



Effect of zinc supplementation on relative expression of immune response genes in neonates with sepsis: A preliminary study

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Background & objectives: Zinc alters gene expression mainly by binding to a site on the transcription factor. Genome-wide expression studies have shown early repression of genes related to zinc and immunity in adult patients with sepsis. The present study was conducted to evaluate the role of zinc supplementation on relative expression of immune response genes in neonatal sepsis.

Methods: In the present study, a sample of convenience of 22 neonates each was selected from the zinc supplemented and control groups using random numbers for expression of immune-related genes by zinc supplementation. These neonates with sepsis were earlier randomized into two groups: with and without zinc supplementation in addition to standard antibiotics and supportive care. Relative expression of immune response genes were analyzed for 22 neonates in each group using quantitative real-time PCR for calprotectin (*S100A8/A9*), tumor necrosis factor-alpha (*TNF-α*), interleukin-6 (*IL-6*), toll-like receptor-4 (*TLR-4*), cluster of differentiation 14 (*CD14*) and lipopolysaccharide-binding protein (*LBP*) genes.

Results: An increase in serum zinc levels was observed in zinc-supplemented group compared to controls. *S100A8* gene showed downregulation by three-fold ($P<0.001$) and *S100A9* gene showed upregulation by two-fold ($P<0.05$) in zinc group compared to controls. *CD14* gene showed upregulation by one-fold in zinc-supplemented group compared to controls ($P<0.05$). No significant fold changes were observed with respect to *TNF-α*, *IL-6*, *LBP* and *TLR-4* genes between the two groups.

Interpretation & conclusions: The results of our preliminary study showed that the zinc supplementation might modulates the relative expression of immune-related genes involved in sepsis pathway among neonates. However, studies with larger sample size are needed to be done to provide a better picture on the outcome by gene expression in neonatal sepsis by zinc supplementation.

Key words Calprotectin - fold change - gene expression - immune response genes - neonatal sepsis - zinc supplementation

The expression of genes represents the body's specific and complex response to stimuli during severe infection, trauma, *etc.* Gene expression is primarily regulated by nutrition and it interacts with transcription

factors where zinc occupies a specific site to indirectly interact with secondary mediators¹. RNA polymerases require Zn ions for RNA synthesis essential for gene expression². In paediatric sepsis and septic shock,

genome-wide expression studies showed that genes were repressed and associated with adaptive immunity and zinc biology³. It was observed that children with septic shock who did not survive, had lower serum levels of zinc⁴. A murine model has demonstrated that zinc depletion leads to increased mortality from sepsis⁵.

Calprotectin (S100A8/S100A9) acts as an endogenous activator of toll-like receptor-4 (TLR-4) and promotes inflammatory responses⁶. Calprotectin prevents microbial growth by zinc competition. Histidine-rich regions of calprotectin mediate zinc chelation which is an antimicrobial mechanism in host defense⁷. The sensitivity of immune cells to lipopolysaccharides (*LPS*) and cluster of differentiation 14 (*CD14*) gene expression is increased by lipopolysaccharide-binding protein (*LBP*) that correlates with cellular responsiveness to *LPS*. *LBP* gene expression was found to be induced in multiple organs following injury, and *CD14* upregulation was associated with the severity of infection and mortality⁸. Protein kinase C activates Ca^{++} ions that lead to heterodimerization of S100A8/S100A9. Activation of p38 mitogen-activated protein kinases phosphorylates S100A9. Intracellularly, this complex binds to actin resulting in cytoskeletal reorganization and when released extracellularly, it mediates inflammatory mediators⁹.

A link was found between S100A8 and S100A9 protein expression and sepsis pathophysiology⁹. The study showed that increase in S100A8/A9 protein complex occurred systemically during *Escherichia coli*-induced abdominal sepsis in mice¹⁰. The response was associated with elevated levels of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and enhanced bacterial dissemination. Both *S100A8/A9* expression increase response to bacterial products such as *LPS*¹¹. There are insufficient data correlating calprotectin gene and genetic variations in diseases¹². This study was undertaken to find the therapeutic efficiency of zinc in modifying the relative expression of immune response genes in neonatal sepsis compared to controls.

