Pak. J. Biotechnol. Vol. 15 (1) 129-134 (2018) ISSN Print: 1812-183
www.pjbt.org ISSN Online: 2312-7791

TLR2 AND TLR4 AS A BIOMARKER OF BACTERIAL SEPSIS SYNDROME IN ADULT AND CHILDREN PATIENTS IN IRAQ

Farah Q. Younis, Ali Hussein. Alwan, Neihaya H. Zaki

Department of Biology, College of Science University of Mustansiriya, Iraq.
E.mail: farahyounis89@gmail.com

Article received 8.1.2018, Revised 10.2.2018, Accepted 19.2.2018

ABSTRACT

This study aimed to evaluate the using of Toll-like receptors (TLR2 and TLR4) gene expression as an early biomarkers for diagnosis of bacterial septic syndrome in children and elderly. The causative agents of infection were determined by blood culture. Tumor Necrosis Factor alpha (TNF-α), Interleukin 10 (IL-10), and Soluble Human Leukocyte Antigen - antigen D Related (sHLA-DR) was measured by enzyme-linked immunosorbent assay (ELISA) and TLR2, TLR4 expression was determined by quantitative real-time PCR. We included 75 patients was diagnosed with sepsis syndrome.The age range of patients (13 days-92 years) with mean 56.3±13.9 and matched to 55 healthy volunteers. Depending on age, patients were divided into 4 age groups (group A <1 year, group B 1-13 year, group C 14-40 year and group D >40 year). The results indicated that the levels of TNF-α, IL-10 and sHLA-DR in all age groups were significantly increased ($P<0.05$) except the level of TNF-α in group B ($P=0.123$) compared with control groups. A positive correlation has observed between levels of TNF-α and sHLA-DR with patients age (*P*-value=0.044 and *P*-value= 0.00013),respectively. TLR2 and TLR4 mRNA expression was significantly increased in all age groups with significant difference between group A and groups D. TLR2 expression highly increased in G+ve infection, while TLR4 expressed highly in G-ve infection. We conclude that TLR2 and TLR4 expression in bacterial sepsis patients indicates the strong possibility of using them as biomarkers in the early diagnosis of bacterial sepsis syndrome, in children and elderly patients

Keyword: TLR2, TLR4, Soluble HLA-DR, Sepsis biomarker, bacterial sepsis

INTRODUCTION

The human innate immune system is the first defense line against invasive pathogens. Infant's immune system is differing from that of adult but both very young children, and older adults both have a relatively poor immune system (Simon *et al*., 2014). Both immune responses (innate and adaptive) are irregulated by aging, which contributes to increase the occurrence of infection in the elderly (Starr and Saito, 2014). Bacterial sepsis syndrome is a systemic inflammatory response to an infectious stimulus orchestrated by the bulk of pro-inflammatory mediator production as a response to pathogen associated molecular patterns (PAMP) such aslipopolysaccharide (LPS) and peptidoglycan which the main component of bacterial cell wall of G-ve and G+ve bacteria (Rittirsch *et al*., 2008).The innate immune response very important in the defense against bacterial pathogens in early phases of infection. Pattern recognition receptors (PRRs) are part of these defense, which expressed on leukocytes, and one of the most important PRRs is Toll-like receptors (TLRs), which is important as sentinel receptors have increased appreciation that which recognized PAMPs (Zhang *et al*., 2010). LPS is primarily sensed by TLR4 while, peptidoglycan, has been sensed by TLR2 (Kumar *et al*., 2011). A TRL cascade of cellular signals leads to activation of pro-inflammatory cytokines such TNFα, and anti-inflammatory cytokines like IL-10 and this TLR sactivation not only leads to inducing of inflammatory responses but also to development of antigen-specific adaptive immunity (Lu, 2009).

