Plasma Intermedin Level Indicates Severity and Treatment Efficacy of Septic Shock in Sprague-Dawley (SD) Rats

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Source of support: Departmental sources

Background: The aim of this study was to investigate the value of plasma intermedin (IMD) in assessing severity and treatment efficacy of septic shock.

Material/Methods: Healthy male Sprague-Dawley (SD) rats were chosen and divided into a normal control group (n=15) and a shock model group (n=27) that received intravenous injection of lipopolysaccharide (LPS). Then, 3 specimens were taken from each group. The shock model group rats were divided into an LPS group and a treatment group with 12 rats each. The treatment group received intravenous injection of compound sodium lactate solution. Plasma IMD and IMD1-47 mRNA expressions were compared and analyzed.

Results: Mean arterial pressure (MAP) was lower while white blood cell count and TNF-α were higher in the shock model group than in the normal control group ($P<0.05$). After 10 h and 20 h, the treatment group had lower plasma IMD and IMD1-47 mRNA expressions compared with the LPS group ($P<0.05$). Plasma IMD and IMD1-47 mRNA expressions in the LPS group after 20 h were significantly higher than after 10 h ($P<0.05$). IMD was positively correlated with interleukins (IL-3, IL-6, and IL-8), white blood cell count, and body temperature (all $P<0.05$), but were negatively correlated with systolic pressure (r=-0.8474, $P=0.0040$).

Conclusions: Plasma IMD level can effectively reflect the severity of septic shock and can be used as an important indicator of septic shock treatment effectiveness.

MeSH Keywords: Aftercare • Receptors, Interleukin-3 • Severity of Illness Index • TNF-Related Apoptosis-Inducing Ligand

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/897885

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Background

Septic shock is an acute and overwhelming inflammatory response that results from the dysregulation of the innate immune response after infection [1]. As a special type of sepsis, septic shock is also known as a systemic inflammatory response syndrome (SIRS) caused by infection, and the dysregulation of cytokines and inflammatory mediators play a crucial role in the occurrence and development of septic shock [2,3]. In general, the incidence of severe injection is much higher, reflecting the relative percentage of patients who develop organ dysfunction and thus are at greater risk of septic shock [4]. The mortality rate of patients with septic shock is much higher than that of patients with nonseptic critical illness and of the general population [5]. Furthermore, previous evidence demonstrated that septic shock has been associated with oxidative damage [6]. Traditional predictive markers for the diagnosis of septic shock include CRP, interleukin-6 (IL-6), and interleukin-8 (IL-8) [7]. Although somewhat effective in diagnosis, these factors have only limited value in early diagnosis and prognosis of septic shock, and more sensitive indicators are urgently needed.

Intermedin (IMD), also named as adrenomedullin-2, a member of the calcitonin/calcitonin gene-related peptide (CGRP) family, exerts a wide range of biological effects, especially in maintaining cardiovascular homeostasis [8]. The possible mechanism may be related to the upregulation in cAMP level in the circulatory system, and regulation of the proliferation of the smooth muscle and up-regulating NO system [9]. IMD also protects the mammalian vasculature, myocardium, and kidney from acute ischemia-reperfusion injury, chronic oxidative stress, and pressure-loading [10]. Severe infection may induce inflammatory response, as well as cell apoptosis, suggesting that IMD may serve as a sensitive indicator of septic shock [11]. IMD1-47 is deemed as a novel CGRP generated from prepro-IMD by proteolytic cleavage at the NH_2-terminal [12]. As one of the IMD members, it may have the same functions as IMD; therefore, we suspected that IMD level can serve as an important indicator for septic shock treatment effectiveness.

Septic shock rat models were established and plasma IMD levels were examined to verify the feasibility of plasma IMD level as an effective indicator for septic shock severity and treatment efficacy.

