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Reduced frequency of two activating KIR genes in patients with sepsis

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ABSTRACT

Natural killer (NK) cell activity is regulated by activating and inhibitory signals transduced by killer cell immunoglobulin-like receptors (KIR). Diversity in KIR gene repertoire among individuals may affect disease outcome. Sepsis development and severity may be influenced by genetic factors affecting the immune response. Here, we examined sixteen KIR genes and their human leucocyte antigen (HLA) class I ligands in critical patients, aiming to identify patterns that could be associated with sepsis. Male and female patients (ages ranging between 14 and 94 years-old) were included. DNA samples from 211 patients with sepsis and 60 controls (critical care patients with no sepsis) collected between 2004 and 2010 were included and genotyped for KIR genes using the polymerase chain reaction method with sequence-specific oligonucleotide (PCR-SSO), and for HLA genes using the polymerase chain reaction method with sequence-specific primers (PCR-SSP). The frequencies of activating KIR2DS1 and KIR3DS1 in sepsis patients when compared to controls were 41.23% *versus* 55.00% and 36.49% *versus* 51.67% ($p = 0.077$ and 0.037 respectively before Bonferroni correction). These results indicate that activating KIR genes 2DS1 and 3DS1 may more prevalent in critical patients without sepsis than in patients with sepsis, suggesting a potential protective role of activating KIR genes in sepsis.

1. Introduction

Sepsis can be defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. During sepsis, early activation of immune cells occurs, and the imbalance between pro-inflammatory and anti-inflammatory pathways culminate in organ dysfunction [1,2]. The precise mechanisms by which sepsis produces an uncontrolled inflammatory response and multiple organ dysfunction remain unknown, but several studies have suggested the involvement of genetic factors [3,4].

Natural killer (NK) cells are key lymphocytes involved in early immune response against infected or transformed cells in clinical conditions including infections, tumors, and allogeneic hematopoietic cell transplantation, while remaining tolerant to self [5,6]. NK cells can play a role in sepsis stimulating both pro- and anti-inflammatory responses, for example by activating phagocytic cells at the site of infection and producing and releasing interferon-γ (INF-γ), leading to macrophage activation. NK cells can also be activated by antigen-presentation cells, such as dendritic cells, thus amplifying the inflammatory response [7,8].

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The activity of NK cells is regulated by the balance between signals transduced from activating and inhibitory cell surface receptors, including killer immunoglobulin-like receptors (KIR), which are members of the immunoglobulin superfamily [9,10]. Inhibitory KIR molecules bind to target HLA class I molecules and prevent the attack of NK cells on normal cells. When an activating KIR binds to its ligand, activating signals are generated, leading to the destruction of target cell [11–13]. Genes encoding KIR are located on human chromosome 19q13.4 in the leucocyte receptor complex. To date, seventeen KIR receptors have been characterized in humans: eight inhibitory types (2DL1-3; 2DL5A and B; 3DL1-3), seven activating types (2DS1-5, 3DS1, 2DL4), and two pseudogenes that do not encode a functional KIR receptor (2DP1 and 3DP1). Of these, four are always present and are considered framework genes (2DL4, 3DL2, 3DL3, 3DP1) [13–16]. The ligands for KIR are the classical HLA class I molecules HLA-A, -B and -C. Based on the dimorphism in the epitope at position 80, all HLA-C alleles can be divided into two groups: C1 group carrying asparagine, and the C2 group carrying lysine at this position. The receptors KIR2DL2, KIR2DL3 and KIR2DS2 bind HLA-C1 ligands, whereas KIR2DL1 and KIR2DS1 bind HLA-C2 ligands. The inhibitory KIR3DL1 recognizes HLA-B Bw4 allotypes and KIR3DL2 binds HLA-A3 and HLA-A11 [17].

