GENETIC FACTORS OF SEPSIS IN CHILDREN

Sorin Buzinschi
Faculty of Medicine, University of Transylvania, Clinical Hospital of Children Brasov

ABSTRACT

The genetic fingerprint holds a major relevance in the apparition, evolution and prognosis of infections. The paper focuses on polymorphisms for one nucleotide (SNPs) of the genes which encode proteins with the main role in recognizing pathogens (Toll-like receptors: TLR2, TLR4, TLR5) or in the inflammatory response (TNFα, IL6, IL10) and which determine the severity and the type of the infection. The allelic configuration constitutes an important risk element for corioamniotitis, prematurity, sepsis and death. It can also be a growth factor in resistance to common infections. Post-septic immunodepression influences late mortality of children who have suffered from infectious shock.

Key words: genetic polymorphism, infections, death risk

GENETIC FACTORS OF SEPSIS IN CHILDREN

The sepsis represents a manifestation of the systemic inflammatory response generated by an infection. In its severe form it determines generalized organic dysfunction, peripheral perfusion disorder, hypotension, compromises tissues energogenesis and death. In severe infections of the premature infants the death rate ranges from 20% to 50% (1). In USA the death rate in severe sepsis of adults ranges from 20% to 70%, with an expense of 50.000$ per patient (2). Recent hypotheses reveal that the clinical state, the treatment response, after-effects of an infection, severe evolution and death are influenced by individual fluctuation of the genetic background (3,4,5,6,7). In clinical praxis some children brought in at ED suffering from community-acquired pneumonia show slight manifestations and can be treated at home, while other children affected by the same germs show severe respiratory failure and septic shock. In similar treatment circumstances most of the patients who suffer from sepsis have a positive evolution, but there is a small, yet significant, percent of cases which aggravate (sometimes unexpectedly) with multiple organ failure and death. Unfavorable evolution factors taken into account were the virulence of the germs, tardy treatment access, related pathology, but the doubt concerning the quality of care was often invoked. The first queries concerning the profound causes of these processes were initiated by Sorensen et al. (8) (9). Using data obtained from The Danish Adoption Register the authors noticed that the adopted subjects have a five times greater risk of death caused by infection, if the biological parent died due to an infection before turning 50. Conformable data regarding monozygotic twins indicate that there is a genetic aspect which exposes them similarly to infections and death. In some diseases like tuberculosis, leprosy, Helicobacter pylori infection, chronic infection with hepatitis C virus and the phenotypic response to immunization there is an ample conformity in monozygotic twins unlike dizygotic ones (11). Trough in-depth studies of the human genome (**whole genome association study**) it was shown the population's great variability, many of the genes being polymorphic because of small differences in the nucleotide sequence (16). Related with the genetic epidemiology data, research has been conducted in molecular biology. The studies focus on the polymorphism for one nucleotide (SNPs), as a reason for the growth of the critical risk during infections. The genetic polymorphism bears an influ-

Coresponding author:
Phd. Sorin Buzinschi, Faculty of Medicine, University of Transylvania, Clinical Hospital of Children, 45 Nicopole Str., Brasov
e-mail: sbuzinschi@yahoo.com

ence on both susceptibility and resistance to infections.

Knowledge of factors with major impact upon the occurrence and evolution of sepsis may help understand the evolution of sepsis in the neonatal, pediatric or adult intensive care unit, applying differentiated therapeutic measures.

Mention should be made that most of the data regarding SNPs was collected from adults or children; these may not be entirely concordant with those of the premature infants due to the adjustment of the gene expression during development (12). This is the reason why the research on premature and term newborn, although there is little research work done, carries a high information value.

