Co-Infection of the Hepatitis C Virus With Other Blood-Borne and Hepatotropic Viruses Among Hemophilia Patients in Poland

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Abstract

Background: The prevalence of HCV infection in people with hemophilia is substantially higher than that in the general population (63% - 98%). Multiple transfusions and substitutive therapy have also been linked to a high risk of HBV and HIV transmission. However, the prevalence of other blood-borne viral infections in this population is less well known.

Objectives: This study aimed to assess the prevalence of co-infection with HBV and other blood-borne viruses in Polish HCV-infected hemophiliacs.

Methods: Seventy-one individuals, the majority of whom were male (94.36%), who had congenital bleeding disorders (60 had hemophilia A, five had hemophilia B, and six had other factor deficiencies) and HCV infection, which was defined as the presence of positive anti-HCV antibodies, were included in this study. The study group was divided into two subgroups according to the year in which blood donors were first tested for HBsAg in Poland. The serological markers were screened using commercially available enzyme immunoassays according to the manufacturer's instructions. The molecular tests were performed using real-time PCR technology with commercial assays according to the manufacturer's instructions.

Results: The spontaneous elimination rate of HCV RNA was 29.6%. The HCV genotype 1 was detected in 28 patients (65.1%), genotype 2 in one patient (2.3%), genotype 3 in 11 patients (25.6%), genotype 4 in two patients (4.7%), and a mixed infection with genotypes 1 and 4 was detected in one person (2.3%). Fifty-three patients (74.6%) were anti-HBc positive. Among the seven HBsAg(+) patients, three individuals were HBV-DNA positive. No occult hepatitis B was detected. In six HBsAg positive patients, the HCV RNA was positive, while one patient was also infected with HIV. The prevalence rate of past infection with HAV in the study group was 30.9%, with a tendency for a higher prevalence in older patients. The prevalence of CMV and EBV infection was high and similar to that seen in the general population. All the patients were HGV and HTLV-1 negative.

Conclusions: The diagnostics and management of infections with hepatotropic viruses, particularly HBV, are neglected in hemophilic patients. All patients with coagulation disorders and a history of exposure to non-inactivated blood products should be screened for blood-borne infections. The prevalence of other potentially blood-borne viral infections exhibited a pattern similar to that observed in the general population.

Keywords: Hemophilia A, Hemophilia B, Hepatitis C, Coinfection, Blood-Borne Pathogens

1. Background

Hemophilic patients are particularly vulnerable to blood-borne infections, among them hepatotropic viruses. The prevalence of HCV infection in this group is substantially higher than that seen in the general population, with different studies finding the prevalence to be 63% - 98% (1-5). This is due to the lack of effective virus inactivation procedures for blood products, as well as the lack of donor testing, prior to the 1990s. In addition, multiple transfusions and substitutive therapy have been linked to a high risk of HBV transmission and HIV transmission in some countries (2, 4, 6-8). However, the prevalence of other blood-borne viral infections in this population has been less frequently investigated (9, 10).

Currently, the risk of virus transmission during blood product transfusions is marginal due to several factors. First, the manufacturing process for blood products now includes purification and virus inactivation procedures.

Moreover, donor testing for HBV and HBV vaccinations is widely implemented in the majority of countries. Safe recombinant coagulation factor preparations are also now being used more frequently (11, 12).

The prevalence of hemophilia in Poland is approximately 1:12,300 inhabitants (13). The analysis presented in this study was part of a wider study assessing the staging of chronic liver disease in Polish HCV-infected hemophilic patients (HemC). An assessment of the prevalence of concomitant blood-borne infections, particularly HBV, was carried out, since this is an additional factor influencing the natural history of HCV infections.

2. Objectives

The aim of this study was to assess the prevalence of co-infection with HBV, other blood-borne viruses, and hepatotropic viruses in Polish HCV-infected hemophiliacs.

3. Methods

The study group consisted of 71 individuals, the majority of whom were male (67/71, 94.36%), with congenital blood coagulation disorders and HCV infection, which was defined as the presence of positive anti-HCV antibodies. The patients were recruited from several centers in Poland. They were randomly selected hemophiliacs who had responded positively to an invitation to be tested for the diagnosis of liver disease. The study group's characteristics are presented in Table 1. The study group was divided into two subgroups according to the year in which blood donors were first tested for HBsAg in Poland (i.e., 1972).

