EFFECT OF KILLER IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) IN THE RESPONSE TO COMBINED TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C

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Abstract

KIR receptors are related to the activation and inhibition of NK cells, and may play an important role in the innate response against infection with such viruses as HCV. We examined whether the different combinations of KIR receptors with their HLA class I ligands influenced the response to combined treatment (Peg-IFN-α and ribavirin) in patients infected by Hepatitis C virus (HCV). A total of 186 consecutive patients diagnosed with chronic HCV infection were analyzed. Seventy seven exhibited HCV RNA levels at 6 months post treatment and were called non-responders (NR) while 109 cleared viral RNA and were named sustained viral responders (SVR). Patients were typed for HLA-B, HLA-Cw, KIR genes and for HCV genotype. In our study, the frequency of KIR2DL2 allele was significantly increased in NR (p<0.001, OR=1.95) as well as KIR2DL2/KIR2DL2 genotype (p<0.005, OR=2.52). In contrast, KIR2DL3 (p<0.001) and KIR2DL3/KIR2DL3 genotype (p<0.05, OR=0.54) were significantly increased in the SVR. Different combinations of KIR2DL2 and KIR2DL3 alleles with their ligands were analyzed. The KIR2DL2/KIR2DL2-HLA-C1C2 genotype was significantly increased in the NR (p<0.01, OR=3.15). Additionally, we found a higher frequency of the KIR2DL3/KIR2DL3-HLA-C1C1 genotype in the SVR group (p<0.05, OR=0.33). These results were not affected by the HCV genotype. Conclusions: Patients who carried the KIR2DL2/KIR2DL2-HLA-C1C2 genotype were less prone to respond to treatment. However, the KIR2DL3/KIR2DL3-HLA-C1C1 genotype clearly correlated with a satisfactory response to treatment, defined by the clearance of HCV-RNA.

Keywords: HCV, KIR receptors, Combined treatment, SVR, Peg-IFN, Rivabirin.
INTRODUCTION

Hepatitis C virus (HCV) infection is a common chronic disease affecting over 170 million people worldwide (48). Around 80% of these individuals evolve to chronic infection, and 10-20% of patients develop cirrhosis over a 20 year-period. A minority (2%) progress to hepatocellular carcinoma (HCC) annually (18). Several host factors including age, body mass index (BMI), gender, fibrosis, cirrhosis or absence of cirrhosis and several viral factors including viral genotype and viral load can influence the response to treatment (5, 32, 43). Pegylated interferon-alpha (Peg-IFN-α) plus ribavirin (combined therapy) constitutes the most effective therapy for the treatment of chronic hepatitis C (12). Since this treatment carries serious side effects, it is necessary to identify those patients who can clear HCV infection in order to reduce the period of this aggressive therapy.

Natural Killer (NK) cells are a type of lymphocytes that play an important role in the host defense against HCV infection (14). NK cell activity is determined by the balance of different signals received and the equilibrium between inhibitory and activating receptors (2). Some receptors are specific for Human Leukocyte Antigens class I (HLA class I) molecules (3). NK cells check the surface of the surrounding cells, detect the presence of their HLA class I molecules and then discriminate between healthy, infected or transformed cells (9). When NK cells contact target cells, the resulting interactions of their receptors produce either activating or inhibitory signals. If the expression of HLA class I molecules on the target cell is absent or reduced, the inhibitory signal is not generated (25).

Killer cell immunoglobulin-like receptors (KIRs) are members of a group of regulatory molecules expressed on NK cells and a subset of T cells (30). This family of polymorphic genes is located on chromosome 19 (19q13.4), within the leukocyte receptor
complex (LCR). The LCR also encodes a number of genetically and functionally related genes. KIR receptors with long cytoplasmic tails are inhibitors, based on the presence of immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic domains. KIR receptors with short tails interact with adaptor molecules such as DAP-12 (DNAX activation protein) which contain immunoreceptors tyrosine-based activation motifs (ITAMs) and transmit activating signals (24). Several inhibitory KIR receptors have been well defined. KIR2DL1 binds the subset of HLA-Cw molecules with lysine at position 80 of the heavy chain (HLA-C2 group). KIR2DL2 and KIR2DL3 bind the subset of HLA-Cw molecules with asparagine at position 80 (HLA-C1 group) (34).

