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ORIGINAL ARTICLE

Fc Gamma Receptor IIA (CD32A) R131 Polymorphism as a Marker of Genetic Susceptibility to Sepsis

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Abstract—Sepsis is a devastating disease that can affect humans at any time between neonates and the elderly and is associated with mortality rates that range from 30 to 80 %. Despite intensive efforts, its treatment has remained the same over the last few decades. Fc receptors regulate multiple immune responses and have been investigated in diverse complex diseases. FcγRIIA (CD32A) is an immunoreceptor, tyrosine-based activation motif-bearing receptor that binds immunoglobulin G and C-reactive protein, important opsonins in host defense. We conducted a study of 702 patients (184 healthy individuals, 171 non-infected critically ill patients, and 347 sepsis patients) to investigate if genetic polymorphisms in the CD32A coding region affect the risk of septic shock. All individuals were genotyped for a variant at position 131 of the FcγRIIA gene. We found that allele G, associated with the R131 genotype, was significantly more frequent in septic patients than in the other groups ($p=0.05$). Our data indicate that FcγRIIA genotyping can be used as a marker of genetic susceptibility to sepsis.

KEY WORDS: infection; inflammation; biomarkers; critical care; genetic susceptibility.

INTRODUCTION

Sepsis is a complex disease characterized by massive systemic inflammatory responses of infectious origin that lead to a multitude of clinical manifestations that frequently culminate in multiple organ dysfunction or failure. Sepsis is the leading cause of death in intensive care units and represents a constant source of concern for health systems around the world, mainly because of its high incidence and associated hospital costs [1, 2].

The genetic variability of septic patients is a factor that has been extensively investigated in the last decades, as sepsis is a heterogeneous disease affecting different subpopulations of critically ill individuals and is characterized by high individual presentation. Genetic association with different immune profiles or clinical outcomes might help clinicians to diagnose and treat sepsis, contribute to a better understanding of its pathophysiology, and open new avenues for drug development [3–6].

Fc receptors (FcRs) are major components of the immune system that elicit pleiotropic effector responses including the production of inflammatory mediators, phagocytosis, antibody-dependent cellular cytotoxicity, and chemotaxis, among others [7, 8]. FcγRII (CD32) is

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an IgG receptor widely expressed by neutrophils, monocytes, macrophages, dendritic cells, and platelets, and comprises two subclasses: FcγRIIA and FcγRIIB [7]. FcγRIIA (CD32A) is an immunoreceptor tyrosine-based activation (ITAM)-containing receptor that bears either an arginine (R131) or a histidine (H131) at position 131 of the mature protein [9]. The arginine residue decreases the affinity of FcγRIIA for IgG2 [10], a subclass of IgG that binds to carbohydrate portions in bacterial capsules. As a consequence, the lower affinity affects phagocytosis mediated by IgG2 [11]. Interestingly, FcγRIIA-H131 displays a high affinity for C-reactive protein [12], an acute-phase protein produced in high amounts during infection.

The purpose of this study was to investigate the prevalence of two well-characterized FcγRIIA alleles in a cohort of septic patients compared with healthy subjects to determine their potential as biomarkers for sepsis.

PATIENTS AND METHODS

Study Design

Blood samples were collected at the Intensive Care Unit of Hospital São Lucas (septic patients: case, and non-infected patients: control 1) and at the Research Unit of Paternity (healthy individuals: control 2), both from the Pontifical Catholic University of Rio Grande do Sul. Severe sepsis and septic shock were defined according to the criteria of the ACCP/SCCM Consensus Conference Committee proposed in 1992 [13].

Exclusion criteria included human immunodeficiency virus infection, patients in immunosuppressive therapy, patients aged under 16 years, non-Caucasian ancestry, and pregnant or lactating women.

All subjects were from southern Brazil and were composed of a singular genetic background: the majority of subjects had European ethnicity (Portuguese, Spanish, Italian, and German ancestry), and a small number of individuals had African genetic traits [14].

The study was approved by the ethics committees of the Hospital das Clinicas de Porto Alegre and Hospital das Clinicas da Universidade de Sao Paulo (protocol no. 205/13), being performed in accordance with the Declaration of Helsinki. All subjects or patient surrogates received detailed explanations and provided written consent prior to inclusion in this investigation.

