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Impact of Different Sources of Infection on Therapy Response in Chronic Hepatitis C

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ABSTRACT

Introduction: Prior to the 1990s, the most common sources of HCV infections were blood transfusions, unsafe injections and I.V drug use. Screening of blood products for HCV has eradicated transfusion-transmitted hepatitis C in most countries since 1992–in Bosnia and Herzegovina, however, since 1995, due to the war. **Aim**: To investigate the impact of the source of HCV infection on the therapeutic response in patients treated for chronic HCV infection with dual combined therapy. **Methods**: We diagnosed chronic HCV infections amongst 246 patients over a period of five years and selected them according to the reported source of infection. Pegylated interferon alfa 2a or alfa 2b with ribavirin was administered during the time that was genotype-dependent. HCV RNA levels in sera were measured by real time PCR. Liver histology was evaluated in accordance with the level of necroinflammation activity and the stadium of fibrosis. **Results**: Regardless of the genotype of the virus and the source of infection, SVR was achieved in 67% of the patients. Therapeutic response (ETR) was not achieved in 25% of the patients who were infected with an untested blood transfusion and 6% of the patients who had had wartime surgery. Amongst the different sources of infections, patients with a war-surgery source of infection responded better to therapy than those with a blood transfusion source of infection ($p = 0.023$). A blood transfusion source of infection implies a larger fibrosis stage than in blood donors; ($g = 1.177$; $s^2 = 0.577$). A blood transfusion source of infection implies a significantly larger necroinflammatory activity than in blood donors; ($g = 1.456$; $s^2 = 0.618$). **Conclusions**: An untested blood transfusion was a significant risk factor for more advanced liver diseases in regards to necroinflammatory activity and the fibrosis stage. This source of infection was also a risk factor for low responses to antiviral therapy. At the same time, I.V. drug users had more progressive necroinflammatory activity, but a high therapeutic response to antiviral therapy.

Key words: blood transfusion, viral hepatitis C, therapy response

1. INTRODUCTION

Exposure to blood is the most efficient way for HCV transmission. Blood transfusion was a major risk factor in most countries before the screening of blood donors began in 1990. In some studies, approximately 10% of all blood recipients were infected with HCV. (1, 2). Screening of all donors for anti-HCV antibodies and, later, for HCV RNA has virtually eliminated posttransfusion HCV infection (3, 4)

Due to the war in Bosnia and Herzegovina, HCV testing of blood transfusion started in 1995. In the period between 1992 and 1995, more than 100,000 blood transfusions that were untested for HCV were administered only in Sarajevo. Currently, the risk of acquiring HCV via a blood transfusion where appropriate screening policies are implemented is less than 1 in a million per unit transfused.(4)

Transmission of HCV via blood-derived products (such as concentrated clotting factors to be used in hemophiliacs) is equally effective. Interferon based antiviral therapy in chronic hepatitis C is genotype-dependent, which means that sustained virological response (SVR), which is an undetectable HCV RNA in the patients' blood after six months following the cessation of therapy, depends on the viral genotype.

In patients with genotype 1 and 4 SVR could be achieved in 50% of patients, and in genotype 2 and 3, SVR can be achieved in approximately. 80% of the patients.

Following the conventional interferon which didn't achieve the required therapeutic effect, combined antiviral therapy with pegylated in-

terferon and ribavirin was a step forward in achieving sustained virological response (SVR), which is consider therapeutic success. In addition to the virus genotype, some other factors like gender, age, metabolic syndrome, alcohol consumption are considered to be a risk factor for less response to antiviral therapy. Those patients are a group of patients that are marked as "difficult to treat". The source of infection was not recognized as a factor that influences the therapeutic result in dual antiviral therapy.

2. MATERIALS AND METHODS

Examinees

The group of respondents with chronic liver disease of viral C etiology consisted of 246 patients of both genders; 186 (70%) were male and 78 (30%) were female. The age of the patients hospitalized at the Gastroenterohepatology Clinic of the University Hospital Sarajevo ranged from 20 to 65 years.

Special attention was paid to the medical hystory of the disease and possible risk behaviors which could be the reason for HCV infection. A questionaire was administered to all the patients, providing data and explaining the possible methods of HCV infection.