Material & Methods

The study was conducted in the departments of Neonatology and Biochemistry, Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry, India, from May 2013 to November 2016. Ethical approval was obtained from the Institutional Ethics Committee for conducting

the study. Neonates with clinical signs of sepsis such as fluctuation in temperature, poor feeding, reduced activity, apnoea, tachycardia, grunting, sclerema and hypotension were screened¹³. Neonates of age <28 days with more than 50 per cent enteral feeds, gestational age ≥ 32 wk, two screening tests positive (microESR >15 mm in the first hour, increased band cell count >20 per cent and C-reactive protein >4 mg/dl)^{14,15} or positive blood culture were included in the study. Neonates already treated for sepsis, Apgar score <6 at five minutes and those with major congenital malformations were excluded.

Two hundred and fifty two neonates with clinical symptoms of sepsis were screened¹⁶. Detailed history regarding gender, maturity, Apgar score, time of onset of symptoms, birth weight and blood culture positivity were recorded. One hundred and eighty five neonates were randomized using sequential numbers kept in opaque-sealed envelopes and allocated into two groups - control (n=94) and zinc-supplemented (n=91). After taking consent from the parents, blood sample (1.5 ml) was collected from the neonates in the zinc-supplemented and control groups at baseline and after 10th day of intervention. A zinc sulphate dry syrup formulation (20 mg/ml) (Dr. Reddy's Laboratories, Hyderabad) was administered orally for cases in a dose of 3 mg/kg twice a day for 10 days in addition to antibiotics. Zinc was administered with adequate care to avoid spillage by neonatal intensive care unit (NICU) staff. Blinding was not done for zinc administration. However, the person estimating zinc levels was blinded from the study. The neonates were observed closely for any side effects. The control group received standard antibiotic treatment and supportive care. The data were analyzed for inflammatory mediators in relation to outcome and it was found that as the infection was controlled with therapy, the inflammatory markers were also significantly decreased with a better outcome with zinc supplementation^{16,17}.

Due to limited availability of resources, a sample of convenience of 22 neonates in each group was selected from the earlier study group on zinc supplementation. To minimize selection bias, random numbers were used for recruiting the neonates (Figure). Primary outcome for this study was fold change in the expression levels of immune-related genes with zinc supplementation. Tolerance and adverse reaction to zinc supplementation were recorded as the secondary outcome.

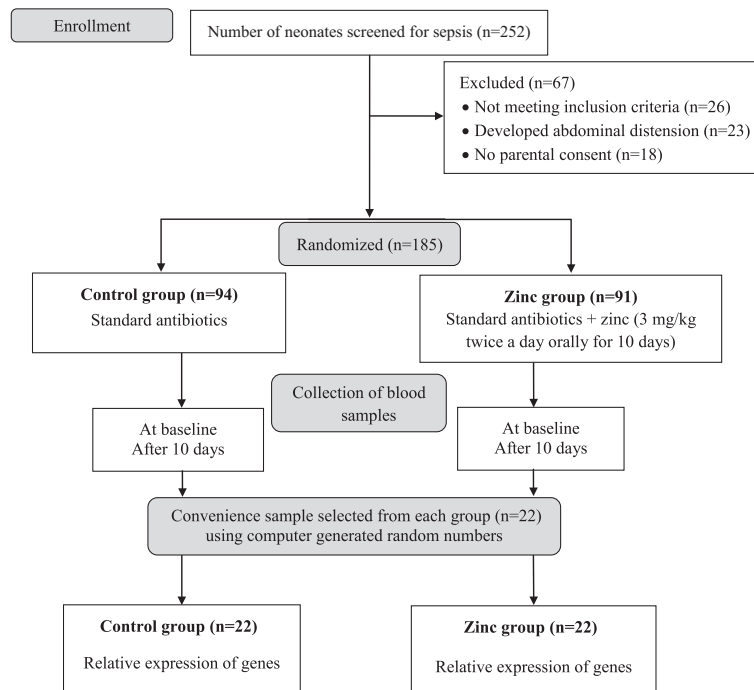


Figure . Study flow chart.

Serum was separated and stored in polypropylene tubes at -20°C for serum zinc assay. Using dry ice, the serum samples were transported to the Division of Nutrition Laboratory, Indian Council of Medical Research, New Delhi, for estimating serum zinc levels by Inductively Coupled Plasma-Mass Spectrometry.

RNA was isolated by guanidium thiocyanate-phenol-chloroform extraction (TRIZOL method). The concentration and the purity of isolated RNA were detected using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The purity of RNA was assessed by the ratio of absorbance at 260 and 280 nm. The ratio was observed to be between 1.8 and 2. The isolated RNA was converted to cDNA using cDNA synthesis kit (Roche, USA). CFX96 Real-Time PCR Detection System (Bio-Rad, USA) was used for evaluating gene expression levels using SYBR Green I and TaqMan probes (Roche, USA).