Studies that have associated KIR receptor genotypes with diseases have identified mainly viral infections and autoimmune diseases (22, 45). The importance of NK cells in the resolution of viral infections has prompted studies that correlate KIR receptors and their ligands with outcomes (11). Some studies identify a relationship between KIR receptor genotypes and outcomes with several infectious agents, such as the Human Immunodeficiency Virus (HIV) (27, 29), Cytomegalovirus (CMV) (6), Hepatitis B Virus (HBV) (28), and Hepatitis C Virus (HCV) (21, 26).

Recently, a protective association of the inhibitory receptor KIR2DL3 in combination with HLA-C^Asn80 (HLA-C1) on the course of HCV infection was described (21). The prevalence of KIR2DL3 and its ligand HLA-C1 is increased in individuals who eliminate HCV spontaneously, in contrast to those who remain chronically infected. The protective effect of KIR2DL3 / HLA-C^Asn80 was observed only among individuals who carried both homozygous genes and had received a low HCV exposure dose. Recently, we found that the frequency of HLA-Bw4^I80 ligand and the activating receptor KIR3DS1 was
increased in HCV healthy carriers compared to patients who had developed hepatocellular carcinoma (26).

The aim of this study was to investigate the influence of KIR genes and KIR-HLA combinations on the response to combined therapy with pegylated interferon (Peg-IFN-\(\alpha-2b\)) and ribavirin in a group of patients with HCV infection.

**PATIENTS AND METHODS**

**Patients**

A group of 186 consecutive, unrelated Caucasian patients diagnosed with chronic HCV infection were enrolled in the study between January 2004 and December 2005. They were diagnosed by the Gastroenterology Service of the Hospital Universitario Central de Asturias (HUCA) in Oviedo (Spain) and by the Clínica Universitaria de Navarra (CUN) in Pamplona (Spain). All patients were positive for anti-HCV antibodies and HCV RNA in serum.

The patients received the same standard treatment of Peg-IFN-\(\alpha-2b\) (1.5\(\mu\)g/Kg/week) and ribavirin (<65 Kg, 800 mg/day; 65-85 Kg, 1000mg/day; >85 Kg, 1200 mg/day). The duration of the treatment was 24 weeks for HCV genotypes 2 or 3 and 48 weeks for HCV genotypes 1 or 4. All patients were followed for at least 6 months post-treatment in order to establish the response, in accordance with virological criteria (10). Patients were classified into two treatment outcomes: patients who did not mount a sufficient anti-HCV response (non-responders, NR), as defined by a consistent positive viral load during treatment, its end or 6 months post treatment. Another group of patients were classified as sustained virological responders (SVR). The SVR was defined by
consistent undetectable HCV RNA levels in serum during 6 months post treatment.

The protocol was approved by the Ethics Committee of both hospitals, and all patients gave written informed consent before enrollment.

**Laboratory methods**

*Immunological tests.*

Genomic DNA was extracted from peripheral blood with the Magtration-MagaZorb® DNA Common Kit-200 N using Magtration System 12GC (Precision System Science Co., Ltd., Woerstadt, Germany). The HLA-B, HLA-Cw and KIR genes were typed using LIFECODES HLA-SSO and KIR-SSO typing kits (Tepnel Lifecodes Corporation, Stamford, UK) based on Luminex xMAP technology (Luminex Corp., Austin, TX, USA), according to the manufacturer’s instructions. Ambiguities in KIR typing were resolved by PCR-SSP in accordance with the method previously described (16, 19).

*Microbiological tests.*

Serum samples were obtained at least every month before, during and after the treatment. These samples were frozen at -80°C within 4 hours of collection. The RNA viral levels in serum and HCV genotype were determined. The HCV genotype was identified by the VERSANT® HCV Genotype 2.0 Assay (LiPA; Bayer HealthCare, Tarrytown, NY, USA). HCV RNA was quantified during the treatment and the follow-up period by real time PCR (Cobas Taqman 48, Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions.
Statistical analysis