Patients were selected according to the hepatitis C viral infection genotype and treated according to two current therapeutic protocols:

Genotypes 1 and 4: Pegylated interferon alpha 2a 40 KD, 48 weeks with ribavirin (1000-1200 mg/daily)

Genotypes 2 and 3: Pegylated interferon alpha 2a 40 KD, 24 weeks with ribavirin (800 mg/daily) (5).

Working method

Biochemical analyses and other tests

The following laboratory tests using standard methods were performed for all the respondents: functional liver tests , serological analyses; detection of nucleic acid of hepatitis C virus by polimerase chain reaction (PCR) qualitatively and quantitatively along with genotyping of C virus which determines the duration of therapy.

Routine hematological and biochemical tests were performed for all the patients, in order to exlude different comorbidity and concominant diseases.

Fibrosis and architectural disorder of liver tissue samples obtained from liver biopsy under ultrasound control were analyzed by way of pathohistological examination in order to asses stage of liver disease (5).

Percutaneous liver biopsy

Analysis of liver tissue in the cylinder of at least 20 mm in length was mandatory.

Routine preparation of samples for histopathological interpretation began with instant fixation in 10% neutral "buffered" formalin. Following standard and special methods of colouring (PAS, D-PAS, Ganori, Van Gieson, Trichrom Masson), the grade of necroinflammatory activity and the stage of fibrosis in the liver were determined using classification according to Ishak and associates.(6)

Serological analyses and monitoring of viremia

Detection of HCV antibodies was done by Enzyme immunoassays EIA, and a definitive confirmation of the findings was done by Recombinant immunoblot assay RIBA.

HCV RNA test was done by molecular analysis of AMPLICOR and COBAS AMPLICOR HCV MONITOR test v2.0.; Confirmation of viral infection and detection of outcome of therapy was achieved by this analysis.

AMPLICOR HCV MONITOR test v2.0. was used for quantification of the quantity of the virus (viral load) and monitoring of the patients' response to therapy. Qualitative AMPLICOR and COBAS AMPLICOR HCV test with the lowest detection level of 50 IU/ml was used for assessment of sustained virological response (SVR).(7)

Direct sequencing was method for determination of viral genotype.

Statistical analysis

Data was statistically processed by means of descriptive statistics, in order to determine mean value (X), standard deviation (SD) and standard error of the mean (SEM) for each group. In order to determine the existence of differences and also the level of significance of the differences, the variance inside the groups was analyzed. For variables that did not belong to the same population, a student t-test was employed to determine the statistically significant difference between the groups. The results of the preliminary analysis served to provide the basis for the choice of methods in further statistical analysis. In order to establish partial quantitative differences of the variables, the pondered mean difference, MD was used, as well as its significance. Another effective measure used in this research was the risk ratio, RR, with Yates-corrected p-value. It enabled a magnitude quantification effect on the source of infection on ETR in HCV patients. Values of $p<0.05$ were taken as significant.

3. RESULTS

Regardless of the genotype, sustained virological response (SVR) was achieved in 67 % of the patients. At the same time, 25% of the patients who were infected with an untested blood transfusion and 6% of the patients who had had wartime surgery did not achieve the end of treatment response (ETR).

Among the different risk groups, patients with the "wartime surgery" infection source responded better to therapy than blood transfusion ($p = 0.023$). Narcotics also

Effect of infection source on ETR	Patients with HCV		RR (95%CI)	P Yates-corrected chi-squared test
	Positive	Negative		
Unknown	23	88	3.419 (3.006, 3.888)	0.091
War related	2	31		
War related	2	31	0.242 (0.137, 0.430)	0.023
Blood transfusion	1	3		
Narcotics	2	24	0.308 (0.173, 0.548)	0.049
Blood transfusion	1	3		

Table 1. Effect of infection source on ETR. Interpretation of RR: the probability that patients with an unknown source of infection will not respond to therapy is 3.419 times greater than in patients with war related source of infection.. According to the p-value, results for infection source War related vs. Blood transfusion and Narcotics vs. Blood transfusion are statistically significant.

responded much better to therapy than blood transfusion at the end of therapy (p = 0/049) (Table 1). In regards to fibrosis stage (blood transfusion as the infection source implies larger fibrosis stage than in blood donors; g = 1/177; s^2 = 0.577) (Table 2). A large positive difference was also found in the necroinflammatory activity for blood transfusion-infected patients compared to blood donors (blood transfusion and narcotics as the infection source implies significantly larger necroinflammatory activity than in blood donors; g = 1.456; s^2 = 0.618) (Table 3).