Displaying specificity for HLA class I allotypes, and extensive polymorphism variation, KIR gene variation affects resistance and susceptibility to a great number of diseases in which the involvement of the immune system is determinant. Genetic susceptibility or resistance to infectious and inflammatory disorders, together with environmental and host risk factors, is thought to determine disease progression [18,19]. There are a number of studies describing a relationship between KIR genes and several diseases but no previous studies have has examined the possible role of KIR in sepsis. In the present study, we examined sixteen KIR genes and their HLA class I ligands in a group of 271 critical patients aiming at the identification of patterns in KIR genotypes and HLA ligands that could be associated with sepsis.

2. Methods

2.1. Patient and control samples

DNA samples of two hundred and seventy-one critical care patients of both genders, with ages ranging from 14 to 94 years old, admitted to the Intensive Care Unit, São Lucas Hospital, Pontifical Catholic University of Rio Grande do Sul, in Porto Alegre, Brazil, in the period between 2004 and 2010 were included. Their blood was collected into tubes containing EDTA (ethylenediamine tetraacetic acid) and DNA extracted using a salting-out procedure accordingly to Lahiri and Nurnberger [20]. Samples were properly kept labelled and stored at −80 °C until analysis. Only samples with a DNA concentration superior of 20 ng/μL were analyzed.

Subjects were southern Brazil residents, with a majority of subjects with European ethnicity, and a smaller number of individuals with African genetic traits [21], and divided into two groups: sepsis – patients who met sepsis criteria – and controls – critical care patients with no sepsis. Social and demographic data, including age, sex, mortality at the ICU and hospital, SOFA e APACHE II scores were obtained from all patients, in addition to the occurrence septic shock and death. Confidentiality was observed for all samples.

Sepsis was defined as a "clinical syndrome defined by the presence of both documented or suspected infection and a systemic inflammatory response (SIRS)". Signals and symptoms for sepsis were considered as described by Levy and colleagues [22]. Severe sepsis was defined as sepsis accompanied by hypoperfusion or organ dysfunction, while septic shock is defined as severe sepsis that demands sustained use of vasopressive drugs.

Exclusion criteria included human immunodeficiency virus infection; patients in immunosuppressive therapy; non-Caucasian ancestry; pregnant or lactating women. Patients readmitted to the ICU were excluded.

2.2. KIR genotyping

KIR was genotyped using the polymerase chain reaction method with sequence-specific oligonucleotide (PCR-SSO, One Lambda® Inc, California. USA) for 16 KIR genes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DP1, 3DP1 and 3DS1). The KIR-SSO Genotyping Test applies Luminex technology (One-Lambda® Inc, Canoga Park, CA, USA) and was performed according to the manufacturer's instructions. Briefly, genomic DNA, specific primers and manufacturer's solutions were mixed and the DNA amplified by the GeneAmp PCR system 9600 (Perkin-Elmer, Norwalk, CT, USA). Resulting products were visualized under UV light after electrophoresis in 1% agarose gel containing ethidium bromide. Internal control was included in each PCR reaction.

2.3. HLA genotyping

HLA genotyping was performed using the polymerase chain reaction method with sequence-specific primers (PCR-SSP). HLA typing Cw epitope C1 (Cw 01, 03, 07 {01– 06}, 08, 12 {02, 03, 06}, 14, 16 {01, 03, 04}), and C2 (Cw 02, 04, 05, 06, 0707, 12 {04, 05}, 15, 1602, 17, 18) were performed using PCR-SSP, as described by Jones et al. [17]. HLA-Bw4, A3 and A11 genotyping were also performed using PCR-SSP, accordingly to Bunce et al. [23].

2.4. Ethics

This study was approved by the Research Ethics Board of the Porto Alegre Clinical Hospital (HCPA, CAEE 10555212.6.0000.5327, protocol number 13-0038) and was conducted in accordance with the Declaration of Helsinki. All subjects or their surrogates received detailed explanations and provided written consent prior to inclusion in this investigation.