Material and Methods

Ethics statements

All animals were bought from the Laboratory Animal Center of Linyi People's Hospital. All animal procedures were conducted after approval from the Medical Ethics Review Board of Linyi People's Hospital. The experimental environment was set in accordance with China's national standard for Experimental Animal Environment and Facilities (GB14925-2001). Animal feeding management and animal experimental operation were in line with Beijing Experimental Animals Management regulations.

Animals grouping

Healthy male SD rats (n=42) weighing 210~250 g were divided into a normal control group (n=15) and a shock model group (n=27). After the shock model was successfully constructed and samples were obtained, 3 rats in each group were randomly chosen and scarified. The remaining rats were divided into 3 groups: the normal control group (n=12), the lipopolysaccharide (LPS) group (n=12), and the treatment group (n=12) (the shock model group was divided into an LPS group and a treatment group).

Construction of septic shock model and sample collection

The rats were fasted for 12 h, and intraperitoneal injection of 36 mg/kg of pentobarbital sodium (Guangzhou Land Biotech Corp., China) was performed for anesthesia with room temperature maintained at 26°C and body temperature at 37°C. The rats were fixed on the operating table and a 3-cm incision was made in the groin. Blunt dissection was performed to separate subcutaneous connective tissues and to expose the femoral artery and arteriovenous. Catheterization was performed in the femoral artery to connect the 4-channel polygraph (ABI, USA) to monitor mean arterial pressure (MAP). After blood pressure was stable for 30 min, rats in the shock model group were given intravenous injection of 10 mg/kg LPS (Sigma, USA), while rats in the normal control group were given equivalent intravenous injection of 10 mg/kg normal saline (Guangzhou Land Biotech Corp., China) for 5 min. The standard for successful septic shock model construction was: after LPS was injected for 1 h, MAP ≤60 mm Hg or the decrease of MAP ≥40 mm Hg; symptoms of agitation, skin flooding, cyanosis, and less urine appeared. After the model was constructed for 1 h, 1 ml of blood was drawn in the common carotid artery from rats in both the normal control group and the shock model group. An equivalent normal saline was filled to ensure enough blood volume. Half of the 1-ml blood sample was put into a low-temperature and high-speed centrifuge at a speed of 600 g for centrifugation for 15 min. The supernatant was extracted and stored in the refrigerator at –20°C for TNF-α detection with an ELISA kit (Sigma, USA) in strict accordance with the specifications. After blood anticoagulation, a 0.5-ml blood sample was preserved in the refrigerator at –20°C for examination of total number of white blood cells using an automatic whole blood chart instrument (ABI, USA). After going through

Table 1. Primer design.

Primer group		Sequence	Product length (bp)
IMD1-47	Upstream	5'-CCTCACTTCGGCCTGTAGTCT-3'	297
	Downstream	5'-ACCCACCTCAGCCATAACTGT-3'	
β-actin	Upstream	5'-ATCTGGACCACACCTTC-3'	291
	Downstream	5'-AGCCAGGTCCAGACGC-3'	

the above-mentioned procedures and specimen collection, 3 rats from each of the 2 groups were chosen and killed; their lungs, hearts, kidneys, livers, gastrointestinal tract, and other organs were dissected. An MBL2000-type optical microscope (Julabo Technology Co., Ltd., China) was used for pathological analysis. The remaining 12 rats in the normal control group were left without any treatment. Twenty-four rats in the shock model group were randomly divided into an LPS group (n=12) and a treatment group (n=12). Intravenous injection of 25 ml/kg compound sodium lactate solution was performed on every rat in the treatment group for treatment within 30 min; the same treatment was performed every other day within a cycle of 7 days. Rats in the LPS group were treated with equivalent normal saline. During the experiment, the 3 groups of rats were normally fed.

Plasma IMD expressions by ELISA

After the shock models in normal control group, LPS group, and treatment group were set for 10 h and 20 h, 2 ml of blood was drawn in the common carotid artery at 2 different stages. Then the blood was evenly mixed with 30 μl of 10% EDTA-Na$_2$ (Guangzhou Land Biotech Corp., China) and 40 μl of aprotinin (Santa Cruz, USA) in the tube and centrifuged at 4°C at 600 g for 10 min to separate the plasma. The plasma was preserved at -80°C. An ELISA kit was used for testing contents of IMD in the plasma in strict accordance with the specifications.

