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EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
*World Journal of Critical Care Medicine*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

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Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai,
Hong Kong, China
Fax: +852-6555-7188
Telephone: +852-3177-9906
E-mail: bpgoffice@wjgnet.com
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REVIEW

# Metabolic theory of septic shock

Jay Pravda

Jay Pravda, Inflammatory Disease Research Centre, West Palm Beach, FL 33420, United States
Author contributions: Pravda J is the sole author of this manuscript and solely responsible for its content; Pravda J performed all the research, collected, analyzed and interpreted all the data; Pravda J conceived of and developed the Metabolic Theory of Septic Shock; Pravda J prepared and wrote the manuscript and performed all critical revisions; Pravda J certifies that the Metabolic Theory of Septic Shock is the product of his original research and Pravda J has overall responsibility for this manuscript.
Correspondence to: Jay Pravda, MD, MPH, Inflammatory Disease Research Centre, West Palm Beach, P.O. Box 32632, FL 33420, United States. jaypravda@yahoo.com
Telephone: +1-682-2513030 Fax: +1-888-7005813
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## Abstract

Septic shock is a life threatening condition that can develop subsequent to infection. Mortality can reach as high as 80% with over 150000 deaths yearly in the United States alone. Septic shock causes progressive failure of vital homeostatic mechanisms culminating in immunosuppression, coagulopathy and microvascular dysfunction which can lead to refractory hypotension, organ failure and death. The hypermetabolic response that accompanies a systemic inflammatory reaction places high demands upon stored nutritional resources. A crucial element that can become depleted early during the progression to septic shock is glutathione. Glutathione is chiefly responsible for supplying reducing equivalents to neutralize hydrogen peroxide, a toxic oxidizing agent that is produced during normal metabolism. Without glutathione, hydrogen peroxide can rise to toxic levels in tissues and blood where it can cause severe oxidative injury to organs and to the microvasculature. Continued exposure can result in microvascular dysfunction, capillary leakage and septic shock. It is the aim of this paper to present evidence that elevated systemic levels of hydrogen peroxide are present in septic shock victims and that it significantly contributes to the development and progression of this frequently lethal condition.



**Key words:** Septic shock; Hydrogen peroxide; Hypermetabolic; Sepsis; Systemic inflammatory response syndrome

**Core tip:** For decades septic shock has been attributed to an over-active immune response. However, immune modulation has failed to reduce mortality, casting doubt on a direct causal role for the immune response in the development of septic shock. A closer look suggests that septic shock is the result of a generalized build-up of hydrogen peroxide, a toxic cellular by-product generated as a consequence of the hypermetabolic state that accompanies a systemic immune response. This finding points to the systemic accumulation of hydrogen peroxide as a significant risk factor for the development of septic and non-septic shock syndromes.

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## INTRODUCTION

Sepsis is a life threatening condition that is associated with a systemic inflammatory response to a microbial infection[1]. Sepsis is the most common cause of mortality in the intensive care unit with a fatality rate that can rise to 80% for those developing multiple organ failure. The progression of an exaggerated systemic inflammatory response is thought to be responsible for the eventual development of septic shock and death[2]. However, multiple therapeutic efforts aimed at controlling the immune

response with the intent of interrupting the process leading to organ failure have been uniformly unsuccessful[1]. This simple fact has prompted a reappraisal of the role played by the immune system in the development of this condition that kills more than 150000 Americans yearly; more than breast, colon, prostate and brain cancer combined[3,4].

Although immune activation is clearly evident, recent evidence suggests that the immune response may not be the direct mediator of the pathologic process that leads to septic shock. Studies conducted to define the circulating leukocyte transcriptome have revealed that there is no qualitative difference in the immunogenetic response when comparing burn or blunt trauma patients with complicated or uncomplicated outcomes. In other words, severely injured patients who die from their injuries have the same immunogenetic response as patients who recover; the only difference being the duration and intensity of systemic inflammation[5].

The lack of a unique immunogenetic response suggests that septic shock is the phenotypic expression of a separate process that is initiated simultaneously with systemic immune activation. The multiple organ involvement, which can lead to death within a few days, suggests that this concomitant process is systemic in nature and initiated in parallel with inflammatory response. Moreover, the microvascular edema associated with multiple organ failure, which persists despite efforts at immunosuppression, suggests that a non-immune mediated angiopathic agent is being released into the systemic circulation[1].

The high (8%) increased mortality rate for each hour of delay before instituting antibiotics after the onset of hypotension suggests that the duration of this parallel process is closely linked with a greater risk of an adverse outcome and down regulating the immune response with successful therapy simply allows this parallel process to turn off[6].

In other words, survival is closely correlated with the early down regulation of a systemic process closely linked to systemic immune activation suggesting depletion of a crucial biochemical element that is critical for survival. Put differently, if catabasis (immune down regulation) is achieved by successful antibiotic therapy prior to depletion of this critical element the patient will survive, if not the patient is at high risk for organ failure, septic shock and death.

## HYPERMETABOLIC RESPONSE

A key systemic process that is turned on and up-regulated with systemic inflammation is cellular metabolism, which becomes hypermetabolic from the onset of sepsis[7]. The sustained high fever, highly amplified protein synthesis, tachycardia and tachypnea characteristic of a septic immune response requires supra-physiological energy supplies. It is estimated that basal energy requirements for a septic patient can reach up to 10000 calories daily[8]. This hypermetabolic state not only requires increased nutrient intake but also generates a large amount of toxic cellular by-products as a result of increased electron transport chain (ETC) activity required to synthesize sufficient adenosine triphosphate (ATP) to support a prolonged hypermetabolic state. This critical need for supplemental nutrients often cannot be met as it occurs at a time when caloric intake is curtailed as a result of the severe illness afflicting the patient[8]. This suggests the progressive depletion of an element whose principal function is to metabolize a toxic cellular waste product that, upon accumulation, leads to organ dysfunction, microangiopathic edema and refractory hypotension, the characteristic pathologic findings in septic shock.

An important toxic product that is continuously generated as a result of cellular metabolism is hydrogen peroxide ($H_2O_2$), which is formed as a result of several metabolic activities including protein synthesis (disulfide bond formation), DNA recycling (Xanthine oxidase), ATP synthesis (ETC activity) and fatty acid oxidation (peroxisomal metabolism)[9-12]. Most $H_2O_2$ is degraded to water via the enzymatic action of glutathione peroxidase (GPx), a selenium containing enzyme that has an obligate requirement for the co-factor glutathione (GSH) in order to metabolize $H_2O_2$. The biochemical reaction is: $2\ GSH + H_2O_2 \rightarrow GS\text{-}SG + 2H_2O$ in which two molecules of GSH are converted to one molecule of glutathione disulfide (GS-SG) and two molecules of water. Glutathione is consumed during this process and must be replenished in order for the cell to prevent accumulation of $H_2O_2$ to toxic levels[13,14].

Replenishment of glutathione, however, is not favored during periods of sustained hypermetabolism and caloric insufficiency, which frequently accompany critical illnesses such as sepsis leading to depletion of glutathione reserves.

Within 48 h of diagnosis critically ill children with sepsis were found to have a 60% decrease in whole blood GSH synthesis, suggesting depletion of whole body GSH stores[15,16]. Systemic GSH depletion is supported by studies showing over 50% decrease in lung and skeletal muscle GSH in septic and critically ill patients[17,18]. The critical importance of glutathione was demonstrated by a study which documented significantly decreased erythrocyte glutathione in septic non-survivors *vs* survivors ($P < 0.0001$)[19]. This suggests high levels of circulating $H_2O_2$ capable of permeating erythrocyte cell membranes and oxidizing (and depleting) intracellular glutathione in septic shock non-survivors. Elegant studies have also demonstrated a significantly higher mitochondrial respiratory rate in non-survivors at three months following sepsis suggesting that failure to down regulate the hypermetabolic state (and excess $H_2O_2$ production) is independently associated with higher mortality even after surviving the initial infectious insult[20].

Generalized depletion of body stores can result in cellular deficiency of GSH leading to a toxic accumulation of $H_2O_2$. A highly toxic oxidizing agent, $H_2O_2$ is the principal mediator of cellular oxidative damage. It does so by generating hydroxyl radical (OH*), the most

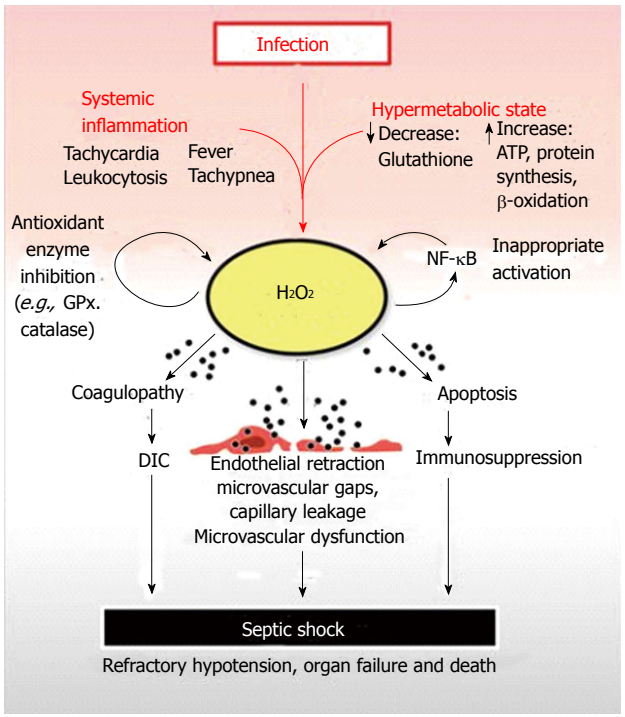


**Figure 1 Septic shock begins with a systemic inflammatory reaction to an infection.** A contemporaneous increase in metabolism is initiated, which can deplete reserves of critical nutrients such as glutathione. Glutathione is crucial for the neutralization of $H_2O_2$, a toxic, membrane-permeable oxidizing agent generated as a by-product of cellular metabolism. Depletion of cellular glutathione results in elevation of $H_2O_2$ which can diffuse out of organ parenchymal cells and into capillary endothelium before reaching the bloodstream. Once in the systemic circulation, excess $H_2O_2$ is distributed throughout the body resulting in systemic oxidative damage to plasma components, organs and blood vessels. The net result is $H_2O_2$ induced coagulopathy, immunocyte apoptosis and microvascular dysfunction leading to disseminated intravascular coagulation, immunosuppression, organ failure and septic shock respectively. $H_2O_2$ inhibits GPx and catalase, which are critical anti-oxidant enzymes required for $H_2O_2$ neutralization. This prevents restoration of normal plasma and tissue redox balance while exacerbating oxidative tissue damage. $H_2O_2$ can also activate nuclear factor-κB (NF-κB) contributing to the inappropriate activation of this master pro-inflammatory transcription factor observed in septic shock. The pathologic activation of NF-kB contributes to elevated tumor necrosis factor-alpha levels, another potent generator of intracellular $H_2O_2$. GPx: Glutathione peroxidase.

potent reactive oxygen radical known in biological systems. Hydroxyl radical will indiscriminately disintegrate proteins, peroxidize lipids and oxidatively damage DNA leading to cell death[21,22].