Soluble HLA-DR is one of the numerous markers that have been tested for their capacity to predict mortality in septic patient's molecules acts a central role in the specific immuneresponse to infection. The important of soluble HLA-DR molecules can function as ligands for super-antigens and, thus, may play a role in the "detoxification" of super-antigens (Perry *et al*., 2004).

Studies that combine children and elderly with bacterial sepsis are generally low.This study came to illustrate differences between the markers in their immune response to sepsis infection and determine a better biomarker for early diagnosis of bacterial septic syndromes in Iraqi's patients.

MATERIALS AND METHODS

Study Design and Population: Seventy five blood samples were collected from different patients who diagnosed cliniclly by a specialized physician with sepsis from intensive care units of Baghdad Hospitals, Iraq, during the period between March 2016 to October 2016. Patients are characterized by having more than two criteria of sepsis with mean 56 ± 13 year. Control group consists of 55 healthy volunteers, with mean of age 35.7 ±17.2 years. The patients have been divided into 2 main groups' adults and children and each group was subdivided into two groups according to their ages. For adults, sepsis has been defined by presents of two from the four following criteria: fever (>38˚C or <36˚C), respiratory rate of >20 breaths/minute or arterial carbon dioxide tension ($PaCO_2$) <32mm Hg, heart rate of >90 beats/ minute, and abnormal white blood cell count (>12,000/µL or <4,000/µL or >10% immature for-ms). For children, sepsis was defined as two of the four following criteria: temperature >38.5˚C <36 ˚C, respiratory rate >2 (SD) above normal for age in the absence of external stimulus, heart rate >2 standard deviations (SD) above normal for age, and white blood cell count elevated or depressed for age, or >10% bands (Rudd *et al*., 2017**).**

Sample Collection: Five ml of blood from adults and 3 ml form children has been collected by vein puncture after prepared it by using a bacterial disinfectant 2% solution. Peripheral venous blood samples were collected from each participant by using sterile single use needles. This blood was used for blood cultures, cytokines measurement and RNA extraction.

Cytokines and soluble HLA-DR measurement: Serum IL-10, TNF-α and soluble HLA-DR concentrations were determined by ELISA using a commercial human ELISA kit (R&D system Inc., USA) for IL-10 and TNF-α and (My BioSource Company, USA) for sHLA-DR, in accordance with the manufacturer's instructions. Concentrations were calculated by using of the mean optical density of two wells and comparison with a standard curve.

RNA extraction: RNA was extracted manually by TRIzole provides by (Invitrogen Life Technologies, USA). An efficient method for purifying total RNA from whole blood, and also the procedure of extraction according to the manufacturer's instructions. RNA concentration was measured by nanodrop spectrophotometer (Quawell Q5000, USA) and the purity detected by noticing the ratio of optical density (O.D.) at wevelength 260/280.

Quantitative real-time PCR: Gene expression of TLR2 and TLR4 were determined by using a KAPA SYBR FAST one-step qRT-PCR kit (Canada) and is a sensitive and convenient solution for real-time PCR using RNA as a template. The kit comprises KAPA SYBR FAST master mix (2 X) and KAPA RT mix (50 X). The KAPA RT mix comprises wild-type M-MuLV reverse transcriptase and RNase inhibitor, and is optimized for rapid one-step, one tube RNA quantification.The result is a unique enzyme, specifically designed for qPCR using SYBR Green I dye chemistry.

RT-PCR was performed using designated primers for TLR2(forward:**5'**TGTGGATGGTGTGGGTCTTG**3'**, Reverse: 3'ATATGCAGCCTCCGGATTGT5'), TLR4 (forward:5'ATATTGACAGGAAACC-CCATCCA3', Reverse: 3'AGAGAGATTGAGTAGGGGCATTT5'), with house keeping gene (GAPDH) (forward: 5'ATCACT GCCACCCAG- AAGACTG3', reverse: 3'AGGTTTTT-CTAGACGGCAGGTCAG5') (Alpha DNA technologies). Relative gene expression to an internal calibrator was determined using the $2^{-\Delta\Delta CT}$ method. The C_t value of the target genes was normalized (ΔCt) to the C_t value of the TLR 2 and TLR4 genes of the samples.