We used the chi-square ($\chi^2$) test to examine whether there were observable differences between qualitative factors and the t-Student test for quantitative factors in independent samples. The different factors that may contribute to the non-response to the treatment were compared using a backward stepwise logistic regression analysis. The effect of the KIR-HLA genotypes on the clearance of HCV-RNA in serum during the treatment was estimated by the Kaplan-Meier method and compared using the log-rank test. In order to perform this statistical analysis, we have defined the HCV-RNA death event as the mean between the last positive and the first negative HCV-RNA result, under the prerequisite that the patient remained negative through the end of the period of treatment (31). Sera were collected at intervals of 4 weeks and samples with undetectable levels of HCV-RNA during treatment from patients with “Breakthrough effect” (39) were not considered for statistical analysis. Data was censored when patients finished their treatment. A p value <0.05 was considered significant. The statistical analysis was calculated using the SPSS 15.0 program (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients characteristics

The clinical and demographic characteristics of the 186 patients are shown in Table 1. Seventy-seven patients (41.4%) were listed as non-responders to the treatment and 109 patients (58.6%) comprised the SVR group because they exhibited no detectable HCV RNA levels throughout the 6 months post treatment. The distribution of viral genotypes in our cohort showed that HCV genotype 1 was the most prevalent in our study.
population, and was significantly more resistant to this treatment regimen (p<0.001, OR=4.22, 95% CI=2.07-8.78). In contrast, HCV genotype 3 was significantly more susceptible to this treatment regimen (p<0.001, OR=0.12, 95% CI=0.05-0.33).

Moreover, BMI was significantly higher in the NR group than in SVR group (p<0.05).

**HLA and KIR distribution in the cohort study**

The frequencies of HLA-B and HLA-Cw alleles in our patients were not significantly different between the treatment outcomes. We then examined the frequency of the different KIR genes (Figure 1). No statistically significant differences were found for most allele combinations except for the KIR2DL2 and KIR2DL3 genes. Considering both genes as alleles of the same locus (20, 46), KIR2DL2 was significantly increased in NR (p<0.001, OR=1.95, 95% CI=1.26-3.03), whereas KIR2DL3 was significantly increased in the SVR (p<0.001) (Table 2).

We also analyzed the distribution of the homozygous and heterozygous genotypes of both alleles. When the KIR2DL2 allele was homozygous, we found significant differences between the treatment outcomes. The KIR2DL2/KIR2DL2 genotype significantly correlated with NR (p<0.005, OR=2.52, 95% CI=1.31-4.86). In contrast, the frequency of the KIR2DL3/KIR2DL3 genotype was significantly associated with viral clearance (p<0.05, OR=0.54, 95% CI=0.28-0.99). No significant differences were found between treatment outcomes when both alleles were heterozygous.

Next, we analyzed the different interactions of KIR2DL2 and KIR2DL3 alleles with their HLA-Cw group 1 (HLA-C1) ligands. When KIR2DL2 was homozygous and its ligand HLA-C1 was heterozygous (KIR2DL2/KIR2DL2-HLA-C1C2), we observed a significant
increase in poor response to treatment (p<0.01, OR=3.15, 95% CI=1.32-7.51). On the contrary, the KIR2DL3/KIR2DL3-HLA-C1C1 genotype was associated with at least 6 month clearance of HCV (p<0.05, OR=0.33, CI=0.1-0.99). We analyzed the remaining combinations of heterozygous KIR2DL2 and KIR2DL3 alleles with their ligands but no significant differences were observed.

We then decided to analyze the progressive effect of KIR2DL3/KIR2DL3-C1C1 on the response to the treatment. Patients were sorted according to KIR2DL3 and HLA-C genotype. The first group contained patients who had the KIR2DL3/KIR2DL3-HLA-C1C1 genotype, the second one contained patients who had the KIR2DL3/KIR2DL3-HLA-C1C2, KIR2DL2/KIR2DL3-HLA-C1C1, or KIR2DL2/KIR2DL3-HLA-C1C2 genotype, and the last group consisted of the remaining patients who were homozygous for KIR2DL2, homozygous for HLA-C2, or patients who had KIR2DL2/KIR2DL2-HLA-C2C2 genotype. This analysis revealed a linear trend between the number of KIR2DL3-HLA-C1 interactions and the odds of a SVR ($\chi^2$ for trend=4.736; p<0.05).