Compounding the cellular cytotoxicity of $H_2O_2$ is its ability to freely diffuse through biological membranes allowing it to permeate other cellular compartments and diffuse to the extracellular space from where it can pass through the capillary endothelium into the blood stream[16,23]. Thus, the end result of a systemic GSH deficiency is the systematic discharge of excess $H_2O_2$ by all organs of the body into the bloodstream where it can damage distant capillary beds leading to systemic microcirculatory dysfunction, microangiopathic edema and refractory hypotension, a hallmark of septic shock.

This is supported by studies showing decreased human endothelial cell levels of GSH and eventual death after *in vitro* exposure to plasma from septic shock patients[24]. This implies a membrane diffusible agent capable of oxidizing intracellular GSH suggesting that a toxic level of plasma $H_2O_2$ was the offending oxidizing agent mediating this effect. This is consistent with the well documented oxidative damage and dose dependent cytotoxicity that occurs during human endothelial cell exposure to $H_2O_2$[25,26].

In other studies high levels of urinary $H_2O_2$ were found to correlate with a fatal outcome in patients with sepsis and adult respiratory distress syndrome suggesting an important role for $H_2O_2$ in the pathogenesis of septic shock[27]. Taken together the evidence suggests that $H_2O_2$ exerts a significant microangiopathic effect contributing to the development of microcirculatory dysfunction and the progression to refractory hypotension and fatal septic shock.

## MECHANISM OF DISEASE

The above evidence supports a pathogenesis of septic shock which is initiated by the systemic depletion of glutathione as the crucial event responsible for the accumulation of $H_2O_2$ in tissues. Subsequent diffusion of $H_2O_2$ into the blood stream leads to systemic elevation of this highly toxic oxidizing agent resulting in the microvascular dysfunction and organ failure observed in septic shock (Figure 1).

At the onset, a systemic inflammatory response is accompanied by a generalized hypermetabolic state which provides the energy needed to sustain the highly upregulated immune response switched on by the presence of a pathogen. The abrupt global increase of cellular bioenergetic reactions to several times their normal basal state presents the cell with a surge of toxic metabolic by-products that must be neutralized to avoid accumulation and cell death. Hydrogen peroxide, a toxic reactive oxygen species, is a significant metabolic by-product that is generated in increased amounts when cellular processes such as protein synthesis, DNA recycling and ATP production are upregulated during periods of hypermetabolism that accompany systemic inflammation.

The majority of cellular $H_2O_2$ is neutralized by GPx, a selenium containing enzyme, which utilizes the tripeptide co-factor glutathione as a donor of reducing equivalents during the enzymatic conversion of $H_2O_2$ to water. GSH is consumed in this reaction and must be replenished in order to prevent accumulation of $H_2O_2$ within the cell. However, during periods of high $H_2O_2$ production the availability of glutathione may be insufficient to keep up with demand leading to net $H_2O_2$ accumulation and glutathione depletion resulting in severe cellular dysfunction and organ failure.

Excess $H_2O_2$ can easily diffuse out of pericapillary parenchymal cells through capillary endothelium and into the blood stream. This augments endothelial generated $H_2O_2$ resulting in oxidative damage and microangiopathic dysfunction. The inability to buffer cellular $H_2O_2$ signals a systemic failure of reductive (anti-oxidant)

capacity as the excess oxidant load is discharged into the blood stream. Over time plasma reductive capacity is exhausted leading to severe disruption in plasma redox potential, which studies have shown is strongly associated with an unfavorable outcome[28].

## HYDROGEN PEROXIDE CAN REPRODUCE CLINICAL ABNORMALITIES OBSERVED IN SEPTIC SHOCK

### *Microcirculatory dysfunction*

The capillary bed is not simply a conduit for the passage of cells. It is a highly dynamic and integrated system of endothelial cells that continuously interacts with its surrounding environment through a variety of displayed receptors and elaborated mediators whose functions includes vasoregulation, coagulation factors, barrier maintenance, immune cell recruitment and oxygen transport[29]. Microcirculatory dysfunction is now considered to play a central role in the pathogenesis of sepsis and microvascular leakage has a defining role in its outcome[1,29].

Histological analysis of microvasculature in a baboon model of lethal *Escherichia coli* (*E. coli*) sepsis revealed large gaps between endothelial cells accompanied by a significant increase in endothelial permeability[30,31]. These changes are also observed upon exposure of human umbilical vein endothelial cells (HUVEC) to $H_2O_2$. Studies have demonstrated an 18x increase (from 20 to 360 gaps/mm$^2$) in inter-endothelial cell gaps within 30 min of HUVEC exposure to $H_2O_2$. A time and dose dependent $H_2O_2$ induced endothelial contraction to about 60% of normal planar surface area was also observed[32,33]. This provides a microanatomical basis by which excess $H_2O_2$ can account for the life threatening massive edema observed both in humans and experimental models of sepsis[29,30].

Accompanying endothelial cell retraction during $H_2O_2$ exposure is the loss of tight junction proteins at the sites of gap formation, which strongly correlated with increased paracellular permeability[34-36]. Extensive cytoskeletal disruption and rearrangement was also shown to occur after endothelial cell exposure to $H_2O_2$[37-39]. Endothelial shape changes have been observed to occur in experimental models of sepsis and several studies have reported these pathological changes upon endothelial cell exposure to $H_2O_2$[30,40-43].

The net effect of continuous $H_2O_2$ exposure on the systemic microvasculature is severe disruption. Barrier function is compromised, intercellular communication is blunted and signal transduction is abrogated. This leads to microvascular edema, arteriovenous shunts and vasodysregulation as a result of cumulative oxidative damage sustained from continued penetration of $H_2O_2$ into endothelial cells. This is supported by studies of low dose $H_2O_2$ perfusion into isolated rat lung, which increased pulmonary vascular bed permeability and capillary filtration coefficient[41].

Studies of bovine brain microvascular endothelial cells exposed to $H_2O_2$ revealed increased paracellular permeability of the blood brain barrier (BBB) with loss of tight junctional proteins (44)[44]. $H_2O_2$ can by-pass the normally protective BBB by simply diffusing into tissues and cells[15]. This can result in dysfunction of cerebral microvasculature and could account for the early mental changes observed in patients with sepsis as a result of impaired synaptic transmission[8].

### *Immune activation*

Numerous genes are activated during a systemic immune response in a critically ill or septic individual. Studies in healthy human volunteers receiving low dose endotoxin identified over 4500 activated genes, most of which were involved in the innate or adaptive immune response (5)[5]. The simultaneous activation of this many genes is facilitated by preformed cytoplasmic signal transcription factors that serve as rapid response mediators to injury and infection. Nuclear factor kappa B (NF-κB) is a transcription factor that plays a central role in the activation and regulation of multiple genes that control immune and inflammatory reactions[45]. NF-κB is significantly elevated in adults and children with sepsis[46-48]. NF-κB is also a highly redox sensitive transcription factor capable of being activated by low levels of $H_2O_2$[49,50] and has been proposed as a biomarker for oxidative stress[51]. This suggests that high levels of ambient $H_2O_2$ may be involved in the inappropriate activation of NF-κB observed in septic shock[45].

A central role for the innate immune system is suggested by the neutrophilic infiltration into multiple organs observed in septic shock[45]. $H_2O_2$ is a highly potent neutrophilic chemo-attractant that can establish a chemotactic gradient as it diffuses out of parenchymal cells into the adjacent microvasculature. Circulating neutrophils can track this $H_2O_2$ gradient and enter the organ parenchyma *via* diapedesis. The net result is neutrophil infiltration into the parenchyma of multiple organs[52-54].

### *Coagulopathy*

Intravascular activation of the coagulation cascade with generation of fibrin and formation of diffuse microvascular thrombi is a pathologic and physiologic hallmark of sepsis[55]. This presents clinically as disseminated intravascular coagulation (DIC) and is found in up to 50% of patients with sepsis[56]. DIC leads to abnormal bleeding and intravascular clotting, obstructing limb and organ blood flow, and is a strong predictor of mortality[56].

Endothelial derived tissue factor (TF) is the major physiological route by which fibrin generation is initiated in sepsis. Importantly, this process is triggered only at sites of vascular injury or endothelial disruption where plasma clotting factors can encounter the TF protein that activates this extrinsic clotting pathway[56,57]. Studies utilizing immunohistochemistry in a lethal *E. coli* baboon sepsis model preferentially localized TF and TF mRNA at arterial branch areas, which is compatible with enhanced contact by a plasma derived oxidizing agent (*e.g.*, $H_2O_2$) at these sites of altered blood flow[30].

$H_2O_2$ can induce vascular injury by peroxidation of cell membrane lipids and studies have shown a marked increase in endothelial cell TF and TF mRNA after 1 and 5 min exposure to Xanthine oxidase, a $H_2O_2$ generating enzyme[10,58]. This indicates that TF is highly sensitive to $H_2O_2$ induced upregulation, which suggests with a contributory role for $H_2O_2$ in sepsis- associated DIC. Consistent with this mechanism is a case report describing a fatal case of sepsis with DIC and multiorgan failure in a previously healthy 37-year-old man after receiving several intravenous infusions of $H_2O_2$[59].