DNA Extraction and Genotyping

A total of 702 samples were collected including 184 samples from healthy individuals, 171 from non-infected critically ill patients, and 347 samples from patients with sepsis. Genomic DNA was extracted from leukocytes by a standard method [15].

The genotyping protocol previously described by Ahlgrimm *et al.* [16] was used with minor changes. In brief, DNA was diluted in water to a final concentration of 10 ng/μL per reaction, and mutation tests were performed using the TaqMan® (Invitrogen) single nucleotide polymorphism (SNP) Genotyping Assay C9077561-20 for the polymorphism rs1801274, which detects the FcγRIIA-R/H131 allele.

The reaction was optimized for a total of 3 μL genomic DNA mixed with 6.25 μL TaqMan Universal Master Mix and 0.312 μL TaqMan SNP Genotyping Assay Mix. After an initial step at 95 °C for 10 min, amplification was performed using 40 cycles of denaturation (92 °C, 15 s), annealing (60 °C, 1 min), and extension (60 °C, 1 min). The PCR was performed using the real-time PCR (RT-PCR) StepOne kit (Invitrogen, USA).

DNA Sequencing

For validation of the probes used in the genotyping tests, we carried out the sequencing of some samples. PCR of the *CD32* gene region containing the rs1801274 SNP was performed with 1 μL of genomic DNA, 1× PCR buffer, 0.1 mM of dNTPs, 4 mM of $MgCl_2$, 0.5 pM of each primer, and 0.5 U of Taq DNA polymerase (Life Technologies, USA) and distilled water. Primer sequences are described in Table 1.

All reactions were performed in a Veriti Thermal Cycler (Applied Biosystems, USA) with an initial step at 95 °C for 5 min, followed by 40 cycles at 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s, followed by 1 cycle at 72 °C for 10 min. After purification with EXO-SAP and quantification with Low Mass DNA Ladder (Invitrogen), sequencing was performed using the ABI3500 Genetic Analyzer using BigDye Terminator v3.1 (Applied Biosystems).

Sequences were analyzed by comparison to reference sequences described in the dbSNP Database (accession number rs1801274) and confirmed by reverse-strand sequencing.

Table 1. PCR Primers Used for rs1801274 Amplification

SNP		Primer sequence (5′>3′)	Amplicon size (bp)	Tm
rs1801274	F	CATCTTGGCAGACTCCCCATACC	348	55 °C
	R	GTACCTCTGAGACTGAAAAACCCTTGG		

SNP single nucleotide polymorphism, *Tm* melting temperature, *bp* base pair

Statistical Analysis

Statistical analysis was performed using the SPSS 18 statistical package (SPSS 18.0 for Windows, Chicago, IL, USA) for the Pearson chi-squared test or the Student *t* test. A p value ≤0.05 was considered statistically significant.

RESULTS

A total of 518 critically ill patients were included in our study, as well as 184 healthy individuals. We genotyped 347 critically ill patients with sepsis, 171 critically ill patients without sepsis (non-infected group), and 184 healthy individuals. The characteristics of these patients are summarized in Table 2.

To validate the results obtained by RT-PCR, we randomly chose some samples for DNA sequencing. At least three samples of each genotype were analyzed per group. All sequencing and genotyping results matched perfectly and were aligned with the Hardy–Weinberg equilibrium (data not shown).

The comparison of genotypic and allelic CD32A frequencies did not show any differences among the study groups (Table 3) or between different degrees of sepsis (Table 4).

Our mortality results, however, indicated that although allele A did not appear to interfere with the development of sepsis, the presence of allele G appears to increase the risk of evolution to this picture (p=0.050) (Tables 5 and 6). These results, along with observations made by many other groups in inflammatory and autoimmune diseases [17–21], indicate the role of FcγRIIA-R131 as a susceptibility factor and indicator of a poor prognosis of sepsis.

DISCUSSION

Recently, genetic variations in sepsis patients have been extensively investigated by the scientific community [4, 22–26]. Most studies focused on components of innate immunity and the coagulation system. Toll-like receptors [27, 28], their intracellular signaling molecules [29, 30], cytokines [31–36], chemokines [37], transcription factors [38], and many other molecules have been studied [39–45].