Statistical analysis: Student *t*-test was used when comparing two groups and ANOVA/Bonferroni test when comparing more than two groups. Data were expressed as mean±S.E. Correlations were determined using a Spearman correlation test. *P*-values lower than 0.05 were considered statistically significant. The data were analyzed by the statistical software (SPSS 22.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

In our trial, the relationship between sepsis and age, grow in blood culture, type of causatve pathogen, cytokines (TNF-α, IL-10, sHLA-DR) and TLR expression in patients were investigated.

The study included 75 patients with suspicion of septicemia who admitted to ICU and hospital lounges, and 55 healthy controls were enrolled in the study. Table 1 showed the demographic characteristics of the patients.

Blood culture was positive in 49/75 (65.3%) of sep-tic patient's. All infections were due to only one organism. About the types of bacteria that isola-ted from positive culture, G-ve constituted 59.1 %, while G+ve constituted 40.8 %. A total of 49 orga-nisms were isolated, the commonest organism was *E.coli* 17 (34.6 %) followed by *S.aureus* 14 (28.5 %). Thee isolated and identified

bacteria which resulted from blood culture showed in table 2.

Characteristic	Adult	Children
No.	41	34
Age (yr)	56.5 ± 22.26	2.6± 3.18
Male / female	28/13	17/17
Infection leads to sepsis		
Ural Sepsis	18 (43.9%)	-
Immune suppression patient	4 (9.7%)	10 (29.4%)
Wound infection	6 (14.6%)	-
Post operation sepsis	5 (12.1%)	-
Renal failure, Hepatic failure, Brain failure	8 (19.5%)	-
Neonatal sepsis	-	10(29.4%)
Meningitis	-	5 (14.4%)
Respiratory tract infection	-	5 (14.7%)
Gastrointestinal infection	-	4 (11.7%)

Table 1: Demographic data of patients with suspected sepsis

Table 2: Types of isolated bacteria in patients enrolled in the present study

Type of Bacteria	No. (%)
G-ve	**29 (59.1)**
E.coli	17 (34.6)
A.baumannii	4 (8.1)
K.pneumonia	4 (8.1)
P.aeroginosa	3 (6.1)
C.freundii	1 (2)
G+ve	**20 (40.8)**
S.aureus	14 (28.5)
E.fecalis	3 (6.2)
S.pneumoniae	3 (6.1)
Total	49 (100)

As a result of our study, G-ve bacteria were the most predominant causative pathogens of septicemia in the present study (59.1 %), while the percentage of G+ve was 40.8 %. As the findings of our study, Sahoo *et al*.(2016) demonstrated that G+ve organisms were found to be 30.8 % among all isolates causing sepsis, whereas the G-ve bacteria were 69.2 % of all isolates. *E. coli* was most common causative of sepsis in patients (34.6 %) followed by *S. aureus* with (28.5 %). This result was identical to Kotgire *et al*.(2017) who found that *E. coli* constituted 34.6 % and *S. aureus* (23.1 %). Also, in another study it ws found that *S. aureus* and *E. coli* were the prevalent isolates from the total isolate that constitute for G+ve and G-ve, respectively (Zhang *et al*., 2015).

Level of IL-10, TNF-α and sHLA-DR in serum of patients with different age groups: According to their ages, the patients have been divided into 4 groups (<1 year (group A), 1-13 year (groups B), 14-40 year (groups C), >40 year (groups D).

TNF-α and IL-10 concentration was significantly increased*P*-value< 0.05, *P*-value< 0.001 in all age groups compared with their controls. Except level of TNF-α in group C (P=0.123). As shown in table 3.

Table 3: Level of IL-10, TNF-α and sHLA-DR in patient's serum with different age groups.