### Immunosuppression

Septic patients experience a considerable decline in lymphocyte numbers through apoptosis in the latter stages of sepsis and this is a significant contributing factor to the immunosuppression experienced by septic individuals[60]. Studies have shown that $H_2O_2$ is a potent apoptosis inducing agent[61]. B lymphocytes treated with agents that inhibit GSH synthesis experience a 95% decline in GSH concentration in 12 h. This is followed by a rise in intracellular $H_2O_2$ after which apoptosis occurs. By 72 h nearly 50% of B cells have died *via* apoptosis[62]. T cells are also highly sensitive to the effects of GSH depletion. Studies have recorded a 30% decline in circulating T lymphocytes within 4 wk after glutathione levels declined to suboptimal levels in healthy volunteers[63]. This supports a role for $H_2O_2$ in the development of sepsis induced immunosuppression.

### Erythrocyte rigidity

Red blood cell deformability is markedly reduced in sepsis and studies have demonstrated a significant reduction in red blood cell deformability upon exposure to $H_2O_2$[64]. A direct relationship was found between oxidant induced changes in erythrocyte deformability and severity of multi-organ failure in septic individuals[65]. This suggests that plasma derived $H_2O_2$ is a source of oxidant-induced RBC membrane damage.

### Circulating endothelial cells

Circulating endothelial cells (CEC) are a reliable, sensitive and specific indicator of vascular damage[66]. These cells rarely exist in the peripheral blood of healthy individuals[67]. Patients with s evere sepsis and septic shock have significantly higher numbers of CECs indicating widespread vascular damage[68,69]. Studies have shown that human endothelial cell detachment is produced by exposure to $H_2O_2$[43]. The presence of CECs in patients with sepsis but without shock suggests that endothelial damage precedes the development of organ damage[68]. This is compatible with $H_2O_2$ release from organ parenchymal cells into the capillary vascular bed causing microvascular dysfunction and edema with subsequent development of organ failure.

### Sepsis associated encephalopathy

Sepsis associated encephalopathy (SAE) is a diffuse cerebral dysfunction occurring in the setting of sepsis but without direct infection of the central nervous system[70]. SAE is characterized by alterations in mental status and motor activity that can range from inattention, disorientation and delirium to agitation, hypoactivity and coma[71,72]. Delirium is frequently the first manifestation of sepsis and often precedes organ failure[73,74]. SAE is reported to occur in up to 70% of septic patients (71).

Neurons are especially sensitive to $H_2O_2$ induced oxidative damage. Studies have shown a concentration dependent cell death starting at 10 μmol/L when neurons are exposed to $H_2O_2$[75]. The tripeptide glutathione is critically important in order to prevent oxidative damage of the brain due to $H_2O_2$[76]. Glutathione is composed of amino acids glycine, cysteine and glutamate. Cysteine is the rate limiting substrate for neuronal glutathione synthesis and transsulfuration of homocysteine is a major source of cysteine in most cells. However, the brain' s neuronal transsulfuration pathway is thought to be a negligible source of cysteine due to low activity of neuronal cystathionine-gamma-lyase (EC 4.4.1.1), a crucial enzyme in the transsulfurationn pathway leading to the synthesis of cysteine[77,78]. Neurons, therefore, rely mainly on the absorption of extracellular cysteine provided by astrocytes for the synthesis of glutathione[77]. Thus, the dependence of brain neurons on extracellular cysteine in order to synthesize glutathione severely limits their ability to upregulate antioxidant defenses in response to $H_2O_2$ mediated oxidative stress. This makes brain neurons highly vulnerable to $H_2O_2$ oxidative damage and dysfunction. This is consistent with the encephalopathy that is reported to occur after accidental ingestion of $H_2O_2$[79]. Encephalopathy was also a manifestation after intravenous administration of $H_2O_2$ during alternative medicine therapy[59].

The main interaction site of neurons and astrocytes is the synaptic cleft[80]. Astrocytes export glutathione directly into the synaptic cleft. Ectoenzymes present in the synapse enzymatically release cysteine from glutathione after which cysteine is transported into neurons by the membrane bound EAAT3 transporter (excitatory amino acid transporter 3)[77-82]. $H_2O_2$ can react non-enzymatically with cysteine in the synaptic cleft to produce cystine[83]. This removes cysteine from the synapse and prevents its importation into the neuron resulting in oxidative stress by decreasing the synthesis of neuronal glutathione. The presence of thiols (*i.e.*, cysteine) in the synaptic cleft suggests that this region can function as a sink for $H_2O_2$ resulting in disruption of synaptic transmission as a result of peroxidation of synaptic cellular membranes.

Thus, circulating $H_2O_2$ can permeate the brain during the initial hypermetabolic systemic inflammatory response syndrome (SIRS) phase of sepsis and disrupt brain function in the early stages of disease. Due to their limited capacity to detoxify $H_2O_2$, brain neurons are the first cells to be affected by $H_2O_2$ induced oxidative stress[84]. This is consistent with the observation that encephalopathy is often the first sign of sepsis.

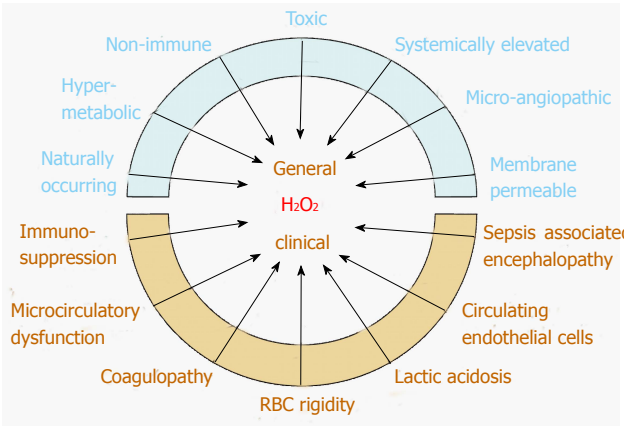


**Figure 2 Pathologically elevated serum $H_2O_2$ levels can account for the general physiological, histological and clinical abnormalities observed in septic shock.** Red blood cell glutathione accounts for a major portion of serum redox buffering capacity and is depleted in septic shock non-survivors vs. survivors. Brain neuron function is highly vulnerable to $H_2O_2$ oxidative stress and is manifested by electroencephalographic changes, which can appear before clinical encephalopathy is evident. Studies show that septic shock survivors upregulate serum antioxidant capacity (which decreases $H_2O_2$), while non-survivors are unable to do so. This suggests that elevated $H_2O_2$ is a necessary concomitant to the development of septic shock and recovery is preceded by decreasing $H_2O_2$. The individual clinical course, bookended by these extremes of $H_2O_2$, is influenced by parameters such as individual antioxidant capacity, susceptibility to oxidative stress, co-morbidities, age, general health and organ system involved.

## LACTIC ACIDOSIS

Sepsis related lactic acidosis is generally attributed to tissue hypoxia. Although tissue hypoxia can result in lactic acidosis it is unsuitable as a general mechanism to explain the appearance of lactic acidosis in septic patients when tissue oxygenation can be normal or even increased[85].

Under normal circumstances pyruvate, the end product of glycolysis in the cytoplasm, is transported into mitochondria where it is oxidized by the Krebs cycle. Lactate synthesis increases when the rate of pyruvate formation in the cytoplasm exceeds its rate of oxidation by the mitochondria. The excess pyruvate in the cytoplasm is then converted to lactate by lactate dehydrogenase and released into the blood stream resulting in lactic acidosis.

Inhibition of Krebs cycle enzymes will decrease pyruvate oxidation resulting in lactic acidosis. This has been observed with inherited deficiency of alpha-ketoglutarate dehydrogenase resulting in severe congenital lactic acidosis[86]. Alpha-ketoglutarate dehydrogenase is also highly sensitive to oxidative inhibition by hydrogen peroxide[87]. Rising systemic concentrations of $H_2O_2$ in sepsis can account for the observed lactic acidosis with normal tissue oxygen perfusion. This has been termed cytopathic hypoxia. In this case the lactic acidosis is an epiphenomenon of a much more serious underlying metabolic abnormality and treatment of the acidosis does not resolve the inhibition of the Krebs cycle.

## DISCUSSION

A hypermetabolic state can develop very quickly after a generalized septic or non-septic insult to the body. At the heart of the hypermetabolic state is a significantly increased bioenergetic response resulting mainly from enhanced ETC activity. The ETC is an assembly of intramitochondrial protein complexes that converts the energy of high-energy electrons into a form that is used to synthesize ATP, a high energy molecule that powers most energy requiring biosynthetic reactions and physiological functions. Thus, the high energy demands of body systems resulting from a generalized septic or non-septic insult are principally met by increased ATP production, which is manifested as a hypermetabolic state and recognized by the same parameters used to define a SIRS such as increased body temperature, heart rate, respiratory rate and increased white blood cell count.

A principle metabolic by-product of ETC activity is hydrogen peroxide; a highly toxic oxidizing agent. Hydrogen peroxide is produced when electrons spontaneously escape from the ETC and combine with available vicinal oxygen to generate superoxide that is enzymatically converted to $H_2O_2$ by superoxide dismutase. The increased amount of $H_2O_2$ generated during a hypermetabolic state can overwhelm the cell's anti-oxidant enzymatic defenses resulting in net intracellular $H_2O_2$ accumulation. The excess $H_2O_2$ can oxidatively inhibit enzyme systems including those needed to neutralize $H_2O_2$ resulting in a positive bio-feedback loop and a vicious cycle of ever increasing intracellular $H_2O_2$[88]. Glutathione functions as a cofactor for GPx, which enzymatically neutralizes $H_2O_2$. GPx is inhibited by the rising concentrations of $H_2O_2$, which explains why exogenously supplied N-acetylcysteine has no effect on the course of septic shock since glutathione cannot be utilized by GPx to neutralize $H_2O_2$[88,89].

Hydrogen peroxide is biomembrane permeable and can diffuse into the bloodstream where it is distributed to all organs of the body generating a state of severe systemic oxidative stress. Studies have documented high levels of $H_2O_2$ in the blood and urine of septic patients[27,90]. This can result in the multi-organ failure and microangiopathic dysfunction characteristic of septic shock. Genetic variation in glutathione levels as well as age related decline has been reported[91-93]. This may compromise the ability to neutralize $H_2O_2$ and predispose individuals to vasoplegic (*i.e.*, septic) shock and multi-organ failure during acute hypermetabolic periods, especially in older individuals. Studies have shown that glutathione is essential for cell survival[94].