Detection of IMD mRNA Expression

The primer design is shown in Table 1. After the models in the normal control group, LPS group, and treatment group were set for 10 h and 20 h, 1 ml of blood was drawn in the common carotid artery. Then the blood was centrifuged at 4°C at 600 g for 10 min to separate the plasma. TRIzol (Invitrogen, USA) was used in One Step Method to extract total RNA from the sample tissue; 2 μg of RNA was reversed into single-strand cDNA with MMLV reverse transcriptase and oligo (dT) 15 primer. A Prism 7000 Fluorescence Quantitative PCR Instrument (ABI, USA) was used to detect the mRNA expression of IMD1-47. The reaction system was 20 μL, including 0.4 pmols of upstream and 0.4 pmols of downstream primers of IMD1-47 mRNA, 10 μL of 2× real-time PCR buffer of SYBR Green I fluorescent dyes

(Roche, Germany), 2 μL of cDNA template (Applied Biosystems, USA), and 1.25 U of Taq DNA polymerase. The reaction condition was and 5 min of denaturation at 95°C, 94°C for 30 s, 57°C for 30 s, and 72°C for 40 s with 30 cycles. With β-actin as the internal reference, 2 μL of PCR products were added to 1 μL of 200 nmol/L β-actin under the same PCR condition as above. The relative expression of IMD1-47 mRNA was calculated by IMD1-47/β-actin value. Each experiment was performed 3 times.

Evaluation of therapeutic efficacy

Twelve rats in the treatment group were normally fed for 7 days. An RBP-1B-type Photoelectric Method Rat Tail Measuring Instrument (ABI, USA) was used to measure the systolic pressure on the 1st, 3rd, 5th, and 7th days. Then, 2.5 ml of blood was drawn from the common carotid artery and equivalent normal saline was injected to ensure enough blood volume; 1.5 ml of blood was centrifuged at 600 g for 15 min. The supernatant was extracted and preserved at −20°C. An ELISA kit (Sigma, USA) was used to test interleukin-3 (IL-3), IL-6, and IL-8 in strict accordance with specifications. Then, 2.5 ml of blood was evenly mixed with 30 μl of 10% EDTA-Na$_2$ and 40 μl of aprotinin in the test tube. After that, 0.5 ml was preserved at −20°C for testing total number of white blood cells with an automatic whole blood drawing instrument; 2 ml was centrifuged at 4°C at 600 g for 10 min to separate the plasma. The plasma was preserved at −20°C. An ELISA kit was used for testing contents of IMD in the plasma in strict accordance with the specifications.

Statistical analysis

SPSS 20 statistical software was used for statistical analysis. Quantity One Basic software was used to analyze gray value of electrophoresis bands of different groups. Measurement data are expressed as mean ± standard deviation ($\bar{\chi}$±SD); comparisons between 2 groups were examined by t test and comparisons among groups were conducted with variance analysis. The count data are expressed as percentages or rates; comparisons between 2 groups were examined by chi-square test and correlation analysis was performed with Pearson correlation analysis. $P<0.05$ means the difference was statistically significant.

Table 2. Comparisons of physiological characteristics between two groups after modeling for 1 h.

Items	Normal control group n=15	Shock model group n=27	P
Average MAP (mm Hg)	128.33±13.22	94.77±15.16	< 0.001
White blood cell count (×10⁹/L)	7.64±3.24	9.56±2.18	0.027
TNF-α (ng/ml)	2.60±1.24	3.43±1.15	0.035

