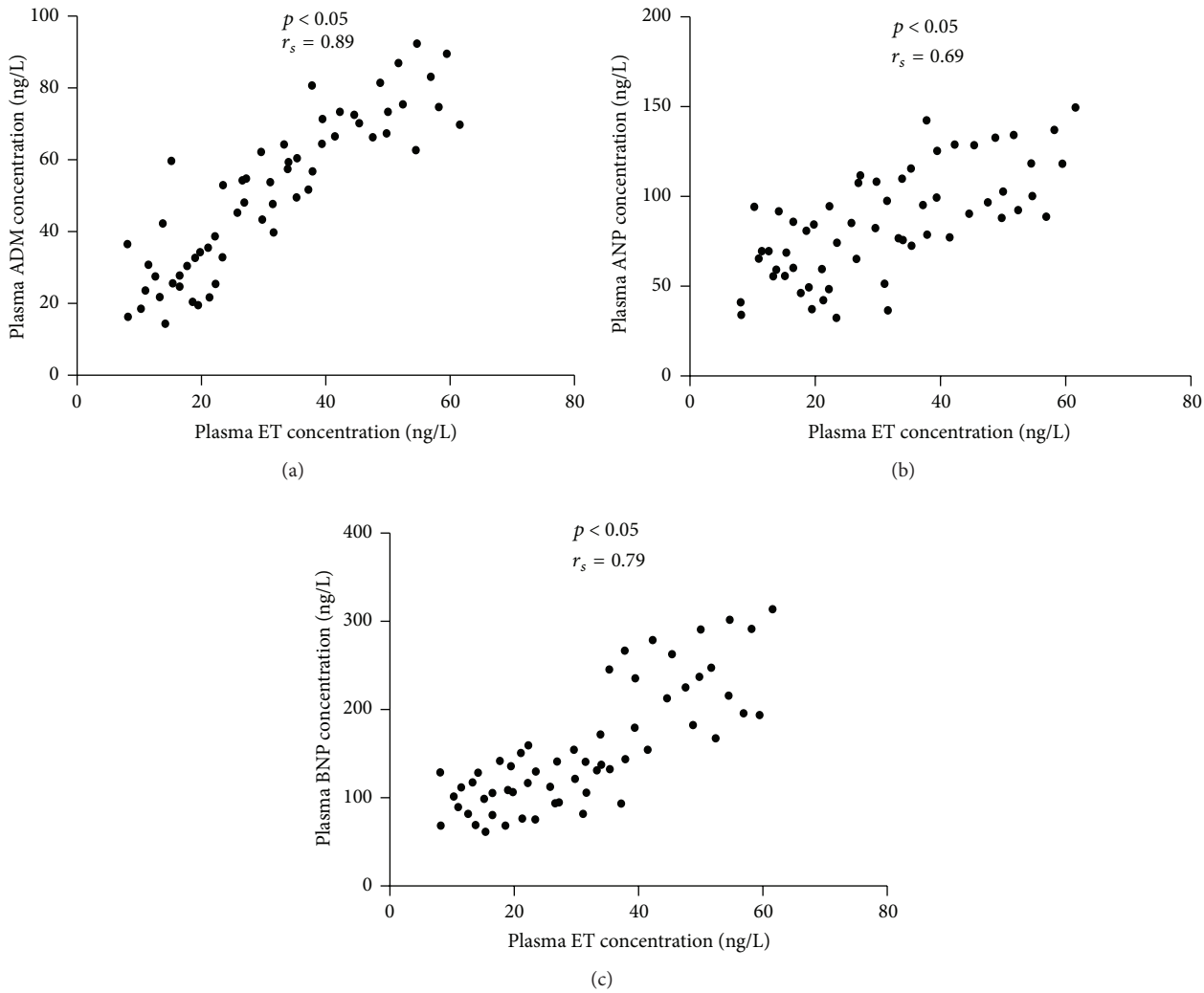

Figure 2: Relationship of plasma ET concentration to values of ADM (a), ANP (b), and BNP (c) in all uroseptic patients.

Table 3: Parameters of uroseptic patients depending on the disease severity after URS.

Parameters	Sepsis ($n = 25$)	Severe sepsis ($n = 24$)	Septic shock ($n = 10$)
Age (years)	44.2 ± 12.9	45.8 ± 12.2	45.1 ± 13.5
Gender (male : female)	12 : 13	13 : 11	4 : 6
WBC ($\times 10^9$/L)	13.7 ± 9.6	17.6 ± 6.1	17.5 ± 5.4
ET (ng/L)	21.9 ± 10.0	35.3 ± 15.1*	44.4 ± 11.4*
CRP (mg/L)	89.5 ± 46.8	116.8 ± 50.9	183.1 ± 51.1*#
LAC (mmol/L)	2.1 ± 0.7	2.6 ± 0.8	2.9 ± 0.8*
PCT (ng/mL)	0.50 ± 0.23	0.62 ± 0.22	0.74 ± 0.19*
Scr (μmol/L)	73 ± 11	76 ± 13	80 ± 15
Ccr (mL/min)	101 ± 8	98 ± 11	95 ± 14

URS: ureteroscopy; WBC: white blood cell count; ET: endotoxin; CRP: C-reactive protein; LAC: lactate; PCT: procalcitonin; Scr: serum creatinine; Ccr: creatinine clearance.
* $p < 0.05$, compared with sepsis; # $p < 0.05$, compared with severe sepsis.

Figure 1(d) shows the plasma concentrations of ADM, ANP, and BNP in uroseptic patients depending on the disease severity. The mean value of ADM was significantly higher in septic shock (70.05 ± 21.21 ng/L) than in severe sepsis (55.90±15.31 ng/L) and sepsis (37.75±18.84 ng/L) ($p < 0.05$). There was also a significant difference in mean ADM value between severe sepsis and sepsis ($p < 0.05$). Similar changes were found in mean values of ANP and BNP.

Scatterplots of Figure 2 show relationship of plasma ET concentration to plasma concentrations of ADM (a), ANP (b), and BNP (c) in uroseptic patients. Plasma ET concentration was positively related to plasma concentrations of ADM, ANP, and BNP.

The ROC curves of WBC, ET, CRP, LAC, PCT, ADM, ANP, and BNP for urosepsis are shown in Figure 3. The AUCs are listed in Table 5. The AUC of ADM was 0.811 which was higher than those of WBC (0.712), ET (0.719), CRP (0.758), LAC (0.787), PCT (0.793), ANP (0.728), and BNP (0.764).

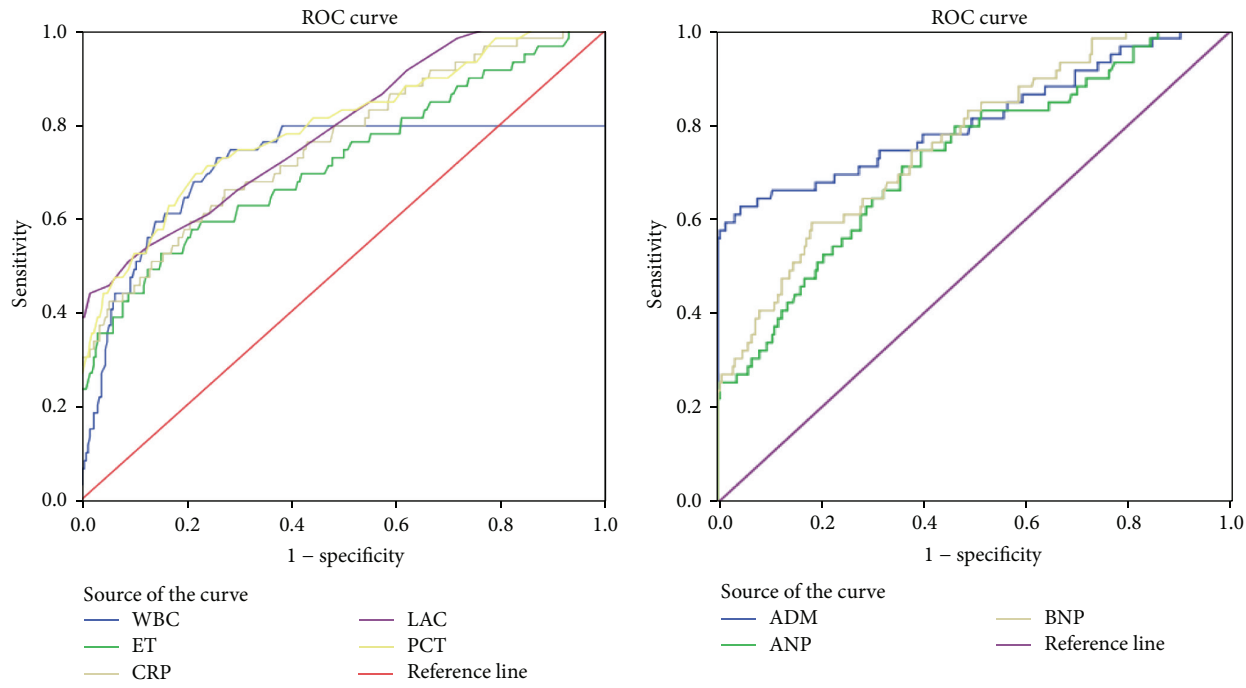

Figure 3: The ROC curves of ET, WBC, CRP, LAC, PCT, ADM, ANP, and BNP for predicting URS-induced urosepsis in UUO patients.

Table 4: Stepwise multiple regression analysis of significant factors for ADM, ANP, and BNP in uroseptic patients.

Variables	ADM (ng/L)			ANP (ng/L)			BNP (ng/L)		
	B	*t*	*p*	*B*	*t*	*p*	*B*	*t*	*p*
WBC ($\times 10^9$/L)	0.020	0.106	0.916	−0.297	−0.694	0.491	−0.567	−0.689	0.494
ET (ng/L)	0.847	4.835	0.000	0.979	2.445	0.018	3.797	4.947	0.000
CRP (mg/L)	0.034	1.058	0.295	0.100	1.367	0.177	−0.120	−0.858	0.395
LAC (mmol/L)	4.404	1.964	0.055	7.101	1.386	0.172	12.994	1.323	0.192
PCT (ng/mL)	13.557	1.550	0.127	−7.611	−0.381	0.705	−16.361	−0.427	0.671

ADM: adrenomedullin; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; WBC: white blood cell count; ET: endotoxin; CRP: C-reactive protein; LAC: lactate; PCT: procalcitonin.

Table 5: The AUCs of WBC, ET, CRP, LAC, PCT, ADM, ANP, and BNP.