2.5. Statistical analysis

Comparison of the KIR gene frequency with the control group was executed by Pearson chi-square with continuity correction, and in a few, where the expected difference between the two groups was small, Fisher's exact test was employed. Significance value was calculated using SPSS for Windows version 21.0 (SPSS Inc., Chicago, IL, USA). Results were also analyzed after Bonferroni corrections due to multiple comparisons. A p value ≤ 0.05 after correction was considered statistically significant. We assessed the Hardy–Weinberg equilibrium for KIR3DS1 and KIR3DL1 genotypes (considering the copy number of KIR3DS1) in the groups of subjects by using X^2 test. Linkage Disequilibrium was calculated as per the Lewontin's principle using MASS KIR Analyzer software. The strong positive LD has been assigned to the KIR genes with a LD score ranging between 0.8 and 1 [24].

3. Results

Two hundred and seventy-one patients met inclusion criteria and were divided into sepsis (211 patients diagnosed with sepsis) and control (60 critical care patients with no sepsis) groups. Statistical analysis indicated no differences between groups regarding age and gender. Demographic data are shown in Table 1.

Table 1
Demographic and clinical information on patents with sepsis and controls.

Descriptives		Sepsis (n = 211)	Controls (n = 60)	Total (n = 271)
Age, in years (±SD)		55.33 (±20.26)	46.37 (±19.43)	53.34 (±20.4)
Gender (%)		F, 49.3%	F, 53.33%	F, 50.2%
		M, 50.7%	M, 46.67%	M, 49.8%
APACHE II mean (±sd)		20.64 (±7.45)	14.82 (±7.02)	19.35 (±7.74)
SOFA-1, mean (±sd)		7.56 (±3.40)	5.18 (±3.11)	6.17 (±3.53)
No. of organs in dysfunction, mean (±sd)		4.07 (±1.36)	3.17 (±1.40)	3.87 (±1.46)
No. of organs in failure, mean (±sd)		0.83 (±1.35)	Zero	0.65 (±1.21)
Cause of ICU admission	Respiratory	80 (38.10%)	13 (21.67%)	93 (34.32%)
	Cardiovascular	12 (5.70%)	12 (20.00%)	24 (8.86%)
	Neurologic	7 (3.3%)	14 (23.33%)	21 (7.75%)
	Renal	2 (1.00%)	3 (3.33%)	5 (1.84%)
	Gastrointestinal	6 (2.90%)	6 (10.00%)	12 (4.43%)
	Sepsis	84 (39.81%)	Zero	84 (30.99%)
	Abdominal Surgery	20 (9.50%)	6 (10.00%)	26 (9.59%)
	Trauma surgery	Zero	4 (6.67%)	4 (1.85%)
	Unknown	Zero	2 (3.33%)	2 (0.74%)
Death during hospital stay		117 (56.00%)	9 (15.25%)	126 (46.49%)
Death during ICU hospitalization		95 (45.00%)	4 (6.67%)	99 (36.53%)

APACHE II, SOFA-1, number of organs in dysfunction and number of organs in failure during ICU hospitalization are shown as mean (±) standard deviation SD. Causes of ICU hospitalization, deaths during ICU hospitalization and hospital stay are shown as total number (%).

KIR gene frequency distribution results among patients with sepsis and controls are shown in Table 2. The frequencies of KIR genes in control group were similar to those found in previous studies of Brazilian populations conducted by our research group and others [24–28]. As expected, framework genes KIR 2DL4, 3DL2, 3DL3 and 3DP1 were present in all individuals. There was a significantly lower frequency KIR3DS1 in septic patients in comparison to controls (36.49% *versus* 51.67%; $p = 0.037$) before Bonferroni correction. Also, the frequency KIR2DS1 in septic patients in comparison to controls (41.23% *versus* 55.00%; $p = 0.077$) fell just short of significance before Bonferroni correction.