MAP – mean arterial pressure; TNF-α – tumor necrosis factor-α.
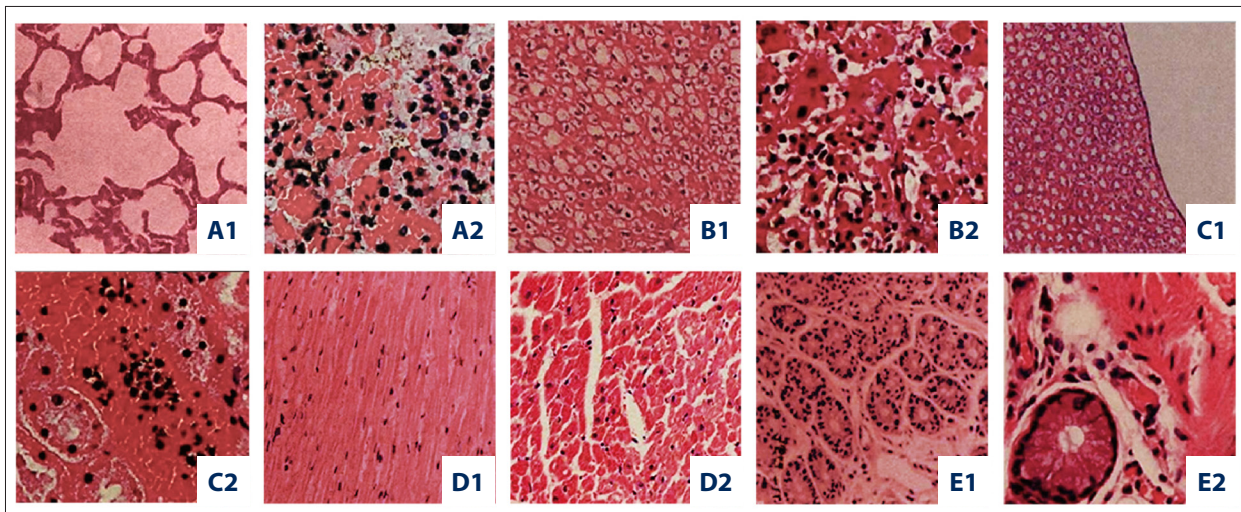
Figure 1. Comparisons of pathological sections between 2 groups 1 h after modeling. (**A1–E1**) Lung, liver, kidney, myocardium, and intestinal tissues of the normal control group; (**A2–E2**) Lung, liver, kidney, myocardium, and intestinal tissues of the shock model group.

Results

The establishment of rat septic shock model

Physiological characteristics after the model had been established for 1 h in the 2 groups are shown in Table 2. MAP in the shock model group was significantly lower than that in the normal control group (*P*<0.05), while more white blood cells and higher TNF-α expressions were observed in the shock model group (both *P*<0.05). Results of organ pathology analysis of the 2 groups are shown in Figure 1. Compared with those of the normal control group, diffuse bleeding lesions appeared in lungs, livers, kidneys, myocardium, and intestinal tissues of the shock model group (all *P*<0.05).

Plasma IMD expressions at different stages of shock

Plasma IMD in the 2 groups at shock stages of 10 h and 20 h are shown in Figure 2. After the model had been established for 10 h and 20 h, plasma IMD expressions in the LPS group and treatment group were significantly higher than those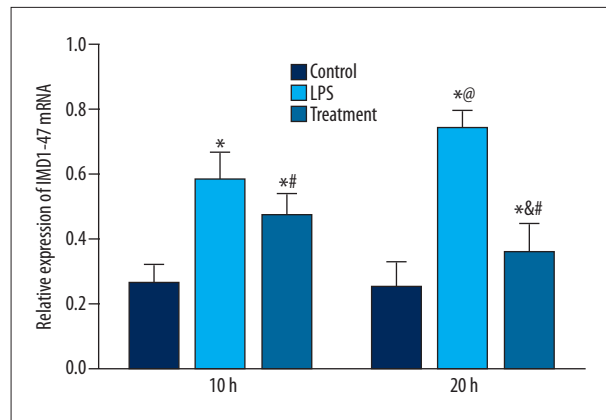 of the normal control group (both *P*<0.05), but plasma IMD expressions in the treatment group was significantly lower than those of the LPS group (*P*<0.05). After LPS group modeling for 20 h, plasma IMD expressions was significantly higher than that for 10 h (*P*<0.05). After treatment group modeling for 20 h, plasma IMD content was significantly lower than that for 10 h (*P*<0.05).