## CONCLUSION

Taken together, the evidence suggests that septic shock is a primary radical induction process that has its origins early in the development of sepsis with the accumulation and generalized dispersal of cytotoxic levels of $H_2O_2$. This arises secondary to glutathione depletion as a result of a systemic inflammatory mediated hypermetabolic state. Studies have shown that systemic inflammation significantly reduces GSH levels, and GSH deficient animals

subjected to shock develop hypotension, kidney and liver failure, increased organ bacteria and dramatic increases in mortality rates[95-97].

The near universal requirement of glutathione for cellular function and the pathological accumulation of $H_2O_2$ that ensues when glutathione is deficient can affect every organ in the body. Studies have shown that $H_2O_2$ can reproduce the clinico-pathological abnormalities observed in septic shock (Figure 2).

Kept in check, the high membrane diffusability of $H_2O_2$ allows it to fulfill its physiological role as a cellular messenger but also creates the potential for a pathophysiological response during times of metabolic stress when reductive (anti-oxidant) mechanisms can become overwhelmed as a consequence of hyper-metabolic $H_2O_2$ production[98]. This is further exacerbated by nutritional deficits that may arise during the course of acute illness in addition to the effect of glutathione deficiency itself, which as master antioxidant of the cell, supplies reducing equivalents to maintain proteins in their reduced (and functional) state[14].

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BRIEF ARTICLE

# Arterial *vs* venous blood gas differences during hemorrhagic shock

Kristopher Burton Williams, Ashley Britton Christmas, Brant Todd Heniford, Ronald Fong Sing, Joseph Messick

Kristopher Burton Williams, Ashley Britton Christmas, Brant Todd Heniford, Ronald Fong Sing, Joseph Messick, Department of Surgery, Carolinas HealthCare System, Charlotte, NC 28204, United States

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Correspondence to: Ronald Fong Sing, DO, FACS, FCCM, Department of Surgery, Carolinas HealthCare System, 1000 Blythe Boulevard, Charlotte, NC 28203, United States. ron.sing@carolinashealthcare.org
Telephone: +1-704-3551311 Fax: +1-704-3555619

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## Abstract

**AIM:** To characterize differences of arterial (ABG) and venous (VBG) blood gas analysis in a rabbit model of hemorrhagic shock.

**METHODS:** Following baseline arterial and venous blood gas analysis, fifty anesthetized, ventilated New Zealand white rabbits were hemorrhaged to and maintained at a mean arterial pressure of 40 mmHg until a state of shock was obtained, as defined by arterial pH $\leqslant$ 7.2 and base deficit $\leqslant$ -15 mmol/L. Simultaneous ABG and VBG were obtained at 3 minute intervals. Comparisons of pH, base deficit, $pCO_2$, and arteriovenous (a-v) differences were then made between ABG and VBG at baseline and shock states. Statistical analysis was applied where appropriate with a significance of $P < 0.05$.

**RESULTS:** All 50 animals were hemorrhaged to shock status and euthanized; no unexpected loss occurred. Significant differences were noted between baseline and shock states in blood gases for the following parameters: pH was significantly decreased in both arterial (7.39 ± 0.12 to 7.14 ± 0.18) and venous blood gases (7.35 ± 0.15 to 6.98 ± 0.26, $P < 0.05$), base deficit was significantly increased for arterial (-0.9 ± 3.9 mEq/L *vs* -17.8 ± 2.2 mEq/L) and venous blood gasses (-0.8 ± 3.8 mEq/L *vs* -15.3 ± 4.1 mEq/L, $P < 0.05$). $pCO_2$ trends (baseline to shock) demonstrated a decrease in arterial blood (40.0 ± 9.1 mmHg *vs* 28.9 ± 7.1 mmHg) but an increase in venous blood (46.0 ± 10.1 mmHg *vs* 62.8 ± 15.3 mmHg), although these trends were non-significant. For calculated arteriovenous differences between baseline and shock states, only the $pCO_2$ difference was shown to be significant during shock.

**CONCLUSION:** In this rabbit model, significant differences exist in blood gas measurements for arterial and venous blood after hemorrhagic shock. A widened $pCO_2$ a-v difference during hemorrhage, reflective of poor tissue oxygenation, may be a better indicator of impending shock.



**Key words:** Hemorrhagic shock; pH; Base deficit; Arterial blood gases; Venous blood gases

**Core tip:** Recent studies regarding early goal directed therapy and damage control resuscitation have indicated a potential role for calculated arteriovenous $pCO_2$ differences in monitoring resuscitative efforts. In a rabbit model of hemorrhagic shock, we demonstrate significant derangements between arterial and venous blood and, while not a novel concept, explore the potential of central venous $pCO_2$ as an indicator of hemorrhagic shock. Our results demonstrate a widened arteriovenous $pCO_2$ difference is significantly associated with hemorrhagic shock and may be a more reliable

indicator of inadequate tissue perfusion and therefore impending circulatory collapse.

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## INTRODUCTION

Circulatory collapse is a definitive indicator of the shock state, but may manifest late during hemorrhage leading to delayed diagnosis, resuscitation, and treatment when clinical metrics of circulatory collapse (hypotension, tachycardia, decreased organ perfusion, altered mental status, *etc.*) are the sole measures of a patient's physiologic status. Robust compensatory responses to injury in young, healthy patients can delay treatment of hemorrhage even further as clinical parameters defining shock may not be evident until later stages in the clinical course. Any delay in diagnosis and treatment during massive hemorrhage will likely result in increased morbidity and mortality, fueling the search for adequate trauma resuscitation protocols, such as damage control resuscitation, as well as reliable early markers of impending or ongoing shock[1].

Serologic markers including pH, base deficit, central venous oxygen saturation and lactate have been used to identify and quantitate shock[2-6]. Arterial blood gas analysis is considered the gold standard to determine oxygenation and acid-base status in the acutely injured as well as critically ill and repeat testing offers a means of monitoring resuscitation efforts. However, serious, albeit rare, complications of arterial cannulation (pseudoaneurysm, hematoma, hemorrhage, limb ischemia, infection, neurologic injury)[7] have led to a search for less invasive means of detecting impending shock, quantitating the degree of shock as well as measuring adequate resuscitation. As such, many studies have examined the reliability and accuracy of central venous blood gas in acid-base monitoring as an alternative to arterial blood gas analysis[8-11]. In previous animal models of severely reduced cardiac output, venous hypercarbia has been shown to correlate with inadequacy of tissue perfusion[12,13] and changes in venous blood were noted to occur with greater magnitude and earlier in the process of clinical deterioration than those of arterial blood[14,15]. Similar discrepancies in arterial and venous $pCO_2$ have been reported in human studies of shock states, as well as the paradox of venous acidemia occurring simultaneously with arterial alkalemia, and have been suggestive of the role of serum $pCO_2$ differences as an indicator of tissue perfusion[16-20]. In a recent clinical study highlighting the importance of serum $pCO_2$ in surgical outcomes, Silva *et al*[21] showed a preoperative arteriovenous $pCO_2$ gap greater than 5.0 mmHg in high risk patients to be predictive of increased in-hospital mortality, circulatory shock, renal failure, intensive care unit (ICU) infection, and length of stay. These previous studies suggest the usefulness of venous blood gas analysis in identifying hemorrhagic shock earlier than other serum markers obtained from arterial blood analysis as well as the potential to accurately monitor adequate resuscitative efforts.

The purpose of this study was to examine the effectiveness of venous blood gas analysis in comparison to the gold standard of arterial blood gas analysis in a rabbit model of hemorrhagic shock.

## MATERIALS AND METHODS

Following approval by the Institutional Animal Care and Use Committee of the Carolinas Medical Center, fifty New Zealand white rabbits weighing 3 to 6 kg were anesthetized with 1.0 to 1.5 mL/kg of sodium pentobarbital (25 mg/mL) through an ear vein. Anesthesia was maintained throughout the experiment with 0.5-1.0 mL/kg of intravenous sodium pentobarbital (12.5 mg/mL) as needed, determined by response to a pain stimulus. Adequately anesthetized animals then underwent a tracheotomy and endotracheal ventilation. Tidal volumes of 10 mL/kg were administered by a mechanical ventilator (Siemens 900C Servo ventilator, Berlin, Germany) and fraction of inspired oxygen ($FiO_2$) was maintained at 0.5.

In all animals, bilateral groin dissection was performed to adequately expose femoral vasculature. Venous access was obtained *via* right femoral vein using a 5.0 French catheter advanced into the level of the right atrium and was utilized for drug infusion as well as withdrawal of venous blood samples. Arterial access was secured *via* left femoral artery utilizing a 3.5 French catheter advanced into the distal abdominal aorta for monitoring of blood pressure, heart rate and arterial blood sampling.

Following baseline arterial and venous blood gas measurements (Radiometer analyzer, ABL-520 #2, Copenhagen, Denmark), animals were hemorrhaged to a mean arterial pressure of 40 mmHg as determined by a multichannel recorder (MT95k2, Astro-Med, Inc., West Warwick, RI). Simultaneous arterial and venous blood gases were obtained every 3 min until hemorrhagic shock was observed, as defined by an arterial pH less than 7.2 and a base deficit greater than or equal to -15 mmol/L. Once the shock state was obtained, animals were then euthanized by intravenous administration of sodium pentobarbital. To minimize procedural variation, all animals were anesthetized, instrumented, hemorrhaged, and euthanized using identical technique by the same investigator.