The distribution of the frequencies of KIR3DL1 and KIR3DS1 alleles and the interaction with their ligands (HLA-B Bw4 allotypes) was compared with outcome. No associations were found between these genes and the response to the treatment (Data not shown).

**Influence of KIR2DL genotype in combination with other risk factors in the non-response to the treatment.**

As previously mentioned, the response to treatment is particularly ineffective for the most prevalent HCV genotype 1 (23), but patients with HCV genotype 2 or 3 are expected
to have a higher probability of treatment success. Other factors that could contribute to the achievement of positive outcome are gender, age, BMI, extent of fibrosis, HCV-RNA levels and presence or absence of cirrhosis. Using a backward stepwise logistic regression model, we initially analyzed the influence of these host and viral variables with KIR and HLA-Cw genetic factors on the non-response to treatment. The resulting comparative model is represented in Table 3. The KIR2DL2/KIR2DL2-HLA-C1C2 genotype had a correlation with poor outcome (p<0.01, OR=4.12, 95% CI=1.68-10.1). Moreover, consistent with previous studies, HCV genotype 1 (p<0.005, OR=3.32, 95% CI=1.49-7.42) contributed clearly towards the non-response of this treatment. Significant interactions between the different variables analyzed were not detected, including the BMI that was statistically significant in the univariate analysis. In this multivariate analysis KIR2DL3/KIR2DL3-HLA-C1C1 genotype was not significantly associated with non-response to treatment.

**Effect of the KIR-HLA genotype in the clearance of HCV RNA at the end of the treatment**

We determined the HCV-RNA levels in serum of our patient cohort during the treatment period. For the Kaplan-Meier analysis, patients were divided into two groups: the first group was composed of patients with HCV genotype 1 or 4 (n=137) and the second one of patients with HCV genotypes 2 or 3 (n=49) (Fig. 2). In group 1, 70.6% of the patients who carried the KIR2DL3/KIR2DL3-HLA-C1C1 genotype had a complete response and cleared the HCV-RNA (log Rank, p=0.062), which showed a trend for a protective effect of this KIR-HLA genotype. In contrast, most patients who had the
KIR2DL2/KIR2DL2-HLA-C1C2 genotype did not clear HCV-RNA (log Rank, \( p<0.01 \)) by the end of the treatment. Group 2 patients infected with HCV genotypes 2 or 3 exhibited similar associations between genotypes and outcomes. All patients who had the KIR2DL3/KIR2DL3-HLA-C1C1 genotype cleared the HCV-RNA (log Rank, \( p<0.05 \)) after 24 weeks of treatment, whereas patients who had the KIR2DL2/KIR2DL2-HLA-C1C2 genotype did not (log Rank, \( p<0.005 \)). Patients with KIR2DL3/KIR2DL3-HLA-C1C1 genotype cleared the HCV-RNA at a higher frequency than those patients who carried the KIR2DL2/KIR2DL2-HLA-C1C2 genotype.

Moreover, at the end of the follow-up period, 65.4% of patients who had KIR2DL2/KIR2DL2-HLA-C1C2 genotype were poor responders, whereas 79.2% of all patients who had KIR2DL3/KIR2DL3-HLA-C1C1 were able to clear HCV-RNA in serum by the end of treatment. In a previous analysis, we did not find statistical differences when patients were divided in Early Viral Responders (EVR), SVR and NR (Data not shown).

In conclusion, KIR-HLA genotype can influence the clearance of HCV RNA at the end of the treatment independently of HCV genotype.

**DISCUSSION**

Several studies have identified specific genetic factors that play an important role in the persistence of HCV infection (41). It has also been observed that some HLA alleles clearly influence the resolution of other viral infections (1). Thus, HLA molecules have been the focus of numerous studies for establishing possible associations with the persistence or the elimination of HCV (8, 40). Some associations between certain KIR receptors with their HLA ligands and the progression of HCV infection have also been
described (26).

In the present study, we analyzed the influence of KIR receptors on the response to combined treatment. While the number of patients was relatively small, we considered the homogeneity of the group important. In spite of these limitations, we observed that KIR2DL3 was associated with a sustained viral immune response while KIR2DL2 had clearly correlated with a non-response. Furthermore, we found that KIR2DL3/KIR2DL3-HLA-C1C1 was associated with sustained virological response, whereas KIR2DL2/KIR2DL2-HLA-C1C2 had a significantly increased frequency in the NR population.