Relative quantification using cycle threshold ($\Delta\Delta\text{Ct}$) method: The samples were subjected to relative gene expression analysis for assessing the change in the expression level of target gene as a result of zinc supplementation in real-time thermal cycler. Data analysis was done by the cycle threshold ($\Delta\Delta\text{Ct}$) method¹⁸.

The primer sequences for the acute inflammatory response genes *SI00A8/SI00A9*, *TNF- α* , *IL-6*, *CD14*, *TLR-4* and *LBP* were designed using Universal Probe Library (UPL)^{6,8,11,12}. TaqMan probes (Roche, USA) were used for the acute inflammatory response genes *SI00A8/SI00A9*, *CD14*, *TLR4* and *LBP*. DNA binding dye SYBR Green I was used for *TNF- α* and *IL-6* genes in CFX96 Real-Time PCR Detection System (Bio-Rad, USA). The data were normalized using beta actin as the reference housekeeping gene.

The ΔCt value for each sample was determined by calculating the difference between the Ct value of the target gene and the endogenous reference gene. This was determined for each control as well as the test sample. The $\Delta\Delta\text{Ct}$ value for each sample was determined by subtracting the ΔCt value of the test sample from the ΔCt value of the control sample. PCR efficiencies of target gene and endogenous gene were compared, and the normalized level of target gene expression (fold changes) was calculated using the formula: normalized target gene expression level in sample = $2^{-\Delta\Delta\text{Ct}}$ ¹⁸.

Statistical analysis: Analysis was done using SPSS (PASW) version 19 (IBM Corp., Armonk, NY, USA). Analysis of co-variance was used to find out the

adjusted mean difference compared to baseline between both the groups and gene expression was expressed as mean±standard deviation (SD). Data for fold change was represented as median and quartiles and analyzed using Mann-Whitney U test.

Results

Baseline data such as sex, mean birth weight, maturity, age at enrolment and serum zinc concentration were comparable between the zinc and control groups (Table I). The mean duration of hospital stay was also similar in both the groups.

Relative gene expression in quantitative polymerase chain reaction (qPCR): The adjusted mean differences at 95 per cent confidence interval in mRNA expression levels in the two groups are shown in Table II. The fold change of immune response genes were expressed as median (interquartile range) (Table III). There was significant fold change with regard to *S100A8*, *S100A9* and *CD14* genes whereas the other genes did not show significant change.

Effect of zinc supplementation on relative expression of immune response genes and fold change: The expression levels of immune response genes were normalized to the ratio of housekeeping gene beta-actin. In qPCR, it was found that *S100A8* and *S100A9* gene expression levels were similar at baseline in both the groups. After day 10, *S100A8* gene was found to be downregulated by three-fold in zinc group ($P<0.01$) and *S100A9* gene was observed to be upregulated by two-fold in zinc group compared to the control groups ($P<0.05$). The expression levels of *TNF- α* and *IL-6* genes did not show any significant change compared to baseline in both the groups. In the present study, no significant difference was observed in the mRNA

expression of *LBP* gene. The expression levels of *CD14* gene did not exhibit any significant differences in zinc group compared to controls. Fold change of *CD14* gene was found to be significantly upregulated by one-fold in zinc group ($P<0.05$). Baseline expression levels of *TLR-4* gene were similar in both the groups. But post zinc treatment, significant mRNA expression of *TLR-4* gene was found in zinc group compared to controls ($P<0.002$). When it was expressed as fold change, no significant difference was found between groups.

Discussion

Pathophysiology of sepsis and septic shock involves complex cytokine and inflammatory networks. Nuclear factor κ B (NF- κ B) mediates the transcription of a large number of genes that play vital role and improve outcome of sepsis. Suppressing NF- κ B activity in sepsis model reduces acute inflammatory processes and organ dysfunction, thereby serving as a therapeutic target in patients with sepsis. A previous study has observed higher levels of NF- κ B activity that leads to increased mortality rate and worst clinical outcome¹⁹. We have earlier reported that zinc supplementation in neonatal sepsis has reduced mortality and improved neurodevelopment outcome at one year corrected age^{16,17}.