### Statistical analysis

Data was stored and analyzed using SAS software version 9.3 (SAS Inc., Cary, North Carolina). Obtained arterial and venous blood gases were compared to baseline measurements with regard to pH, base deficit and $pCO_2$. Arteriovenous differences for each parameter (pH, base deficit, $pCO_2$) were then calculated at baseline and shock. Statistical analysis was performed using the unpaired t-test

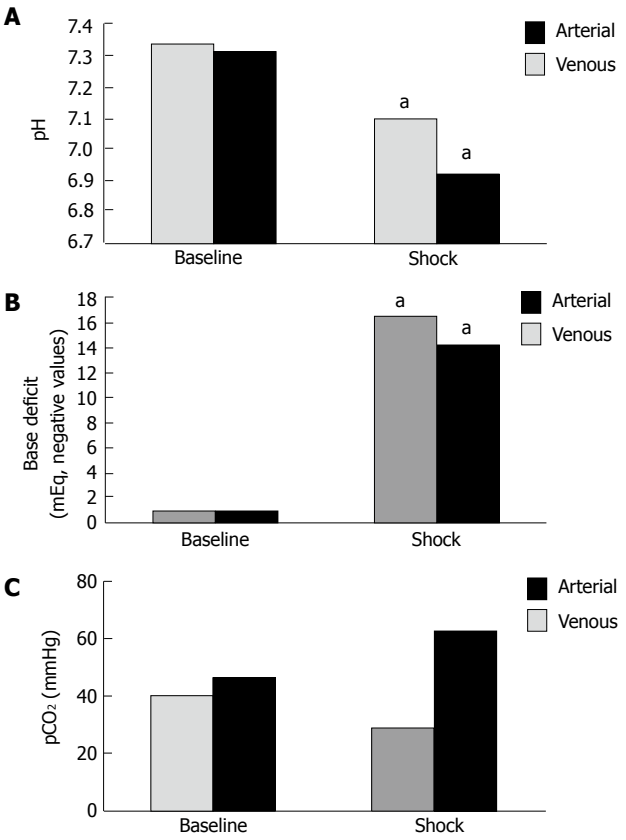


**Figure 1 Arterial and venous blood gas at baseline and shock states.** [a]*P* < 0.05 *vs* control group. A: pH; B: Base deficit; C: $pCO_2$.

or Wilcoxon rank sum test where appropriate. For all comparisons, statistical significance was set at a *P* value of less than 0.05.

RESULTSAll 50 animals underwent successful administration of anesthesia, groin dissection, instrumentation, hemorrhage to a state of shock and euthanization without any unexplained or premature losses. Data are expressed as mean ± SD.

### *Arterial and venous blood gases at baseline and the hemorrhagic shock state*

Mean values for pH were significantly decreased from baseline to shock ($P < 0.05$) in both arterial (7.39 ± 0.12 to 7.14 ± 0.18) and venous (7.35 ± 0.15 to 6.98 ± 0.26) blood gases (Figure 1A). Figure 1B compares mean values obtained for base deficit at the 2 physiologic states; a significant increase ($P < 0.05$) was seen in arterial (-0.9 ± 3.9 mEq/L *vs* -17.8 ± 2.2 mEq/L) and venous (-0.8 ± 3.8 mEq/L *vs* -15.3 ± 4.1 mEq/L) base deficit during shock. In comparing $pCO_2$ at baseline and shock, a non-significant decrease was observed in arterial $pCO_2$ (40.0 ± 9.1 mmHg *vs* 28.9 ± 7.1 mmHg, $P > 0.05$), while venous blood samples demonstrated a non-significant trend towards increased $pCO_2$ (46.0 ± 10.1 mmHg *vs* 62.8 ± 15.3 mmHg, $P > 0.05$), as shown in Figure 1C.

### *Blood gas arteriovenous differences at baseline and the hemorrhagic shock state*

Arteriovenous differences in pH, base deficit, and $pCO_2$ at baseline and the hemorrhagic shock state are represented. No significant differences were seen in calculated differences at baseline for pH (0.04 ± 0.03), base deficit (0.01 ± 3.07 mEq/L), or $pCO_2$ (5.8 ± 7.5 mmHg), although venous pH demonstrated a larger non-significant trend toward acidosis and a larger non-significant base deficit was seen for arterial samples. In the shock state, a significant difference was noted for arteriovenous $pCO_2$ difference (34.0 ± 3.10 mmHg, $P < 0.05$), however, calculated differences for pH (0.16 ± 0.08) and base deficit (2.56 ± 3.10 mEq/L) were not significant.

## DISCUSSION

Our results demonstrated significant parallel trends of acidosis and increased base deficit in both arterial and venous blood during hemorrhagic shock in a rabbit model (Figure 1A and B). The arteriovenous $pCO_2$ difference during shock was also statistically significant as venous hypercarbia was observed with simultaneous arterial hypocarbia (Figure 1C).

During hemorrhagic shock, oxygen delivery to tissues is reduced due to lack of red blood cell mass and, subsequently, hemoglobin concentration is insufficient to meet tissue oxygen demands[22]. Contributing to the drop in oxygen carrying capacity, decreased cardiac output secondary to reduced venous return slows the delivery and elimination of venous $CO_2$ in the lungs and augments ongoing venous hypercarbia[13,18,23]. Reduced oxygen delivery to tissues results in a shift from aerobic toward anaerobic cellular metabolism effecting subsequent production of organic acids, such as lactate, and ensuing acidosis and hypercarbia[17]. When the oxygen supply can be restored quickly, metabolic function can return to normal; however, when the oxygen insufficiency is prolonged, cells become irreversibly damaged and are unable to function in normal energy metabolism[24]. Serum and tissue acidosis develop in direct proportion to the amount and acuity of hemorrhagic shock[2,3,6,14,25].

Studies in both animal models and humans have demonstrated a pronounced dissociation between the arterial and venous $pCO_2$ during periods of decreased oxygen delivery as a consequence of decreased cardiac output, such as cardiac tamponade[15], severe hemorrhagic shock[2,14], hemodynamic instability[5,17,18,20] or septic shock[16,19]. Carbon dioxide accumulates very rapidly during hemodynamic compromise, as with massive blood loss, before significant amounts of organic acids are detectable in blood since the normal liver is capable of upregulating lactate metabolism early in the hemorrhagic process[15,21]. Mixed venous $CO_2$ ($CvCO_2$) can be represented according to the Fick equation, $CvCO_2 = VCO_2/Q + CaCO_2$, where $VCO_2$ represents $CO_2$ production in tissues, Q signifies cardiac output, and $CaCO_2$ denotes the arterial $CO_2$ content. Carbon dioxide is released into the circulation at the tissue-venous interface, represented by $VCO_2/Q$, and is eliminated at the alveolar-arteriole interface in the lungs, represented by $CaCO_2$. Under conditions of normal cardiac output and venous return,

there is adequate ventilatory elimination of $CO_2$ that is produced in the tissues and acid-base equilibrium is established. However, as cardiac output and venous return decrease, the increased $CO_2$ produced from anaerobic tissues cannot be effectively eliminated by the lungs, resulting in a disconnect between arterial and venous vascular trees whereby arterial blood gases reflect $CO_2$ exchange at the alveolar-arterial level while venous blood gases are indicative of acid-base status and oxygenation at the level of the tissues. Examining the Fick equation, it can be seen that as cardiac output (Q) declines with simultaneous increased tissue $CO_2$ production ($VCO_2$), mixed venous $CO_2$ ($CvCO_2$) will increase. This was demonstrated in our rabbit hemorrhage model in which the animals were adequately ventilated but hypoperfused, resulting in hypercarbia detected in venous but not arterial blood gas analysis, representing insufficient oxygen delivery to tissues. Therefore, venous blood gas values may better reflect insufficient oxygen delivery to the tissues and subsequent impending shock. Although our results did not show a statistically significant difference in the arteriovenous pH gradient, the venous samples were markedly more acidic than the arterial samples taken during the shock state (Figure 1A).

The clinical utility of arteriovenous $pCO_2$ differences in goal directed therapy (GDT) has recently been addressed in the literature. In a series of septic ICU patients resuscitated to a mixed venous oxygen saturation goal of 70% or greater, Vallée *et al*[16] demonstrated those patients with arteriovenous $pCO_2$ differences greater than 6 mmHg had higher lactate concentrations and lower lactate clearance rates than those with arteriovenous $pCO_2$ differences less than 6 mmHg, subsequently reflecting the status of global tissue perfusion. They further demonstrated lower cardiac indexes in those patients with arteriovenous $pCO_2$ values greater than 6 mmHg following "adequate" resuscitation to a mixed venous oxygen saturation of 70% as compared to the cohort of patients with arteriovenous $pCO_2$ values less than 6 mmHg. Similarly, Futier *et al*[26] demonstrated larger arteriovenous $pCO_2$ differences to be significantly associated with post-operative complications in "adequately resuscitated" patients (to a mixed venous oxygen saturation goal greater than 71%) undergoing major abdominal surgery. These clinical results indicate further optimization of GDT may be obtained through a combination of mixed venous oxygenation and arteriovenous $pCO_2$ difference analysis, potentially playing vital roles in progressive damage control resuscitation models in trauma[1].

Some shortcomings of the current study deserve discussion. First, this study was not conducted in a spontaneously-breathing animal model, effectively eliminating the possibility of respiratory compensation which likely will occur in cases of acute injury and hemorrhage. To address this criticism, Mathias *et al*[15] performed acid-base comparisons between arterial and venous blood gases in a spontaneously-breathing, under-anesthetized canine model of acute cardiac tamponade and found a similar paradox of venous acidosis and hypercarbia with concomitant arterial alkalemia and hypocarbia, even in the early stages of decreased cardiac output (20%) prior to any reduction in arterial blood pressure. Although the criticisms of performing these studies in animal models with blunted or absent respiratory compensatory mechanisms are valid concerns, the results of Mathias *et al*[15] indicate the paradoxical acid-base trends are still evident in a minimally-anesthetized, spontaneously-breathing, non-ventilated animal model. Also, performing these studies in live animal models without sufficient sedation or supportive measures, such as ventilatory support, would certainly be considered distressful to the animals. A second shortcoming of this study is the lack of a temporal metric for the onset of acid-base changes in our animal model, as well as a lack of serum lactate analysis. It would be beneficial to define the chronological relationship of arterial hypocapnea, venous hypercapnea and acidosis in comparison to the accumulation of lactate during the course of hemorrhagic shock in the rabbit model to better define the usefulness of venous blood gas in the course of hemorrhagic shock.

Our study in a rabbit model indicates hemorrhage shock results in significant acidosis and base deficit in both arterial and venous blood with a significant arteriovenous $pCO_2$ difference of venous hypercarbia and arterial hypocarbia, consistent with previously reported disparities between arterial and venous $pCO_2$ in the setting of severely hypoperfused states. These results indicate that venous blood gas analysis may be a superior indicator of cellular hypoperfusion in hemorrhagic shock, as evidenced by pronounced hypercarbia, and may be more reflective of tissue oxygenation compared to arterial blood gas analysis. Further studies are needed to determine if venous blood gas analysis is a more rapid indicator of impending circulatory collapse or is a more accurate gauge of adequate resuscitative efforts.