Variables	Area	SE	*p*	95% CI	
				Lower	Upper
WBC ($\times 10^9$/L)	0.712	0.049	0.000	0.615	0.809
ET (ng/L)	0.719	0.041	0.000	0.639	0.799
CRP (mg/L)	0.758	0.037	0.000	0.686	0.830
LAC (mmol/L)	0.787	0.034	0.000	0.720	0.854
PCT (ng/mL)	0.793	0.035	0.000	0.724	0.862
ADM (ng/L)	0.811	0.038	0.000	0.737	0.885
ANP (ng/L)	0.728	0.037	0.000	0.655	0.801
BNP (ng/L)	0.764	0.034	0.000	0.697	0.831

WBC: white blood cell count; ET: endotoxin; CRP: C-reactive protein; LAC: lactate; PCT: procalcitonin; ADM: adrenomedullin; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide.

Kaplan-Meier curves for ADM, ANP, and BNP are depicted in Figure 4. The cutoff values of ADM, ANP, and BNP were 41.925, 68.565, and 128.575 ng/L, respectively, for prognosis in uroseptic patients. The survival rates were 64.9%, 66.7%, and 62.9%, respectively, whose values of the three peptides were above the cutoff values, whereas the survival rates were 90.9%, 94.1%, and 91.7%, respectively, whose values of the three peptides were below the cutoff values. There was a significant difference in survival rates between the groups above and below the cutoff values ($p < 0.05$).

4. Discussion

Ureteral calculi represent a common condition that urologists encounter in everyday practice. Ureteroscopy is one of the

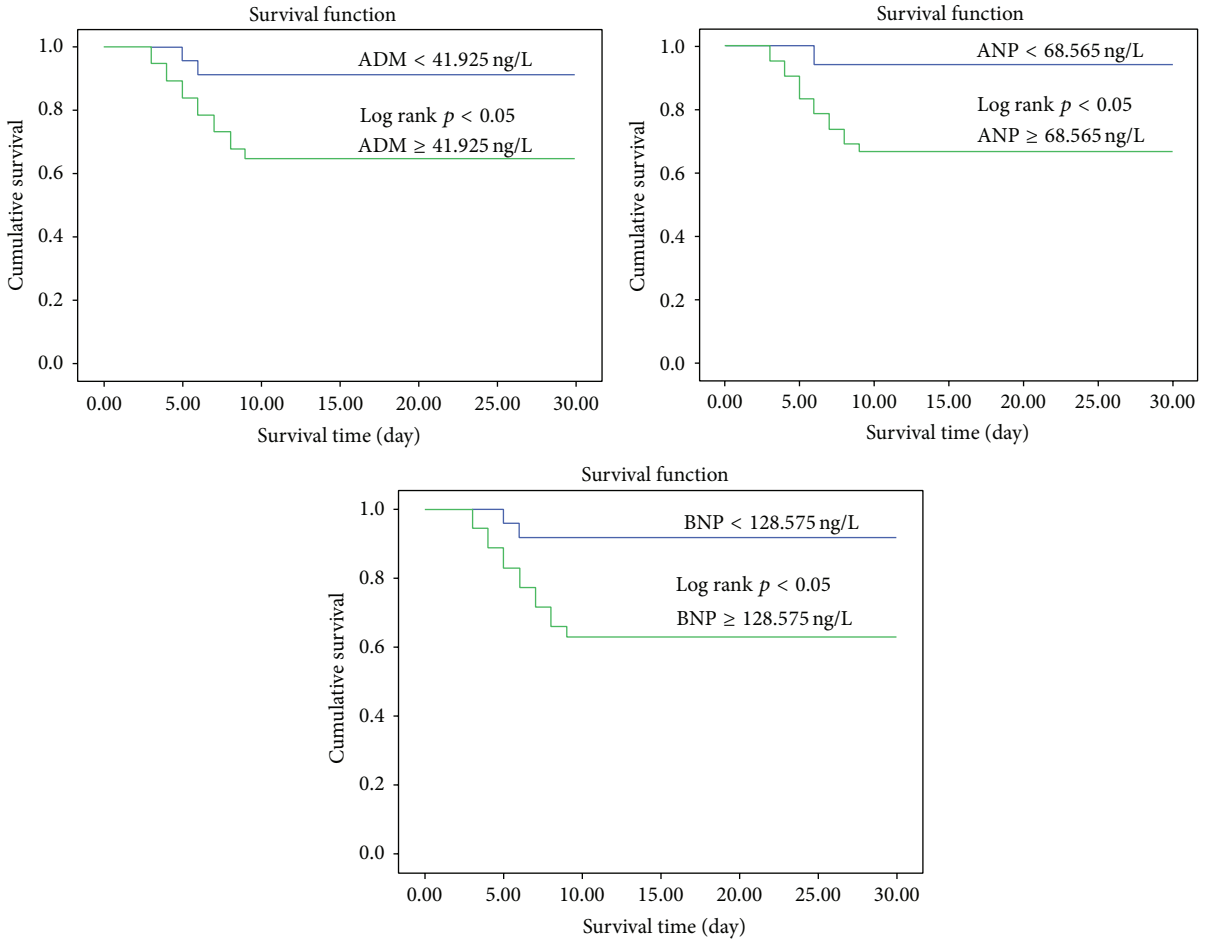

Figure 4: Kaplan-Meier curves for 69 uroseptic patients subdivided into two groups according to the cutoff values of ADM, ANP, and BNP in plasma.

most important treatment options for ureteral calculi that do not pass spontaneously or are unlikely to do so [19]. This procedure carries the risk of postoperative urosepsis affecting UUO patients undergoing ureteroscopy and laser lithotripsy [20]. Urosepsis, uroseptic shock, and the ensuing multiple organ failure continue to be the most common causes of death in critically ill patients of urological department admitted to intensive care unit (ICU) [21]. In urosepsis, as in other types of sepsis, the severity of sepsis depends mostly upon the host response [22]. Human ADM, consisting of 52 amino acids, has a ring structure formed by a disulfide bond and an amidated carboxyl terminus and belongs to a family of calcitonin gene-related peptides [23]. Nowadays, it has been demonstrated that ADM can be synthesized by various other tissues including endothelial and vascular smooth muscle cells, myocardium, and central nervous system [24]. It has multiple functions in a wide range of tissues and acts mainly as a vasodilatory and proliferation-inhibitory factor in cardiovascular system [25]. It was recently reported that ADM plays a central role in initiating the hyperdynamic response during the early stages of sepsis and was a useful predictor for development of severe sepsis and septic shock [10, 26]. The ANP and BNP are similar to ADM in cardiovascular effects including natriuresis, diuresis, and vasodilatation, thereby reducing fluid volume and blood pressure [13, 27]. ANP is a 28-amino-acid peptide chiefly synthesized and released by atrial myocytes in response to atrial distension and stretch, whereas BNP is a 32-amino-acid peptide synthesized and released by ventricular myocytes in response to ventricular stretch or pressure overload [28]. Pro-ANP is a valuable biomarker for prediction of severity of septic patients [29, 30]. The plasma BNP concentrations were increased in patients with severe sepsis or septic shock and poor outcome was associated with high BNP levels [31, 32].

In the current study, the uroseptic rates were significantly higher and higher in proportion to the irrigation pressure. This result was further confirmed by logistic regression analysis of risk factors for urosepsis which showed irrigation pressure was an independent risk factor. Therefore, this result may provide a guide that it is necessary to perform URS under lower irrigation pressure in the clinical practice. Moreover, stone size was another independent risk factor for urosepsis. It can be easily explained that the operation time is longer with bigger stone size.

In our study, plasma levels of ADM, ANP, and BNP were higher in uroseptic patients after URS than before URS

and in controls, as well as WBC and plasma concentrations of ET, CRP, LAC, and PCT, but Scr and Ccr remained unchanged. No significant differences were found between uroseptic patients before URS and controls. Moreover, the plasma concentrations of ADM, ANP, and BNP were related to the severity of disease, as well as plasma concentrations of ET, CRP, LAC, and PCT [9, 33, 34]. It can be inferred that WBC and plasma concentrations of ET, CRP, LAC, and PCT had some predictive value for urosepsis and disease severity, in agreement with the literature [35]. ADM, along with ANP and BNP, may participate in initiating the hyperdynamic response during the early stages of sepsis because of their similar physiological functions, in agreement with the literature [16, 26]. Endotoxin was identified as the most important factor in uroseptic patients. This result was confirmed by stepwise multiple regression analysis of independent parameters related to plasma concentrations of ADM, ANP, and BNP. Plasma endotoxin concentration was not only correlated with values of ADM but also related to the values of ANP and BNP in uroseptic patients. We can infer that URS may cause endotoxin absorption which is proportional to the irrigation pressure. The elevated plasma endotoxin concentration may subsequently result in ADM secretion and myocardial cell injury which are responsible for the elevated plasma levels of ADM, ANP, and BNP. Indeed, the mechanism of ADM secretion in large part relates to the effects of lipopolysaccharide (LPS) stimulation which is the most important ingredient of endotoxin. Moreover, it can be inferred that urosepsis secondary to endotoxin absorption can lead to endotoxic cardiomyopathy.

Biomarkers play important roles in diagnosis, differential diagnosis, risk stratification, therapeutic monitoring, and prognosis in sepsis. Many features of pathophysiologic progression correlate with the severity and outcome of the disease and become candidate prognostic biomarkers [36]. Our study showed that the older biomarkers of WBC and plasma concentrations of ET, CRP, LAC, and PCT were predictive indicators of urosepsis according to their AUCs. Our data seem to be compatible with the previous report [35]. The predictive value of PCT is still superior to the other biomarkers. However, the most valuable predictor is ADM and ANP and BNP also have some predictive value in urosepsis according to their AUCs, in agreement with the literature [10, 30, 32].

Several clinical studies have demonstrated that ADM, ANP, and BNP are predictors of adverse outcome in patients with sepsis, but most of these studies were conducted in the ICU and contained relatively small sample sizes. The prognostic value of ADM, ANP, and BNP in uroseptic patients induced by URS in the urological department is still undefined.

Our results suggest that the prognostic value of ADM is superior to ANP and BNP, and ADM, ANP, and BNP are robust independent predictors of in-hospital death in uroseptic patients. In uroseptic patients with ADM, ANP, and BNP levels above the cutoff values of our study, the in-hospital mortality was 35.1%, 33.3%, and 36.1%, respectively, with all patients dying within the first 10 days. In uroseptic patients with ADM, ANP, and BNP levels below the cutoff values, survival rates were 90.9%, 94.1%, and 91.7%, respectively, at the 30-day follow-up. These results demonstrate that ADM, ANP, and BNP are strong predictors of adverse outcome in patients with urosepsis.

5. Conclusions

In summary, with the physiological roles of ADM taken together, our study shows that ADM, along with ANP and BNP, may participate in initiating the hyperdynamic response during the early stages of sepsis in uroseptic patients. In addition, ADM, ANP, and BNP are strong predictors of adverse outcome in patients with urosepsis.