THE IMMUNE SYSTEM IN NEWBORN AND CHILDREN

The fetal immune system develops in a sterile and protected environment, coexisting with the maternal immune system. In relation with the environment, the newborn relies on the inborn immune system. The immune capacity grows through progressive development of the adaptive immune response. Neonatal inborn immunity acts **before** the microbial exposure through (13) (14): surface barriers (skin, mucous membranes), immune cells "sentinel", pathogen detection system, proinflammatory proteins, defense proteins and peptides and through immunoglobulins received from the mother. IgG immunoglobulins are actively carried through placenta, their density in term newborn drawing near to or exceeding the density in the maternal blood. In premature newborn the level of transferred immunoglobulins is low in direct ratio with the gestational age. Inborn immune system responses, dynamic regardless of age, allow the host **to recognise pathogens** and to produce fast inflammatory response which comprises cytokine and chemochine production and effector molecules (15). The two functional components of the inborn immune system make use of a large number of receptors, accessory proteins, signaling molecules, and transcription factors involved in protein synthesis. The molecular basis of these processes consists of the expression of some genes which entail the synthesis of certain proteins which determines the response to antigen. The genetic susceptibility in sepsis manifests both in the antigen identification phase and in body response stage; the gene polymorphism which encodes proteins involved in the recognition of the pathogens and gene polymorphism of the inflammatory response factors are differentiated. In other words, the susceptibility to infections is genetically controlled (11). Detailing the genetics of these polymorphisms exceeds the boundaries of pediatrics and it reduces the possibility of having a global view on this subject as complicated as it is.

THE PHASES OF THE INFLAMMATORY RESPONSE

Identifying the invading pathogens relies on the recognition receptors from the cell surface (1)(17) (18,19) called Toll-like receptors (TLR). After the linkage between TLR and molecules of the infectious agent, complex sequential cell signal take place, which determine the activation of the nuclear factor NFkB. By accessing the ADN "library" it allows the activation of the transcription of genes which produce cytokines and other immune effectors which initiate the mechanisms of antimicrobial action. There is also a growth in complement and coagulation protein production. The anti-inflammatory cytokines, which appear in a subsequent phase, bring the cytokine level back to the basal level and initiate tissue repair. Severe sepsis issues from losing the balance between the antagonistic and proinfalmmatory cytokines in favour of the proinflamatory ones, or from the prolonged and massive stimulation carried out by the infectious agent.

The sensors of the inflammatory response TRLs are distributed on the surface of the macrophages, dendritic cells and neutrophils. Unlike cell B and T receptors, which are in a perpetual change, TLRs do not modify, being preserved evolutionally for the recognition of the bacterial antigens such as lipopolysaccharide LPS, bacterial flagellin, viral RNA or viral DNA sequences. LPS, the major component of the cell wall of Gram-negative germs is the most powerful stimulator of the inborn immune response (6). TLR2 and TLR4 play a major part in indentifying the Gram-negative and Gram-positive bacteria constituents (5).

TLR2 recognises Gram-positive constituents such as peptidoglycan, lipoteichoic acid and some lipoproteins. TLR2 polymorphisms represent a risk factor in infection with these bacteria, as it is shown in research on patients suffering from severe infections with Staphyloccocus aureus (16). Experimental research on necrotizing enterocolitis indicated the growth of the TLR2 and TLR4 expression and NFkB factor in the intestinal epithelium before the formation of histologic lesions (14).

TLR4 produces signals for a pro-inflammatory response in infections with Gram-negative germs,