HLA gene frequencies for sepsis and controls are shown in Table 3. There were no statistically significant differences in HLA-A, HLA-B or HLA-C between groups, or when analyzing these genes for homozygosis and heterozygosis. In addition, no significant differences were found for C1, C2, Bw4, A3 and A11, although the p value for the association between KIR2DS1 + C2+ fell just short of significance (73.3% for controls *versus* 64,45% for sepsis, $p = 0.075$), before Bonferroni correction. No differences between groups were found after Bonferroni correction. Moreover, no differences between groups were found when comparing the inhibitory and activating KIR genes frequencies in the presence of their ligands (Tables 4 and 5). Statistical analysis found no association between the prevalence of KIR genes and clinical outcomes, such as organ dysfunction, hospital and ICU stay and/or mortality, or even patient gravity, measured through SOFA-1 and APACHE II.

Linkage disequilibrium showed similar observations as obtained on the basis of allele frequency and KIR profiles (Table 6). Both populations are in Hardy-Weinberg equilibrium for the KIR3DS1/KIR3DL1: controls ($p = 0.248$) and sepse ($p = 0.09$). The qui-square was not significant between groups ($p = 0.055$) (Table 7). Genotypic frequency (GF), derived from carrier frequencies (CF), was calculated with the formula $GF = 1 - \sqrt{(1 - CF)}$. This transformation was based on the assumption of Hardy–Weinberg equilibrium and it was performed to KIR3DL1, KIR3DS1, KIR2DL2 and KIR2DL3 since they behave as alleles (Table 8).

4. Discussion

Genetic variations among patients with sepsis have been reported over the past few years [3,4,29–35], with major focus on the clotting and innate immune systems, aiming at better

Table 2
Killer cell immunoglobulin-like receptor (KIR) gene frequencies in controls (n = 60) and patients with sepsis (n = 211).

Gene	Control N (%)	Sepsis N (%)	p value[a]	p value[b]
KIR2DL1	57 (95.00)	199 (94.31)	0.999	0.999
KIR2DL2	33 (55.00)	119 (56.40)	0.964	0.999
KIR2DL3	52 (86.67)	187 (88.63)	0.851	0.999
KIR2DL4	60 (100.00)	210 (99.5)	0.999	0.999
KIR2DL5	41 (68.33)	118 (55.92)	0.116	0.999
KIR2DP1	57 (95.00)	199 (94.31)	0.999	0.999
KIR2DS1	33 (55.00)	87 (41.23)	0.077	0.999
KIR2DS2	32 (53.33)	115 (54.50)	0.989	0.999
KIR2DS3	22 (36.67)	64 (30.33)	0.439	0.999
KIR2DS4	56 (93.33)	195 (92.42)	0.999	0.999
KIR2DS5	24 (40.00)	75 (35.55)	0.631	0.999
KIR3DL1	57 (95.00)	196 (92.89)	0.771	0.999
KIR3DL2	60 (100.00)	211 (100.00)	NC	NC
KIR3DL3	60 (100.00)	211 (100.00)	NC	NC
KIR3DP1	60 (100.00)	210 (99.5)	0.999	0.999
KIR3DS1	31 (51.67)	77 (36.49)	0.037	0.518

Activating genes KIR2DS1 and KIR3DS1 are less frequent in patients with sepsis.
NC, not calculated.
[a] Chi-Square test or Fisher's exact test.
[b] p value after Bonferroni correction.

Table 3
Frequencies of KIR ligands in controls and septic patients.

Gene	Controls N (%)	Sepsis N (%)	*p* value[a]	*p* value[b]
A3	9 (15.00)	40 (18.95)	0.608	0.999
A11	4 (6.67)	5 (2.37)	0.113	0.678
Bw4	40 (66.67)	131 (62.08)	0.619	0.999
C1	49 (81.67)	175 (82.94)	0.833	0.999
C2	47 (78.33)	149 (70.66)	0.250	0.999
C1/C1	13 (21.67)	62 (29.38)	-	-
C2/C2	11 (18.33)	36 (17.06)	0.496	0.999
C1/C2	36 (60.00)	113 (53.56)	-	-

C1 group: HLA-Cw 01, 03, 07 (01–06), 08, 12 (02, 03, 06), 14, 16 (01, 03, 04).
C2 group: HLA-Cw 02, 04, 05, 06, 07, 12 (04, 05), 15, 16, 17, 18.
Bw4: HLA-B 13, 27, 44, 51, 52, 53, 57, 58.
[a] Chi-Square test or Fisher's exact test.
[b] *p* value after Bonferroni correction.