Polymorphisms with an exchanged single nitrogenous base (SNPs) occur throughout the genome and can alter the expression or function of their gene products [46]. This type of polymorphism is the most common genetic variation in the general population. SNPs occur in approximately 1:1000 base pairs and the most common is the substitution of cytosine for thymine (C>T). It is estimated that 10 % of all SNPs in the human genome are functional. A number of studies have investigated multiple SNPs from multiple genes with the hope of identifying biomarkers in complex diseases.

Table 2. Demographic Data of Patients According to Presence or Absence of Sepsis

Polymorphism	Demographic data	Non-infected	Sepsis	*p* value
CD32	*Number*	*171*	*347*	
	Female sex, *n* (%)	79 (46.2)	156 (45.0)	0.851[a]
	Age, mean (SD)	51.75 (20.37)	56.19 (19.62)	0.017[b]
	Survival, *n* (%)	134 (78.4)	164 (47.3)	<0.001[a]
	Septic shock, *n* (%)	0 (0.0)	245 (70.6)	

Entries in italics represents $p<0.05$
SD standard deviation
[a] Chi-squared test
[b] Student's *t* test

Table 3. Comparison of Genotypic and Allelic Frequencies in Patients With or Without Sepsis and in Healthy Individuals

CD32 allele	Non-infected patients ($n=171$) n (%)	Sepsis patients ($n=347$) n (%)	Healthy subjects ($n=184$) n (%)	p value[a]
AA	50 (29.2)	74 (21.3)	49 (26.6)	0.197
AG	75 (43.9)	172 (49.6)	93 (50.5)	
GG	46 (26.9)	101 (29.1)	42 (22.8)	
A	175 (51.2)	320 (46.1)	191 (51.9)	0.127
G	167 (48.8)	374 (53.9)	177 (48.1)	

[a] Chi-squared test

Elucidation of the role of these genetic variations in the inflammatory process and in the course of infection might enhance our understanding of the molecular events that occur during sepsis and help improve its diagnosis and treatment. Indeed, the search for reliable sepsis biomarkers is one of the greatest goals in critical care medicine, because clinicians are challenged daily to differentiate sepsis from other excessive inflammatory processes [47]. In many cases, antibiotics are administered in the absence of infection, when the risk of death is high and the physician cannot exclude possible infection. This common scenario generates unnecessary costs and might lead to antibiotic-resistant bacteria.

FcγRIIA (CD32A) polymorphisms have been described in association with infectious, inflammatory, and autoimmune diseases. Examples include systemic lupus erythematosus [48], sickle cell disease [49], fulminant meningococcemia in children [50], malaria [51], human immunodeficiency virus infection [52], and Epstein–Barr virus infection [53]. Homozygosity for FcγRIIA-H131 was associated with a higher risk of pneumococcal community-acquired pneumonia [54–56], and neutrophils from subjects homozygous for FcγRIIA-R131 exhibited a significantly reduced uptake of opsonized pneumococci, group B streptococci, neisseriae, and staphylococci compared with FcγRIIA-H131 cells [57]. In our study, we aimed to identify an FcγRIIA polymorphism that was more prevalent in sepsis when compared with healthy individuals or non-infected patients or that could be associated with sepsis mortality. As blood cultures are only positive for bacteria in 30 % of sepsis cases, we did not focus on specific etiologic agents. Our goal was to identify an FcγRIIA polymorphism that could serve as a biomarker for bacterial sepsis in general, independently of the source of infection or deeper microbiological classification. We found that the presence of allele G in the FcγRIIA gene was associated with an increased risk of sepsis.

The mechanisms that are triggered by FcγRIIA during sepsis are complex. Because FcγRIIA is an ITAM-bearing receptor and the R131 polymorphism has a lower affinity for IgG2 [58], this polymorphism might induce a weaker inflammatory response than the H131 genotype. However, many previous studies by our group indicated that ITAM-bearing receptors also trigger inhibitory signals under special conditions [59–63]; thus, it is very difficult to predict the signaling mechanisms elicited by FcγRIIA polymorphisms during sepsis.