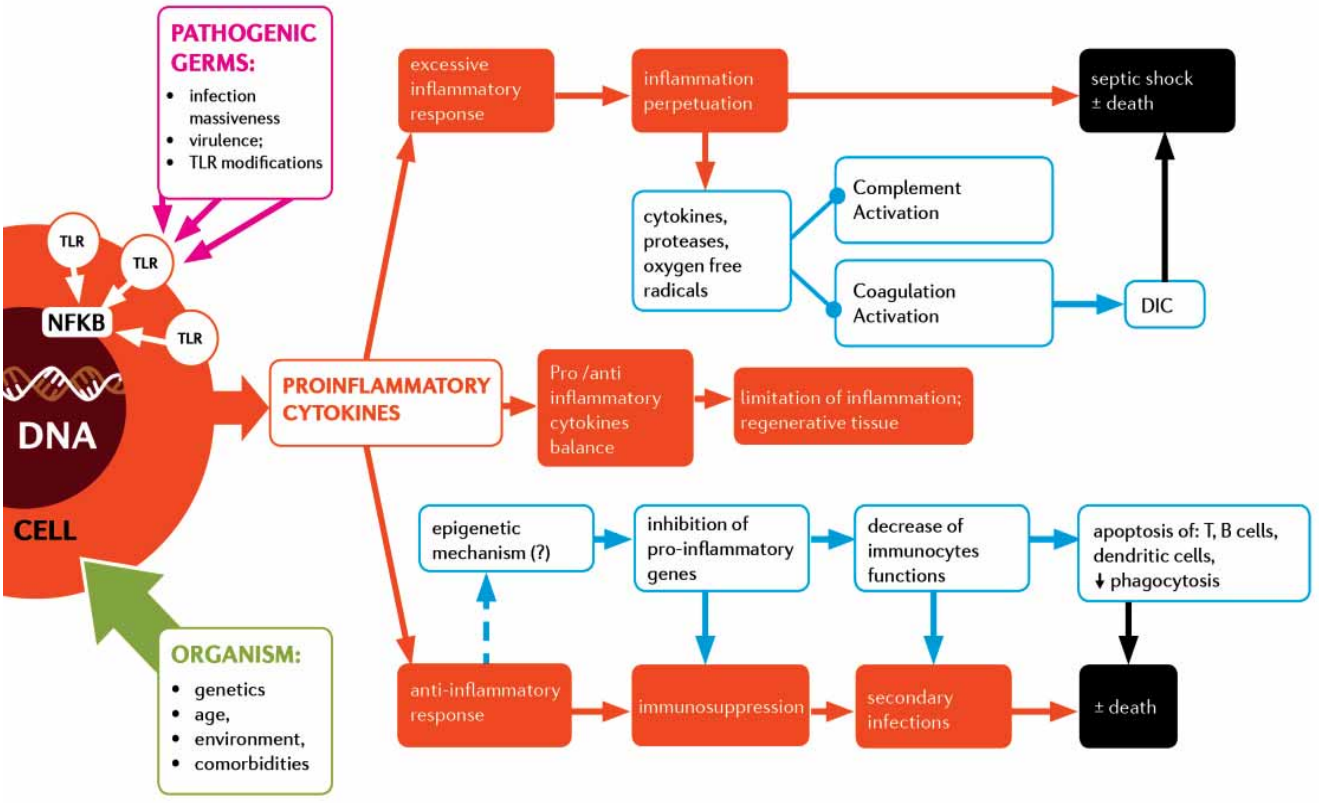

FIGURE 1. *Body's response to septic shock (Angus et al., modified) (43)*

the inductive factor being the existence of LPS. The TLR polymorphisms have been linked to sepsis/ septic shock with Gram-negative germs and high death rate in newborn and premature infants. Mention should be made that the predisposition to Gram-negative bacteria infections, the high frequency of the septic shock and the severity of the systemic inflammatory response (5). TLR4 is able to recognise specific proteins of the respiratory syncytial virus (33).

TLR5 recognises the bacterial flagellin; it contributes to the activation of NFkB and release of proinflammatory cytokine. In a research on 535 premature newborn, Maziad et al. (17) examined the role of the SNPs of the genes which mediate the immune response to bacterial infections. Among other genetic configurations, SNP for TLR5 has been related with E. coli infections or Ps aeruginosa, both germs being flagellated.