Table 4
Inhibitory KIR gene frequencies in the presence or absence of their ligands in controls and septic patients.

Genes	Controls N (%)	Sepsis N (%)	*p* value[a]	*p* value[b]
KIR2DL1 + C2+	44 (73.33)	136 (64.45)	0.318	0.999
KIR2DL1 + C2/C2	10 (16.67)	36 (17.06)	0.999	0.999
KIR2DL2 + C1+	25 (41.67)	102 (48.34)	0.482	0.999
KIR2DL2 + C1/C1	8 (13.33)	33 (15.64)	0.814	0.999
KIR2DL3 + C1+	43 (71.67)	159 (74.36)	0.795	0.999
KIR2DL3 + C1/C1	13 (21.67)	58 (27.49)	0.460	0.999
KIR3DL1 + Bw4+	37 (61.67)	120 (56.87)	0.606	0.999
KIR3DL2 + A3+	9 (15.00)	40 (18.96)	0.608	0.999
KIR3DL2 + A11+	4 (6.67)	5 (2.37)	0.113	0.999

C1 group: HLA-Cw 01, 03, 07 (01–06), 08, 12 (02, 03, 06), 14, 16 (01, 03, 04).
C2 group: HLA-Cw 02, 04, 05, 06, 07, 12 (04, 05), 15, 16, 17, 18.
Bw4: HLA-B 13, 27, 44, 51, 52, 53, 57, 58.
[a] Chi-Square test or Fisher's exact test.
[b] *p* value after Bonferroni correction.

Table 5
Activating KIR gene frequencies in the presence or absence of their ligands in controls and septic patients.

Genes	Controls N (%)	Sepsis N (%)	*p* value[a]	*p* value[b]
KIR2DS2 + C1+	24 (40.00)	97 (45.97)	0.500	0.999
KIR2DS2 + C1/C1	8 (13.33)	33 (15.64)	0.814	0.999
KIR2DS1 + C2+	24 (40.00)	57 (27.01)	0.075	0.450
KIR2DS1 + C2/C2	6 (10.00)	14 (6.63)	0.403	0.999
KIR3DS1 + Bw4+	21 (35.00)	51 (24.17)	0.131	0.999

C1 group: HLA-Cw 01, 03, 07 (01–06), 08, 12 (02, 03, 06), 14, 16 (01, 03, 04).
C2 group: HLA-Cw 02, 04, 05, 06, 07, 12 (04, 05), 15, 16, 17, 18.
Bw4: HLA-B 13, 27, 44, 51, 52, 53, 57, 58.
[a] Chi-Square test or Fisher's exact test.
[b] *p* value after Bonferroni correction.

Table 6
Linkage disequilibrium analysis in eleven KIR genes of critical care patients.