Moreover, FcγRIIA binds to CRP, an acute-phase protein that recognizes pathogenic microbes and damaged cells; activates complement; and promotes the clearance of apoptotic cells [64]. However, its mechanisms of action, including Fc receptor biology, are largely unknown. CRP

Table 4. Comparison of Genotypic and Allelic Frequencies in Septic Patients Requiring Vasopressors (Septic Shock) or Not (Severe Sepsis)

CD32 allele	Severe sepsis patients ($n=102$) n (%)	Septic shock patients ($n=245$) n (%)	p value[a]
AA	24 (23.5)	50 (20.4)	0.286
AG	44 (43.1)	128 (52.2)	
GG	34 (33.3)	67 (27.3)	
A	92 (45.1)	228 (46.5)	0.739
G	112 (54.9)	262 (53.5)	

[a] Chi-squared test

Table 5. Comparison of Genotypic and Allelic Frequencies of CD32 by Patient Survival

CD32 allele	Death ($n=220$) n (%)	Survival ($n=298$) n (%)	p value[a]
AA	49 (22.3)	75 (25.2)	0.713
AG	106 (48.2)	141 (47.3)	
GG	65 (29.5)	82 (27.5)	
A	204 (46.4)	291 (48.8)	0.451
G	236 (53.6)	305 (51.2)	

[a] Chi-squared test

Table 6. Comparison of the Presence and Absence of Alleles A and G in Critically Ill Patients With or Without Sepsis

CD32 allele	Non-infected patients ($n=171$) n (%)	Sepsis patients ($n=347$) n (%)	p value[a]
AA+AG	125 (73.1)	246 (70.9)	0.607
GG	46 (26.9)	101 (29.1)	
GG+AG	121 (70.8)	273 (78.7)	0.050*
AA	50 (29.2)	74 (21.3)	

Presence of G: non-infected 121/394 (31 %), sepsis 273/394 (69 %). Absence of G: non-infected 50/124 (40 %), sepsis 72/124 (60 %)
*$p<0.05$ compared with non-infected patients
[a] Chi-squared test

exists in conformationally distinct forms, which explain its various functions [65, 66]. A recent report described that CRP-FcγRIIA interactions mediate potent antineutrophil and antiplatelet adhesion functions, limiting inflammation and thrombosis [67]. In addition, some FcγRIIA-mediated responses triggered by CRP are allele specific [68].

Our findings, thus, put in evidence the importance of Fc receptors in sepsis and emphasize the complex biological functions of these molecules in the presence of overwhelming infection, regardless of the source of infection or bacterial agent. Our strongest limitations are the number of patients included and the lack of mechanistic assays, so we believe that further studies are necessary to confirm our data, investigate the role of other Fc receptor polymorphisms in sepsis, and conduct *in vitro* studies to provide a broader view of this fascinating topic. A greater understanding of these phenomena might contribute to developing personalized medicine for sepsis patients [69] and bring new hope to the critically ill.

CONCLUSION

The identification of genetic variants associated with altered susceptibility and clinical manifestations in sepsis will permit the early identification of infected patients or those at a higher risk of death and allows tailored treatment, avoiding unnecessary or detrimental drug administration. Further studies are necessary to clarify why the R131 polymorphism is prevalent in septic patients and the molecular events that follow its activation.

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Compliance with Ethical Standards. The study was approved by the ethics committees of the Hospital das Clinicas de Porto Alegre and Hospital das Clinicas da Universidade de Sao Paulo (protocol no. 205/13), being performed in accordance with the Declaration of Helsinki. All subjects or patient surrogates received detailed explanations and provided written consent.

Conflict of Interest. The authors have no financial or ethical conflicts of interest.

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REFERENCES

1. Martin, G.S., D.M. Mannino, S. Eaton, and M. Moss. 2003. The epidemiology of sepsis in the United States from 1979 through 2000. *The New England Journal of Medicine* 348(16): 1546–54.
2. Hotchkiss, R.S., and I.E. Karl. 2003. The pathophysiology and treatment of sepsis. *The New England Journal of Medicine* 348(2): 138–50.
3. Holmes, C.L., J.A. Russell, and K.R. Walley. 2003. Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. *Chest* 124(3): 1103–15.
4. Namath, A., and A.J. Patterson. 2009. Genetic polymorphisms in sepsis. *Critical Care Clinics* 25(4): 835–56. **x**.
5. Shimada, T., S. Oda, T. Sadahiro, M. Nakamura, Y. Hirayama, E. Watanabe, et al. 2011. Outcome prediction in sepsis combined use of genetic polymorphisms—a study in Japanese population. *Cytokine* 54(1): 79–84.