Earlier studies have reported on gene expression in various diseases^{20,21}. Shanley *et al*²² reported genome level longitudinal expression profiles in children with septic shock from whole blood derived RNA characterized by differential regulation of 2142 and 2504 gene probes on days 1 and 3. The gene regulation was involved in various signalling pathways and gene networks²². Gene expression analysis performed in ICU patients with severe

Table I. Demographic characteristics of zinc and control groups

Characteristics	Control group (n=22)	Zinc group (n=22)
Male, n (%)	19 (86.4)	17 (77.3)
Preterm, n (%)	19 (86.4)	15 (68.2)
Birth weight (kg), mean±SD	1.72±0.3	1.85±0.6
Apgar score at five minutes, median (range)	8 (6-9)	8 (6-9)
Blood culture positive, n (%)	6 (27.3)	7 (31.8)
Serum zinc levels (μ g/l), mean±SD		
Baseline	794.7±189.22	811.2±129.9
10 th day of supplementation	824.9±155.2	901.15±146.85***

*** $P<0.001$ compared to baseline

Table II. mRNA expression levels of immune response genes

Genes	Control group (n=22)		Zinc group (n=22)		Adjusted mean difference (baseline) 95% CI	P
	Mean±SD					
	Baseline	10 th day	Baseline	10 th day		
<i>SI00A8</i>	2.89±0.83	4.89±0.81	2.79±0.82	3.32±0.82	1.58 (1.08-2.08)	<0.001
<i>SI00A9</i>	3.53±1.09	1.41±0.93	4.34±0.89	1.71±0.86	0.15 (0.74-0.43)	0.59
<i>TNF-α</i>	4.25±1.46	4.63±1.28	4.51±1.88	4.37±1.81	0.30 (0.65-1.26)	0.52
<i>IL-6</i>	3.97±1.15	4.32±1.37	4.38±1.42	4.85±1.18	0.58 (1.37-0.20)	0.14
<i>LBP</i>	4.11±1.13	5.97±1.23	4.39±1.38	5.78±1.17	0.31 (0.33-0.96)	0.33
<i>CD14</i>	4.77±1.21	6.25±1.14	4.45±1.34	5.99±1.06	0.21 (0.46-0.88)	0.52
<i>TLR-4</i>	4.95±1.58	5.68±0.96	4.86±1.53	6.52±0.97	0.86 (1.39-0.33)	0.002

Table III. Fold change of the immune response genes

Immune response genes	Median (Q1, Q3)		P
	Control group (n=22)	Zinc group (n=22)	
	Fold change		
<i>SI00A8</i>	4.69 (2.36, 7.14)	1.86 (0.57, 3.43)	0.001
<i>SI00A9</i>	1.61 (0.73, 3.59)	3.38 (2.0, 5.25)	0.026
<i>TNF-α</i>	1.89 (0.45, 3.95)	1.14 (0.45, 3.21)	0.62
<i>IL-6</i>	1.50 (0.51, 4.07)	1.39 (0.88, 4.17)	0.81
<i>LBP</i>	5.62 (1.67, 7.69)	3.42 (1.21, 5.19)	0.12
<i>CD14</i>	1.23 (0.71, 3.90)	3.50 (1.87, 5.56)	0.03
<i>TLR-4</i>	2.90 (1.28, 6.96)	3.66 (1.30, 5.76)	0.84

TNF-α, tumor necrosis factor-α; *IL-6*, interleukin-6; *TLR-4*, toll-like receptor-4; *CD14*, cluster of differentiation 14; *LBP*, lipopolysaccharide binding protein

sepsis was compared with healthy controls using gene expression mRNA copy number in another study. It was found that immune regulatory genes provided novel robust tool for host response to an infection²³. The genes upregulated in septic shock patients were involved in multiple signalling pathways and gene networks related to inflammation and immunity²⁴. The downregulated gene expression patterns correspond to zinc-related biology²⁵. On the other side, expression studies in adults with sepsis and septic shock did not reveal repressed genes related to zinc biology although upregulated genes were associated with adaptive immune system²⁶.

Calprotectin sequesters micronutrient zinc and its availability to microbes is limited and termed as nutritional immunity. Equimolar concentrations of MRP8 and MRP14 showed a potent growth inhibitory

effect that was reversed by 30 mM zinc. Sequestration of zinc by calprotectin allows *Salmonella typhimurium* to thrive in the inflamed gut²⁷.