Age group	No.	IL-10 Mean ± S.E	P.value	TNF-α Mean ± S.E	P. value	sHLA-DR Mean ± S.E	F
Group A	24	49.50 ± 9.36	**0.000****	56.1 ± 5.74	**0.001****	9.06 ± 1.75	**0.031***
	18	3.84 ± 0.49		28.63±4.10		4.49 ± 0.16	
Group B	10	41.16 ± 13.04	**0.033***	62.48 ± 11.71	**0.123**	9.19 ± 2.63	**0.128**
	7	3.32 ± 0.89		39.80 ±2.04		4.59 ± 0.16	
Group C	12	77.97 ± 21.43	**0.011***	77.97 ± 9.08	**0.009***	15.97 ± 2.36	**0.021***
	8	2.54 ±0.49		43.90 ± 4.00		8.52 ± 0.45	
Group D	29	60.33±12.24	**0.000****	81.19 ± 9.62	**0.000****	20.00 ± 1.88	**0.000****
	22	2.91 ± 0.56		34.00 ± 3.28		9.30 ± 0.47	

*** *P*-value<0.05 ** *P*-value<0.001**

sHLA-DR concentration was significantly increased *P*-value< 0.05, *P*-value< 0.001 in age groups (group A, group C and group D) compared with their controls,but no significant has been shown in age group B when it's compared with their control group. As shown in table 3.

Artero *et al*. (2012) indicated to a direct relationship between progress age and the occurrence of sepsis, with a sharp increase in incidence in elderly people, and also the incidence of sepsis in infants is also elevated. A previous work reported when the patients are reached at the age of 65 and above, the relative risk for sepsis was 13 times higher than younger patients (Burrell *et al*., 2016).

In the infant sepsis the group A who >1year was 32% of all patients and 70 %of children's groups and this result similarity with Wacker*et al*., who

finding that 76% of children less than1 year of age from their cases of sepsis are most likely to be infected[Wacker*et al*., 2013]. Angus *et al*. (2001) found that the incidence of sepsis was highest in infants (5.3/1,000 aged,1y), and also, rising sharply in the elderly (26.2/1,000 aged 85y) and also the number of cases also increased with increasing age, so that, this result in line with our study**.** Children from 1-4 years old are clearly different than adults in terms of underlying disease, mortality, and sites of infection, and also those younger than 35 years of age, in fact, these two groups more similar to each other than they are to either infants or adults over the age of 60 years (Watson *et al*., 2003)**.**

The results of our study proved that pro-inflammatory and anti inflammatory cytokines levels were significantly higher in septic patients compared with healthy controls. As well; the level of IL-10 significantly higher in the elderly compared to children patients.These results were in accordance with previous studies who reported that no age-related difference in TNF-α, or IL-10 on admission in hospitalized patients with *S. pneumonia* infection; and also the elderly patients had significantly higher levels of TNF-αcomparing with younger patients, suggesting once again a prolonged period of inflammatory response in elderly (Bruunsgaard *et al*., 1999 ; Krabbe *et al*., 2004).Other investigators found that all cytokine levels were measured significantly higher in septic patients compared with healthy individuals and the monitoring of serum cytokines levels were a good marker for the immune status (House and Descotes, 2007)**.**

Soluble HLA-DR levelhad significantly higher in all age groups except group B. Our results accordance with Perry *et al*. (2004) who has been reported that sHLA-DR increased in the infectious and inflammatory diseases or diseases that activated immune profile. Another study found increasing of soluble HLA-DR in adult patients after surgery prior to clinical signs of bacterial sepsis (Hietbrink, 2008). High levels of sHLA-DR has been shown in septic patients compared with healthy controls ans this may be due to the bacterial infection and their response to inflammation**.** Rebmann*et al*.(2002) indicated that soluble forms of HLA in serum and other body fluids can perform functions as immune response modulators. There is an increasing support that the serum-soluble HLA forms play a role in the pathophysiology of many diseases.