## COMMENTS

### Background

Early and adequate tissue perfusion is a key tenet of goal-directed therapy and damage control resuscitation, employed in critical care and trauma practices, respectively. Arteriovenous differences in $pCO_2$ have demonstrated potential in the early detection of insufficient tissue perfusion as well as the quantification of resuscitative efforts.

### Research frontiers

Establishment of an early, reliable, and easily obtainable marker for impending circulatory collapse in hemorrhagic shock would contribute significantly to treatment algorithms, possibly allowing supportive measures (fluid resuscitation, blood product administration, vasopressor circulatory support, *etc.*) to be initiated prior to classic physiologic indicators of circulatory collapse. However, no such definitive marker has been elucidated. In this study, the authors demonstrate significant similarities and differences in arterial and venous blood gas derangements, focusing on the arteriovenous differences noted in a rabbit model of hemorrhagic shock in an effort to further define arteriovenous $pCO_2$ differences as a potential early indicator of inadequate tissue perfusion.

### Innovations and breakthroughs

Previous studies have examined arterial and venous blood gas derangements (pH, base deficit, lactate levels, oxygen saturation) in states of hypoperfusion in animal models as well as humans. Paradoxical venous hypercarbia with arterial hypocarbia associated with decreased cardiac output has also been reported in

the literature, suggesting a role for $pCO_2$ monitoring in cases of hypoperfused states. In this study, authors conclusively demonstrated a widened $pCO_2$ difference (venous hypercarbia with concomitant arterial hypocarbia) is associated with hemorrhagic shock in a novel rabbit model.

### Applications

The results of this study, viewed in light of recent work regarding venous blood gas analysis in hypoperfused states, further supports the prospect that central venous blood gas $pCO_2$ differences may indicate effectiveness of resuscitative efforts in the acutely injured hemorrhagic state. Certainly, further human studies in the setting of acute hemorrhage deserve attention so that a more rapid, accurate and easily obtainable mechanism of resuscitation may be elucidated.

### Terminology

The term arteriovenous $pCO_2$ difference is used to describe the absolute value of the quantifiable variance between arterial blood gas $pCO_2$ and venous blood gas $pCO_2$. This is represented in units of mmHg, a standard unit of measurement for partial pressure.

### Peer review

This is a well-written manuscript which analyzes the effects of hemorrhagic shock on arterial and venous blood gases in a rabbit animal model. An added caveat is the significant widened arteriovenous $pCO_2$ difference seen in the shock state. The manuscript also reviews pertinent publications on the subject, highlighting recent clinical studies which suggest a role for arteriovenous $pCO_2$ differences in monitoring resuscitation. Although not novel, the results certainly provide further evidence that widened $pCO_2$ differences are indicative of worsening shock and may therefore possibly be another tool in our armament to monitor resuscitation.

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BRIEF ARTICLE

# Variable change in renal function by hypertonic saline

Jesse J Corry, Panayiotis Varelas, Tamer Abdelhak, Stacey Morris, Marlisa Hawley, Allison Hawkins, Michelle Jankowski

Jesse J Corry, Panayiotis Varelas, Tamer Abdelhak, Stacey Morris, Marlisa Hawley, Allison Hawkins, Department of Neurology, Henry Ford Hospital, Marshfield, WI 54449, United States
Jesse J Corry, Panayiotis Varelas, Tamer Abdelhak, Department of Neurosurgery, Henry Ford Hospital, Marshfield, WI 54449, United States
Michelle Jankowski, Department of Biostatistics, Henry Ford Hospital, Marshfield, WI 54449, United States
Author contributions: Corry JJ contributed to study concept and design, data review, statistical analysis and review, manuscript authorship; Varelas P and Abdelhak T participated in data review, manuscript authorship and editing; Morris S, Hawley M and Hawkins A contributed to data collection, manuscript editing; Jankowski M contributed to statistical analysis, manuscript methods authorship and editing; all authors approved final revision.
Correspondence to: Jesse J Corry, MD, Department of Neurology, Henry Ford Hospital, Marshfield, 000 N. Oak Avenue, 4F3, Marshfield, WI 54449,
United States. corry.jesse@marshfieldclinic.org
Telephone: +1-715-079562 Fax: +1-715-3875727
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## Abstract

**AIM:** To investigate the effects of hypertonic saline in the neurocritical care population.

**METHODS:** We retrospectively reviewed our hospital's use of hypertonic saline (HS) since March of 2005, and prospectively since October 2010. Comparisons were made between admission diagnoses, creatinine change (Cr), and HS formulation (3% NaCl, 3% NaCl/sodium acetate mix, and 23.4% NaCl) to patients receiving normal saline or lactated ringers. The patients ($n$ = 1329) of the retrospective portion were identified. The data presented represents the first 230 patients with data.

**RESULTS:** Significant differences in Acute Physiology and Chronic Health Evaluation Ⅱ scores and Glasgow Coma Scale scores occurred between different saline formulations. No significant correlation of $Cl^-$ or $Na^+$ with Cr, nor with saline types, occurred. When dichotomized by diagnosis, significant correlations appear. Traumatic brain injury (TBI) patients demonstrated moderate correlation between $Na^+$ and Cr of 0.45. Stroke patients demonstrated weak correlations between $Na^+$ and Cr, and $Cl^-$ and Cr (0.19 for both). Patients receiving HS and not diagnosed with intracerebral hemorrhage, stroke, subarachnoid hemorrhage, or TBI demonstrated a weak but significant correlation between $Cl^-$ and Cr at 0.29.

**CONCLUSION:** Cr directly correlates with $Na^+$ or $Cl^-$ in stroke, $Na^+$ in TBI, and $Cl^-$ in other populations. Prospective comparison of HS and renal function is needed.



**Key words:** Hypertonic saline solution; Sodium chloride; Acute kidney injury; Cerebral edema; Critical care

**Core tip:** This work adds to the literature that changes in $Na^+$ and $Cl^-$ in the neurocritical care population correlate to adverse changes in renal function. It is critical for the neurointensivist to remain cognizant of this when choosing whether or not to use hypertonic saline, and what to monitor when doing so. Unlike previous work, this data suggests some diseases may have more or less a change in renal function from $Na^+$ or $Cl^-$. This argues for further study of how the formulations of these fluids may change outcome in the neurocritically ill.

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## INTRODUCTION

A nearly ubiquitous problem in the neurocritical care population is cerebral edema (CE). Cerebral edema has been implicated in delayed neurological deterioration, and worse outcome, through the elevation of intracerebral pressure (ICP)[1]. The role of CE in outcome is contentious, with evidence suggesting that the extent of CE may, and may not, correlate with outcome[2-6]. Animal models of CE demonstrate increased water content of edematous tissue correlates with inflammation and neuronal death[7]. Potentially, reduction of this edema may reduce the degree of neuronal death, potentially improving outcome and decreasing hospital length-of-stay.

The medical management of CE is not without problems. Mannitol use is common, but is complicated by deleterious effects on renal function, fluctuations in intravascular volume, and pH. Over time, mannitol' s slow elimination from the cerebrospinal fluid may require progressively higher doses to control ICP and rebound CE[8,9]. Increasingly, hypertonic saline (HS) is being used to abate cerebral edema. Used in bolus or a continuous infusion fashion, HS has been shown to be safe and effective in reducing ICP in patients with traumatic brain injury (TBI), subarachnoid hemorrhage (SAH), and stroke[10,11]. HS shifts fluid from endothelium and surrounding tissues into the vascular compartment, normalizing the endothelia volume, increasing capillary diameter, and reducing resistance to flow[12]. Edema can be reduced in this manner. Further, hypertonic fluids produce smooth muscle vasodilation improving regional blood flow[12]. HS is relatively inexpensive. The early use of HS may reduce secondary cell injury caused by cerebral edema[13]. These characteristics make HS an ideal therapeutic option in conditions such as SAH, intracerebral hemorrhage (ICH), stroke, and TBI.

However, the use of HS has not demonstrated any survival or outcome benefit despite reductions in ICP[12,14]. Further, HS may be associated with increased risk-of blood-stream infections, and possibly increased risk-of nosocomial and urinary tract infections[14]. A growing body of evidence suggests a possible link between HS use, renal dysfunction, and mortality[15-17]. We hypothesize the use of HS correlates to adverse changes in renal function.

## MATERIALS AND METHODS

### Study setting

The Henry Ford Neurocritical Care Unit (NCCU) is a 16 bed unit with a yearly census over 1000 patients. The Henry Ford Neurocritical Database records data on stroke, TBI, ICH, SAH, SE, and spinal cord injury patient populations admitted to the NCCU. The data has been prospectively collected since October of 2010, with data added retrospectively from March of 2005 (when the first neurointensivist joined the staff) until October 2010. Between March 2005 and October 2010, 1329 patients of the retrospective cohort meet the inclusion criteria. The data presented represents the first 230 patients with data.

### Study design

With institutional review board approval, we mined the Henry Ford Neurocritical Database to identify all patients from March of 2005 to October 2010 with the aforementioned diagnosis who received HS. These patients were cross matched with the institution's pharmacy database to ascertain which saline formulation patients in the retrospective cohort received. In this retrospective sample, if patients were identified who received HS, and were not in the NCCU database, their data was retrospectively collected. Data was collected from admission until NCCU discharge, death, or post admission day (PAD) 13. Variables sought included: (1) IVF formulation: Normal saline (NS), ringer's lactate (LR), HS (3% NaCl, 3% NaCl:Na acetate, 23.4%); (2) physiologic: Mean arterial pressure; serum sodium, creatinine, chloride, $HCO_3$, BUN, creatinine; admission weight; (3) clinical: Admission Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE Ⅱ) scores, admission Glasgow coma scale in all patients, Hunt and Hess grade in SAH patients, NIH stroke scale in stroke patients, presence of external ventricular drain, and duration of ICU stay. APACHE Ⅱ scores were retrospectively calculated on all patients in the retrospective cohort; and (4) demographics: Age; sex; race; presence of hypertension, diabetes, pre-existing of renal insufficiency and etiology of renal insufficiency, history of coronary artery disease or congestive heart failure. Patients received various formulations of HS at the discretion of the attending NCCU staff. Correlations to renal function, as measured by Cr, to the formulation of saline used, and to changes in serum sodium and chloride levels were made. Patients receiving only LR o rNS served as a comparison group.