SI00A8 mRNA and protein levels were reduced during recovery in septic shock patients. An increase in *SI00A9* mRNA levels in whole blood leukocytes from septic shock patients predicted hospital-acquired infections²⁸. A decrease in mRNA levels was observed at days 7-10 in patients who did not progress to late sepsis. However it remained to be seen in patients who later acquired opportunistic infections²⁸. In our study, *SI00A8* gene showed downregulation by three-fold and *SI00A9* gene exhibited upregulation by two-fold in zinc group compared to controls. No significant differences were observed in *TNF-α* and *IL-6* cytokine gene expression in zinc supplemented group compared to controls.

LBP gene expression was induced in multiple organs with injury and *CD14* upregulation was associated with mortality in rats with infection and severely injured patients⁸. In neonates with sepsis, LBP and soluble CD14 levels were significantly increased compared to healthy controls. Thus highly elevated plasma LBP levels persist for more than 24 h and provide the clinicians with longer duration to identify early-onset sepsis in the newborn²⁹. In our study, significant differences were not found in the mRNA expression of *LBP* and *CD14* genes between the two groups. The fold change of *LBP* gene was downregulated insignificantly in zinc group compared to controls but *CD14* gene was significantly up regulated in the zinc group.

In patients with sepsis, *CD14*, *TLR-2* and *TLR-4* genes showed increased expression compared to

controls. Mortality was related with downregulation of *TLR-2* and expression of *CD14* on monocytes was correlated with reduced cytokine inducibility. It was suggested that *CD14* and *TLR-2* were key factors in monocyte hyporesponsibility during severe sepsis³⁰. In the present study, post treatment, significant mRNA expression levels of *TLR-4* gene were found in zinc group compared to control group. When data were expressed as fold change, *TLR-4* gene did not show any significant difference.

Despite small sample size, the data obtained from our study showed significant change in the expression of some of the immune related genes with zinc supplementation. The study was under powered to look at the outcome with change in gene expression.

We could analyze gene expression only for 22 samples in each group due to resource constraints and this was the limitation of the study. Further studies with a large sample size should be carried out in future to get a clear clinical picture of the expression of immune-related genes on the outcome of neonatal sepsis. Our preliminary results showed that zinc supplementation might modifies the expression of immune related genes in newborns with sepsis.

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Conflicts of Interest: None.

References

- Cvijanovich NZ, King JC, Flori HR, Gildengorin G, Wong HR. Zinc homeostasis in pediatric critical illness. *Pediatr Crit Care Med* 2009; 10 : 29-34.
- Hempe JM, Cousins RJ. Cysteine-rich intestinal protein binds zinc during transmembrane zinc transport. *Proc Natl Acad Sci U S A* 1991; 88 : 9671-4.
- Wong HR. Genome-wide expression profiling in pediatric septic shock. *Pediatr Res* 2013; 73 : 564-9.
- Wong HR, Shanley TP, Sakthivel B, Cvijanovich N, Lin R, Allen GL, *et al*. Genome-level expression profiles in pediatric septic shock indicate a role for altered zinc homeostasis in poor outcome. *Physiol Genomics* 2007; 30 : 146-55.
- Ehrchen JM, Sunderkötter C, Foell D, Vogl T, Roth J. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. *J Leukoc Biol* 2009; 86 : 557-66.
- Benedyk M, Sopalla C, Nacken W, Bode G, Melkonyan H, Banfi B, *et al*. HaCaT keratinocytes overexpressing the S100 proteins S100A8 and S100A9 show increased NADPH oxidase and NF-kappaB activities. *J Invest Dermatol* 2007; 127 : 2001-11.
- Loomans HJ, Hahn BL, Li QQ, Phadnis SH, Sohnle PG. Histidine-based zinc-binding sequences and the antimicrobial activity of calprotectin. *J Infect Dis* 1998; 177 : 812-4.
- Wang SC, Klein RD, Wahl WL, Alarcon WH, Garg RJ, Remick DG, *et al*. Tissue coexpression of *LBP* and *CD14* mRNA in a mouse model of sepsis. *J Surg Res* 1998; 76 : 67-73.
- El Gazzar M. Immunobiology of S100A8 and S100A9 proteins and their role in acute inflammation and sepsis. *Int J Immunol Immunother* 2015; 2 : 013.
- van Zoelen MA, Vogl T, Foell D, Van Veen SQ, van Till JW, Florquin S, *et al*. Expression and role of myeloid-related protein-14 in clinical and experimental sepsis. *Am J Respir Crit Care Med* 2009; 180 : 1098-106.
- Wong HR, Cvijanovich N, Allen GL, Lin R, Anas N, Meyer K, *et al*. Genomic expression profiling across the pediatric systemic inflammatory response syndrome, sepsis, and septic shock spectrum. *Crit Care Med* 2009; 37 : 1558-66.
- Li Q, Meng H, Zhang L, Xu L, Chen Z, Shi D, *et al*. Correlation between single nucleotide polymorphisms in a calprotectin subunit gene and risk of periodontitis in a Chinese population. *Ann Hum Genet* 2007; 71 : 312-24.
- Gerdes JS. Clinicopathologic approach to the diagnosis of neonatal sepsis. *Clin Perinatol* 1991; 18 : 361-81.
- Gerdes JS, Polin R. Early diagnosis and treatment of neonatal sepsis. *Indian J Pediatr* 1998; 65 : 63-78.
- Kumar R, Musoke R, Macharia WM, Revathi G. Validation of c-reactive protein in the early diagnosis of neonatal sepsis in a tertiary care hospital in Kenya. *East Afr Med J* 2010; 87 : 255-61.
- Banupriya N, Vishnu Bhat B, Benet BD, Sridhar MG, Parija SC. Efficacy of zinc supplementation on serum calprotectin, inflammatory cytokines and outcome in neonatal sepsis - A randomized controlled trial. *J Matern Fetal Neonatal Med* 2017; 30 : 1627-31.
- Banupriya N, Bhat BV, Benet BD, Catherine C, Sridhar MG, Parija SC. Short term oral zinc supplementation among babies with neonatal sepsis for reducing mortality and improving outcome - a double-blind randomized controlled trial. *Indian J Pediatr* 2018; 85 : 5-9.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; 25 : 402-8.
- Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. *Am J Physiol Lung Cell Mol Physiol* 2006; 290 : L622-45.
- Chu A, Foster M, Hancock D, Bell-Anderson K, Petocz P, Samman S. *TNF-alpha* gene expression is increased following zinc supplementation in type 2 diabetes mellitus. *Genes Nutr* 2015; 10 : 440.
- Erez O, Romero R, Tarca AL, Chaiworapongsa T, Kim YM, Than NG, *et al*. Differential expression pattern of genes