The study was conducted in accordance with the declaration of Helsinki for human research, and the protocol was accepted by the local ethics committee of Wroclaw Medical University (opinion number: KB871/2012). All the subjects signed informed consent forms prior to enrolling in the study.

The clinical data collected included the type of coagulation disorder in patients with hemophilia, the severity based on the clotting factor level (14), the types of blood products used in the treatment of hemophilia, additional factors relevant to the transmission of blood-borne viral infections (e.g., intravenous drug use, invasive medical procedures), and the history of HBV vaccinations.

3.1. Serological Tests

The serological testing included: anti-HCV (Monolisa Anti-HCV Plus version 2, Bio-Rad), HIV Ag/Ab (Genscreen Ultra HIV Ag-Ab, Bio-Rad), HBsAg (Murex HBsAg version 3,

Table 1. Demographic and Virologic Characteristics of the Study Group[a]

Characteristics	Results
Mean age	40.4 ± 12.5
Range	24 - 73
Study subgroups	
A (before 1972) (n = 29)	53.06 ± 9.1
B (1972 - 1990) (n = 42)	31.86 ± 4.6
Sex	
Male	67 (94.4)
Female	4 (5.6)
Type of congenital blood coagulation disorder	
Hemophilia A	60 (84.5)
Hemophilia B	5 (7)
Other[b]	6 (8.5)
HCV status	
HCV-RNA positive	50 (70.4)
HCV-RNA negative	21 (29.6)
HCV genotypes	
Genotype 1	28 (65.1)
Genotype 2	2 (2.3)
Genotype 3	11 (25.6)
Genotype 4	2 (4.7)
Mixed 1/4	1 (2.3)
HCV viral load, IU/mL[c]	
$< 8 \times 10^5$	11 (37.93)
$8 \times 10^5 - 5 \times 10^6$	9 (31.03)
$> 5 \times 10^6$	9 (31.03)
Type of blood product transfused	
Whole blood	23 (32.39)
Human plasma	54 (76.05)
Cryoprecipitate	64 (90.14)
Plasma-derived clotting factors	69 (97.18)
Recombinant clotting factors	54 (76.05)
Additional factors relevant to blood-borne pathogens infection	
IVDU	0
History of surgical procedures	46 (64.8)

[a]Values are expressed as No. (%) or mean $\pm$ SD.
[b]Von Willebrand disease, factor XI deficiency, factor V deficiency.
[c]Quantitative viral load testing was performed on 29 (58%) patients with detectable HCV RNA.

Murex Biotech Limited), anti-HBc (ETI-AB-Corek Plus, DiaSorin S.p.A.), anti-HBe (ETI-AB-EBK Plus, DiaSorin S.p.A.),

anti-HBs (Monolisa Anti-UBs Plus, Bio-Rad), anti-EBV IgG (Epstein-Barr Virus (VCA) IgG Elisa, NovaTec Immunodiagnostica), anti-CMV IgG (Platelia CMV IgG, Bio-Rad), anti-HGV (AccuDiagTM HGV Ab ELISA, Diagnostic Automatation, INC.), anti-HTLV I + II (Murex HTLV I + II, DiaSorin S.p.A), and anti-HAV IgG (ETI-AB-HAVK Plus Anti-HAV, DiaSorin S.p.A.).

3.2. Molecular Tests

The HCV RNA was assessed quantitatively using a real-time PCR technique (Real-TM Quant DX, Sacace Biotechnologies) with a Rotor-Gene 3000 analyzer (Corbett Research). The analytical sensitivity (limit of detection (LOD)) of the assay was 13 IU/mL. The linear range of the measurement was 13 - 108 IU/mL. In relevant cases, the HCV genotype was determined using a HCV Genotype 2.0 Assay (LiPA) (Siemens Healthcare Diagnostics). The HBV DNA was assessed quantitatively using a real-time PCR technique (GeneProof Hepatitis B Virus (HBV) PCR kit, GeneProof) with a Rotor-Gene 3000 analyzer (Corbett Research). The analytical sensitivity (limit of detection (LOD)) of the assay was 26 IU/mL.

3.3. Statistical Analysis

For the statistical analysis in this study, the chi-square test was used. A value of $P \leq 0.05$ was considered to be statistically significant. All calculations were performed using Statistica 64 version 10 for Windows (StatSoft Inc., Tulsa, OK, USA).