6. Sipahi, T., H. Pocan, and N. Akar. 2006. Effect of various genetic polymorphisms on the incidence and outcome of severe sepsis. *Clinical and Applied Thrombosis/Hemostasis: Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis* 12(1): 47–54.
7. Daeron, M. 1997. Fc receptor biology. *Annual Review of Immunology* 15: 203–34.
8. Guilliams, M., P. Bruhns, Y. Saeys, H. Hammad, and B.N. Lambrecht. 2014. The function of Fcgamma receptors in dendritic cells and macrophages. *Nature Reviews Immunology* 14(2): 94–108.
9. Raaz-Schrauder, D., A.B. Ekici, L.E. Munoz, L. Klinghammer, R.E. Voll, J.H. Leusen, et al. 2012. Patients with unstable angina pectoris show an increased frequency of the Fc gamma RIIa R131 allele. *Autoimmunity* 45(7): 556–64.
10. Warmerdam, P.A., J.G. van de Winkel, A. Vlug, N.A. Westerdaal, and P.J. Capel. 1991. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. *Journal of Immunology* 147(4): 1338–43.
11. Salmon, J.E., J.C. Edberg, N.L. Brogle, and R.P. Kimberly. 1992. Allelic polymorphisms of human Fc gamma receptor IIA and Fc gamma receptor IIIB. Independent mechanisms for differences in human phagocyte function. *The Journal of Clinical Investigation* 89(4): 1274–81.
12. Zuniga, R., G.S. Markowitz, T. Arkachaisri, E.A. Imperatore, V.D. D'Agati, and J.E. Salmon. 2003. Identification of IgG subclasses and C-reactive protein in lupus nephritis: the relationship between the composition of immune deposits and FCgamma receptor type IIA alleles. *Arthritis and Rheumatism* 48(2): 460–70.
13. Bone, R.C., R.A. Balk, F.B. Cerra, R.P. Dellinger, A.M. Fein, W.A. Knaus, et al. 1992. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 101(6): 1644–55.
14. Parra, F.C., R.C. Amado, J.R. Lambertucci, J. Rocha, C.M. Antunes, and S.D. Pena. 2003. Color and genomic ancestry in Brazilians. *Proceedings of the National Academy of Sciences of the United States of America* 100(1): 177–82.
15. Lahiri, D.K., and J.I. Nurnberger Jr. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Research* 19(19): 5444.
16. Ahlgrimm, M., M. Pfreundschuh, M. Kreuz, E. Regitz, K.D. Preuss, and J. Bittenbring. 2011. The impact of Fc-gamma receptor polymorphisms in elderly patients with diffuse large B-cell lymphoma treated with CHOP with or without rituximab. *Blood* 118(17): 4657–62.
17. Brambila-Tapia, A.J., and I.P. Davalos-Rodriguez. 2009. Fcgamma receptor polymorphisms and systemic lupus erythematosus. *Revista de Investigacion Clinica; Organo del Hospital de Enfermedades de la Nutricion* 61(1): 66–72.
18. Tanaka, Y., Y. Suzuki, T. Tsuge, Y. Kanamaru, S. Horikoshi, R.C. Monteiro, et al. 2005. FcgammaRIIa-131R allele and FcgammaRIIIa-176V/V genotype are risk factors for progression of IgA nephropathy. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association—European Renal Association.* 20(11): 2439–45.
19. Aksu, K., G. Kitapcioglu, G. Keser, A. Berdeli, G. Karabulut, S. Kobak, et al. 2008. FcgammaRIIa, IIIa and IIIb gene polymorphisms in Behcet's disease: do they have any clinical implications? *Clinical and Experimental Rheumatology* 26(4 Suppl 50): S77–83.
20. Kobayashi, T., S. Ito, K. Yamamoto, H. Hasegawa, N. Sugita, T. Kuroda, et al. 2003. Risk of periodontitis in systemic lupus erythematosus is associated with Fcgamma receptor polymorphisms. *Journal of Periodontology* 74(3): 378–84.