IMD mRNA Expression

Relative expressions of IMD1-47 mRNA in each group after the model had been established for 10 h and 20 h are shown in Figure 3. After modeling for 10 h and 20 h, plasma IMD1-47 mRNA expressions in the LPS group and treatment group were significantly higher than those in the normal control group, while plasma IMD1-47 mRNA expressions in the treatment group were significantly lower than those in the LPS group (all *P*<0.05). After LPS group modeling for 20 h, plasma IMD1-47 mRNA expressions were significantly higher than that for 10 h (*P*<0.05). After treatment group modeling for 20 h, plasma IMD1-47 mRNA expressions were significantly lower than that for 10 h (*P*=0.003).
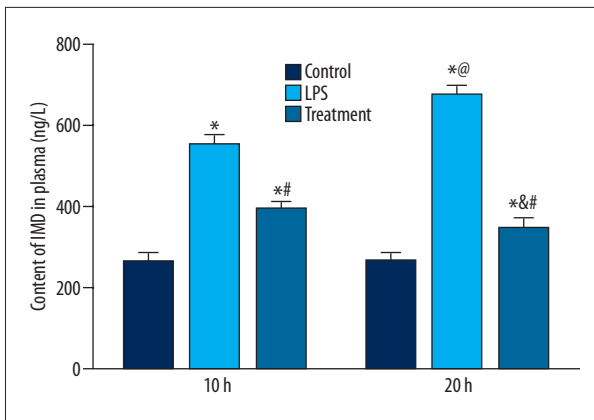
Figure 2. Comparisons of plasma intermedin (IMD) contents among the 3 groups at 10 h and 20 h after modeling. * Compared with the normal control group 10 h and 20 h after modeling, *P*<0.05; # compared with the treatment group and lipopolysaccharide (LPS) group 10 h and 20 h after modeling, *P*<0.05; @ in LPS group, compared with 10 h after modeling, *P*<0.05; & in treatment group, compared with 10 h after modeling, *P*<0.05.

Figure 3. Comparisons of relative expressions of plasma intermedin (IMD) 1-47 mRNA among groups 10 h and 20 h after modeling. * Compared with the normal control group after modeling for 10 h and 20 h, *P*<0.05; # compared with lipopolysaccharide (LPS) group 10 h and 20 h after modeling, *P*<0.05; @ in LPS group, compared with 10 h after modeling, *P*<0.05; & in treatment group, compared with 10 h after modeling, *P*<0.05.

Table 3. Growth condition of rats in different groups.

Items	Normal control group (n=12)	LPS group (n=12)	Treatment group (n=12)
Survival rate (%)	100	0*	83.33
Weight (g)	218.24±25.56	188.57±25.61*	193.22±22.55*

* Compared with the normal control group, *P*<0.05.

Comparison of the rat growth among different groups

The growth condition of rats is shown in Table 3 after 7 days of normal feeding. No rats died in the normal control group, with a survival rate of 100%. Two rats died in the treatment group, with a survival rate of 83.33%. The survival rates in the normal control group and treatment group showed no significant difference (*P*>0.05). All rats died in the LPS group, with a survival rate of 0%, which was significantly lower than those in the normal control group and treatment group (*P*<0.05). Surviving rats in the normal control group and treatment group had normal activities and diets. Average weight of surviving rats in the normal control group was significantly higher than that in the treatment group (*P*<0.05).

Analysis of therapeutic evaluation of plasma IMD level

Correlation between plasma IMD level and endotoxin indices in the treatment group is shown in Table 4. From the 1st, 3rd, 5th, and 7th days, plasma IMD expressions decreased gradually and the corresponding contents of IL-3, IL-6, IL-8, white blood cell count, and body temperature decreased, while the systolic pressure increased gradually (all *P*<0.05). IMD expressions were positively correlated with contents of IL-3, IL-6, IL-8, white blood cell count, and body temperature (*P*=0.0031; *P*=0.0014; *P*=0.0114; *P*=0.0026; *P*=0.0177), while IMD expressions were negatively correlated with systolic pressure (*P*=0.0040) (Table 5).