Genes	KIR3DL1	KIR2DL1	KIR2DL3	KIR2DS4	KIR2DL2	KIR2DL5	KIR3DS1	KIR2DS1	KIR2DS2	KIR2DS3	KIR2DS5	KIR2DL4	KIR2DP1
KIR3DL1	–												
KIR2DL1	1	–											
KIR2DL3	0.11	0.72	–										
KIR2DS4	1	1	0.17	–									
KIR2DL2	0.50	1	0.9	0.38	–								
KIR2DL5	1	0.24	0.54	1	0.45	–							
KIR3DS1	1	0.30	0.16	1	0.19	1	–						
KIR2DS1	1	0.17	0.16	1	0.23	0.97	0.98	–					
KIR2DS2	0.53	1	0.91	0.41	1	0.41	0.18	0.21	–				
KIR2DS3	0.38	1	0.34	0.33	0.85	0.97	0.24	0.19	0.76	–			
KIR2DS5	0.89	0.03	0.03	0.90	0.13	0.97	0.74	0.91	0.08	0.09	–		
KIR2DL4	1	1	0.98	0.97	1	1	1	1	1	1	1	–	
KIR2DP1	1	1	0.72	1	1	0.24	0.30	0.17	1	1	0.03	1	–
KIR3DP1	1	1	0.98	0.97	1	1	1	1	1	1	1	0.57	1

LD values have been calculated as per the Lewontin's principle. 1 represents a perfect LD; 0.80–0.99 represents a strong LD; values tending towards zero suggest a weak LD.

Table 7
Association analysis between copy number of KIR3DS1 in septic patients and controls.

	Controls	Sepse	p value
3DL1/3DL1	29 (48.3)	133 (63.3)	
3DL1/3DS1	28 (46.7)	63 (30.0)	0.055
3DS1/3DS1	3 (5.0)	14 (6.7)	

Both populations are in Hardy-Weinberg equilibrium: controls ($p = 0.248$) and sepsis ($p = 0.09$). The Pearson qui square test indicated no significant difference between groups, although it fell short of significance ($p = 0.055$).

understanding possible genetic predispositions for sepsis, sepsis-related organ dysfunction and death. NK cells are a very important part of the innate immune system, playing a key role during early infection events due to their ability to deliver responses that can lead to effective clearing of pathogens. They can be rapidly recruited into infected organs and tissues by chemoattractant factors produced by activated resident macrophages and dendritic cells, which are also a major source of interferon INFα/β that induces NK cell proliferation and activation. Once activated, NK cells help to limit the dissemination of pathogen infection before adaptive immune system activation is initiated, through recognition and elimination of non-self after interaction with their receptors and posterior cytokines release, such as tumor necrosis factor, that will amplify the inflammatory response [8,36,37].

The number and type of KIR genes arranged on the haplotypes vary greatly. A database and online repository for immune gene frequencies in worldwide populations reports 573 different KIR genotypes [38]. This wide variation produces substantial differences between individuals in their KIR gene content. Genetic susceptibility or resistance to infectious diseases, in conjunction with environmental and host risk factors, is thought to determine disease progression.

It has been proposed that variations in the expression of KIR repertoire on NK cells and their corresponding ligands are associated with several infectious disorders [39], including [40,41], human immunodeficiency virus (HIV) [42–45], hepatitis C virus [46], and tuberculosis [47–49]. Braun et al. described a lower prevalence of the activating gene KIR2DS3 in patients with tuberculosis when compared to controls, which can be due to the large diversity of their study population, but also can be related to a protective role of the presence of KIR2DS3 [49].

Several diseases with autoimmune basis, such as lupus, psoriasis vulgaris, psoriatic arthritis, rheumatoid arthritis, diabetes, among others, have been associated with KIR genes, especially with the presence of activating genes, lack of their inhibitors and interaction of KIR/HLA [39,50–52]. Although we did not observe an association of any genes with its respective ligand, association between KIR2DS1 + C2+ was more prevalent in controls and fell just short of significance before Bonferroni correction.

The lower prevalence of activating genes KIR2DS1 and KIR3DS1 in sepsis than in critical patients with no sepsis, suggests that the presence of these activating genes may act as a protective factor against sepsis in critical care patients. This seem to be true for other infectious diseases. A previous study published by Bonagura and colleagues suggested that the presence of activating receptors KIR2DS1 and KIR3DS1 may protect from developing a rare disease of the larynx and upper airway caused by an HPV strain, HPV-6/11, recurrent respiratory papillomatosis (RRP) [53].