Correlation between cytokines and sHLA-DR and age: The results of our study indicated to a significant positive correlation (*P*-value <0.05) between levels of both TNF-α and sHLA-DR in patients serum and their age. As shown in figure 1-A, the concentration TNF-α was increased with increasing of age (R= 0.2332, *P*-value =0.044) and also increasing of sHLA-DR in serum of patients with increasing of their ages (R =0.42832, *P*-value = 0.00013). No correlation was observed between the level of IL-10 of patient's serum and their age (Figure 1-B).

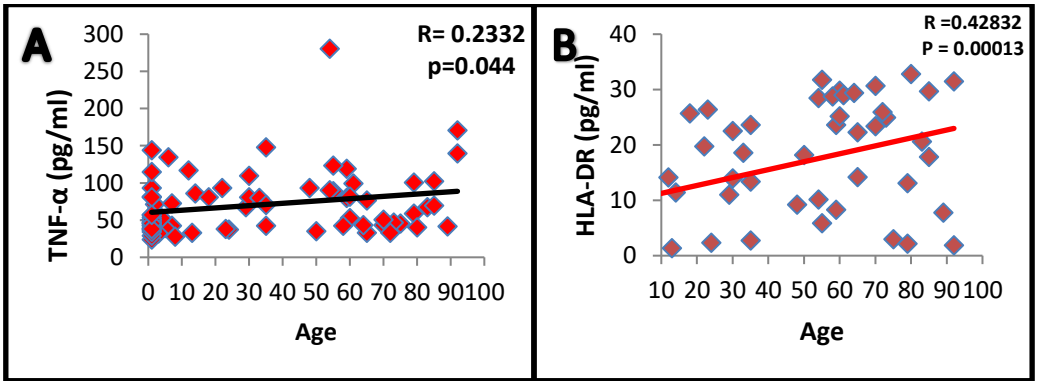

Figure 1: A:correlation between TNF-α concentration and patients age. B: correlation between sHLA-DR concentration and patients age

The data of this study demonstrated a positive correlation between TNF-α, sHLA-DR in the patient's serum and their age. The expression of HLA-DR in both term and preterm newborns with or without signs of infection showed a lower level in comparison with adults during the first day of life (p<0.0001). Prematurity associates with lowering expression of neonates with gestational age less than 32 weeks (p=0.0008) (Zhuang *et al*., 2017). Thevaranjan *et al*. (2017) was reported that ageing is relatedto increasing of inflammatory activity in the blood, including increased circula-

ting levels of TNF-α compared with the young group. While Kleiner *et al*. (2013) did not show any differences between young children and adults, but were all upregulated in children between TNF-α levels and showed more complex profiles.

Expression of TLR2 and TLR4 in different age groups: As shown in table 4, TLR2 and TLR4 mRNA have been expressed in all age groups. TLR2 expression has been shown with high level in group D (4.458 **±**0.650) and the lowest level of expression showed in group A (2.436 **±**0.298), with a significant difference among all groups (*P*-value < 0.049). TLR4 expression has been shown with high level in group D(7.669 **±** 0.803) and the lowest level of expression showed in group B (1.575 **±** 0.296), and also a significant difference among all groups (*P*-value <0.000).