Discussion

In this study, plasma IMD mRNA and cytokines levels had stronger expressions as the severity of septic shock increased; while the expressions decreased after treatment was given, which to some extent suggests plasma IMD level can indicate severity and treatment effect of septic shock.

Generally speaking, the pathogenesis of shock is complex, and is related to a variety of organs in the body, especially to the pathological process of cardiovascular system, cytokines, and inflammatory factors [13,14]. These factors include a variety of endogenous damage factors such as endothelin and

Table 4. Plasma IMD level and endotoxin indices changes in treatment group at different times.

Group	IMD content (ng/L)	IL-3 content (ng/ml)	IL-6 content (ng/ml)	IL-8 content (ng/L)	White blood cell count (×10⁹/L)	Temperature (°C)	Systolic pressure (mmHg)
Day 1	457.6±51.55	0.17±0.09	0.29±0.13	2.58±1.01	10.27±1.51	40.8±0.8	62.36±12.55
Day 3	402.9±55.43	0.14±0.08	0.23±0.07	2.49±1.34	9.35±1.64	40.2±0.7	75.69±14.78*
Day 5	338.3±56.72*	0.08±0.01*	0.21±0.08	1.33±0.61*	8.63±1.45*	39.7±0.5*	93.08±17.65*
Day 7	284.5±52.12*	0.05±0.02*	0.18±0.05*	0.86±0.14*	8.04±1.26*	39.4±0.5*	99.62±16.88*
F	26.500	5.643	3.375	4.913	5.123	8.437	14.100
P	<0.001	0.002	0.027	0.005	0.004	<0.001	<0.001

* Compared with one-day treatment, $P<0.05$.

Table 5. Correlation between plasma IMD level and endotoxin indices in treatment group.

	IL-3	IL-6	IL-8	White blood cell count	Body temperature	Systolic pressure
IMD	0.8597*	0.8944*	0.7790*	0.8680*	0.7411*	−0.8474*

* $P<0.05$.

angiotensin [15], while endogenous defense factors like adrenomedullin (ADM) and calcitonin gene-related peptide CGRP play protective roles in the body [16,17]. Several studies have demonstrated that CGRP is released into the circulation during the pathogenesis of septic shock in humans [18,19]. As a novel member of the CGRP family, IMD has been reported to have better effect in decreasing blood pressure and increasing heart rate through intravenous injection compared with CGRP and ADM, which helps to identify IDM as an effective endogenous defense factor in shock [20,21].

Our experimental results show that with aggravation of septic shock, high expressions of IMD mRNA and cytokines levels occur, together with a low level of MAP and high white blood cell copy count and TNF-α, suggesting that an obvious inflammatory response appears in the process of septic shock. Severe infection may cause cell damage and the damaged endothelial cells can promote the secretion of inflammatory cytokines TNF-α and IL-6 due to increased expression of intercellular adhesion factor and mediated inflammatory cell dissociation [22,23]. The factors that mediate the apoptosis include the lipid peroxidation of oxygen free radicals, energy metabolism disorder, the activation of neutrophils [23], which may influence the growth of the body and even damages tissues or organs. Our experimental results confirmed that diffuse bleeding lesions occur in the lungs, liver, kidneys, cardiac muscles, and intestinal tissues of the shock model group compared with the normal control group. Accordingly, plasma IMD level presented high expression before treatment. Studies

have confirmed that in the course of shock, plasma IMD level is positively correlated with the severity of shock [24,25].