### Statistical analysis

Intervariable associations were calculated between using Pearson's correlation coefficients. The *P* values for these correlation coefficients were computed using clustering methods that take into account the multiple measures from the same patient. This was done with the entire sample as well as within each saline type and within each diagnosis type.

## RESULTS

### Who received HS solutions?

Table 1 summarizes the baseline characteristics of this cohort. There were no significant differences in diagnosis between groups. There were significant differences in the APACHE Ⅱ scores and Glasgow Coma Scale (GCS) scores between the different formulations of HS. Significant differences emerged in admission $Na^+$, A-a gradient, APACHE Ⅱ score, and GCS. In pairwise comparisons, patients receiving HS demonstrated higher APACHE Ⅱ scores and lower GCSv scores. 3% patients uniformly scored lower on GCS components compared to LR/NS patients, and lower in the GCSm and GCSe

**Table 1 Baseline characteristics by saline type**

| Variable | 23.40% (*n* = 22) | 3% (*n* = 13) | NS/LR (*n* = 194) | *P* value |
|---|---|---|---|---|
| Diagnosis | | | | 0.078 |
| ICH | 15 (68) | 7 (54) | 67 (35) | |
| Other | 3 (14) | 3 (23) | 47 (24) | |
| SAH | 4 (18) | 2 (15) | 43 (22) | |
| Stroke | 0 (0) | 0 (0) | 28 (14) | |
| TBI | 0 (0) | 1 (8) | 9 (5) | |
| Age (yr) | 61.9 ± 15.6 | 59.2 ± 19.6 | 56.8 ± 16.2 | 0.356 |
| HCT (mmHg) | 38.2 ± 6.9 | 38.1 ± 6.2 | 38.1 ± 7.0 | 0.999 |
| WBC ($10^9$/L) | 12.5 ± 6.2 | 11.6 ± 5.2 | 11.4 ± 9.1 | 0.865 |
| Temperature (℃) | 37.3 ± 0.9 | 37.2 ± 0.7 | 36.8 ± 2.5 | 0.514 |
| HR | 81.3 ± 20.3 | 86.5 ± 19.1 | 82.6 ± 16.7 | 0.668 |
| RR | 18.1 ± 4.6 | 17.5 ± 7.3 | 18.6 ± 4.4 | 0.633 |
| MAP | 98.3 ± 18.2 | 100.8 ± 33.2 | 106.5 ± 24.8 | 0.267 |
| Na | 141.5 ± 6.4 | 8.2 ± 5.3 | 139.0 ± 4.1 | 0.037 |
| K | 3.9 ± 0.6 | 3.7 ± 0.5 | 4.0 ± 2.0 | 0.888 |
| Glasgow coma Scale (verbal) | 2.6 ± 1.7 | 2.3 ± 1.5 | 3.4 ± 1.7 | 0.010 |
| 1 | 10 (45) | 6 (46) | 53 (27) | |
| 2 | 1 (5) | 2 (15) | 10 (5) | |
| 3 | 2 (9) | 1 (8) | 15 (8) | |
| 4 | 5 (23) | 3 (23) | 29 (15) | |
| 5 | 4 (18) | 1 (8) | 87 (45) | |
| Glasgow Coma Scale (motor) | 5.0 ± 1.5 | 3.9 ± 1.8 | 5.3 ± 1.3 | 0.002 |
| 1 | 1 (5) | 1 (8) | 5 (3) | |
| 2 | 1 (5) | 3 (23) | 4 (2) | |
| 3 | 2 (9) | 2 (15) | 16 (8) | |
| 4 | 2 (9) | 1 (8) | 13 (7) | |
| 5 | 4 (18) | 2 (15) | 27 (14) | |
| 6 | 12 (55) | 4 (31) | 129 (66) | |
| Glasgow coma Scale (eyes) | 2.9 ± 1.3 | 2.0 ± 1.0 | 3.3 ± 1.1 | < 0.001 |
| 1 | 5 (23) | 5 (38) | 28 (14) | |
| 2 | 4 (18) | 4 (31) | 15 (8) | |
| 3 | 2 (9) | 3 (23) | 31 (16) | |
| 4 | 11 (50) | 1 (8) | 120 (62) | |
| A aGrad[1] | 6.1 ± 195.3 | 173.7 ± 141.2 | 125.3 ± 154.9 | 0.024 |
| pH[2] | 7.4 ± 0.1 | 7.4 ± 0.1 | 7.4 ± 0.1 | 0.306 |
| $PCO_2$[3] | 27.0 ± 1.7 | 27.0 ± 3.0 | 26.0 ± 3.3 | 0.756 |
| $PaO_2$ | 209.2 ± 114.6 | 189.5 ± 136.8 | 150.8 ± 115.1 | 0.053 |
| Baseline Cr | 1.3 ± 1.4 | 0.9 ± 0.3 | 1.4 ± 1.8 | 0.655 |
| APACHE Ⅱ | 14.9 ± 7.6 | 17.5 ± 5.2 | 10.7 ± 6.5 | < 0.001 |

[1]23.4%, *n* = 19; 3%, *n* = 12; normal saline/ringer's lactate (NS/LR), *n* = 177; [2]23.4%, *n* = 19; 3%, *n* = 10; NS/LR, *n* = 111; [3]23.4%, *n* = 3; 3%, *n* = 3; NS/LR, *n* = 83. ICH: Intracerebral hemorrhage; TBI: Traumatic brain injury; SAH: Subarachnoid hemorrhage; HCT: Hematocrit; MAP: Mean arterial pressure; APACHE Ⅱ: Admission acute physiology and chronic health evaluation Ⅱ scores.

compared to 23.4% patients (Table 2). This would suggest patients receiving HS, particularly 3% solutions, had a greater illness burden at admission.

### *What are the effects of HS on renal function?*

Table 3 summarizes the effects of HS on renal function in this cohort. No significant correlation occurred with $Na^+$ or $Cl^-$ with Cr when grouped according to saline type. The correlation between $Na^+$ and Cr within each of the saline types does not differ much from the overall correlation, except for within the 3% saline group. The correlation for the 3% saline group is 0.256 but this was not statistically significantly different from zero ($P = 0.26$).

**Table 2 Pairwise comparisons for those that were significant**

| Dependent | 23.4% *vs* 3% | 23.4% *vs* NS/LR | 3% *vs* NS/LR |
|---|---|---|---|
| $Na^+$ | 0.035 | 0.016 | 0.503 |
| A aGrad | 0.370 | 0.009 | 0.306 |
| Apache Ⅱ | 0.261 | 0.005 | < 0.001 |
| GCS v | 0.579 | 0.034 | 0.019 |
| GCS m | 0.027 | 0.294 | < 0.001 |
| GCS E | 0.028 | 0.123 | < 0.001 |

NS/LR: Normal saline/ringer's lactate; APACHE Ⅱ: Admission acute physiology and chronic health evaluation Ⅱ scores; GCS: Glasgow coma scale.

**Table 3 Correlations between $Cl^-$, $Na^+$ and Cr**

| Population | $Na^+$ and Cr | | | $Cl^-$ and Cr | | |
|---|---|---|---|---|---|---|
| | *n* | Corr | *P* value | *n* | Corr | *P* value |
| Overall | 230 | 0.025 | 0.63 | 229 | 0.074 | 0.16 |
| Saline type | | | | | | |
| 23.40% | 22 | 0.037 | 0.58 | 22 | 0.042 | 0.65 |
| 3% | 13 | 0.256 | 0.26 | 13 | 0.181 | 0.43 |
| NS/LR | 194 | 0.026 | 0.65 | 194 | 0.097 | 0.09 |
| Diagnosis type | | | | | | |
| ICH | 89 | 0.145 | 0.10 | 89 | 0.058 | 0.55 |
| Other | 53 | 0.096 | 0.09 | 53 | 0.287 | < 0.001 |
| SAH | 50 | 0.125 | 0.08 | 50 | 0.085 | 0.28 |
| Stroke | 28 | 0.187 | < 0.001 | 28 | 0.185 | 0.001 |
| TBI | 10 | 0.447 | 0.048 | 10 | 0.361 | 0.1 |

NS/LR: Normal saline/ringer's lactate; ICH: Intracerebral hemorrhage; TBI: Traumatic brain injury; SAH: Subarachnoid hemorrhage.

The same holds true for the correlation between $Cl^-$ and Cr, it is greater in the 3% saline group but still not statistically significantly different from zero ($r = 0.18$, $P = 0.43$). When the correlations were dichotomized by the diagnosis, significant findings appear.

The strongest correlations were found in TBI, patients given HS and not diagnosed with TBI, stroke, SAH, or ICH (other), and stroke. With respect to TBI, a moderate correlation was found between rise in Cr and $Na^+$. For stroke, weak correlations between rise in Cr and both increases in $Na^+$ and $Cl^-$ occurred. Patients in this "other" category demonstrated a significant, yet weak, correlation between increases in $Cl^-$ and Cr.

## DISCUSSION

Even small increases in creatinine the first two days following admission are predictive of mortality[16,18]. Thus, therapies precipitating kidney injury are concerning. Presentation or development of diminished renal function is a predictor of poor outcome and mortality in stroke, ICH and SAH[19-25]. In the case of ICH, this has been associated with hemorrhage volume and GCS[21,22,26]. Similarly, development of renal dysfunction during hospitalization has been linked to increased mortality and is associated with lower GCS and higher APACHE Ⅲ score in TBI[27,28].

Frequently neurointensivists are asked to control cerebral edema *via* the use of mannitol and HS. Superi-

ority of one agent remains a matter of debate. Not yet recruiting at the time of this manuscript, investigators at Massachusetts General Hospital are investigating if induced, sustained hypernatremia to a goal of 150-160 mmol/L following traumatic brain injury will decrease the rate of cerebral edema formation and improve patient outcomes[29]. One study, sponsored by Indiana University, currently enrolling is looking at 20% mannitol *vs* 3% saline for the treatment of intracranial hypertension[30].