21. van der Pol, W.L., M.D. Jansen, J.B. Kuks, M. de Baets, F.G. de Leppers-van Straat, J.H. Wokke, et al. 2003. Association of the Fc gamma receptor IIA-R/R131 genotype with myasthenia gravis in Dutch patients. *Journal of Neuroimmunology* 144(1–2): 143–7.
22. Suffredini, A.F., and S.J. Chanock. 2006. Genetic variation and the assessment of risk in septic patients. *Intensive Care Medicine* 32(11): 1679–80.
23. Sutherland, A.M., and K.R. Walley. 2009. Bench-to-bedside review: association of genetic variation with sepsis. *Critical Care* 13(2): 210.
24. Kumpf, O., and R.R. Schumann. 2008. Genetic influence on bloodstream infections and sepsis. *International Journal of Antimicrobial Agents* 32(Suppl 1): S44–50.
25. Chung, L.P., and G.W. Waterer. 2011. Genetic predisposition to respiratory infection and sepsis. *Critical Reviews in Clinical Laboratory Sciences* 48(5–6): 250–68.
26. Esposito, S., A. Zampiero, L. Pugni, S. Tabano, C. Pelucchi, B. Ghirardi, et al. 2014. Genetic polymorphisms and sepsis in premature neonates. *PloS One* 9(7), e101248.
27. Nachtigall, I., A. Tamarkin, S. Tafelski, A. Weimann, A. Rothbart, S. Heim, et al. 2014. Polymorphisms of the toll-like receptor 2 and 4 genes are associated with faster progression and a more severe course of sepsis in critically ill patients. *The Journal of International Medical Research* 42(1): 93–110.
28. Lin, Y.T., A. Verma, and C.P. Hodgkinson. 2012. Toll-like receptors and human disease: lessons from single nucleotide polymorphisms. *Current Genomics* 13(8): 633–45.
29. Yin, J., C.L. Yao, C.L. Liu, Z.J. Song, C.Y. Tong, and P.Z. Huang. 2012. Association of genetic variants in the IRAK-4 gene with susceptibility to severe sepsis. *World Journal of Emergency Medicine* 3(2): 123–7.
30. Toubiana, J., E. Courtine, F. Pene, V. Viallon, P. Asfar, C. Daubin, et al. 2010. IRAK1 functional genetic variant affects severity of septic shock. *Critical Care Medicine* 38(12): 2287–94.
31. Zhang, A.Q., W. Pan, J.W. Gao, C.L. Yue, L. Zeng, W. Gu, et al. 2014. Associations between interleukin-1 gene polymorphisms and sepsis risk: a meta-analysis. *BMC Medical Genetics* 15: 8.
32. Kornblit, B., L. Munthe-Fog, H.O. Madsen, J. Strom, L. Vindelov, and P. Garred. 2008. Association of HMGB1 polymorphisms with outcome in patients with systemic inflammatory response syndrome. *Critical Care* 12(3): R83.
33. Kothari, N., J. Bogra, H. Abbas, M. Kohli, A. Malik, D. Kothari, et al. 2013. Tumor necrosis factor gene polymorphism results in high TNF level in sepsis and septic shock. *Cytokine* 61(2): 676–81.
34. Pappachan, J.V., T.G. Coulson, N.J. Child, D.J. Markham, S.M. Nour, M.C. Pulletz, et al. 2009. Mortality in adult intensive care patients with severe systemic inflammatory response syndromes is strongly associated with the hypo-immune TNF-238A polymorphism. *Immunogenetics* 61(10): 657–62.
35. Accardo Palumbo, A., G.I. Forte, D. Pileri, L. Vaccarino, F. Conte, L. D'Amelio, et al. 2012. Analysis of IL-6, IL-10 and IL-17 genetic polymorphisms as risk factors for sepsis development in burned patients. *Burns: Journal of the International Society for Burn Injuries* 38(2): 208–13.
36. Paskulin, D.D., P.R. Fallavena, F.J. Paludo, T.J. Borges, J.B. Picanco, F.S. Dias, et al. 2011. TNF-308G>a promoter polymorphism (rs1800629) and outcome from critical illness. *The Brazilian Journal*