Previous studies have suggested a model in which KIR-HLA combinations inhibit NK cells with different intensities (44, 46). According to this model, KIR2DL1-HLA-C2 has the strongest capacity of inhibition, followed by KIR2DL2-HLA-C1, and finally, by KIR2DL3-HLA-C1. The weaker inhibition of KIR2DL3 may result in a greater activation of NK cells and, consequently, a more efficient resolution of viral infection. The effects of additional NK activating receptors, such as NKG2D, may lead to a higher efficacy of control against HCV infection. The two KIR2DL1 (present in 98% of the individuals) and KIR2DL2 receptors may, however, induce a more intense inhibition of NK cells, and therefore reduce the efficacy of resolving HCV infection. Moreover, the different KIR2DL-HLA-C interactions may further transmit inhibitory signals with different strengths (37).

In agreement with these studies, we suggest that the KIR2DL3/KIR2DL3-HLA-C1C1 genotype combination influences the generation of a sustained virological response, since the inhibitory signals produced by KIR2DL1 and KIR2DL2 are absent. Individuals who have the KIR2DL3/KIR2DL3-HLA-C1C1 genotype, more effectively activated NK
cells in response to the administration of Peg-IFN-α during treatment, although NK cell functions have been impaired during chronic HCV infection (15). Furthermore, several studies have described HCV mechanisms that inhibited the responses of NK cells and correlated with the establishment of a chronic infection (17, 42). Nevertheless, recent findings demonstrate that a sustained response to combined treatment for patients with HCV chronic infection is closely associated with increased NK cells in the liver (49). Other KIR2DL-HLA genotypes have at least one stronger inhibitory signal and their response to treatment was less effective. Within these genotypes, we suggest that the KIR2DL2/KIR2DL2-HLA-C1C2 genotype has the most inefficient response to treatment, because cumulative inhibitory signals are produced by KIR2DL1 and KIR2DL2. This stronger inhibition could be enhanced by the treatment because Peg-IFN-α induces the expression of HLA class I molecules (38), and favours the interaction between the infected cells and NK cells.

We further observed that the homozygous KIR2DL3 allele in combination with homozygous HLA-C1 was significantly more frequent in patients who responded to antiviral treatment. The non-responding patients had a higher frequency of the homozygous KIR2DL2 allele with its heterozygous ligand, supporting our hypothesis. As previously mentioned, Khakoo et al (21) described that the presence of the homozygous KIR2DL3 allele and its ligand is correlated to spontaneous clearance of HCV. Our study shows a similar association but in this case KIR2DL3/KIR2DL3-HLA-C1C1 is significantly associated with a complete response to the antiviral therapy, while the presence of the genotype KIR2DL2/KIR2DL2-HLA-C1C2 correlates with an inadequate response to the treatment and with persistent viral RNA. **Despite the observation that**
KIR2DL2/KIR2DL2-HLA-C2C2 genotype carries the strongest inhibitory signal mediated by KIR2DL1-HLA-C2, we did not detect statistical differences related to non response to treatment. Possible explanations include its low frequency in our population and/or the absence of the additional KIR2DL2-HLA-C1 inhibitory signal. Moreover, the bound peptides in MHC class I play an important role in the balanced recognition of NK cells, and KIR2DL1 recognition of this complex may be reduced by some modified peptides during tumor transformation or viral infections (13).

The monitoring of HCV RNA levels in serum throughout the treatment period revealed a high percentage of patients with KIR2DL3/KIR2DL3-HLA-C1C1 who exhibited a complete response. In contrast, the majority of individuals with the KIR2DL2/KIR2DL2-HLA-C1C2 genotype were unable to clear the HCV RNA. These data also suggested that the treatment in the presence of the KIR2DL2/KIR2DL2-HLA-C1C2 genotype will be less effective in resolving HCV infection.