Actually, the increase of IMD level in the course of septic shock is beneficial for body protection. According to the results, at the beginning of treatment, IMD level had a high expression. A study conducted by Wang et al. also concluded that the secretion of CGRP was increased in the animal models of septic shock [26], suggesting that animal models of septic shock had higher IMD levels. The level of IMD decreased as the body recovered. From the perspective of regulation of IMD on the body, as the expression of IMD is regulated by the NOS signal, the upregulation of IMD suggests that the efficacy of the NOS regulation pathway is improved, contributing to the mediation of ADM factor, which is one of the protective mechanisms of IMD [27]. The increase of IMD level also increases the expression of NO. Through regulating the pathway activity of NO-cAMP, the IMD can down-regulate the metabolic level in the course of shock, thus protecting the body [28]. IMD is also involved in the regulation pathway of CL/RAMPs receptor binding, which plays an important role in maintaining the stability of the internal environment and enhancing effective perfusion of cells and damaging lipid peroxidation [29]. Generally speaking, IMD exerts a similar biological effect as the CGRP family does to activate the cAMP signaling pathway to prevent cell damage in the course of shock. A study conducted by Sexton et al. also reported that CGRP treatment appears to be beneficial in animal models of sepsis [30].

Conclusions

Above all, with the application of rat shock model, our results supported that the expression level of IMD can act as an indicator for the severity and prognostic evaluation of septic shock. However, our results were not evidenced by clinical experimental samples. Compared with those of rats, actual situations of human patients are much more complex; therefore, the diagnostic role of IMD level in the severity and prognostic evaluation of septic shock remains to be proved by further evidence. However, the correlation between IMD and septic shock was largely demonstrated, and its potential clinical utility as an indicator of severity and treatment efficacy of septic shock, as well as showing its role in shock, are promising.

Competing interests

None.

References:

1. Huet O, Chin-Dusting JP: Septic shock: desperately seeking treatment. Clin Sci (Lond), 2014; 126: 31–29

2. Pavare J, Grope I, Gardovska D: Prevalence of systemic inflammatory response syndrome (SIRS) in hospitalized children: A point prevalence study. BMC Pediatr, 2009; 9: 25

3. Ismail J, Sankar J: Systemic Inflammatory Response Syndrome (SIRS) and sepsis – an ever-evolving paradigm. Indian J Pediatr, 2015; 82: 675–76

4. Martin GS: Sepsis, severe sepsis and septic shock: Changes in incidence, pathogens and outcomes. Expert Rev Anti Infect Ther, 2012; 10: 701–6

5. Linder A, Guh D, Boyd JH et al: Long-term (10-year) mortality of younger previously healthy patients with severe sepsis/septic shock is worse than that of patients with nonseptic critical illness and of the general population. Crit Care Med, 2014; 42: 2211–18

6. Andresen M, Regueira T, Bruhn A et al: Lipoperoxidation and protein oxidative damage exhibit different kinetics during septic shock. Mediators Inflamm, 2008; 2008: 168652

7. Zhao Y, Li C, Jia Y: Evaluation of the Mortality in Emergency Department Sepsis score combined with procalcitonin in septic patients. Am J Emerg Med 2013; 31: 1086–91

8. Ni X, Zhang J, Tang C, Qi Y: Intermedin/adrenomedullin2: an autocrine/paracrine factor in vascular homeostasis and disease. Sci China Life Sci, 2014; 57: 781–89

9. Zhou YB, Sun HJ, Chen D et al: Intermedin in paraventricular nucleus attenuates sympathetic activity and blood pressure via nitric oxide in hypertensive rats. Hypertension, 2014; 63: 330–37

10. Holmes D, Campbell M, Harbinson M, Bell D: Protective effects of intermedin on cardiovascular, pulmonary and renal diseases: comparison with adrenomedullin and CGRP. Curr Protein Pept Sci, 2013; 14: 294–329

11. Gille-Johnson P, Hansson KE, Gardlund B: Severe sepsis and systemic inflammatory response syndrome in emergency department patients with suspected severe infection. Scand J Infect Dis, 2013; 45: 186–93