4. Results

All the individuals in the study group had been exposed to non-virus-inactivated blood products. The majority of them were treated with plasma-derived clotting factors and cryoprecipitate (97.18% and 90.14%, respectively). Moreover, 46 patients (64.8%) reported a history of surgical interventions, 22 individuals in the older group (75.9%) and 24 (57.1%) in the younger group, although the difference was not statistically significant (P = 0.1).

Positive HCV RNA was detected in 70.4% of the study group. A higher, albeit not statistically significant, percentage of spontaneous HCV RNA clearance was observed in the younger group when compared to the patients born prior to 1972 (38.1% vs 17.3%, P = 0.058).

HCV genotype results were available for 43 subjects. The prevalence of the HCV genotypes in the study group is presented in Table 1.

4.1. HBV Infection and HBV/HCV co-Infection Analysis

Among the 71 patients included in the study group, 53 (74.6%) patients were found to be anti-HBc positive. The HBV serological status data are presented in Table 2. Among the seven HBsAg(+) patients, only three individuals were HBV-DNA positive. Further, no patients were undergoing treatment with antivirals. No occult hepatitis B was detected (defined as detectable HBV DNA in serum with negative HBsAg). Twenty-six individuals from the study group had been vaccinated against HBV during their lifetime. No patients had been vaccinated as a newborn and accurate data concerning the age of vaccination were not available. Fifty percent of the vaccinated patients (n = 13) had never been infected with HBV, although 11 cases were anti-HBc positive and two of them replicated HBV (HBsAg positive). The HCV RNA was positive in six HBsAg positive patients, and one of those patients was also infected with HIV. The replication of both HBV and HCV was detected in two individuals. Table 3 presents the patients' HBV infection and HCV replication status according to their year of birth.

Table 2. The HBV Serological Status of the Study Group

HBV Infection Markers Pattern	Status of HBV Infection	No. (%)
anti-HBc(+), **HBsAg** (+), **anti-HBs**(-)	Active infection	7 (9.9)
anti-HBc(+), **HBsAg** (-), **anti-HBs**(+)	Past/occult infection	42 (59.2)
anti-HBc(+), **HBsAg** (-), **anti-HBs**(-)	Past/occult infection or falsely positive anti-HBc[a]	4 (5.6)
anti-HBc(-), **HBsAg** (-), **anti-HBs**(+)[b]	Immune post-vaccination	13 (18.3)
anti-HBc(-), **HBsAg** (-), **anti-HBs**(-)	Susceptible to infection	5 (7.0)

[a]A total anti-HBc enzyme immunoassay for the qualitative determination of the total number of antibodies to the hepatitis B core antigen (anti-HBc) in human serum/plasma was performed. The diagnostic specificity of the assay was 99.83% and the diagnostic sensitivity was 100%.
[b]Post-vaccination group.

4.2. Markers of Selected Blood-Borne and/or Hepatotropic Viruses

In the four subgroups established according to the patients' year of birth, the rates of anti-VCA EBV IgG, anti-CMV IgG, and anti-HAV IgG varied. In the oldest subgroup (i.e., those born before 1960), all the patients exhibited positive antibodies against CMV and EBV. The serology test results are summarized in Table 4.

Table 3. Year of Birth and HBV Infection and/or HCV Replication Status[a]

	Group A (Born Before 1972)	Group B (Born Between 1972 and 1990)	P Value
anti-HBc(+)	27 (93.1)	26 (61.9)	0.003
anti-HBc(+), HBsAg(-), anti-HBs(+)	20 (69.0)	22 (52.4)	0.16
anti-HBc(+), HBsAg(-), anti-HBs(-)	2 (6.9)	2 (4.8)	0.7
anti-HBc(+), HBsAg(+), anti-HBs(-)	5 (17.2)	2 (4.8)	0.083
anti-HBc(-), HBsAg(-), anti-HBs(+)	1 (3.4)	12 (28.6)	0.007
HCV-RNA(+)	24 (82.7)	26 (61.9)	0.058

[a]Values are expressed as No. (%).