Analysis of the risk factors in the NR to the treatment showed significant associations only with the BMI, the HCV genotype, the KIR genotype and the KIR-HLA genotype. Previous studies have shown that HCV genotypes influence the response to treatment (50). For example, HCV type 1 is associated with a poor response to antiviral treatment (36), similar to our results. Several studies suggest that HCV genotype 1 is more resistant to treatment due to the interactions of certain viral proteins, such as NS5A, with the IFN-α signalling pathway (35). In fact, 5 HCV infected patients with KIR2DL3/KIR2DL3-HLA-C1C1 did not resolve their infections after therapy in our study which suggests that additional modalities for resolving HCV infections are warranted. On the other hand, anti-viral treatment usually is efficacious against HCV genotypes 2 and 3,
and justifies a shorter treatment schedule (7).

T CD8+ lymphocytes, as NK cells, are crucial in the defense against viral infections. With regard to T CD8+ lymphocytes and HLA class I molecules, several studies indicate a central association with the resolution of viral diseases (33). On the other hand, T CD8+ lymphocytes can express KIR genes, and the signals from these receptors can contribute to the control of viral infections (4).

In conclusion, the balance of activating and inhibitory signals on the NK cells and the T CD8+ lymphocytes, which is modulated by treatment and conditioned by genetics, help define the antiviral response. In addition to viral genotype, KIR receptors play an important role in the immune response against HCV, and may be key factors which modulate the progression of the infection and the response to treatment.

Despite the small number of subjects included in this study, we were able to demonstrate significant effects of KIR receptors and its ligands on the response to chronic HCV treatment. Nevertheless, additional genetic and functional studies will be necessary in order to clarify the involvement of KIR receptors in the HCV infection.

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REFERENCES


progression to AIDS. Nat. Genet. 31:429-434.


Table 1. Clinical and HCV characteristics of infected patients included in the present study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All Patients (n=186)</th>
<th>SVR group (n=109)</th>
<th>NR group (n=77)</th>
<th>p value(1)</th>
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<tbody>
<tr>
<td>Age, mean ± SD, years</td>
<td>47.2 ± 12.9</td>
<td>43.6 ± 7.5</td>
<td>49.5 ± 10.4</td>
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<tr>
<td>Gender distribution (n (%))</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>135 (72.6)</td>
<td>83 (76.1)</td>
<td>52 (67.5)</td>
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<tr>
<td>Female</td>
<td>51 (27.4)</td>
<td>26 (23.9)</td>
<td>25 (32.5)</td>
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<tr>
<td>Weight, mean±SD, Kg</td>
<td>70.5±13</td>
<td>68.3±11</td>
<td>72.1±13</td>
<td>-</td>
</tr>
<tr>
<td>BMI, mean±SD, Kg/m²</td>
<td>23.2±3.6</td>
<td>22.7±3.4</td>
<td>24.1±4.1</td>
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<td>Viral load before treatment, median, 10^6 IU/mL</td>
<td>5.3 (4.67)</td>
<td>5.38 (4.67)</td>
<td>5.17 (3.9)</td>
<td>-</td>
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<tr>
<td>Analytical levels before treatment, mean ± SD, IU/L</td>
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<td></td>
<td></td>
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<tr>
<td>AST&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.6±16.3</td>
<td>59.8±14.5</td>
<td>68.7±17.5</td>
<td>-</td>
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<tr>
<td>ALT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>108.5±19.4</td>
<td>98.5 ± 18.9</td>
<td>114.3 ± 20.5</td>
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<td>γGT&lt;sup&gt;d&lt;/sup&gt;</td>
<td>76.5±24.3</td>
<td>64.7±23.6</td>
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<td>Ferritin, ng/mL</td>
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<td>Platelets, 10&lt;sup&gt;9&lt;/sup&gt; U/ml</td>
<td>221.5±57.6</td>
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<td>Cholesterol</td>
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<td>173.4±32.8</td>
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<td>Cirrhosis, (n (%))</td>
<td>5 (2.7)</td>
<td>2 (1.8)</td>
<td>3 (3.9)</td>
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<tr>
<td>Source of HCV infection</td>
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<td>Blood transfusion (n (%))</td>
<td>61 (32.8)</td>
<td>33 (30.3)</td>
<td>28 (36.4)</td>
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<td>Drug users</td>
<td>55 (29.6)</td>
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<td>19 (24.7)</td>
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<td>40 (36.7)</td>
<td>30 (38.9)</td>
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<td>HCV Genotype (n (%))</td>
<td>126 (67.7)</td>
<td>61 (55.9)</td>
<td>65 (84.4)</td>
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<td>126 (67.7)</td>
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<td>65 (84.4)</td>
<td>&lt;0.001</td>
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<td>4 (3.7)</td>
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<td>3</td>
<td>44 (23.6)</td>
<td>39 (35.8)</td>
<td>5 (6.5)</td>
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<td>4</td>
<td>11 (5.9)</td>
<td>5 (4.6)</td>
<td>6 (7.8)</td>
<td>-</td>
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</table>