Table 4: Expression of TLR2 and TLR 4 in patients according to their age

Age group	No.	TLR 2 (fold change±S.E)	*P*. value	TLR 4 (fold change± S.E)	*P*. value
Group A	21	2.436 ±0.298	**0.049***	2.497 ± 0.281	**0.000****
Group B	5	2.763 ±0.697		1.575 ± 0.296	
Group C	8	3.021 ±0.664		3.255 ± 0.616	
Group D	26	4.458 ±0.650		7.669 ± 0.803	

*** *P*-value < 0.05 ***P*-value< 0.000**

The results of our study showed increasing of the gene expression for TLR2 and TLR4 in age groups with significant differences between group A and group D. This result consistent with the results of many researchers (Akira and Takeda, 2004; Zhang *et al*., 2010) who found that TLR2 and TLR4 genes expression in peripheral blood significantly increasing in neonatal infections.This result indicates that the status of infection is a main parameter affecting in the expression of TLR2 and TLR4, which can use it as a septic syndrome biomarkers. De Gaudio *et al*. (2009) has been reported that expression of TLRs and their function due to aging affects not only the magnitude but also the quality of the host immune response to pathogens by the altered inflammatory and priming environment.

Expression of TLR 2 and TLR 4 according to type of bacteria: Our results exhibited that the expression of TLR2 significantly increased in septic patients (*P*-value<0.000) who infected by G+ve compared with G-ve 5.570 ± 0.712 , 2.333 ± 0.225) respectively. On the other hand,the results showed that the expression of TLR4 significantly increases in septic patients (*P*-value<0.000) who infected by the G-ve compared with G-ve (8.120 ± 0.684, 2.231 ± 0.257) respectively.

Furthermore, TLR2 was increased in cases of infection with G+ve bacteria, while TLR4 generally increased in the cases of infection with G-ve bacteria. This result agreed withDas (2000) who reported that TLR-4 is one of the receptor which specifically for LPS and signal transduction cascades activation may be lipoteichoic acid. Moreover, the specificity of TLR-2 is much less, and responds to a different numberof antigens including peptidoglycan and G+ve. This evidence suggests that it is important to look at each TLR separately and in their combination of the possibility responses to specific microorganisms to discover specific protein pathways for the different types of pathogens that will enable us to understand the pathways of the innate immune system responds to different stimuli**.**

CONCLUSION

Our findings showed a significant increase in the gene expression of TLR2 and TLR4 in different age groups of patients and also, between the children who under 1 year of age and those above 40 years. Also, The TLR2 gene expression increased in patients infected with G+ve bacteria in comparison with G-ve and increasing of TLR4 gene expression in patients with G-ve compared with G+ve. This result indicates to the strong possibility of using TLR2 and TLR4 vital indicators in the early diagnosis of bacterial sepsis syndrome, especially in children and elderly patients, and to determine the type of bacterial infection.

ACKNOWLEDGMENT

The authors would like to thank Al-Mustansiriyah University (www.uomustansiriyah.edu.iq), in Baghdad. Iraq for its support in the present work.

REFERENCES

Akira, S. and K. Takeda, Toll-like receptor signalling. Nat. Rev. Immunol. 4(7): 499-511 (2004).

Angus, D.C., W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo and M.R.Pinsky, Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associa-

ted costs of care. Crit. Care Med. 29(7): 1303 -10 (2001).

Artero, A., R. Zaragoza and J.M. Nogueira, Epidemiology of severe sepsis and septic shock. In Severe Sepsis and Septic Shock-Understanding a Serious Killer. InTech. (2012).

Bruunsgaard, H., P. Skinhøj, J. Qvist and B.K. Pedersen, Elderly humans show prolonged in vivo inflammatory activity during pneumococcal infections. J. Infect. Dis. 180(2): 551-4 (1999)

Burrell, A.R., M.L. McLaws, M. Fullick, R.B. Sullivan, and D. Sindhusake, Sepsis Kills: early intervention saves lives. Med. J. Aust. 204(2): 73 (2016).

Das U.N, Critical advances in septicemia and septic shock. Critical care. 4(5):290 (2000).

De Gaudio, A.R., S. Rinaldi, C. Chelazzi, and T. Borracci, Pathophysiology of sepsis in the elderly: clinical impact and therapeutic considerations. Curr. Drug Targets. 10(1): 60-70 (2009).

Hietbrink, F., Kinetics of the excessive cellular innate immune response after injury (Doctoral dissertation, Utrecht University. (2008).