Mannitol appears to reduce ICP through reducing brain water content[31]. However, its use may result in kidney injury and rebound edema[8,9]. Increasingly HS saline is being used in various formulations either as a preventative or acute therapy[12,32]. HS appears to have a number of beneficial effects. In TBI, the use of 23.4% NaCl results in ICP reductions and elevations in cerebral perfusion pressure with commensurate elevations in brain tissue oxygenation[33,34]. These reductions in ICP are most notable in patients with the greatest elevations in ICP. Further, in cerebral hemorrhages ≥ 30 mL, the early use of HS to target a serum sodium between 145-155 mmol/L demonstrates both absolute and relative reductions of cerebral edema when compared to normonatremic patients[32].

These effects appear to be the result of a combination of actions including reduction in brain water content *via* osmotic forces, reductions in peripheral vascular resistance, and arteriolar vasodilatation with improvement in capillary blood flow[12,13,35]. Further, animal models treated with HS have demonstrated reduced aquaporin 4 expression on astrocytes with attenuation of brain water content[13]. In addition, increasing evidence demonstrates HS possesses immune-modulating properties *via* reduction in cytokine production and neutrophil activation[36,37]. Finally, animal models have demonstrated reductions in neuronal apoptosis.

Despite the ample experimental evidence, the clinical use of HS has not demonstrated any survival or outcome benefit and is not without risk[12,14]. Hypernatremia is associated with insulin resistance, reduced hepatic gluconeogenesis and lactate clearance, delirium, rhabdomyolysis, and reduced cardiac function[38,39]. Not surprisingly, ICU acquired elevations or reductions in serum sodium, dysnatremia, are common in neurosurgical and trauma patients, and associated with kidney injury[40]. Further, when compared to normonatremic patients, dysnatremia is associated with increased disease severity, longer length-of-stay, and mortality[40-42].

Our group inquired whether the formulation of saline used affected renal function in the neurocritical care population. The question of IVF formulation affecting patient outcome has been debated for some time. Evidence is increasingly suggesting formulation of saline and/or rapid change in serum sodium or chloride may adversely affect renal function[15-17]. A study in healthy subjects has demonstrated greater natriuresis and sooner time to first post-bolus micturition in those receiving LR *vs* NS[43]. Huang *et al*[16] reported the use of HS in burn patients produced significant increases renal, pulmonary, and cardiac failure compared to LR use. Patients receiving HS had less urine output. Further, the development of renal failure was heralded by a greater initial rise and slower subsequent fall in serum sodium levels during the first week of admission. Although this study was in burn patients, its findings are provocative. More recently, a study evaluating the effects of a $Cl^-$ restrictive *vs* $Cl^-$ liberal usage in critical ill patients demonstrated more acute kidney injury and greater use of renal-replacement therapy in the $Cl^-$ liberal group[44]. Though the populations of these studies differ from the neurocritical care population, this suggests the formulation of saline may have an effect on renal function.

With respect to our initial question, does saline type affect renal function; we found no such correlation in our sample. We found patients receiving HS have higher disease severity as assessed by lower GCS and higher APACHE Ⅱ scores. Not surprisingly, we found sicker patients more frequently received HS in this sample. This correlation has been previously reported[15]. This makes intuitive sense, with evidence suggesting early use of HS may limit the development of CE[32]. Similar to the findings of Aiyagari *et al*[15], Froelich *et al*[17] reported adverse changes in renal function with serum $Na^+$ > 155 mmol/L. This was not associated with the use or formulation of HS, a finding noted in our study too.

Unexpectedly, when we dichotomized by diagnosis, we found weak to moderate correlations between admitting disease and changes in Cr associated with hyperchloremia or hypernatremia. This was most noted in TBI, stroke, and non-vascular NCCU diagnosis; trends were also noted in ICH and SAH too. The explanation for this association is uncertain. Previous studies demonstrate correlations to $Na^+$ increase and renal dysfunction[15,17]. Although patients receiving a continuous HS infusion, when compared to a cohort receiving NS, do not have a higher risk of renal dysfunction, a significant correlation between sever hypernatremia and renal dysfunction does exist[17]. This could however reflect a more severe underlying brain injury rather than effect of HS.

Both clinical and experimental literature provides insight as to how $Na^+$ and $Cl^-$ could adversely affect renal function. HS solutions initially cause renal vasodilatation and increased renal blood flow[45]. It is theorized hypernatremia may produce renal injury *via* intravascular dehydration and vasoconstriction[46]. Canine models undergoing rapid renal artery sodium elevations demonstrate reduced renal blood flow and glomerular filtration rate with inhibition of rennin secretion[47]. Clinical studies have demonstrated hypernatremia is associated with elevations in creatinine in approximately 10% of patients[15]. This noted increase parallels elevations in sodium and APACHE Ⅱ scores, and is inversely related to admission GCS scores. However, save for sodium values > 160 mEq/L hypernatremia is not independently associated with mortality[15,17].

While direct proof linking saline-induced hyperchloremia to nephrotoxicity is not available, a strong cir-

cumstantial case can be made[48]. NS, with 154 mmol/L of chlorine can result in hyperchloremia and an acidosis[43,49]. Elevation in chloride can reduce renal blood flow and decreases the excretion of sodium[43,45]. Hyperchloremia appears to cause a renal vasoconstriction specific to renal vasculature and independent of the renal nerve[45]. This reduction in renal blood flow could precipitate renal ischemia and reducing glomerular filtration rate[45,50]. At the macula densa, $Cl^-$ activates tubuloglomerular feedback by precipitating afferent arteriolar vasoconstriction and decreased glomerular filtration rate[51]. Further, animal models suggest $Cl^-$ increases thromboxane synthesis resulting in renal vasoconstriction and reduced renal blood flow[52].

This single center, retrospective study has a number of limitations. First is the choice of serum creatinine as a biomarker of renal function. Though regularly used to infer kidney health and glomerular filtration, it is at best a crude measure of these. Often creatinine may be insensitive to early, deleterious changes in renal function. Next, given the time and cost of collecting retrospective data, this data represents an interim analysis to see if continued collection of these variables was warranted. As such, its small size and single center nature limit its applicability. Other centers with different demographics or practices may have different outcomes from what is represented here. The retrospective design and single center location limits what questions can be asked, data obtained, and the number of patients available. Regarding the disease specific correlations, a number of deficiencies exist. Regarding TBI, this study did not collect data on vasopressor use, blood pressure targets, or volume received, all variables noted to augment renal blood flow[53]. Intense sympathetic stimulation alters prostaglandin-mediated vasodilatation, resulting in reduced glomerular filtration[54]. Data on antecedent medication use was not collected. Could prior use of medications such as angiotensin converting enzyme inhibitors, in the setting of rapid changes in serum $Na^+$ and $Cl^-$, result in diminished renal blood flow? Finally, after dichotomizing by diagnosis, differences in baseline physiologic variables was not assessed. Perhaps these correlations occurred in patients who were inherently more ill when viewed from the perspective of admitting diagnosis. Despite these limitations, this data is provocative in suggesting the admitting disease may affect the physiologic response to a therapy.

This study adds to the literature demonstrating the use of HS is not inherently injurious to renal physiology. Further, we too note the correlation of injury severity to HS use. Finally, our data suggests when viewed from the perspective of admitting diagnosis, HS use may correlate to the development of kidney injury. However, the nature of this correlation needs further exploration. Variables to investigate include rate change of $Na^+$ and $Cl^-$; HS administration times over the course of disease; role of premorbid medications; and regional differences in population makeup. Wide variability exists in the treatment of cerebral edema among intensivists[55]. With no clear "right answer" to the question of cerebral edema, more investigation is needed regarding the risks/benefits of the treatments available and the patients who would be best suited for particular therapies. Prospective comparisons of HS formulation and renal function are needed to further assess if formulation affects outcome and cost. Prospective studies are warranted to better define this association and its effect on outcome.

## COMMENTS

### Background

The treatment and management of cerebral edema is among the duties of a neurointensivist. When and how to treat cerebral edema remain contentious. Further, a neurointensivist must remain cognizant of how their neurocentric therapies may affect the rest of the patient's body.

### Research frontiers

Intravenous fluids and hypertonic saline are ubiquitous in the critical care and neurocritical care setting. Data has previously demonstrated "not all fluids are created equal." Understanding how the formulation of intravenous fluids may affect outcome is critical to providing effective critical care. Discovery of deleterious correlations may help generate prospective, hypothesis driven, studies on patient or disease specific intravenous fluids aimed at improving outcome.

### Innovations and breakthroughs

Prior work has demonstrated that the formulation of hypertonic saline (HS) may not affect renal function. However, the relative change of $Na^+$, and presence in particular of hypernatremia, may correlate with development of kidney injury and worse outcomes. Much of this work was in a mixed critical care or mixed neurocritical care population. This study assessed if not only $Na^+$, but if $Cl^-$, HS formulation, and disease state played a role. The data here presented suggests potential rolls of $Cl^-$ and disease state to adverse renal function. These findings need to be confirmed by larger, prospective trials. Potentially, such findings could form the basis for developing patient or disease specific intravenous fluids aimed at reducing cerebral edema and mitigating adverse renal effects. Further, if borne out in future studies, better understanding of what interactions occur between intravenous fluids, disease state, and comorbidities may allow for development of new therapeutic options in neurocritical care.

### Applications

Data presented here, and in the context of literature to date, may suggest to the bedside clinician to be judicious with the prescription of HS to patients with cerebral edema, to closely monitor renal function, and use $Cl^-$ limiting formulations of HS.

### Terminology

Cerebral edema is the process whereby injured brain develops increase free water by cytotoxic or vasogenic means. Typically, these two pathologies combine in a temporal fashion. Much of the overall change of brain volume is related to this, a concern in the rigid volume provided within the skull. Potentially, cerebral edema may exacerbate inflammation. Hypertonic saline, or HS, are intravenous fluids of higher osmolarity aimed at increasing serum sodium. This has a multitude of effects including: (1) reducing brain free water and edema; (2) reducing aquaporin production and thus water entry into cells preventing/limiting the development of cerebral edema; (3) improving red blood cells malleability and ability to travel through injured tissue; and (4) potential mitigating effects on inflammation. Creatinine, Cr, is a biomarker of kidney health. Though crude, this is a readily available biomarker that can guide the clinicians management of a patient.

### Peer review

This analysis provides some provocative findings that need a larger study to confirm. Further, it summarizes much of the literature on this topic to date.

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

**Patent** (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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