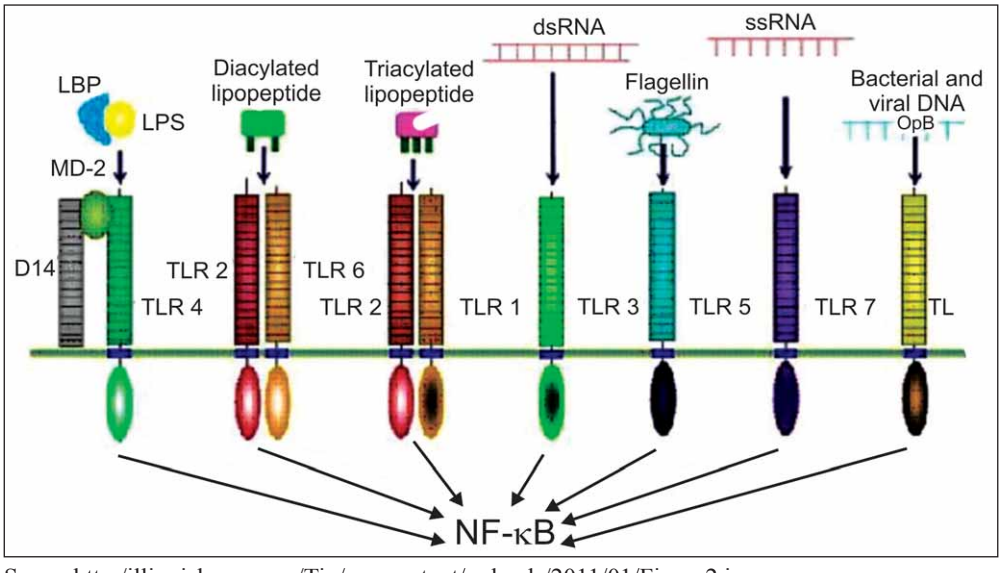

FIGURE 2. *TLRs and its differential activation according to the specific microbial molecules*

Sursa: http:/illinoislyme.com/Tix/wp-content/uploads/2011/01/Figure2.jpg

TLRS CONFIGURATION IN NEWBORN AND PREMATURE INFANTS

The severe evolution of the infections in the neonatal age may be attributed to the non-existence of the immunological memory, to an adaptive skilled immunity and to genetic heterogeneity to which prematurity, cardiac malformations or neurological affections add (34).

Research on monocytes in VLBW highlighted the decrease in expression on the surface of TLR4 monocytes, decrease of NFkB nuclear factor activation and of production of cytokines. TLR5 polymorphism could play a role in the apparition of premature births along with other polymorphisms such as TLR2, TLR4, TLR10 through favoring of chorioamnionitis (17)(20). *In utero* infection favored by these polymorphisms is an important risk factor in early start sepsis, about 80% of the premature births under 30 weeks being related to intrauterine infections.

TABLE 1. *Efects of some genetic polymorphisms in neonatal sepsis*

Authors	Polymorphisms	Efects in neonatal sepsis
Ahrens et al. (21)	TLR4, D14, NOD2, IL6	IL6, predictive changes for Gr+ Sepsis; NOD2 allele and IL6, high incidence sepsis at VLBW
Lorenz et al., 2002 (22)	TLR4	High risk of G- sepsis; risk of premature birth
Zhang et al., 2007 (23)	TLR2, TLR4	TLR2 correlation with G+ sepsis; TLR4 with Gr- sepsis
Krediet et al., 2007 (24)	TLR2, TLR4	TLR2 aberrant immune response at birth
Kollman et al., 2009 (45)	TLRs, IL-1β, IL6, IL-23, Il-10	Decreased response capacity to adults
Maziad et al., 2010 (17)	TLRs, CD14, IL-6, Il-10, IL-1β, TNF-α	Genes TLR2, TLR5,PLA2, IL-10 associated with sepsis TLR2,PLA2 variations, Gr+ infections, IL-10 Gr- infections

Microorganisms which determine vaginal infections or infections of the amniotic fluid trigger the production of the cytokines and antimicrobial peptides, which are involved in premature birth and/or the premature rupture of membranes. Although infections represent an important trigger factor, the modification of the inflammatory cascade through polymorphisms of TLR4, IL-1β, IL4, IL-10, IL-1ra, TNF-α genes may be looked at as one of the genetic premises of the premature birth (19).