5. Discussion

The health consequences of chronic hepatitis C are significant, and they include liver cirrhosis and hepatocellular carcinoma. Co-infection with other hepatotropic viruses, particularly HBV, may adversely modify the course of chronic liver disease and accelerate the progression of liver fibrosis. Fortunately, two significant developments have contributed to the diminished risk of blood-borne virus transmission in multi-transfused patients, including hemophiliacs. Since 1972, blood donors have been tested for HBsAg, while tests for anti-HCV have been performed and inactivation procedures for blood products introduced since 1991. Prior to these developments, the HCV and HBV infection rates in hemophilic patients in Poland were high (95% of those who were anti-HCV positive, 8.7% of HBsAg, and 68% of anti-HBc positive) (2), whereas the rates observed in the general population were significantly lower (anti-HCV was 0.6% - 2.1% and HBsAg was about 1%) (15-19). Our results were comparable with the rates observed among hemophiliacs in the USA and Western European countries (3, 4, 10, 20).

Iran is considered to be a country with a low frequency of HCV infection (21). Viral-inactivated factor concentrates have been available since 1985 and recombinant products since 1992 (22). In 1997, the compulsory screening of blood donors was introduced. However, the prevalence of HCV among hemophilia patients is very high, fluctuating from 13.3% to 80.5%. Yet, the prevalence is significantly lower in southern Iran than in northern and central Iran. The overall prevalence of hepatitis C among hemophiliacs in Iran is said to be 40.8% (23).

Among the blood products used for the treatment of hemophilia, the highest risk of viral infection transmission was found to be related to non-virus-inactivated products (i.e., fresh frozen plasma and cryoprecipitate), which were used in Poland until the early 1990s. After that time, safe, virus-inactivated lyophilized concentrates of clotting factors were introduced. The risk of infection was further minimized following the introduction of molecular tests for blood donors. In Poland, HCV RNA has been tested since 2002, while HBV DNA and HIV RNA have been tested since 2005 (2, 24, 25). All the individuals in our study group had been exposed to non-virus-inactivated blood products in the past. Cryoprecipitate was used in 90.14% of cases and human plasma in 76.05%. The route of HCV infection in this group is therefore highly likely to be transfusion related.

In our patients with positive anti-HCV, the spontaneous elimination rate of HCV RNA (29.6%) was similar to that found in other publications (2, 26, 27). The predominant HCV genotype was 1 (65.1%), which is the most common genotype in the general Polish population (28). In one case, the HCV genotype 2 was detected in a HIV-positive patient who received blood products imported from France.

The prevalence of HBV infection, here defined as positive HBsAg, among hemophiliacs is lower than that of HCV infection, with different studies finding the prevalence to be 7% - 12% (2, 10, 29-31). However, the rate of positive anti-HBc is much higher (2) (74.6% in our study group). A lower prevalence of active infection, here defined as positive HBsAg and/or HBV DNA, is commonly observed for the population infected with HBV after early childhood and in instances of co-infection.

In our study, only three out of seven HBsAg positive patients tested positive for HBV DNA in their serum samples. None of the HBsAg-positive patients was undergoing treatment with antivirals at the time. Inactive carriers represent the largest group of chronic HBV-infected patients. This condition is diagnosed according to the lack of HBeAg and the presence of anti-HBe, undetectable or low levels of HBV DNA in PCR-based tests, normal ALT levels, and minimal or no necroinflammation, minor fibrosis, or even normal histology on biopsy (32, 33).

In patients born after 1972, the anti-HBc rate is significantly lower than in older patients (61.9% vs 93.1%), although it is still high. The introduction of blood donor testing for HBsAg did not eliminate the risk of HBV transmission, which is probably related to other transmission routes, including nosocomial infections. In our study group, 67.6% of subjects had a history of surgical procedures. An epidemic of nosocomial HBV infections appeared in Poland during the 1970s and 1980s (15). A significant improvement in the epidemiological situation was observed following the improvement of nosocomial infec-

Table 4. Serological Markers of Other Viruses According to Patients' Year of birth

	Total, %	Born Before 1960; (n = 14)	Born 1961 - 70; (n = 14)	Born 1971 - 80; (n = 23)	Born After 1980; (n = 20)	P Value (χ^2)
anti-VCA EBV IgG	68 (95.7)	14 (100)	14 (100)	22 (95.6)	18 (90)	0.98
anti-CMV IgG	59 (71.0)	14 (100)	13 (92.8)	16 (69.6)	11 (55)	0.14
anti-HAV IgG	22 (30.9)	11 (78.6)	5 (35.7)	2 (8.7)	4 (20)	< 0.001
HIV Ag/Ab	1		1 (100)			
anti-HTLV I/II total	0					
anti-HGV IgG	0					

tion control standards and the introduction of obligatory vaccinations in neonates (from 1993 to 1996). All the subjects in the study group were born before that time. In addition, only a minority of the patients (26 persons) were vaccinated against HBV, and our study revealed that half of this group had been infected prior to vaccinations being accompanied by proper testing for HBsAg and anti-HBc. A significantly higher successful vaccination rate was observed in the younger patients.