House, R. V and J. Descotes, Cytokines in human health: immunotoxicology, pathology and clinical applications. Humana.366 (2007)

Kleiner, G., A. Marcuzzi, V. Zanin, L. Monasta and G. Zauli, Cytokine levels in the serum of healthy subjects. Mediators Inflamm (2013).

Kotgire, S. and S. Hatkar, Aerobic bacteriological profile and its antimicrobial sensitivity pattern from blood culture specimens in a tertiary care hospital. APALM.4(1): A23-27 (2017).

Krabbe, K.S., M. Pedersen and H. Bruunsgaard, Inflammatory mediators in the elderly. ExpGerontol. 39(5): 687-99 (2004).

Kumar, H., T. Kawai and S. Akira, Pathogen recognition by the innate immune system. Int. Rev. Immunol. 30(1): 16-34 (2011).

Lu, Y. C., Regulation of toll-like receptor signal transduction pathways (Doctoral dissertation, University of Toronto)(2009).

Perry, S.E., S.M. Mostafa, R. Wenstone, A. Shenkin and P.J. McLaughlin, HLA-DR regulation and the influence of GM-CSF on transcription, surface expression and shedding. Int. J. Med. Sci. 1(3): 126 (2004).

Rebmann, V., S. Ugurel, W. Tilgen, U. Reinhold and H. Grosse-Wandilde, Soluble HLA-DR is a potent predictive indicator of disease progression in serum from early-stage melanoma patients. IJC. 100(5): 580-5 (2002).

Rittirsch, D., M.A. Flierl and Ward, P.A. Harmful molecular mechanisms in sepsis. Nat. Rev. Immunol. 8(10): 776 (2008).

Rudd, K.E., L.K. Tutaryebwa, and T.E. West, Presentation, management, and outcomes of sepsis in adults and children admitted to a rural Ugandan hospital: A prospective observational cohort study. PloS one. 12(2): e0171422 (2017).

Sahoo, D., L. Mohanty, S.S. Panda and S.N. Mishra, Bacteriological analysis of blood culture isolates in patients with sepsis in a tertiary care hospital of eastern India. IJCMR. 3: 3448-50 (2016).

Simon, A.K., G.A. Hollander and A. McMichael, Evolution of the immune system in humans from infancy to old age. InProc. R. Soc. B. 282(1821): 20143085 (2015).

Starr ,M.E, and H. Saito, Sepsis in old age: review of human and animal studies. Aging Dis. 2:126 (2014).

Thevaranjan, N., A. Puchta, C. Schulz, Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. Cell Host and Microbe. 21(4): 455-66 (2017).

Wacker, C., A. Prkno, F.M. Brunkhorst, and P. Schlattmann, Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect. Dis;13(5): 426-35 (2013).

Watson, R.S., J.A. Carcillo, W.T. Linde-Zwirble, G. Clermont, J. Lidicker and D.C. Angus, The epidemiology of severe sepsis in children in the United States. AJRCCM 167(5): 695-701 (2003).

Zhang, J.P., Y. Yang, O. Levy and C. Chen, Human Neonatal Peripheral Blood Leukocytes Demonstrate Pathogen-Specific Coordinate Expression of TLR2, TLR4/MD2 and MyD88 During Bacterial Infection *In Vivo*. Pediatr. Res.68(6): 479 (2010).

Zhang, X., D. Liu, Y.N. Liu, R. Wangand L.X. Xie, The accuracy of presepsin (sCD14-ST) for the diagnosis of sepsis in adults: a meta-analysis. CritCare. 19(1): 323 (2015).

Zhuang, Y.,H. Peng, Y. Chen, S.Zhou andY.Chen, Dynamic monitoring of monocyte HLA-DR expression for the diagnosis, prognosis, and prediction of sepsis. Front. Biosci. 22: 1344 (2017).