<sup>1</sup> IR, Interquartile range.
<sup>b</sup> AST, aspartate aminotransferase
<sup>c</sup> ALT, alanine aminotransferase
<sup>d</sup> γGT, gamma-glutamyltranspeptidase
(1) All p values were no significant except as indicate in the table.
Table 2. Comparison of frequency of KIR2DL2 and KIR2DL3 receptors, and their respective ligands, to Viral Response.

<table>
<thead>
<tr>
<th></th>
<th>SVR</th>
<th>NR</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KIR alleles</strong></td>
<td>n=218</td>
<td>n=154</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR2DL2</td>
<td>83 (38.1%)</td>
<td>84 (54.5%)</td>
<td>1.95</td>
<td>1.26-3.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KIR2DL3</td>
<td>135 (61.9%)</td>
<td>70 (45.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HLA-C alleles</strong></td>
<td>n=109</td>
<td>n=77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-C1C1</td>
<td>42 (38.5%)</td>
<td>27 (35%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-C1C2</td>
<td>46 (42.2%)</td>
<td>42 (54.6%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-C2C2</td>
<td>21 (19.3%)</td>
<td>8 (10.4%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td><strong>KIR-HLA combinations</strong></td>
<td>n=109</td>
<td>n=77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2DL2/2DL2+ HLA-C1C1</td>
<td>10 (9.2%)</td>
<td>10 (13%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2DL2/2DL2+ HLA-C1C2</td>
<td>9 (8.3%)</td>
<td>17 (22.1%)</td>
<td>3.15</td>
<td>1.32-7.51</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2DL2/2DL2+ HLA-C2C2</td>
<td>3 (2.7%)</td>
<td>3 (3.9%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2DL3/2DL3+ HLA-C1C1</td>
<td>13 (11.9%)</td>
<td>12 (15.6%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2DL3/2DL3+ HLA-C1C2</td>
<td>17 (15.6%)</td>
<td>11 (14.2%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2DL3/2DL3+ HLA-C2C2</td>
<td>9 (8.3%)</td>
<td>1 (1.3%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2DL3/2DL3+ HLA-C1C1</td>
<td>19 (17.4%)</td>
<td>5 (6.5%)</td>
<td>0.33</td>
<td>0.1-0.99</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2DL3/2DL3+ HLA-C1C2</td>
<td>20 (18.3%)</td>
<td>14 (18.2%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2DL3/2DL3+ HLA-C2C2</td>
<td>9 (8.3%)</td>
<td>4 (5.2%)</td>
<td>-</td>
<td>-</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note. Statistical significance was calculated by using the chi-square test.
For KIR2DL2, a >1 odds ratio indicates a poor responder to treatment. NS, not significant.

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>(95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR2DL2/KIR2DL2-HLA-C1C2</td>
<td>4.12</td>
<td>1.68-10.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HCV genotype 1</td>
<td>3.32</td>
<td>1.49-7.42</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Note. In the initial analysis gender, age (>50 years), BMI (>27 Kg/m²), AST (>2 times normal value), ALT (>2 times normal value), GGT (>2 times normal value), HCV-RNA (>400000 IU/mL) and cirrhosis or absence of cirrhosis factors were also included. In this analysis KIR2DL3/KIR2DL3-HLA-C1C1 genotype was no significant.
Figure 1: KIR allele frequencies in chronic HCV patients compared to treatment outcome.

Note: *(KIR2DL2, p<0.001, OR=1.95, 95% CI=1.26-3.03); §(KIR2DL3, p<0.001)
Figure 2: Percentage of complete response to combined treatment before the follow up period.

Note: For patients with HCV genotypes 2 and 3, 24 weeks of treatment. For patients with HCV genotypes 1 and 4, 48 weeks of treatment.
CR: Complete response