GENETIC VARIATION OF THE GENES INVOLVED IN THE HOST'S RESPONSE TO PATHOGENS

The recognition of the pathogens involves the coordinate intervention of a number of cytokines and chemokines which determine a dual answer:

- the growth of the inflammatory cytokines such as TNF-α, IL-1, IL-6
- subsequently the release of the inflammatory cytokines such as IL-10, IL-17, IL-23

TNF-α plays a key role in the pathogeny of the acute inflammatory response. Apart from the trigger effect of the inflammatory cascade, an important role has been attributed to it regarding the apparition of arterial hypotension, capillary stagnation, adult type respiratory distress syndrome, multiple systemic organic failure in developed-shock process (1)(6)(16). There have been identified some SNPs of the genes that encode TNF-α, resulting in the growth its production from macrophages after the *in vitro* LPS stimulation (26). In experimental circumstances TNF-α density grows 24 times in two hours from LPS inoculation with subsequent proinflammatory cytokine release (26). Clinical research has pointed out that the presence of TNF-α-308A allele, assimilated as a hypersecretory genotype, enhances the risk of death by 3.7 times in adults with septic shock, compared to those who didn't own this variant (27). These results were confirmed in children's meningococcemia, in community-acquired pneumonia, but also in Kawasaki disease, cerebral malaria, and severe infections in HIV patients (6). The presence of the bialleic polymorphism for TNF-α in adult patients with post-surgical septic shock lead to a high mortality rate (98% vs. 62%) (28).

IL-6 b is a cytokine both proinflammatory and anti-inflamtory, depending on the moment of intervention in the chain of changes of the infectious process. As a proinflammatory cytokine IL-6 mediates the occurrence of the fever and of the acute phase reactants, and it's stimulating the effects of B and T lymphocytes. Through its immunomodulatory action, it ensures the transition from the leucocyte populations to those of monocytes, i.e. the transit from inborn immunity to gained immunity. The presence of some allele (IL6-174G) in homozygotic state proved to be predictive in the apparition of Gram-positive sepsis. IL-6 was involved in meningococcal sepsis where it interferes as a major factor in myocardilcal dysfunction (31). In a study on VLBW newborn infants, carriers of IL6-174G allele, who received prophylactic treatment with teico-

planin (targocid), Gram-positive germ infection rate was of 2.4% compared to the uncured group with which the incidence has risen to 16,6% (21) (18). These data suggest that the screening for IL6-174G allele may be considered a base for the selective prophylaxis in newborn infants with high risk.

IL-10, anti-inflammatory cytokine produced by monocytes, reduced the expression of some cytokine such as TNF-α, IL-1α, IL-1β, IL-6, IL-8 and of T helper cells (1), decreases the inflammatory response and contributes to the protection of the body against the destructive effects of the inflammatory mediators (16). IL-10 super-expression induces immunosuppression in bacterial sepsis and augments mortality through inhibition of the microbial clearance (6).

There have been described many other polymorphisms of other factors involved in the local and systemic inflammatory response: Mannose Binding Lectin, IL-1/IL-1RA, Bactericidal Permeability Increasing Protein (BPI), Heat Shock Proteins (HSP), Angiotensin I Converting Enzyme, Interferon-gamma, Protein C, fibrinogen, Plasminogen Activator Inhibitor-1 (PAI-1) (1,4,6,13,16,19,21) Table 2.

POSTINFECTION IMMUNOSUPRESSION

In order to counteract prolonged exposure to germs and their products, such as LPS, the immune system has developed post-septic immunosupression, a mechanism which allows hematopoietic cells to become temporarily low reactive to this kind of stimuli. The compensatory anti-inflammatory response counteracts the dangerous effects of sepsis, but makes patients susceptible to different infections for a prolonged period of time. LPS tolerance requires reprogramming of TLR4 response, prolonged repression of TNF-α and IL-1β genes, changes in the structure of NFkB factor probably through epigenetic mechanisms (2,30). The children who survived severe sepsis detain a high risk of death for the following years. Not taking into consideration premature and 0 to 1 infants, Czaja et al. (32) researched over 7000 cases of severe sepsis, of which 6,8% died during the first hospitalisation or at the next one in 28 days. Almost half of the children were hospitalised again at least once in the next months; for 30% the main diagnostic was connected with respiratory infections. 6,5% of these died during rehospitalisation. The death risk maintains very high in the next two years. The causes of these deaths can be multifactorial, but the fore can be considerd reprogramming of the immune response. In adults hospitalized in the intensive care units death at 28 days raises from 10% in systemic inflammatory response syndrome to 20% in sepsis, 20 to 40% in severe sepsis and 40 to 60% in septic shock. The impact on subsequent evolution is extremely severe, with a 50% decrease of life expectancy in the next 5 years (41). In some research on elders regarding the effects of sepsis in long term survival, Quartin et al (42) noticed a 26% risk of death due to non-septic causes in 1 year and the decrease of hope for expected life span from 8 to 4 years.