Ethical Approval

This study has been approved by the Ethics Committee of Renmin Hospital of Wuhan University, in adherence to China Association for Ethical Studies.

Consent

All subjects agreed to participate in this study and provided written informed consent which was kept on file after the ethics committee approved this consent procedure.

Conflict of Interests

The authors report no conflict of interests regarding the publication of this paper.

Authors' Contribution

Wei Hu was responsible for project development, data collection, data analysis, and paper writing. Pang-hu Zhou was in charge of project development and data collection. Xiao-bin Zhang was responsible for project development. Wei Wang and Lijun Zhang carried out data collection. Wei Hu and Pang-hu Zhou contributed equally to this study.

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Research Article

Systemic Inflammatory Markers Are Closely Associated with Atherogenic Lipoprotein Subfractions in Patients Undergoing Coronary Angiography

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Objective. To investigate the relationship between inflammatory markers and atherogenic lipoprotein subfractions. *Methods*. We studied 520 eligible subjects who were not receiving any lipid-lowering therapy. The inflammatory markers including white blood cell (WBC) count, high-sensitivity C-reactive protein (hs-CRP), fibrinogen, erythrocyte sedimentation rate (ESR), and D-dimer were measured. A multimarker inflammatory index was developed. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) separation processes were performed using Lipoprint System. *Results*. In age- and sex-adjusted analysis, several inflammatory markers (WBC count, hs-CRP, fibrinogen, and ESR) were positively related to circulating non-HDL cholesterol and remnant cholesterol ($p < 0.05$, all). Among lipoprotein subfractions, we observed a positive association of inflammatory markers with very low-density lipoprotein cholesterol, small LDL cholesterol, and LDL score ($p < 0.05$, all). Meanwhile, a negative association was detected between inflammatory markers and mean LDL particle size ($p < 0.05$) or large HDL cholesterol ($p < 0.05$). Moreover, we found that the relationships between multimarker index quartiles and small LDL cholesterol, LDL score, and mean LDL particle size were slightly stronger in patients with CAD. *Conclusions*. Systemic inflammatory markers are positively correlated with small LDL cholesterol and LDL score while being negatively linked with mean LDL particle size and large HDL cholesterol, highlighting the potential contribution to increased cardiovascular risk.

1. Introduction

Coronary artery disease (CAD) represents an important public health burden. Aside from the traditional risk factors, compelling evidence has now accumulated in support of inflammation as an important risk factor [1]. It has a pivotal role in all stages of atherosclerosis from endothelial dysfunction and plaque formation to plaque disruption and thrombosis [2]. Several inflammatory markers such as C-reactive protein (CRP) and fibrinogen have acquired prognostic significance [3, 4].

Although dyslipidemia has been recognized as the major cardiovascular risk factor and lowering of low-density lipoprotein (LDL) cholesterol has convincingly been shown to reduce cardiovascular events, there is still considerable remaining risk [5, 6]. Indeed, LDL particles are a heterogeneous collection of particles which vary in potential pathologic properties such as size, density, and lipid composition [7]. Moreover, high-density lipoprotein (HDL) particles are much more heterogeneous in their size and composition than LDL. This may at least partly explain why attempts to reduce cardiovascular events by pharmacologically increasing HDL plasma levels have failed [8, 9]. Hence, lipoprotein subfractions have emerged as a novel approach to assess the atherogenicity of lipoproteins.

There is a large body of evidence linking inflammatory status and dyslipidemia [10, 11]. Notably, it has been well established that inflammation is also able to affect lipoprotein metabolism [12]. Moreover, our previous data demonstrated positive associations between inflammatory markers and

important cholesterol regulator, proprotein convertase subtilisin/kexin type 9 (PCSK9) [13, 14], which has also been found to be closely related to atherogenic lipoprotein subfractions [15, 16], However, whether there are certain relationships between inflammatory markers and lipoprotein subfractions has not been reported yet. To address this question, we studied five kinds of systemic inflammatory markers (white blood cell (WBC) count and its subsets, high-sensitivity CRP (hs-CRP), fibrinogen, erythrocyte sedimentation rate (ESR), and D-dimer) in a group of subjects scheduled for coronary angiography who were not receiving any lipid-lowering therapy. Specifically, we hypothesized a presence of heterogeneity in the relationship of systemic inflammatory markers with atherogenic lipoprotein subfractions, which would aid our understanding of their interplay in the pathogenesis of atherosclerotic disease.

2. Materials and Methods

2.1. Study Design and Population. The study complied with the Declaration of Helsinki and was approved by the hospital's ethical review board (Fu Wai Hospital, National Center for Cardiovascular Diseases, Beijing, China). Each participant provided written, informed consent before enrollment.

In a group of subjects scheduled for coronary angiography because of angina-like chest pain and/or positive treadmill exercise test or clinically suspected CAD in our department, we selected 520 consecutive individuals who were not treated with lipid-lowering drugs (351 CAD and 169 non-CAD). Inclusion criteria were as follows: (1) having no treatment history of statins and/or other lipid-lowering drugs at least 3 months before entering the study; (2) having detailed clinical, laboratory data and well documented traditional cardiovascular risk factors; (3) having undergone coronary angiography. Exclusion criteria were subjects over 90 years, pregnancy or lactation, psychiatric disorder, the existence of any infectious or systematic inflammatory disease, acute coronary syndrome, serious heart failure or arrhythmia, significant hematologic disorders, thyroid dysfunction, severe liver dysfunction, and/or renal insufficiency and malignant tumors. The flowchart of the current study was shown in Figure 1.

Hypertension was defined as repeated blood pressure measurements ≥140/90 mmHg (at least two times in different environments) or currently taking antihypertensive drugs. Diabetes mellitus (DM) was defined as a fasting serum glucose level ≥126 mg/dL in multiple determinations and/or the current use of medication for diabetes. Dyslipidemia was defined by medical history or the use of lipid-modulating medications in order to reduce lipids or fasting total cholesterol (TC) ≥200 mg/dL or triglyceride (TG) ≥150 mg/dL.

2.2. Biochemical and Clinical Analyses. Fasting blood samples were collected in precooled EDTA tubes at baseline from each patient. After centrifugation at 3000 rpm for 15 min at 4°C, all plasma aliquots were stored in our laboratory at −80°C and were not thawed until use.

The concentrations of lipid profiles were determined by automatic biochemistry analyzer (Hitachi 7150, Tokyo, Japan). In detail, the LDL cholesterol concentration was analyzed by selective solubilization method (low-density lipid cholesterol test kit, Kyowa Medex, Tokyo). HDL cholesterol concentration was determined by a homogeneous method (Determiner L HDL, Kyowa Medex, Tokyo). Non-HDL cholesterol was calculated as TC minus HDL cholesterol. Remnant cholesterol was calculated as TC-HDL-C-LDL-C as previously reported [17]. The WBC count, neutrophil, lymphocyte, and monocyte differentials were determined using an automated blood cell counter (Beckman Coulter Ireland Inc., Mervue, Galway, Ireland). The plasma hs-CRP levels were determined using immunoturbidimetry (Beckman Assay 360, Bera, CA, USA). The fibrinogen levels were quantitatively measured by the method of Clauss and a Stago autoanalyzer with STA Fibrinogen kit (Diagnostica Stago, Taverny, France). The Westergren method was used for the measurement of ESR. Plasma D-dimer level was measured by Stago evolution (France).

2.3. LDL Subfraction Analysis. The cholesterol contents of LDL subfractions were determined electrophoretically by the use of high-resolution 3% polyacrylamide gel tubes and the Lipoprint LDL System (Quantimetrix Corporation, Redondo Beach, CA, USA) according to the manufacturer's instructions as previously described [18]. Seven LDL subfractions were obtained. Subfraction 1 represented large LDL particles, subfraction 2 indicated intermediate LDL particles, and subfractions 3–7 were defined as small LDL particles. The cholesterol mass (mg/dL) of each lipoprotein subfraction, the mean LDL particle size (Å), and the proportion (%) of the cholesterol mass of each lipoprotein subfraction over the TC mass were determined by this assay. The LDL score was calculated as the proportion of small LDL particles to the whole LDL area in our sample [19].

2.4. HDL Subfraction Analysis. Similar to LDL subfraction analysis, the cholesterol contents of HDL subfractions were also determined electrophoretically by the use of high-resolution 3% polyacrylamide gel tubes and the Lipoprint HDL System (Quantimetrix Corporation, Redondo Beach, CA, USA) as in our previously described work [20]. The relative area for each HDL subfraction was determined and multiplied by HDL cholesterol concentration of the sample to yield the amount of cholesterol for each band in mg/dL. Using this assay, HDL was divided into 10 subfractions. Subfractions 1–3 represented large HDL particles, subfractions 4–7 indicated intermediate HDL particles, and subfractions 8–10 meant small HDL particles.

2.5. Statistical Analysis. The data were expressed as the mean ± SD for the continuous variables and the number (percentage) for the categorical variables. Student's t-test or Mann-Whitney U test was used for the comparisons between continuous variables, and the chi-squared test was applied for the categorical variables between CAD and non-CAD group. Correlations between multiple inflammatory markers and

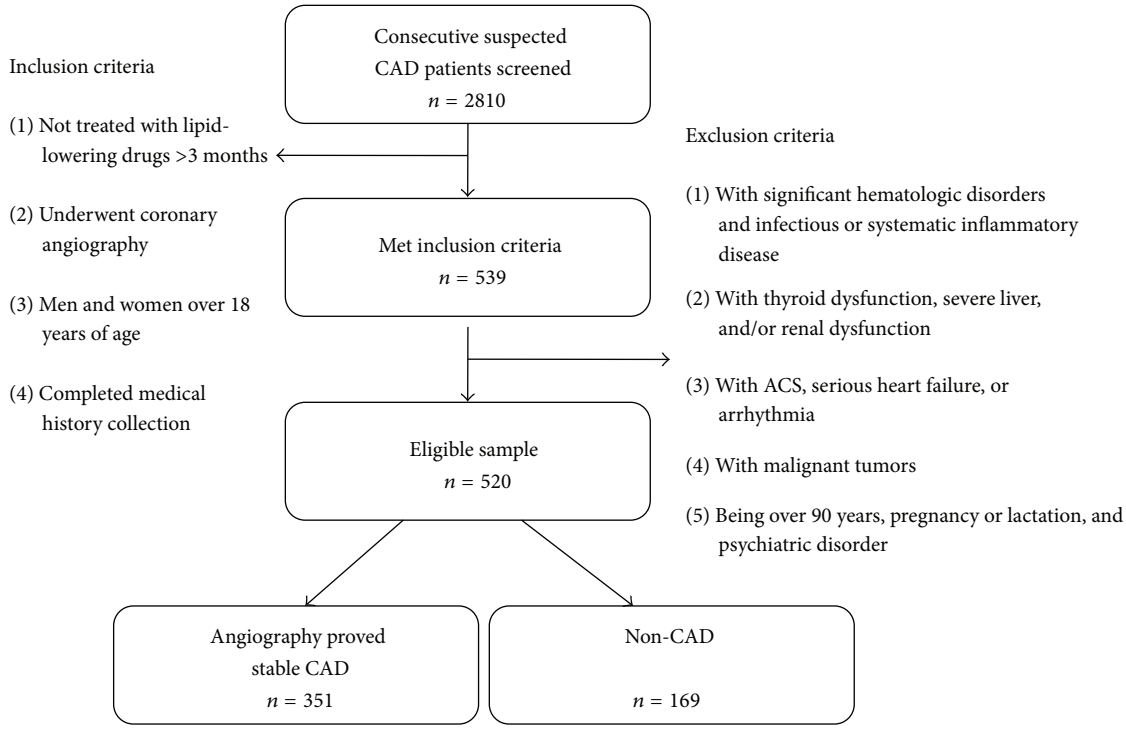

Figure 1: The flowchart of the current study.