We also analyzed if the source of infection (defined as narcotics, war-related, other or unknown) was related to HCV genotype (1a, 1b and 3), age (grouped 1945-1965 versus others) or gender, deploying Chi-square independence test for contingency tables.

In order to obtain relevant results, we selected only the variables with sufficient contingents. It turns out that the source of infection and HCV genotype is dependent (alternative hypothesis accepted with p-value of 0.004), as well as the source of infection and age (alternative hypothesis accepted with p-value<0.0001). When exploring the relationship between the source of infection and gender, the source was additionally grouped as narcotics, other, unknown, and again we detected a dependency, with alternative hypothesis accepted with p-value of 0.0017.(8)

		Blood Donor	Blood Transfusion	Hemodialysis	Narcotics	Sexual	Unknown
Blood Transfusion	g	1.177					
	s2	0.577					
Hemodialysis	g	0.183	-1.043				
	s2	0.502	0.379				
Narcotics	g	0.900	-0.674	0.690			
	s2	0.375	0.203	0.203			
Sexual	g	0.710	-0.885	0.172	-0.527		
	s2	0.709	0.544	0.502	0.367		
Unknown	g	0.660	-0.506	0.530	0.022	0.399	
	s2	0.343	0.175	0.175	0.038	0.342	
War related	g	0.890	-0.897	0.637	-0.144	0.456	-0.125
	s2	0.371	0.204	0.199	0.058	0.364	0.036

Table 2. Effect of Infection source on fibrosis stage A large positive difference in the fibrosis stage for blood transfusion infected patients compared to blood donors (blood transfusion as infection source implies a higher fibrosis stage than in blood donors).

		Blood donor	Blood transfusion	Hemodialysis	Narcotics	Sexual	Unknown
Blood transfusion	g	1.456					
	s2	0.618					
Hemodialysis	g	-0.227	-1.396				
	s2	0.503	0.414				
Narcotics	g	1.028	-0.381	1.235			
	s2	0.378	0.199	0.217			
Sexual	g	0.177	-1.283	0.335	-0.883		
	s2	0.669	0.591	0.506	0.374		
Unknown	g	0.827	-0.321	1.049	-0.008	0.712	
	s2	0.344	0.175	0.178	0.038	0.343	
War related	g	0.654	-0.397	0.851	-0.119	0.548	-0.107
	s2	0.367	0.196	0.203	0.058	0.365	0.036

Table 3. Effect of infection source on necroinflammatory activity. A large positive difference in necroinflammatory activity for blood transfusion infected patients compared to blood donors (blood transfusion and narcotics as infection source implies significantly larger necroinflammatory activity than in blood donors).

4. DISCUSION

Blood transfusion was a major risk for acute hepatitis C in the past, with more than 10% of transfusion recipients acquiring the infection. (9). The screening of blood donors by donor history and elevated serum alanine aminotransferase (ALT) caused a reduction of non-A, non-B posttransfusion hepatitis, even before hepatitis C virus was identified.

Development of donor screening programme for anti-HCV antibodies in 1990., almost eliminated the risk of posttransfusion acute HCV infection (9). Those screening has decreased the risk of transfusion-associated HCV infection to less than 1 case in 103,000 transfused units (10,11,12,13). The use of the polymerase chain reaction (PCR) assay has reduced the risk of acquiring HCV from blood transfusions to 1 in 230,000 donations. (14).