of Infectious Diseases : an Official Publication of the Brazilian Society of Infectious Diseases 15(3): 231–8.
37. Villar, J., L. Perez-Mendez, C. Flores, N. Maca-Meyer, E. Espinosa, A. Muriel, et al. 2007. A CXCL2 polymorphism is associated with better outcomes in patients with severe sepsis. *Critical Care Medicine* 35(10): 2292–7.
38. Thair, S.A., K.R. Walley, T.A. Nakada, M.K. McConechy, J.H. Boyd, H. Wellman, et al. 2011. A single nucleotide polymorphism in NF-kappaB inducing kinase is associated with mortality in septic shock. *Journal of Immunology* 186(4): 2321–8.
39. Huh, J.W., K. Song, J.S. Yum, S.B. Hong, C.M. Lim, and Y. Koh. 2009. Association of mannose-binding lectin-2 genotype and serum levels with prognosis of sepsis. *Critical Care* 13(6): R176.
40. Nakada, T.A., J.A. Russell, J.H. Boyd, R. Aguirre-Hernandez, K.R. Thain, S.A. Thair, et al. 2010. beta2-Adrenergic receptor gene polymorphism is associated with mortality in septic shock. *American Journal of Respiratory and Critical Care Medicine* 181(2): 143–9.
41. Benfield, T., K. Ejrnaes, K. Juul, C. Ostergaard, J. Helweg-Larsen, N. Weis, et al. 2010. Influence of factor V Leiden on susceptibility to and outcome from critical illness: a genetic association study. *Critical Care* 14(2): R28.
42. Zhang, A.Q., L. Zeng, W. Gu, L.Y. Zhang, J. Zhou, D.P. Jiang, et al. 2011. Clinical relevance of single nucleotide polymorphisms within the entire NLRP3 gene in patients with major blunt trauma. *Critical Care* 15(6): R280.
43. Henckaerts, L., K.R. Nielsen, R. Steffensen, K. Van Steen, C. Mathieu, A. Giulietti, et al. 2009. Polymorphisms in innate immunity genes predispose to bacteremia and death in the medical intensive care unit. *Critical Care Medicine* 37(1): 192–201. **e1-3**.
44. Paludo, F.J., J.B. Picanco, P.R. Fallavena, R. Fraga Lda, P. Graebin, T. Nobrega Ode, et al. 2013. Higher frequency of septic shock in septic patients with the 47C allele (rs4880) of the SOD2 gene. *Gene* 517(1): 106–11.
45. Graebin, P., T.D. Veit, C.S. Alho, F.S. Dias, and J.A. Chies. 2012. Polymorphic variants in exon 8 at the 3' UTR of the HLA-G gene are associated with septic shock in critically ill patients. *Critical Care* 16(5): R211.
46. Brookes, A.J. 1999. The essence of SNPs. *Gene* 234(2): 177–86.
47. LaRosa, S.P., and S.M. Opal. 2011. Biomarkers: the future. *Critical Care Clinics* 27(2): 407–19.
48. Vigato-Ferreira, I.C., J.E. Toller-Kawahisa, J.A. Pancoto, C.T. Mendes-Junior, E.Z. Martinez, E.A. Donadi, et al. 2014. FcgammaRIIa and FcgammaRIIIb polymorphisms and associations with clinical manifestations in systemic lupus erythematosus patients. *Autoimmunity* 47(7): 451–8.
49. Kangne, H.K., F.F. Jijina, Y.M. Italia, D.L. Jain, A.H. Nadkarni, M. Gupta, et al. 2013. The Fc receptor polymorphisms and expression of neutrophil activation markers in patients with sickle cell disease from western India. *BioMedicine Research International* 2013: 457656.
50. Bredius, R.G., B.H. Derkx, C.A. Fijen, T.P. de Wit, M. de Haas, R.S. Weening, et al. 1994. Fc gamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. *The Journal of Infectious Diseases* 170(4): 848–53.
51. Zhao, J., L. Ma, S. Chen, Y. Xie, L. Xie, Y. Deng, et al. 2014. Association between Fc-gamma receptor IIa (CD32) gene polymorphism and malaria susceptibility: a meta-analysis based on 6928 subjects. *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 23: 169–75.
52. Forthal, D.N., G. Landucci, J. Bream, L.P. Jacobson, T.B. Phan, and B. Montoya. 2007. FcgammaRIIa genotype predicts progression of HIV infection. *Journal of Immunology* 179(11): 7916–23.