12. Grossini E, Molinari C, Mary DA et al: Intracoronary intermedin 1-47 augments cardiac perfusion and function in anesthetized pigs: Role of calcitonin receptors and beta-adrenoreceptor-mediated nitric oxide release. J Appl Physiol (1985), 2009; 107: 1037–50

13. Geng J, Xiao S, Zheng Z, Song S, Zhang L: Gambogic acid protects from endotoxin shock by suppressing pro-inflammatory factors *in vivo* and *in vitro*. Inflamm Res, 2013; 62: 165–72

14. Merrill JC, You J, Constable C et al: Whole-body deletion of LPS-induced TNF-alpha factor (LITAF) markedly improves experimental endotoxic shock and inflammatory arthritis. Proc Natl Acad Sci USA, 2011; 108: 21247–52

15. Ahn J, Kim J: Mechanisms and consequences of inflammatory signaling in the myocardium. Curr Hypertens Rep, 2012; 14: 510–16

16. Chen FX, Yu YB, Yuan XM et al: Brain-derived neurotrophic factor enhances the contraction of intestinal muscle strips induced by SP and CGRP in mice. Regul Pept, 2012; 178: 86–94

17. Kitamura K, Kangawa K, Kawamoto M et al: Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. 1993. Biochem Biophys Res Commun, 2012; 425: 548–55

18. Lee JK, Jung JS, Park SH et al: Deficiency of alpha-calcitonin gene-related peptide induces inflammatory responses and lethality in sepsis. Cytokine, 2013; 64: 548–54

19. Berg RM, Strauss GI, Tofteng F et al: Circulating levels of vasoactive peptides in patients with acute bacterial meningitis. Intensive Care Med, 2009; 35: 1604–8

20. Takei Y, Inoue K, Ogoshi M et al: Identification of novel adrenomedullin in mammals: a potent cardiovascular and renal regulator. FEBS Lett, 2004; 556: 53–58

21. Yang JH, Qi YF, Jia YX et al: Protective effects of intermedin/adrenomedullin2 on ischemia/reperfusion injury in isolated rat hearts. Peptides, 2005; 26: 501–7

22. Kielar ML, John R, Bennett M et al: Maladaptive role of IL-6 in ischemic acute renal failure. J Am Soc Nephrol, 2005; 16: 3315–25

23. Kinaci MK, Erkasap N, Kucuk A et al: Effects of quercetin on apoptosis, NF-kappaB and NOS gene expression in renal ischemia/reperfusion injury. Exp Ther Med, 2012; 3: 249–54

24. Kjaeve J, Vaage J, Bjertnaes L: Increased microvascular permeability caused by toxic oxygen metabolites is partly reversed by exchanging the perfusate in isolated rat lungs. Acta Anaesthesiol Scand, 1989; 33: 605–9

25. Qin YW, Teng X, He JQ et al: Increased plasma levels of intermedin and brain natriuretic peptide associated with severity of coronary stenosis in acute coronary syndrome. Peptides, 2013; 42: 84–88

26. Wang X, Ebong SJ, Call DR et al: Calcitonin gene-related peptide partially reverses decreased production of chemokines KC and MIP-2 following murine sepsis. Inflammation, 2002; 26: 167–74

27. Yang JH, Pan CS, Jia YX et al: Intermedin1-53 activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas. Biochem Biophys Res Commun, 2006; 341: 567–72

28. Li P, Sun HJ, Han Y et al: Intermedin enhances sympathetic outflow via receptor-mediated cAMP/PKA signaling pathway in nucleus tractus solitarii of rats. Peptides, 2013; 47: 1–6

29. Kuwasako K, Cao YN, Nagoshi Y et al: Characterization of the human calcitonin gene-related peptide receptor subtypes associated with receptor activity-modifying proteins. Mol Pharmacol, 2004; 65: 207–13

30. Sexton PM, Christopoulos G, Christopoulos A et al: Procalcitonin has bioactivity at calcitonin receptor family complexes: Potential mediator implications in sepsis. Crit Care Med, 2008; 36: 1637–40