- encoding for anti-microbial peptides in the fetal membranes of patients with spontaneous preterm labor and intact membranes and those with preterm prelabor rupture of the membranes. *J Matern Fetal Neonatal Med* 2009; 22 : 1103-15.
22. Shanley TP, Cvijanovich N, Lin R, Allen GL, Thomas NJ, Doctor A, *et al.* Genome-level longitudinal expression of signaling pathways and gene networks in pediatric septic shock. *Mol Med* 2007; 13 : 495-508.
 23. Grealy R, White M, Stordeur P, Kelleher D, Doherty DG, McManus R, *et al.* Characterising cytokine gene expression signatures in patients with severe sepsis. *Mediators Inflamm* 2013; 2013 : 164246.
 24. Wong HR. Clinical review: Sepsis and septic shock - the potential of gene arrays. *Crit Care* 2012; 16 : 204.
 25. Wong HR, Cvijanovich NZ, Allen GL, Thomas NJ, Freishtat RJ, Anas N, *et al.* Validation of a gene expression-based subclassification strategy for pediatric septic shock. *Crit Care Med* 2011; 39 : 2511-7.
 26. Rink L, Haase H. Zinc homeostasis and immunity. *Trends Immunol* 2007; 28 : 1-4.
 27. Liu JZ, Jellbauer S, Poe AJ, Ton V, Pesciaroli M, Kehl-Fie TE, *et al.* Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut. *Cell Host Microbe* 2012; 11 : 227-39.
 28. Fontaine M, Pachot A, Larue A, Mougin B, Landelle C, Venet F, *et al.* Delayed increase of *S100A9* messenger RNA predicts hospital-acquired infection after septic shock. *Crit Care Med* 2011; 39 : 2684-90.
 29. Berner R, Fürll B, Stelter F, Dröse J, Müller HP, Schütt C. Elevated levels of lipopolysaccharide-binding protein and soluble CD14 in plasma in neonatal early-onset sepsis. *Clin Diagn Lab Immunol* 2002; 9 : 440-5.
 30. Schaaf B, Luitjens K, Goldmann T, van Bremen T, Sayk F, Dodt C, *et al.* Mortality in human sepsis is associated with downregulation of Toll-like receptor 2 and CD14 expression on blood monocytes. *Diagn Pathol* 2009; 4 : 12.

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