TABLE 2. *Summary of the main genetic polymorphisms involved in sepsis (5,6,16)*

Gene	Polymorphism	Allele Frequency	Consequences
TLR2	-16933 T/A		Bacteria, sepsis, septic shock Gram + Germs (St aureus)
TLR4	299 A/G, 399 T/I	5%	Sepsis, septic shock Gr- germs Severe inflammatory response
TLR5	392 A/T	7.5%	E coli Infections, Ps aeruginosa Encourages chorioamniotite, premature births
TNFα	TNFα 308A	18%	Increase susceptibility and mortality in septic shock, meningococcemia, Negative prognosis in severe infections
IL-6	-174GG other polimorfisme	43%	- Low levels, protective role in sepsis patients - Sepsis in premature, myocardial dysfunction in meningococcemia
IL-10	592A	33%	Low levels → augmentation of death in sepsis

FACTORS WHICH PROVIDE INCREASED RESISTANCE TO INFECTIONS

Many clinical studies have shown that some people show resistance to ordinary infections. Only a few students in a class or a school, who have made contact with β-hemolitic Gr A streptococcus, will develop acute articular rheumatism and acute glomerulonephritis. During the carriage of N meningitides few children will display invasive meningococcal disease. Some factors, known or under observation, offer resistance in malaria, hepatitis B and C, tuberculosis, HIV infection, leprosy. Thus, in infection with hepatitis B virus, the response is extremely variable; only a part of the patients develop chronic hepatitis. The protection is offered by the haplotypes of the HLA Class II, which ensures the presentation of the viral particles and their cle-

arance, as studies applied to the Asian population indicate (35). It has been proven that the presence of the IL28B genotype induced by the viral infection ensures spontaneous clearance of the hepatitis C virus along with IFNα and IFNλ, which bear an anti-HCV effect (3). Only 5-10% of the subjects infected with M tuberculosis develop the disease; vaccination accidents with virulent germs determined a number of diseases but a part of the children were protected naturally. Some of the causes of these individual variations are known and they are connected with the genetic background through the intervention of the lymphocytes CD4, DC8, which produce IFN-γ. Genetic polymorphisms of TNFα, IL-12 and IL-6 might be also involved in endurance to M. tuberculosis infection (39). Detailed studies of **HIV infection** brought light into the role of the membrane receptor CCR5 which allows the mediators of the inflammatory response to enter the cell environment. The HIV virus entrance in the cell is possible by connecting with this receptor. CCR-Δ32 mutation blocks the entrance of the virus by modifying the morphology of the receptor (36). CCRΔ-32 homozygotes are immune to AIDS and the heterozygotes develop the disease slowly. It is interesting to notice that 14-30% of the North-European population owns this mutation (0-5% for African and Asian population).The carriage of this genetic change would derive from the selective resistance to the smallpox virus and other viral diseases like hepatitis C (37), malaria and other bacterial disease. Some authors have considerd in the presence of the CCR-Δ32 gene a result of immunological adaptation of the population through great outbreaks of infectious diseases throughout history (38).

NATURAL SELECTION AND GENES SUSCEPTIBLE OF INFECTIONS

Pediatrics is the first branch of medicine that must deal with immune competence of patients suffering from life-threatening infections. Unfortunately, by reason of the lack of clinically applied genetic studies, until now there are few answers in this area. The evolution of premature infants with sepsis/ septic shock may constitute the first selective barrier of natural selection, although the existence of genetic mark in the evolution of sepsis may be proven in all stages of life. Studies of genetic epidemiology genetics suggest that genetic variations of populations are determined by the co-evolution of the germs and the immune response system. Infectious diseases have exerted a selective major pressure during time. That is why the genes involved in the immune response are the most diverse and the most of the human genome (40). This indicates that there are evolutional advantages in having immune responses to a large range of pathogens. Combinations of "good" and "bad" allele may be decisive factors for survival.