In Bosnia and Herzegovina, due to the war, blood transfusion started to be tested for HCV as of 1995. This effectively demonstrates that for the entire duration of the war, injured persons received blood transfusions that were untested for HCV. According to the Institute for blood transfusions in Sarajevo, 100,000 persons received untested blood transfusions. Since we could not find any similar data in the available literature, we analyzed our patients, who were treated at the Clinic for Gastroenterohepatology for chronic hepatitis C, selecting them based on the source of infection. Among those patients who reported the source of infection as “war-related” presented the group of patients who had surgery due to war injury. This method of infection, as well as IV drug users, was the most frequently reported method of HCV infection. The next most frequently reported methods of infection were blood transfusions, blood donors and other risk groups, such as sexual and haemodyalisis.

Analyzing the serological parameters of the conducted antiviral therapy, SVR in all genotypes were quite high (67%), while end-of-treatment response (ETR) was the lowest in the group of patients infected with blood transfusions (25% did not achieve ETR) and in the group of patients with war injury 6% did not receive ETR.

Amongst the different risk groups of patients, it was evident that patients who were probably infected with untested blood transfusions responded less to therapy

in comparison with "war surgery" (p = 0.023) group or IV drug users (p = 0.049). The next parameter that we analyzed in our patients was the histological changes in liver biopsies specimens, in regards to fibrosis stage and necroinflammatory activity. The degree of the fibrosis stage was higher in blood transfusions in comparison to other risk groups, e.g. blood transfusion as the infection source implies a higher fibrosis stage than in blood donors. At the same time, a large positive difference was detected in necroinflammatory activity for blood transfusion infected patients compared to blood donors.

Blood transfusion and narcotics as an infection source implies a significantly larger necroinflammatory activity than in blood donors. Beside the investigated influences of the source of infection on the course of diseases and therapeutic response, we also investigated the possible relationship between HCV genotype/subtype and the mode of transmission, age and gender.

Seven genotypes and 67 subtypes of hepatitis C virus (HCV) have been proved thus far (15). The most frequent genotypes in our patients were: 1a in 34% of the patients,1b in 32%, genotype 3 in 14% and genotype 4 in 9%. In 11% of the cases it was not possible to detect the genotype. In addition to distinct geographic distributions and clinical disease features associated with different genotypes, several studies have shown that HCV genotype prevalence differs according to the transmission route. Genotypes 3a and 1a are highly represented among HCV-infected injection drug users and genotype 1b constitutes a high proportion of HCV infection cases among patients who received blood transfusions (16).

The results of our study are in accordance with the results of Pawlotsky et al., who also confirmed that the source of infection and the virus genotype are dependent (p = 0.004). In our group of patients, the most frequent genotype 3 was detected in IV drug users, and genotype 1, regardless the subtypes, was the most frequent in blood transfusions. By analysing the age of the investigated group, our patients are predominantly young, with 63% of the patients in the age bracket between 26 to 45 years.

The association of specific genotypes with the mode of transmission is related to age differences among the groups infected with different genotypes. For example, in Europe, patients with HCV genotype 1b infection are generally older than patients infected with HCV genotypes 1a and 3a (17) likely reflecting the changing epidemiology of HCV transmission over time, resulting from the implementation of blood screening practices. Likewise, genotype 1b is the most prevalent genotype among patients infected with HCV in Japan (18) and the infected population is generally older than in the United States (19). This corresponds with an earlier infectious epidemiology in Japan, with iatrogenic transmission as a predominant risk factor for HCV acquisition.

In contrary to that, iatrogenic transmision of other hepatotropic viruses was more frequent in hemodyalisis patients in Croatia (20). The results of our study presented also a dependency among the source of infection and age (p < 0.0001), showing that genotype 3 was mostly represent in younger population, in comparrison to genotype 1. Out of total amount, 70% of the patients from the investigated gruop are males, which is in accordance with gender distribution in other studies.

When exploring the relation between the source of infection and gender, the source of infection was additionally grouped as narcotics, other and unknown. Again, we detected a dependency (p = 0.0017), meaning that in the selected risk groups, the male gender was predominant.

5. CONCLUSIONS

Untested blood transfusion was a risk factor for more advanced liver disease in regards to necroinflammatory activity and the fibrosis stage. This source of infection was also a risk factor for patients who were less responsive to antiviral therapy. At the same time, IV drug users had more progress in necroinflammatory activity but a high therapeutic response to antiviral therapy.

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