lipoprotein subfractions were examined by partial correlation analysis with adjustments for age and sex. The chronic inflammatory activity can be assessed by a number of correlated parameters, such as WBC count, hs-CRP, fibrinogen, and ESR. Hence, we employed a principle component analysis to extract from the individual markers of inflammation (WBC count, hs-CRP, fibrinogen, and ESR) a single weighted multimarker inflammatory index. In the current study, only the first principle component was observed and no additional significant principal components were identified. Accordingly, we developed the overall multimarker inflammatory index by weighting the respective coefficients of each of the four inflammatory markers that contributed to the primary underlying factor (inflammation) as previously reported [21]. The general linear model was used for the comparison of lipoprotein subfractions according to multimarker index quartiles. The categorical variables were compared using the chi-squared test. A p value of less than 0.05 was considered statistically significant. Statistical studies were carried out with the SPSS program (version 19.0, SPSS, Chicago, Illinois, USA).

3. Results

3.1. Baseline Characteristics. A total of 520 individuals were enrolled in the present study. The mean age of the study cohort was 56.6 ± 10.0 years and 335 (64.4%) study participants were male. Among them, 351 (67.6%) had significant angiographically documented CAD as having >50% diameter stenosis in ≥1 major epicardial coronary artery. The main demographic and clinical characteristics of the study subjects are listed in Table 1. As a result, we observed that the CAD group has relatively higher small LDL cholesterol levels (9.0 ± 9.8 versus 7.9 ± 9.3 mg/dL, $p = 0.092$) and LDL score (0.14 ± 0.13 versus 0.12 ± 0.13%, $p = 0.067$) but smaller mean LDL particle size (266.4 ± 5.9 versus 267.2 ± 6.0 Å, $p = 0.079$), although the difference does not reach statistical significance in the current analysis. Meanwhile, the CAD group has dramatically lower large HDL cholesterol (13.5±7.2 versus 15.1 ± 7.7 mg/dL, $p = 0.027$). In addition, several inflammatory markers are increased in patients with CAD, such as WBC count (6.2 ± 1.8 versus 5.9 ± 1.4 ($\times 10^9$/L), $p = 0.076$), neutrophil count (3.8 ± 1.4 versus 3.5 ± 1.1 ($\times 10^9$/L), $p = 0.019$), hs-CRP (2.8±3.1 versus 2.2±2.8 mg/L, $p < 0.001$), and fibrinogen (3.2 ± 0.8 versus 3.0 ± 0.6 g/L, $p = 0.001$).

3.2. Correlations of Multiple Inflammatory Markers to Lipoprotein Subfractions. We next determined the strength of the relationship of multiple inflammatory markers with atherogenic lipoprotein subfractions. As shown in Table 2, after adjusting for age and sex, positive associations were observed between inflammatory markers and very low-density lipoprotein (VLDL) as well as intermediate-density lipoprotein (IDL). Among LDL subfractions, small LDL cholesterol was closely and positively related to WBC count ($p < 0.01$), neutrophil count ($p < 0.05$), lymphocyte count ($p < 0.01$), hs-CRP ($p < 0.01$), fibrinogen ($p < 0.001$), and ESR ($p < 0.05$). Similar results were found between LDL score and inflammatory markers. However, the large LDL cholesterol, which has been supposed to be less atherogenic

Table 1: Baseline characteristics.

Characteristics	All subjects (n = 520)	CAD (n = 351)	Non-CAD (n = 169)	p value
Coronary risk factors				
Age (years)	56.6 ± 10.0	57.9 ± 9.7	54.0 ± 10.2	<0.001
Male, % (n)	64.4 (335)	71.8 (252)	28.2 (99)	<0.001
BMI (kg/m^2)	25.7 ± 3.5	25.9 ± 3.4	25.4 ± 3.5	0.147
Smoking, % (n)	43.5 (226)	50.1 (176)	29.6 (50)	<0.001
Hypertension, % (n)	60.0 (312)	67.0 (235)	45.6 (77)	<0.001
Diabetes mellitus, % (n)	21.7 (113)	25.1 (88)	14.8 (25)	0.009
Dyslipidemia, % (n)	67.7 (352)	70.7 (248)	61.5 (104)	0.045
Family history of CAD, % (n)	16.0 (83)	17.9 (63)	11.8 (20)	0.096
Lipoprotein parameters				
VLDL cholesterol (mg/dL)	44.0 ± 12.3	44.0 ± 10.8	43.9 ± 14.9	0.253
IDL cholesterol (mg/dL)	48.7 ± 13.8	48.3 ± 13.4	49.4 ± 14.6	0.606
LDL cholesterol (mg/dL)	126.1 ± 38.7	125.7 ± 38.4	127.0 ± 39.3	0.723
Large LDL cholesterol	28.7 ± 10.1	28.3 ± 10.1	29.4 ± 10.1	0.277
Intermediate LDL cholesterol	20.5 ± 9.5	20.7 ± 9.5	20.1 ± 9.4	0.466
Small LDL cholesterol	8.6 ± 9.6	9.0 ± 9.8	7.9 ± 9.3	0.092
LDL score (%)	0.13 ± 0.13	0.14 ± 0.13	0.12 ± 0.13	0.067
Mean LDL particle size (Å)	266.7 ± 6.0	266.4 ± 5.9	267.2 ± 6.0	0.079
HDL cholesterol (mg/dL)	43.3 ± 13.9	42.5 ± 14.2	45.1 ± 13.1	0.054
Large HDL cholesterol	14.0 ± 7.4	13.5 ± 7.2	15.1 ± 7.7	0.027
Intermediate HDL cholesterol	20.9 ± 6.5	20.7 ± 7.0	21.2 ± 5.3	0.360
Small HDL cholesterol	8.6 ± 3.3	8.5 ± 3.4	8.8 ± 3.1	0.318
Inflammatory markers				
WBC count (×10^9/L)	6.1 ± 1.7	6.2 ± 1.8	5.9 ± 1.4	0.076
Neutrophil count (×10^9/L)	3.7 ± 1.4	3.8 ± 1.4	3.5 ± 1.1	0.019
Lymphocyte count (×10^9/L)	1.9 ± 0.6	1.9 ± 0.6	2.0 ± 0.5	0.260
Monocyte count (×10^9/L)	0.39 ± 0.29	0.40 ± 0.34	0.37 ± 0.12	0.147
hs-CRP (mg/L)	2.6 ± 9.8	2.8 ± 3.1	2.2 ± 2.8	<0.001
Fibrinogen (g/L)	3.1 ± 0.8	3.2 ± 0.8	3.0 ± 0.6	0.001
ESR (mm/h)	9.8 ± 9.7	10.1 ± 10.2	9.1 ± 8.6	0.463
D-dimer (μg/mL)	0.37 ± 0.44	0.40 ± 0.37	0.33 ± 0.56	0.115

Data are expressed as % (n), median (IQR), or mean ± SD. BMI: body mass index; CAD: coronary artery disease; VLDL: very low-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; WBC: white blood cell; hs-CRP: high sensitivity C-reactive protein; ESR: erythrocyte sedimentation rate.

than small LDL cholesterol, was not significantly linked with any inflammatory markers in the current study ($p > 0.05$, all). We further assessed the correlation between inflammatory markers and mean LDL particle size. Interestingly, our data indicated a definitely negative association (WBC count: $p < 0.01$; lymphocyte count: $p < 0.01$; hs-CRP: $p < 0.05$; fibrinogen: $p < 0.01$; and ESR: $p < 0.05$).

Additionally, in an analysis covering HDL subfractions, multiple inflammatory markers were correlated inversely with large HDL cholesterol (WBC count: $p < 0.01$; lymphocyte count: $p < 0.05$; hs-CRP: $p < 0.05$; fibrinogen: $p < 0.05$; and D-dimer: $p < 0.05$) but not with intermediate HDL cholesterol (only hs-CRP: $p < 0.05$) and small HDL cholesterol ($p > 0.05$, all).

3.3. Relation of Multimarker Inflammatory Index to Lipoprotein Subfractions. Of the individual inflammatory markers, WBC count, hs-CRP, fibrinogen, and ESR were closely related to atherogenic lipoprotein subfractions; therefore, we extracted a multimarker inflammatory index weighting the coefficients of the four individual markers. Consequently, we divided this multimarker index into quartiles. As indicated in Table 3, in a model adjusting for age, sex, body mass index, hypertension, diabetes, smoking, and incidence of CAD, the levels of intermediate LDL cholesterol, small LDL cholesterol, and LDL score were dramatically increased while the mean LDL particle size was decreased according to multimarker index quartiles ($p < 0.01$, all). Besides that, the large HDL cholesterol levels were markedly declined by multimarker index quartiles ($p < 0.05$).

3.4. Subgroup Analysis in Patients with or without CAD. Given that larger percentage of patients with CAD tended to have more severe inflammation (Figure 2), we further

Table 2: Age- and sex-adjusted correlations between lipoprotein subfractions and inflammatory markers.