REFERENCES

1. **Del Vecchio A., Ladisa G., Laforgia N., De Felice C., Priesta G., Latini G.** Genetic polymorphisms in neonatal sepsis *Haematol Reports,* 2006,2(10),31-37
2. **Angus D.C., Linde-Zwirble W..T, Lidiker J.** Epidemiology of severe sepsis in the *United States Crit Care Med,* 2001, 29, 1303-1310
3. **Chapman S.J., Hill A.V.S.** Human genetic susceptibility to infectious disease *Nat Rev Gen,* 2012, 13, 175-188
4. **Namath A., Patterson A.J.** Genetic Polimorphisms in Sepsis Crit *Care Clin,* 2009,25,835-856
5. **Arcaroli J., Fessler M.B., Abraham E.** Genetic polimorphisms and sepsis *Shock* 2005,24,4,300-312
6. **Dahmer M.K., Randolph A., Vitali S., Quasney M.W.** Genetic polimorphisms in sepsis *Pediatr Clin Care,* 2005,6,3, S61-S72
7. **Sutherland A.M., Walley K.R.** Bench-to-bedside review:Association of genetic variations with sepsis *Crit Care,* 2009,13, 210-230
8. **Sorensen T.I., Nielsen G.G., Andersen P.K.** Genetic and environmental influences on premature death in adult adoptees *N Engl J Med,* 1988, 18, 12, 727-732
9. **Petersen L., Nielsen G.G., Andersen P.K., Sorensen T.I.** Case-control study og genetic and environmental influences on premature death of adult adoptees *Genetic Epidemiol,* **2**002,23,2, 123-132
10. **Obel N., Christensen K., Petersen I., Sorensen T.I., Skytthe A.** Genetic and environmental influences on premature death due to **i**nfections assessed in Danish twins, 1943-2001 *Am J Epidemiol,* 2010,1,17,(9),1007-1013
11. **Hill A.V.S.** Evolution, revolution and heresy in the genetics of infectious disease susceptibility. *Phil.Trans R Soc B,* 2012, 367, 840-849
12. **Bizzaro M., Jiang Y., Hussain N., Gruen R.** The impact of Environmental and Genetic Factors on Neonatal Late-Onset Sepsis *J Pediat,* 2011, 158(2),e234-e238
13. **Wynn J.L., Wong H.R.** Parhophysiology and Treatment of Septic Shock in Neonates *Clin Perinatol,* 2010, 37(2), 339-349
14. **Yoon H.S.** Neonatal innate immunity and Toll-like receptor Korean *L Pediat,* 2010, 53(12),985-988
15. **Janeway C.A., Medzhitov R.** Innate immune recognition, 2002, 20, 197-216
16. **Sapru A., Quasney M.W.** Host Genetics and Pediatric Sepsis Open *Infl J,* 2011,4, S 1-M10, 82-100
17. **Maziad A.A., Schaa K., Bell E.F., Dagle J.M., Cooper M.** Role of Polymorphic Variants as Genetic Modulators of Infections in Neonatal *Sepsis Pediatr Res,* 2010, 68(4), 323-329
18. **Levy O.** Genetic Screening for Susceptibility to Infections in the NICU Setting Pediatr Res, 2004, 55, 4, 546-548
19. **Nesin M.** Genetic Basis of Preterm Birth Frontiers Bioscience, 2007, 12,115-124