There is no previous report of association between KIR3DS1 and susceptibility to infectious diseases. However, patients with KIR3DS1 infected with HIV show a delay in AIDS progression, suggesting a more effective performance of immune system in those patients [45]. On the other hand, Zhuang and colleagues [54] have shown that activating genes KIR2DS3 and KIR3DS1 were more prevalent in patients with syphilis than in controls without infectious diseases. Other activating KIR genes, such as KIR2DS3, are significantly higher in hepatitis C, chronic hepatitis B and leprosy [55]. Furthermore, Niepieklo-Miniewska et al. reported that frequencies of the great majority of KIR genes did not differ between patients and controls, except for KIR2DS1, where frequency was significantly lower in patients than in controls, suggesting a protective effect of KIR2DS1 [56]. In our study, sepsis patients in general seem to present phenotypic frequencies in a similar way than general population whilst the controls present a higher frequency for KIR2DS1 and KIR3DS1, suggesting a possible protective effect by these genes [39].

The innate immune system is the first line of defense against pathogens, working to recognize common components of pathogens, so that further immune responses can be signaled in the presence of foreign pathogens. Multiple cell types, including macrophages, dendritic cells, neutrophils and epithelial cells, in addition to NK cells, are involved in the development of an immune response, with each cell type having specific functions. NK cells, although primarily involved with lysis of target cells, can as well act by interacting with antigen presenting cells and T-cells [57]. The differences in KIR frequencies may result in differential cytokine expression, contributing to different outcomes, thus there might be a genetic susceptibility to diseases such as sepsis. Perhaps an earlier and more effective identification of the pathogen can lead to a more effective immune response, protecting the individual against an uncontrolled inflammatory response. Some KIR genes such as KIR3DS1 can bind to HLA-B27 and recruit positive signals, resulting in NK cell activation, and this recognition can regulate the immunomodulatory functions of NK cells. In addition, the finding that the presence of activating gene KIR3DS1 in combination with HLA-B alleles is associated with a delayed progression to acquired immunodeficiency syndrome (AIDS) in individuals with HIV type-1 (HIV-1) suggests a model involving an epistatic interaction between the two loci [45].

Activating and inhibitory KIR display different ligand affinities, and a model of NK cell response has been considered in which KIR variation and the presence of ligands influence the response level in individual patients, within a range that includes excess of activation, balance, excess of inhibition, or undetermined behavior [55]. This concept is applied to the investigation of susceptibility or protection in a wide spectrum of diseases. Differences in KIR gene frequencies and haplotypes may result in differential cytokine expression, contributing to different host responses to infection

Table 8
Genotypic frequencies of KIR genes in septic patients and controls.

		KIR3DL1	KIR3DS1	KIR2DL2	KIR2DL3
Controls	CF	0.9500	0.5167	0.5500	0.8667
	GF	0.78	0.30	0.33	0.64
Sepse	CF	0.9289	0.3649	0.5640	0.8863
	GF	0.73	0.20	0.34	0.67

Genotypic frequency (GF), derived from carrier frequencies (CF), was calculated with the formula $GF = 1 - \sqrt{(1 - CF)}$. This transformation is based on the assumption of Hardy–Weinberg equilibrium.

and disease outcomes, and suggest a genetic influence on susceptibility and pathogenesis.

It is important to keep in mind that sepsis is a heterogeneous disease and its outcome depends on the causative pathogen, microbial load and virulence, and host characteristics, including the genetic composition for other factors involved in the inflammatory process, age, comorbidities, choice of sepsis treatment, and time until diagnostic [58]. Our results provide early information about the role of polymorphisms in KIR during sepsis, suggesting potential prognostic markers that could help predicting sepsis development.

In summary, we verified that activating KIR genes 2DS1 and 3DS1 are more prevalent in critical patients without sepsis than in patients with sepsis, suggesting that the presence of activating genes modulate NK-cell response and may play a role as a protective factor for sepsis in critical care patients. Further research focusing on functional studies is warranted.

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