	WBC count	Neutrophil count	Lymphocyte count	Monocyte count	hs-CRP	Fibrinogen	ESR	D-dimer
VLDL cholesterol (mg/dL)	0.133**	0.112*	0.124**	−0.019	0.129**	0.189***	0.154***	−0.055
IDL cholesterol (mg/dL)	0.055	0.080	0.009	−0.053	0.115**	0.129**	0.090*	−0.042
LDL cholesterol (mg/dL)	0.078	0.079	0.053	−0.028	0.112*	0.090*	0.020	−0.056
Large LDL cholesterol	−0.054	−0.020	−0.075	−0.059	0.008	−0.031	−0.064	−0.008
Intermediate LDL cholesterol	0.094*	0.072	0.102*	0.001	0.153***	0.095*	0.045	−0.077
Small LDL cholesterol	0.128**	0.099*	0.120**	0.007	0.121**	0.141***	0.111*	−0.054
LDL score (%)	0.124**	0.095*	0.111*	0.009	0.099*	0.139**	0.125**	−0.057
Mean LDL particle size (Å)	−0.115**	−0.084	−0.117**	−0.016	−0.096*	−0.117**	−0.111*	0.071
HDL cholesterol (mg/dL)	−0.093*	−0.064	−0.072	−0.059	−0.106*	−0.075	−0.034	0.063
Large HDL cholesterol	−0.123**	−0.089*	−0.104*	−0.052	−0.085	−0.092*	−0.060	0.104*
Intermediate HDL cholesterol	−0.077	−0.055	−0.053	−0.053	−0.090*	−0.047	−0.029	0.042
Small HDL cholesterol	−0.013	−0.006	−0.001	−0.036	−0.085	−0.057	0.004	−0.061

Partial correlations are shown. All the correlations were adjusted for age and sex. VLDL: very low-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; WBC: white blood cell; hs-CRP: high sensitivity C-reactive protein; ESR: erythrocyte sedimentation rate.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

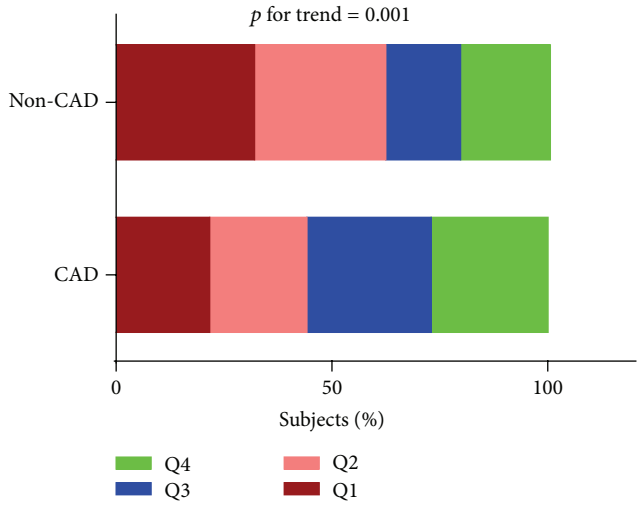

Figure 2: The distribution of subjects in CAD and non-CAD group across multimarker inflammatory index quartiles. Chi-squared test was performed.

performed the subgroup analysis to explore the association between inflammation and atherogenic lipoprotein subfractions in patients with or without CAD. As indicated in Figure 3, we found positive associations of multimarker index quartiles with small LDL cholesterol (CAD: $\beta = 0.183$, $p = 0.001$; non-CAD: $\beta = 0.159$, $p = 0.039$) and LDL score (CAD: $\beta = 0.176$, $p = 0.001$; non-CAD: $\beta = 0.169$, $p = 0.029$) and negative associations with mean LDL particle size (CAD: $\beta = -0.163$, $p = 0.002$; non-CAD: $\beta = -0.160$, $p = 0.039$). Although the relationships were both significant in patients with or without CAD, the former tended to be slightly stronger in the present study.

4. Discussion

The present study confirms the low-grade systemic inflammatory markers are related to features of the circulating cholesterol levels. More importantly, the main and novel findings are that (1) multiple systemic inflammatory markers are positively correlated with the most atherogenic lipoprotein subfractions, such as small LDL cholesterol and LDL score; (2) they are negatively linked with mean LDL particle size; (3) and they are inversely related to the antiatherogenic subfraction, large HDL cholesterol. These findings suggest that the mutual interplay may be a potential major contributor in the development of atherosclerotic disease.

Although the notion that elevated inflammatory markers increase the risk of cardiovascular disease (CVD) has been increasingly recognized [22, 23], underlying mechanisms and pathways remain to be elucidated. Specifically, inflammation and dyslipidemia are well established cardiovascular risk factors and closely associated with each other. However, it remains unclear with regard to which comes first in the atherosclerotic process. As reported, inflammation could affect lipoprotein metabolism [12], which is reflected by decreased plasma HDL-C levels and impaired atheroprotective HDL functions [24]. Additionally, it also has been suggested that the relationship between inflammatory markers and atherosclerosis is independent of plasma lipoprotein levels [25]. Conversely, the presence of dyslipidemia itself may in turn further stimulate the inflammatory process [26]. There are evidences that inflammation could be elicited by modified lipoproteins such as oxidized LDL [27], as well as

Table 3: Relationship of LDL and HDL subfractions with multimarker inflammatory index.

	Multimarker inflammatory index				
	Q1 (n = 130)	Q2 (n = 130)	Q3 (n = 130)	Q4 (n = 130)	p for trend
LDL subfractions					
Large LDL cholesterol (mg/dL)					
Model 1	30.07 ± 0.89	27.92 ± 0.89	28.44 ± 0.89	28.03 ± 0.89	0.289
Model 2	30.36 ± 0.89	27.84 ± 0.89	28.30 ± 0.89	27.96 ± 0.89	0.157
Model 3	30.01 ± 0.91	27.89 ± 0.89	28.53 ± 0.91	28.06 ± 0.90	0.338
Intermediate LDL cholesterol (mg/dL)					
Model 1	18.02 ± 0.83	20.76 ± 0.83	21.19 ± 0.83	22.02 ± 0.83	0.005
Model 2	18.03 ± 0.84	20.62 ± 0.83	21.29 ± 0.84	22.06 ± 0.83	0.005
Model 3	18.06 ± 0.85	20.72 ± 0.83	21.23 ± 0.85	22.01 ± 0.83	0.009
Small LDL cholesterol (mg/dL)					
Model 1	5.87 ± 0.87	8.92 ± 0.87	9.46 ± 0.87	10.89 ± 0.87	0.001
Model 2	5.73 ± 0.87	8.72 ± 0.87	9.69 ± 0.87	11.00 ± 0.87	<0.001
Model 3	5.79 ± 0.90	8.74 ± 0.87	9.64 ± 0.89	10.98 ± 0.87	0.001
LDL score (%)					
Model 1	0.09 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	<0.001
Model 2	0.09 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	<0.001
Model 3	0.10 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.001
Mean LDL particle size (Å)					
Model 1	268.42 ± 0.52	266.38 ± 0.52	266.21 ± 0.52	265.51 ± 0.52	0.001
Model 2	268.54 ± 0.53	266.44 ± 0.52	266.09 ± 0.53	265.45 ± 0.52	<0.001
Model 3	268.39 ± 0.54	266.45 ± 0.52	266.18 ± 0.53	265.49 ± 0.52	0.001
HDL subfractions					
Large HDL cholesterol (mg/dL)					
Model 1	15.29 ± 0.64	13.57 ± 0.64	14.03 ± 0.64	13.02 ± 0.64	0.080
Model 2	15.97 ± 0.61	13.75 ± 0.61	13.44 ± 0.61	12.73 ± 0.61	0.002
Model 3	15.33 ± 0.60	13.62 ± 0.59	13.95 ± 0.60	13.04 ± 0.59	0.049
Intermediate HDL cholesterol (mg/dL)					
Model 1	21.69 ± 0.57	20.70 ± 0.57	20.27 ± 0.57	20.65 ± 0.57	0.329
Model 2	22.07 ± 0.56	20.71 ± 0.56	20.02 ± 0.56	20.52 ± 0.56	0.066
Model 3	21.96 ± 0.57	20.68 ± 0.56	20.08 ± 0.57	20.58 ± 0.56	0.129
Small HDL cholesterol (mg/dL)					
Model 1	8.52 ± 0.29	8.84 ± 0.29	8.49 ± 0.29	8.40 ± 0.30	0.740
Model 2	8.56 ± 0.30	8.79 ± 0.29	8.50 ± 0.30	8.40 ± 0.29	0.815
Model 3	8.63 ± 0.30	8.80 ± 0.29	8.42 ± 0.30	8.37 ± 0.29	0.729

Values were obtained from general linear models. Model 1 was unadjusted. Model 2 was adjusted for age and sex. Model 3 was additionally adjusted for BMI, hypertension, diabetes, smoking, and incidence of CAD. BMI: body mass index; CAD: coronary artery disease; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

the TG-rich lipoprotein remnants [28]. Thus, the interplay between inflammation and lipid metabolism at multiple levels may exacerbate the development of atherosclerosis, resulting in a vicious cycle. However, the traditionally measured cholesterol levels in LDL and HDL particles could not capture all the high LDL or low HDL related risks [29, 30] and other alternative measures reflecting the particle have emerged in multiple studies.

Recently, lipoprotein subfractions have been suggested as a new cardiovascular risk strategy [31]. Experimental and turnover studies have raised the possibility that small LDL may be more atherogenic than buoyant LDL [7]. The Atherosclerosis Risk in Communities study including 11419 participants revealed that small dense LDL cholesterol was associated with the incident CAD [32]. Moreover, Nishikura et al. conducted a study including 190 consecutive CAD patients. During a seven-year follow-up period, small dense LDL cholesterol has been supposed to be a very promising biomarker in predicting future cardiovascular events [33]. Despite the cholesterol levels in LDL subfractions, mean LDL particle size has also been indicated to be closely related to cardiovascular mortality [34]. Currently, the link of HDL subclasses to prognosis remains controversial. Most studies including our data tended to support the idea that decreased large HDL-C level may be more atherogenic than other HDL subfractions [35, 36].