20. **Hartel C.** Genetic association studies in VLBW infants exemplifying susceptibility to sepsis – recent findings and implications for future research *Acta Paediatrica,* 2006, 96,158-165
21. **Ahrens P., Kattner E., Kohler B., Hartel C.** Mutation of Genes Involved in the Innate Immune System as Predictors of Sepsis in Very Low Weight Infants *Pediat Res,* 2004,55,652-656
22. **Lorenz E., Hallman M., Marttila R., Haataja R., Scwartz D.A.** Association beetwen the Asp299Gly polymorphisms in tge Toll-like receptor 4 and premature births in the *Finnish population Pediat Res,* 2002, 52(3), 373-376
23. **Zhang J.P., Chen C., Yang Y.** Changes and clinical significance of Toll-like receptor 2 and 4 expression in neonatal infections *Zhonghua Er Za Zhi (Abstr)***,** 2007, 45(2),130-133
24. **Krediet T.G., Wiertsema S.P., Vossers M.J., Hoeks S.B.** Toll-like receptor 2 polymorphism is associated with preterm birth Pediat Res, 2007, 62(4), 474-476
25. **Louis E., Franchimont D., Piron A.** Tumor necrosis factor (TNF) gene polymorphism influences TNF-α production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healty humans *Clin Exp Immunol,* 1998, 113(3), 401-406
26. **Carrigan A.D., Scott G., Tabrizian M.** Toward Rezolving the Challenges of Sepsis Diagnosis Clinica Chem, 2004, 50, 8, 1301-1314
27. **Mira J.P., Cariou A., Grall F.** Association of TNF2, a TNF-α polymorphism, with septic shock susceptibility and mortality; a multicenter study *JAMA,*1999, 282(6), 561-548
28. **Gau-Jun T., Song-Lih H., Huey-Wen Y.** Tumor necrosis factor gene polymorphism and septic shock in surgical infection *Crit Care Med,* 2000, 28, 8, 2733-2736
29. **Levy O.** Genetic screening for Susceptibility to Infection in the NICU *Setting Pediat Res,* 2004, 55, 4, 546-548
30. **Bierne H., Hamon M., Cossart P.** Epigenetics and Bacterial Infection *Cold Spring Harb Perspect Med,* 2012; doi:10.1101/chsperspect.a010272
31. **Pathan N., Hemingway C.A., Alizadeh A.A., Stephens A.C.** Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock *Lancet,* 2004, 363, (9404), 203-209
32. **Czaja A.S., Zimmerman J.J., Nathens A.B.** Readmission and late mortality after pediatric severe sepsis Pediatrics, 2009, 123(3), 849-857
33. **Kawai T., Akira S.** The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors *Nat Immunol* 2010,11,373-382
34. **Marodi L.** Neonatal Innate Immunity to Infectious Agents Infect *Immunity,* 2006,74,4,1999-2006
35. **Kamatani Y., Ochi H., Kawaguchi T.A.** Genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians Nat Genetics, 2009, 41,591-595
36. ***CCR5** http://en.wikipedia.org/wiki/CCR5
37. **Goulding C., Murphy A., MacDonald G., Barett S.** The CCR5-Δ32 mutation: impact on disease outcome in individuals with hepatitis C infection from a single sourse *Gut,* 2005,54,1157-1161
38. **Galvani A.P., Novembre J.** The evolutionary history of the CCR-Δ32 HIV resistance mutation Microbes Infect, 2005,7,2,302-309
39. **Chackerian A.A., Perera T.V., Behar S.M.** Gamma Interferon-Producing CD4 T Lymphocytes in the Lung Correlate with Resistance to Infection with Mycobacterium tuberculosis Infect Immun, 2001,69,4,2666-2674
40. **Burgner D., Jamieson S.E., Blakwell J.M.** genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better? *Lancet Infect Dis,* 2006,6,(10),653-663
41. **Brun-Buisson C.** The epidemiology of the systemic inflammatory response *Intens Care Med,* 2000,26,Suppl 1, S64-74
42. **Quartin A.A., Schein R.M., Kett D.H., Peduzzi P.N.** magnitude and duration of the effect of sepsis on survival *JAMA,* 1997, 2, 277, 13,1058-1063
43. **Angus D.C., van der Poll T.** Severe Sepsis and Septic Shock *N Engl J Med,* 2013, 369,840-851
44. **Carson W.F., Cavassani K.A., Dou Y., Kunkel S.L.** Epigenetic regulation of immune cell functions during post-septic immunosuppresion *Epigenetics* 2011,6,3,273-283
45. **Kollman T.R., Crabtree J., Rein-Weston A., Blimkie D.** Neonatal innate TLR-mediated responses are distinct from those of adults *J Immunol,* 2009,183,11,7150-7160