53. Diamantopoulos, P.T., V. Kalotychou, K. Polonyfi, M. Sofotasiou, A. Anastasopoulou, A. Galanopoulos, et al. 2013. Correlation of Fc-gamma RIIA polymorphisms with latent Epstein-Barr virus infection and latent membrane protein 1 expression in patients with low grade B-cell lymphomas. *Leukemia & Lymphoma* 54(9): 2030–4.
54. Sole-Violan, J., M.I. Garcia-Laorden, J.A. Marcos-Ramos, F.R. de Castro, O. Rajas, L. Borderias, et al. 2011. The Fcgamma receptor IIA-H/H131 genotype is associated with bacteremia in pneumococcal community-acquired pneumonia. *Critical Care Medicine* 39(6): 1388–93.
55. Bougle, A., A. Max, N. Mongardon, D. Grimaldi, F. Pene, C. Rousseau, et al. 2012. Protective effects of FCGR2A polymorphism in invasive pneumococcal diseases. *Chest* 142(6): 1474–81.
56. Yuan, F.F., K. Marks, M. Wong, S. Watson, E. de Leon, P.B. McIntyre, et al. 2008. Clinical relevance of TLR2, TLR4, CD14 and FcgammaRIIA gene polymorphisms in Streptococcus pneumoniae infection. *Immunology and Cell Biology* 86(3): 268–70.
57. van Sorge, N.M., W.L. van der Pol, and J.G. van de Winkel. 2003. FcgammaR polymorphisms: implications for function, disease susceptibility and immunotherapy. *Tissue Antigens* 61(3): 189–202.
58. Shashidharamurthy, R., F. Zhang, A. Amano, A. Kamat, R. Panchanathan, D. Ezekwudo, et al. 2009. Dynamics of the interaction of human IgG subtype immune complexes with cells expressing R and H allelic forms of a low-affinity Fc gamma receptor CD32A. *Journal of Immunology* 183(12): 8216–24.
59. Pinheiro da Silva, F., M. Aloulou, D. Skurnik, M. Benhamou, A. Andremont, I.T. Velasco, et al. 2007. CD16 promotes Escherichia coli sepsis through an FcR gamma inhibitory pathway that prevents phagocytosis and facilitates inflammation. *Nature Medicine* 13(11): 1368–74.
60. Pasquier, B., P. Launay, Y. Kanamaru, I.C. Moura, S. Pfirsch, C. Ruffie, et al. 2005. Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. *Immunity* 22(1): 31–42.
61. Pinheiro da Silva, F., M. Aloulou, M. Benhamou, and R.C. Monteiro. 2008. Inhibitory ITAMs: a matter of life and death. *Trends in Immunology* 29(8): 366–73.
62. Aloulou, M., S. Ben Mkaddem, M. Biarnes-Pelicot, T. Boussetta, H. Souchet, E. Rossato, et al. 2012. IgG1 and IVIg induce inhibitory ITAM signaling through FcgammaRIII controlling inflammatory responses. *Blood* 119(13): 3084–96.
63. Ben Mkaddem, S., G. Hayem, F. Jonsson, E. Rossato, E. Boedec, T. Boussetta, et al. 2014. Shifting FcgammaRIIA-ITAM from activation to inhibitory configuration ameliorates arthritis. *The Journal of Clinical Investigation* 124(9): 3945–59.
64. Gershov, D., S. Kim, N. Brot, and K.B. Elkon. 2000. C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. *The Journal of Experimental Medicine* 192(9): 1353–64.
65. Schwedler, S.B., J.G. Filep, J. Galle, C. Wanner, and L.A. Potempa. 2006. C-reactive protein: a family of proteins to regulate cardiovascular function. *American Journal of Kidney Diseases : the Official Journal of the National Kidney Foundation* 47(2): 212–22.
66. Boncler, M., D. Dudzinska, J. Nowak, and C. Watala. 2012. Modified C-reactive protein selectively binds to immunoglobulins. *Scandinavian Journal of Immunology* 76(1): 1–10.

67. El Kebir, D., Y. Zhang, L.A. Potempa, Y. Wu, A. Fournier, and J.G. Filep. 2011. C-reactive protein-derived peptide 201–206 inhibits neutrophil adhesion to endothelial cells and platelets through CD32. *Journal of Leukocyte Biology* 90(6): 1167–75.
68. Aas, V., K.L. Sand, H.C. Asheim, H.B. Benestad, and J.G. Iversen. 2013. C-reactive protein triggers calcium signalling in human neutrophilic granulocytes via FcgammaRIIa in an allele-specific way. *Scandinavian Journal of Immunology* 77(6): 442–51.
69. Christaki, E., and E.J. Giamarellos-Bourboulis. 2014. The beginning of personalized medicine in sepsis: small steps to a bright future. *Clinical Genetics* 86(1): 56–61.