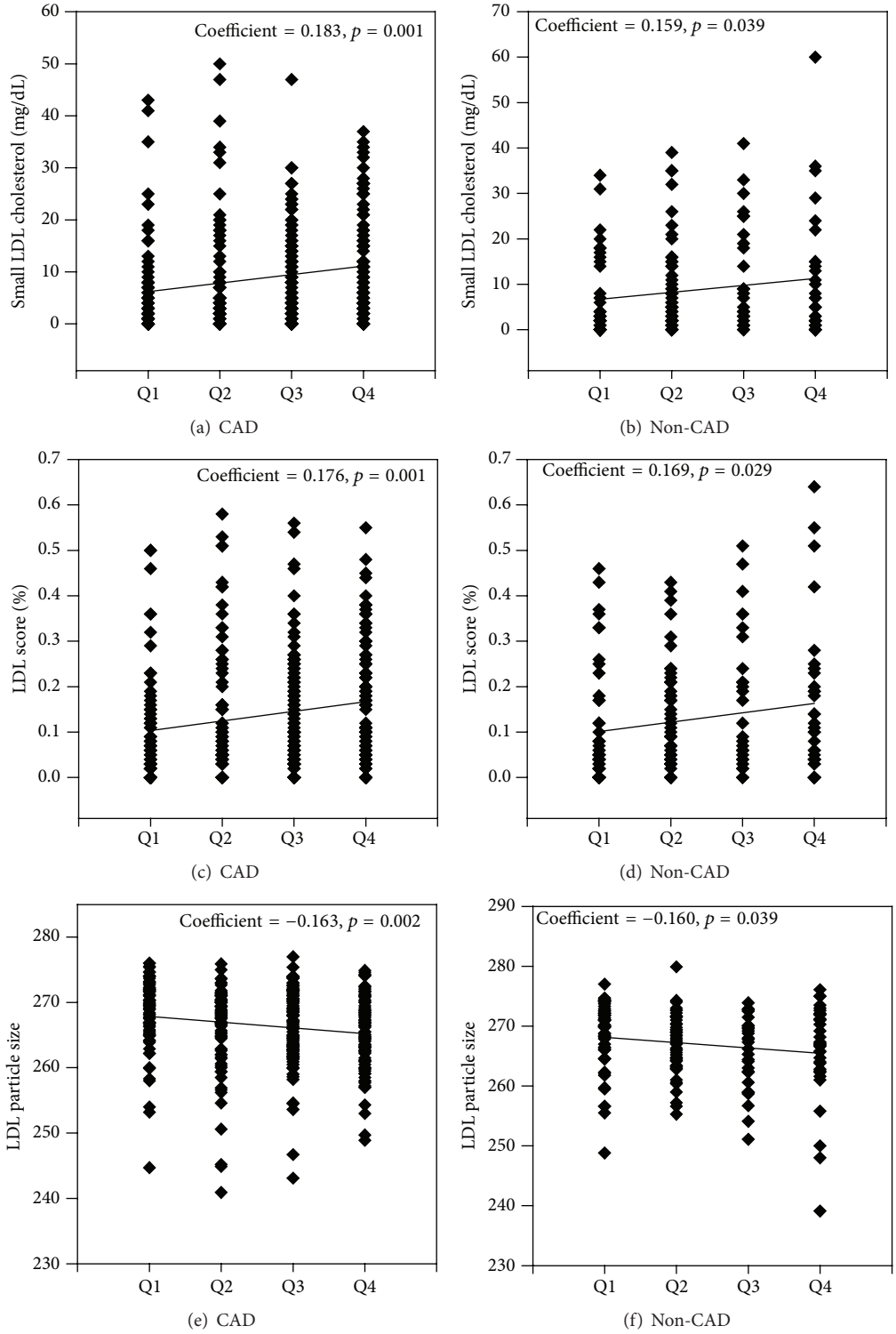

Figure 3: Relation of small LDL-C, LDL score, and mean LDL particle size to the quartiles of multimarker inflammatory index in patients with or without CAD ((a, b) small LDL-C; (c, d) LDL score; (e, f) mean LDL particle size). Simple linear regression analysis was applied.

In view of these heterogeneous and complex relationships, it is important to characterize the role of individual markers of inflammation in relation to different lipoprotein subfractions. In the present study, we employed five kinds of inflammatory markers reflecting the chronic systemic inflammatory activity and found that most of the inflammatory markers are positively associated with atherogenic lipoprotein subfractions in patients that underwent coronary

angiography. Of additional importance, we derived a composite marker of inflammation from a principle component analysis. In doing so, we were able to combine each of the individual markers into a single component, thereby reflecting a general marker of inflammation, permitting us to retain most of the information attributed to each marker. As a result, we observed that the atherogenic lipoprotein subfractions were significantly increased by multimarker index in patients with or without CAD, although it was slightly stronger in the CAD group. These novel findings may suggest that the correlation between inflammatory markers and atherogenic lipoprotein subfractions was stable and persistent and mildly modified by CAD status. Nonetheless, future investigations will have to verify these relationships in different populations and address the exact mechanisms.

The present study is not without limitations. First, it included patients scheduled for angiography. This hospital-based population may not be representative of a random population sample. Second, it was a cross-sectional study, so it was difficult to identify the causal or temporal relationship. Finally, we estimated the lipoprotein subfractions with Lipoprint System and the use of other methods should be investigated in the future.

5. Conclusion

In summary, systemic inflammatory markers are positively correlated with small LDL cholesterol and LDL score while being negatively linked with mean LDL particle size and large HDL cholesterol, highlighting the potential contribution to increased cardiovascular risk.

Conflict of Interests

The authors have no conflict of interests to disclose.

Acknowledgments

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Research Article

Association of Metabolic Syndrome with the Adiponectin to Homeostasis Model Assessment of Insulin Resistance Ratio

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This study aimed at determining whether the adiponectin to HOMA-IR (A/H) ratio is associated with MetS and MetS components and comparing the diagnostic efficacy of adiponectin, HOMA-IR, and the A/H ratio in healthy, middle-aged participants. MetS was assessed in 1628 Kazakh participants (men, 768; women, 860). The associations between adiponectin, HOMA-IR, and the A/H ratio with the components of MetS and MetS were examined using logistic regression analysis and receiver operating characteristic (ROC) curves. Our results show that A/H ratio may be a better diagnostic marker for MetS than either HOMA-IR or adiponectin alone, and it may serve as an important biomarker to determine an increased risk for MetS in healthy middle-aged population.

1. Introduction

Metabolic syndrome (MetS) refers to several interrelated cardiometabolic risk factors including dysglycemia, obesity (particularly central adiposity), elevated blood pressure, elevated triglyceride (TG) levels, and low high-density lipoprotein cholesterol (HDL-C) levels [1, 2]. The prevalence of MetS is approximately 25% in adults, and it is increasing [3, 4]. MetS and its components are associated with an increased risk of type 2 diabetes and cardiovascular disease [5, 6]. The risks of heart disease, stroke, and diabetes are increased 1.5- to 3-fold in people with MetS compared with people without MetS [7]. As a result, MetS is now both public health and clinical problem [8]. Therefore, to decrease the incidence, there is a need to establish a suitable and sensitive screening marker to identify individuals at high risk for MetS.

The accumulated evidence indicates that insulin resistance (IR) with compensatory hyperinsulinemia is an important pathogenic factor for MetS [9, 10], although a precise mechanism linking a specific MetS component with IR is lacking [11, 12]. In epidemiological studies, the homeostasis model assessment-insulin resistance (HOMA-IR) acts as an important index of IR [13, 14].

Adiponectin, which is the most abundant circulating adipokine, is recognized as a critical regulator of insulin sensitivity [15, 16], tissue inflammation [17, 18], and lipid metabolism [19, 20]. Furthermore, a growing body of evidence suggests that decreased serum adiponectin is associated with most of the MetS components and therefore MetS [21, 22].

Hyperinsulinemia might have a negative impact on circulating adiponectin levels, thereby causing IR. HOMA-IR and adiponectin are thought to represent two different and opposite aspects of IR. The adiponectin concentration to HOMA-IR ratio (A/H ratio) is expected to be more sensitive than either parameter alone for the evaluation of MetS risk.

The A/H ratio as an index of MetS was first proposed in 2011 [23]. However, this study included an aged Japanese sample, and the analysis included markers that were measured only once. The association between the A/H ratio and MetS is yet to be confirmed, owing to limited evidence. Therefore, studies are needed to determine if there is a relationship

between the A/H ratio and MetS and if this relationship is stronger than the individual parameters. This study aimed at determining whether the A/H ratio is associated with MetS and comparing the strength of the associations between MetS and adiponectin, HOMA-IR, and the A/H ratio.

2. Materials and Methods

2.1. Ethics Statement. The Institutional Ethics Review Board (IERB) at the First Affiliated Hospital of Shihezi University School of Medicine approved the study (IERB number SHZ2009LL05). Standard university hospital guidelines including informed consent, voluntary participation, confidentiality, and anonymity were followed. All participants provided written informed consent before participation.

2.2. Settings and Participants. The survey was conducted from 2009 to 2013 in Xinyuan County, Xinjiang, which is located approximately 4,400 km (2,739 miles) from Beijing; approximately 98% of the population is Kazakhs. Multistage (prefecture-county-township-village) stratified cluster random sampling was used to select the participants. At the beginning of the study, we chose the Yili prefecture based on the geographical distributions of the minority populations in Xinjiang. We randomly selected one county in Yili prefecture and one township from each county (Nalati Township in Xinyuan County). During the last stage, a stratified sampling method was used to select corresponding villages in each township (3 villages in Nalati Township). We interviewed local Kazakhs aged ≥ 18 years who had resided in the village for at least 6 months. We successfully interviewed a total of 1628 individuals (860 women and 768 men). Exclusion criteria included acute illness within the previous 2 weeks, currently taking medication, cancer, and pregnancy. The overall response rate was 87.0%.

2.3. Anthropometric Measurements and Laboratory Tests. Each participant was interviewed using a structured questionnaire to collect general and demographic information (age and sex) as well as cigarette smoking history (never smoked, ex-smoker, or current smoker). Waist circumference (cm) was measured midway between the lower rib and iliac crest. Weight (kg) and height (m) were measured with the participants in light clothing. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2) and expressed as kg/m^2. Casual blood pressure (BP) was measured 3 times after a 5 min rest in the sitting position using a mercury sphygmomanometer, and an average of 3 measurements was used for analyses. After the physical examination, a blood sample was drawn from the cubital vein in the morning after an overnight fast and was placed in tubes containing heparin sodium. The blood was centrifuged at 2000 rpm for 10 min, and plasma was then separated and stored at −70°C until analysis. Total cholesterol (TC), TG, low-density lipoprotein cholesterol (LDL-C), HDL-C, and fasting blood glucose (FBG) levels were measured using a biochemical autoanalyzer (Olympus AU 2700; Olympus Diagnostics, Hamburg, Germany) in the clinical laboratory at the First Affiliated Hospital of Shihezi University School of Medicine.

The circulating levels of interleukin- (IL-) 6 were determined using ELISA kits (Shanghai Westang Bio-Tech Co. Ltd.). Adiponectin levels were determined using ELISA kits (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). All procedures described in the manufacturer's instructions were followed with quality control parameters within the expected range recommended by the manufacturer. Every tenth sample was duplicated on the same plate. The minimum detectable concentration of IL-6 kit is 0.8 pg/mL with the intra-assay CV < 3% and the interassay CV < 6.9%. The minimum detectable concentration of adiponectin kit is 0.15 ng/mL, with the intra-assay CV ranged from 3 to 6% and the interassay CV < 10%. Insulin level was measured by radioimmunoassay. The HOMA-IR index was defined as follows: fasting insulin (in micro-international units (μIU) per mL) × FBG (in mM)/22.5 [7].