The prevalence rate of past infection with HAV in our patients was 30.9%, which is comparable to that in the general population, with a tendency for a higher prevalence in older patients (34). A comparative seroepidemiological study carried out among the general population proved that the overall anti-HAV prevalence was 35.4% (ranging from 8.1% to 72.2%). In 1998/99, the prevalence was 30.6% (ranging from 11.8% to 75.8%), while in 1990 it was 58.4% (ranging from 10.4% to 93.8%) (35). The risk of transfusion-related infection with HAV is minimal, so the most likely route of HAV transmission is the fecal-oral route. The HAV infection rate in Poland was high until the early 1990s when sanitary conditions were substantially improved, which led to a significant drop in infection rates (36). The higher rate of positive anti-HAV IgG in the youngest patients (those born after 1980) is at least in part due to the introduction of HAV vaccinations; however, the exact number of vaccinated patients was impossible to assess due to a lack of medical documentation.

Although HAV transmission is rarely blood-borne and hemophilic patients are at no greater risk of this infection than the general population, in those already chronically infected with HBV and/or HCV, an additional acute HAV infection may have additional adverse effects on chronic liver disease (37). It is therefore reasonable to recommend routine HAV vaccinations for this population in particular.

Another blood-borne infection (i.e., HIV) accelerates fibrosis progression in chronic hepatitis C, but in the study group analyses only one patient was co-infected with HIV. HIV infection does not represent a significant problem in the Polish population of hemophilic patients due to the unavailability of blood products acquired from donors not tested for HIV in the 1980s, which was an important source of HIV infection in Western Europe and Northern America in those years (2, 8, 38).

The assessment of the prevalence of infection with other potentially blood-borne viruses, namely CMV and EBV, revealed a very high rate of EBV infection in our study group (close to 100%), irrespective of age. The prevalence of CMV infection was high, and it increased with age. This pattern was similar to that found in the general population. However, the epidemiological data are discordant. McVerry et al. proved that, even in the 1970s, the risk of exposure to these infections in patients receiving blood products was not increased (39). Enck et al. demonstrated that the risk of CMV infection was significantly higher in patients with severe hemophilia and a history of multiple blood products transfusions (40). The lack of detail concerning the transfused blood products and their number did not allow for the assessment of the transfusion-related risk of infection with CMV and EBV in our study group. In addition, rather than being limited to the blood-borne route, the transmission routes of these infections are various. Other transmission modes, which are typical for the general population, may play a more significant role in the epidemiology of EBV and CMV in hemophilic patients than the transfusion of blood products.

The prevalence of HGV infection in the Polish population appears to be low. In the only prior Polish study, HGV DNA was detected in only a small percentage (3.2%) of blood donors (25). In our study group, all the patients were HGV-negative, and no cases of HTLV-1 infection were detected. This observation is compatible with European data that shows the infection rate in the general population to be below 0.1% (41).

The most important limitations of our study include the lack of detailed information concerning the exact number of transfusions and type of blood products, for example, the patient diaries did not allow for the assess-

ment of the exact exposure to blood-borne infections in the study group. We could not exclude the passive transfer of antibodies through the blood or blood products, although the analyzed individuals did not receive any transfusions except for recombinant clotting factors shortly before enrolling in the study.

The analysis presented in this study revealed that anti-HCV-positive hemophilic patients, particularly those born prior to 1991, are the group at highest risk of HBV infection through transfusions. However, the diagnostics and management of infection with hepatotropic viruses are neglected in this population, and there is a lack of appropriate screening and vaccinations against HBV and HAV. The consequences of co-infection with other hepatotropic viruses, particularly HBV, may have a clinically significant impact, since such viruses accelerate the progression of liver fibrosis and represent an important risk factor for hepatocellular carcinoma. All patients with coagulation disorders and a history of exposure to non-inactivated blood products should therefore be screened for blood-borne infections. Those patients from populations not covered by obligatory neonate HBV vaccinations should be vaccinated. In the younger population, standard HAV vaccinations are also reasonable.

Acknowledgments

Footnotes

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