2.4. Definition of MetS. MetS was defined using the International Diabetes Federation (IDF) criteria [24], which include central obesity (waist circumference ≥ 90 cm in men or ≥ 80 cm in women, Chinese population waist circumference cutoffs [25]) plus any 2 of the following 4 factors: elevated TG level (>150 mg/dL or 1.69 mmol/L); reduced HDL-C (<40 mg/dL or 1.04 mmol/L in men; <50 mg/dL or 1.29 mmol/L in women); elevated systolic BP (≥130 mmHg) or diastolic BP (≥85 mmHg); and elevated FBG (≥100 mg/dL).

MetS was also defined using the revised National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria [26], which have any three or more of the following: waist circumference ≥ 90 cm in men or ≥80 cm in women (Chinese population waist circumference cutoffs [25]); triglyceride level ≥ 150 mg/dL or taking medication for increased triglycerides; high-density lipoprotein cholesterol (HDL-C) level < 40 mg/dL or taking medication to improve HDL-C; systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or taking antihypertensive agent; fasting glucose level ≥ 100 mg/dL or taking blood glucose-lowering agent.

2.5. Statistical Analysis. Continuous variables are presented as mean ± standard deviation (SD) for clinical characteristics or median (interquartile range) for IL-6, adiponectin, and fasting insulin levels. These variables were compared using unpaired *t*-tests or Mann-Whitney *U* tests. The partial correlation coefficient was used to analyze the association between adiponectin, HOMA-IR, A/H ratio, and other continuous variables of interest, controlling for the effect of age. Multivariable logistic regression analysis with MetS as the dichotomous dependent variable was conducted to determine the association between adiponectin, HOMA-IR, A/H ratio, and MetS. The resulting odds ratios (ORs) and 95% confidence intervals (CIs) are reported. The receiver operating characteristic (ROC) analyses were used to describe the ability of the adiponectin, HOMA-IR, and A/H ratio to differentiate between subjects with and without metabolic syndrome. ROC analyses were also used to evaluate the

Table 1: General characteristics of the study participants according to presence or absence of MetS.

Parameters	Men (n = 768)			Women (n = 860)		
	Without MetS (n = 504)	With MetS (n = 264)	p	Without MetS (n = 593)	With MetS (n = 267)	p
Anthropometric characteristics						
Age (y)	44.44 ± 13.98	50.34 ± 11.47	<0.01	40.17 ± 12.25	49.38 ± 11.48	<0.01
Waist circumference (cm)	83.07 ± 9.31	99.14 ± 8.17	<0.01	77.46 ± 9.67	91.52 ± 8.27	<0.01
BMI (kg/m^2)	23.10 ± 3.09	28.60 ± 3.37	<0.01	22.60 ± 3.43	27.39 ± 3.65	<0.01
Systolic BP (mmHg)	126.80 ± 21.61	144.60 ± 20.34	<0.01	122.60 ± 19.89	144.73 ± 25.60	<0.01
Diastolic BP (mmHg)	81.59 ± 13.33	92.39 ± 12.12	<0.01	78.72 ± 12.26	92.03 ± 12.85	<0.01
Current smoker (n [%])	258 (51.29%)	214 (81.06%)	<0.01	231 (38.89%)	214 (80.15%)	<0.01
Metabolic characteristics						
Total cholesterol (mmol/L)	4.14 ± 0.98	4.75 ± 1.23	<0.01	4.13 ± 1.08	4.57 ± 1.28	<0.01
TG (mmol/L)	1.01 ± 0.48	2.06 ± 1.30	<0.01	0.93 ± 0.42	1.55 ± 0.87	<0.01
HDL cholesterol (mmol/L)	1.42 ± 0.42	1.38 ± 0.57	<0.01	1.60 ± 0.43	1.31 ± 0.49	<0.01
LDL cholesterol (mmol/L)	2.19 ± 0.69	2.53 ± 0.97	<0.01	2.05 ± 0.69	2.49 ± 0.82	<0.01
FBG (mmol/L)	4.47 ± 0.93	5.57 ± 1.47	<0.01	4.28 ± 0.79	5.19 ± 1.22	<0.01
Insulin (μIU/dL)	10.80 (7.01–14.72)	14.95 (12.30–17.00)	<0.01	9.60 (7.20–15.50)	14.61 (8.40–17.50)	<0.01
HOMA-IR	2.05 (1.32–3.00)	3.31 (2.47–4.54)	<0.01	1.81 (1.37–3.08)	3.28 (1.77–4.31)	<0.01
Adiponectin (μg/mL)	5.78 (4.18–6.63)	4.04 (2.67–5.65)	<0.01	6.98 (5.43–8.40)	5.70 (3.93–8.12)	<0.05
A/H ratio	2.43 (1.50–3.95)	1.08 (0.75–1.91)	<0.01	3.30 (2.08–5.00)	2.02 (1.00–3.57)	<0.01
IL-6 (pg/mL)	0.65 (0.16–1.39)	1.58 (1.18–1.97)	<0.01	0.53 (0.15–1.33)	1.60 (0.99–2.01)	<0.01

Values are expressed as means ± SD or number (%), if not stated otherwise. Median values of adiponectin, fasting insulin, IL-6, and HOMA-IR are presented (lower quartile-upper quartile).

SD, standard deviation; BMI, body mass index; TG, triglyceride; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; IL-6, interleukin-6; A/H ratio, adiponectin to homeostasis assessment-insulin resistance.

difference in the contribution of the adiponectin, HOMA-IR, and A/H ratio to the risk levels of each component of MetS. All analyses were performed using SPSS v17.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences with a p value of <0.05 were considered statistically significant.

3. Results

The characteristics of the study population, based on sex and presence of MetS, are provided in Table 1. IL-6 levels, insulin levels, HOMA-IR, and other anthropometric and metabolic characteristics were significantly greater in the MetS group than in the non-MetS group in both men and women (p < 0.05). In contrast, adiponectin levels, the A/H ratio, and HDL-C levels were significantly lower in the MetS group than in the non-MetS group (p < 0.05).

The correlations between the adiponectin levels, HOMA-IR, and A/H ratio and the risk factors of MetS are presented in Table 2. Adiponectin levels and the A/H ratio were negatively correlated with waist circumference, BMI, TC, TG, FBG, insulin, IL-6, and HOMA-IR (all p < 0.05). The correlation coefficients for BMI, waist circumference, TG, FBG, LDL, insulin, IL-6, and HOMA-IR with the A/H ratio were higher than those with adiponectin.

The multivariable adjusted ORs (95% CI) showed that the highest quartiles of adiponectin, HOMA-IR, and the A/H ratio were significantly associated with MetS, compared with the lowest quartiles (Table 3). In models I, II, and III, the adjusted ORs for MetS were higher with the A/H ratio than with adiponectin. In model III, which was adjusted for sex, age, smoking status, LDL-C, TC, and HDL-C, adiponectin (OR, 0.30; 95% CI, 0.19–0.46), HOMA-IR (OR, 3.82; 95% CI, 2.42–6.04), and A/H ratio (OR, 0.25; 95% CI, 0.15–0.40) remained significantly associated with MetS.

Receiver operating characteristic (ROC) analysis was performed to detect the performance of the adiponectin, HOMA-IR, and A/H ratio as a diagnostic marker for MetS defined by the IDF and ATP III (Figure 1). As Figure 1 shows, the area under curve (AUC) of the A/H ratio, HOMA-IR, and adiponectin to detect MetS was 0.727, 0.707, and 0.639, respectively, by IDF criteria and 0.773, 0.747, and 0.715, respectively, by ATP III criteria. In addition we estimated that the best cutoff value for the A/H ratio to identify a risk of MetS was 2.10 (sensitivity, 0.68; specificity, 0.67), by IDF criteria. We estimated that the best cutoff value for the A/H ratio to identify a risk of MetS was 1.89 (sensitivity, 0.76; specificity, 0.67), by ATP III criteria.

The adjusted ORs (95% CI) and AUC to detect the MetS components are shown in Table 4. After adjustment for age, BMI, smoking status, and LDL-C, adiponectin, HOMA-IR, and the A/H ratio were all significantly associated with the MetS components. Except for BP, the ORs for the MetS components were lower for the A/H ratio than for adiponectin. Except for low HDL and abnormal glucose, the

Table 2: Partial correlation analysis among adiponectin, HOMA-IR, A/H ratio, and risk factors of MetS.

	A/H ratio	Adiponectin	HOMA-IR
Waist circumference	−0.220 (<0.001)	−0.212 (<0.001)	−0.210 (<0.001)
BMI	−0.259 (<0.001)	−0.254 (<0.001)	−0.222 (<0.001)
Systolic BP	−0.056 (0.025)	−0.121 (<0.001)	0.050 (0.043)
Diastolic BP	−0.085 (0.001)	−0.132 (<0.001)	0.071 (0.004)
Total cholesterol	−0.076 (0.002)	−0.089 (<0.001)	0.212 (<0.001)
TG	−0.172 (<0.001)	−0.016 (<0.001)	0.222 (<0.001)
HDL cholesterol	0.047 (<0.058)	0.047 (0.061)	−0.052 (0.037)
LDL cholesterol	−0.069 (<0.001)	−0.055 (0.026)	0.212 (<0.001)
FBG	−0.261 (<0.001)	−0.091 (<0.001)	0.398 (<0.001)
Insulin	−0.485 (<0.001)	−0.059 (0.017)	0.875 (<0.001)
IL-6	−0.159 (<0.001)	−0.082 (0.001)	0.151 (<0.001)
Adiponectin	0.618 (<0.001)	—	−0.112 (<0.001)
HOMA-IR	−0.521 (<0.001)	−0.112 (<0.001)	—
A/H ratio	—	0.618 (<0.001)	−0.521 (<0.001)

Values are age- and gender-adjusted Spearman correlation coefficients and p values for correlations of adiponectin, HOMA-IR, and A/H ratio with risk factors of MetS.
BMI, body mass index; BP, blood pressure; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBG, fasting blood glucose; HOMA-IR, homeostasis assessment-insulin resistance; A/H ratio, adiponectin to homeostasis assessment-insulin resistance; MetS, metabolic syndrome.

Table 3: Odds ratios and 95% confidence intervals for the association between metabolic syndrome and various markers.

	Odds ratio (95% CI)		
	Model I	Model II	Model III
Adiponectin			
1st quartile	1	1	1
2nd quartile	0.59 (0.44–0.79)	0.65 (0.44–0.95)	0.66 (0.44–0.99)
3rd quartile	0.29 (0.21–0.40)	0.58 (0.37–0.89)	0.60 (0.38–0.95)
4th quartile	0.28 (0.20–0.40)	0.34 (0.23–0.51)	0.30 (0.19–0.46)
HOMA-IR			
1st quartile	1	1	1
2nd quartile	1.63 (1.11–2.32)	1.62 (1.04–2.52)	1.72 (1.08–2.73)
3rd quartile	2.88 (2.02–4.10)	2.05 (1.33–3.16)	1.83 (1.16–2.88)
4th quartile	7.36 (5.19–10.46)	3.88 (2.52–5.97)	3.82 (2.42–6.04)
A/H ratio			
1st quartile	1	1	1
2nd quartile	0.30 (0.22–0.40)	0.53 (0.35–0.80)	0.52 (0.33–0.83)
3rd quartile	0.25 (0.18–0.16)	0.41 (0.28–0.60)	0.43 (0.28–0.66)
4th quartile	0.11 (0.08–0.16)	0.23 (0.15–0.35)	0.25 (0.15–0.40)

CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; A/H ratio, adiponectin to homeostasis assessment-insulin resistance; MetS, metabolic syndrome; IL-6, interleukin-6.
Model I: adjusted for sex and age.
Model II: adjusted for sex, age, body mass index, smoking status, and low-density lipoprotein cholesterol.
Model III: adjusted for sex, age, body mass index, smoking status, low-density lipoprotein cholesterol, total cholesterol, and high-density lipoprotein cholesterol.

AUCs of the MetS components were higher for the A/H ratio than for adiponectin and HOMA-IR.

4. Discussion

In this study, we found that the A/H ratio is more strongly associated with MetS and most of the MetS components than adiponectin. In addition, the A/H ratio showed greater predictive power than adiponectin and HOMA-IR for the risk of MetS. The A/H ratio is better at correctly classifying subjects with and without MetS than adiponectin or HOMA-IR alone.

Adiponectin is a multifunctional protein with pleiotropic insulin-sensitizing effects and is considered a key molecule in the pathogenesis of MetS [16, 27, 28]. In the present study, adiponectin levels were negatively correlated with waist circumference, BMI, TC, TG, FBG, insulin, IL-6, and HOMA-IR (all $p < 0.05$); when adjusted for sex, age, smoking status, and LDL-C, adiponectin remained significantly associated with MetS and MetS components. These findings are consistent with those of previous reports [21, 29]. Our previous results suggest that decreased adiponectin levels and HOMA-IR might be associated with IR and can predict the course of MetS [30].

The A/H ratio was significantly lower in the MetS group than in the non-MetS group ($p < 0.05$). Furthermore, the A/H ratio was significantly associated with MetS and MetS components after adjustment for sex, age, smoking status, and LDL-C. These results support the suggestion that the A/H ratio could be a powerful index for the evaluation of MetS [23]. However, there are few studies that have compared the strength of the associations between MetS and adiponectin and the A/H ratio or the ability of the A/H ratio to classify subjects with and without MetS. It is important to clarify the diagnostic power of adiponectin and the A/H ratio for future clinical use.

In our study, the A/H ratio showed a greater predicting power than adiponectin. For example, the correlation coefficients for BMI, waist circumference, TG, FBG, LDL, insulin, IL-6, and HOMA-IR with the A/H ratio were higher than those with adiponectin. Except for BP, the ORs for MetS and MetS components were lower for the A/H ratio than for adiponectin. We also conducted ROC analyses with the same participants using the IDF and updated ATP III definition for MetS, and the AUCs of the A/H ratio were higher than those for adiponectin and HOMA-IR by IDF and updated ATP III

Table 4: Odds ratios (95% CI) and ROC analysis for the association between each component of MetS and markers.

	Adiponectin (Q4 versus Q1)		HOMA-IR (Q4 versus Q1)		A/H ratio (Q4 versus Q1)	
	OR* (95% CI)	AUC (SE)	OR* (95% CI)	AUC (SE)	OR* (95% CI)	AUC (SE)
Abdominal obesity	0.37 (0.27–0.51)	0.58 (0.01)	2.74 (2.01–3.72)	0.61 (0.01)	0.20 (0.13–0.31)	0.63 (0.01)
High triglycerides	0.42 (0.28–0.65)	0.64 (0.02)	4.35 (2.82–6.71)	0.67 (0.02)	0.20 (0.13–0.31)	0.69 (0.02)
High blood pressure	0.33 (0.24–0.46)	0.61 (0.01)	1.55 (1.14–2.10)	0.55 (0.01)	0.44 (0.33–0.59)	0.61 (0.01)
Low HDL	0.71 (0.51–0.99)	0.51 (0.02)	1.74 (1.25–2.42)	0.57 (0.02)	0.50 (0.36–0.69)	0.55 (0.02)
Abnormal glucose	0.42 (0.28–0.63)	0.63 (0.02)	10.08 (6.32–16.06)	0.75 (0.02)	0.14 (0.09–0.23)	0.73 (0.02)

CI, confidence interval; AUC, area under the curve; SE, standard error; A/H ratio, adiponectin to homeostasis assessment-insulin resistance; MetS, metabolic syndrome; Q4, highest quartile; Q1, lowest quartile.
*Adjusted for sex, age, smoking status, and LDL cholesterol.

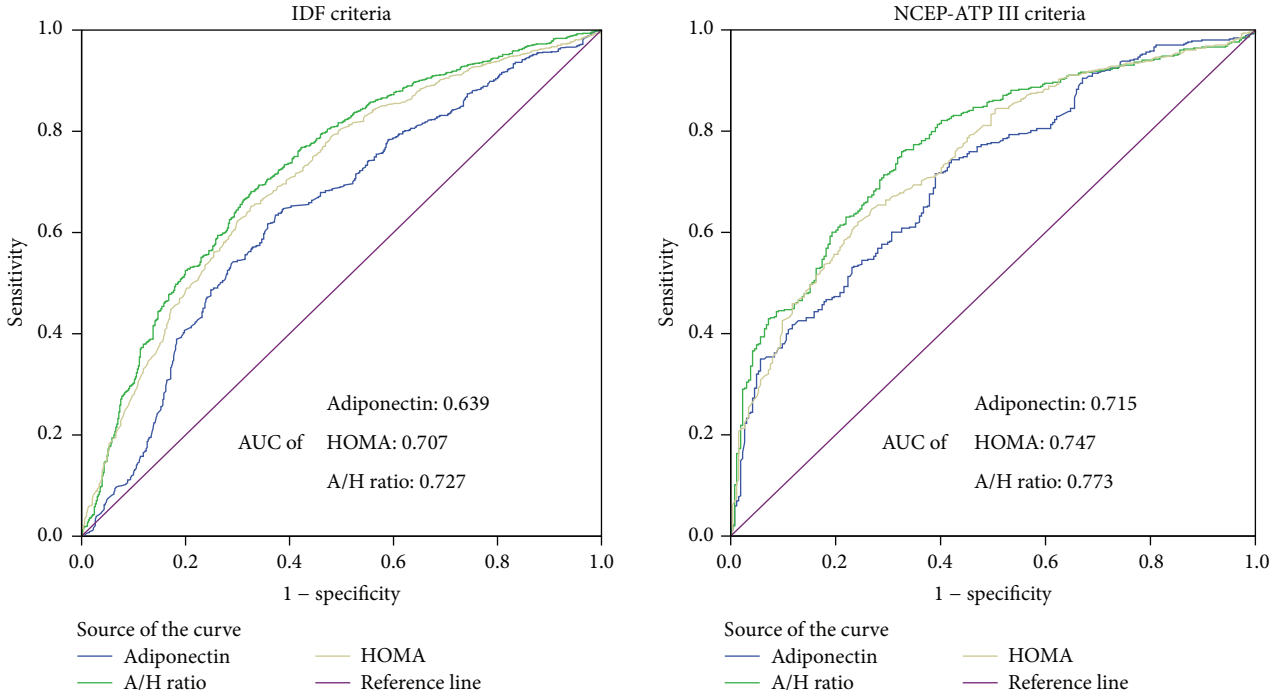

Figure 1: Comparison of predicting powers between adiponectin, HOMA-IR, and A/H ratio for difference metabolic syndrome criteria. HOMA-IR, homeostasis model assessment of insulin resistance; A/H ratio, adiponectin to homeostasis assessment-insulin resistance; ROC, receiver operating characteristic; AUC, area under the curve; IDF, International Diabetes Federation; NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panel III.

definition. In addition, except for low HDL and abnormal glucose, the AUCs of the MetS components were also higher for the A/H ratio than for adiponectin and HOMA-IR.

We performed the additional analysis to obtain the best cutoff for IDF and updated ATP III definition. We estimated that the best value for the A/H ratio to identify a risk of MetS was 2.10 (sensitivity, 0.68; specificity, 0.67), by IDF criteria. We estimated that the best cutoff value for the A/H ratio to identify a risk of MetS was 1.89 (sensitivity, 0.76; specificity, 0.67), by ATP III criteria. The A/H ratio has similar values of sensitivity and specificity with leptin/adiponectin ratio [25, 31].

These results could further explain our finding that the A/H ratio has a significant adjunctive contribution, beyond that of the adiponectin and HOMA-IR alone, to metabolic syndrome.

This study had several limitations. First, the cross-sectional design was not able to determine a causal relationship between MetS or its components and adiponectin HOMA-IR and A/H ratio. Second, we did not evaluate high molecular weight adiponectin, which is considered to be more useful than total adiponectin in evaluating the MetS and IR [31, 32]. Further investigation regarding the role of the ratio of high molecular weight adiponectin and HOMA-IR in MetS is needed.

In conclusion, to the best of our knowledge, this was the first large-scale population-based study to compare the diagnostic efficiency of adiponectin, HOMA-IR, and the A/H ratio in healthy middle-aged participants. We demonstrated that the A/H ratio can act as a marker of MetS and its components, serving as an important surrogate biomarker for MetS risk, and the A/H ratio contributed more to MetS

than either HOMA-IR or adiponectin alone. As a result, this study provides useful information for clinicians to identify individuals at high risk of MetS. These results also show that the A/H ratio is helpful in understanding cardiometabolic diseases.

Abbreviations

BMI:	Body mass index
BP:	Blood pressure
TC:	Total cholesterol
TG:	Triglyceride
HDL:	High-density lipoprotein
LDL:	Low-density lipoprotein
FBG:	Fasting blood glucose
HOMA-IR:	Homeostasis assessment-insulin resistance
A/H ratio:	Adiponectin to homeostasis assessment-insulin resistance
MetS:	Metabolic syndrome
ORs:	Odds ratios
CIs:	Confidence intervals
ROC:	Receiver operating characteristic
AUC:	Area under the curve
IL-6:	Interleukin-6.

Disclosure

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the paper.

Conflict of Interests

None of the authors have any potential conflict of interests associated with this research.

Authors' Contribution

Shu-Xia Guo and Lie-Gang Liu conceived and designed the experiments. Yu-Song Ding, Ru-Lin Ma, Heng Guo, Jing-Yu Zhang, Mei Zhang, Jia-Ming Liu, and Shu-Gang Li performed the experiments. Yu-Song Ding analyzed and interpreted the data. Yu-Song Ding, Shu-Xia Guo, and Wen-Jie Zhang wrote the paper.

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