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BLOOD DONOR SCREENING FOR BLOOD BORN VIRUSES IN POLAND

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ABSTRACT

Blood donor screening of viral markers in Poland is based on serologic testing for anti-HCV, HBsAg, anti-HIV1/2 (chemiluminescence tests) and on nucleic acid testing (NAT) for RNA HCV, RNA HIV-1 and DNA HBV performed in minipools of 6 with real-time PCR (MPX 2.0 test on cobas s201) or with TMA in individual donations (Ultrio Plus or Ultrio Elite). Donors of plasma for anti-D and anti-HBs production are tested for parvovirus B19 DNA. Before implementation tests and equipment are evaluated at the Institute of Hematology and Transfusion Medicine (IHTM).

The last 20 years witnessed a decreasing trend for HBsAg in both first time and repeat donors (1% - 0.3% and 0.1% - 0.02% respectively). Prevalence of anti-HCV repeat reactive results was stable and oscillated around 0.8% for first time donors and 0.2% for repeat donors. Elevated prevalence of seropositive HIV infected donors was recently observed (7.5-9 cases/100,000 donors). Since respective molecular markers implementation HCV RNA was detected on average in 1/119,235 seronegative donations, HIV RNA in 1/783,821 and HBV DNA in 1/61,047. HBV NAT yields were mostly occult hepatitis B (1/80,248); window period cases were less frequent (1/255,146). The efficiency of HBV DNA detection depends on the sensitivity of the HBV DNA screening system.

Key words: *hepatitis B virus, hepatitis C virus, human immunodeficiency virus, parvovirus B19, blood donor screening, NAT*

ABBREVIATIONS:

B19V – parvovirus B19, BTC – Blood Transfusion Center, IDT – individual donation testing, IHTM – Institute of Haematology and Transfusion Medicine, LOD – limit of detection, MP – minipool; MP6, MP24, MP48 – minipool from 6, 24 or 48 donations; NAT – nucleic acid testing, OBI – occult hepatitis B, RR – repeat reactive, ssDNA – single stranded DNA, TMA – transcription mediated amplification, WP – window period.

In the recent years transfusion safety has undergone significant improvement. The progress is mainly related to implementation of nucleic acid testing (NAT). The first section of the article briefly describes the Polish experience in donor screening for blood born viruses with special focus on advancement in NAT technology, which is considered the key factor in blood transfusion safety. The second section of the article presents results of blood born virus testing in Poland.

METHODOLOGY OF BLOOD DONOR SCREENING FOR BLOOD BORN VIRAL INFECTIONS IN POLAND

Prevention of blood born virus transmission is based on specific serological and molecular viral marker testing. Up to 2012 all blood donors were tested for alanine transaminaze (ALT) while now such tests are no longer performed as molecular markers for HBV, HCV and HIV are screened. Serological testing for anti-HCV, anti-HIV -1/2 and HBsAg is now performed with fully automated systems based on chemiluminescence - Abbott Architect (Abbott, USA) and Vidas (Ortho, USA). Poland was one of the first countries to introduce NAT for blood donor screening. In 1999 molecular biology was implemented for HCV RNA screening in plasma for fractionation and for all blood donors in 2002 (1). HIV RNA testing was initiated in 2003 in blood transfusion centers which had implemented HCV screening with HCV/HIV1 Procleix assay (Chiron, United States).

This first transcription mediated assay (TMA) based test was a duplex assay capable of detecting not only HCV RNA but also HIV RNA (2). In 2005, both HIV RNA and HBV DNA were listed as obligatory tests for viral markers in Poland (3). Right from the start NAT screening with PCR was performed in mini-pools (MP) and TMA was applied for individual donations (3). The mini-pools initially consisted of 48 donations and the number was reduced to 24 in 2005. PCR testing was initially performed with Cobas Amplicor and later with Cobas Ampliscreen (Roche, Germany). The nucleic acid isolation step was common for the tested markers in both tests and it was performed manually while amplification and amplicon detection steps with hybridization labeled probes were performed separately for different viruses using Cobas Amplicor Analyser (Roche, Germany). MP6 testing of minipools created by combining plasma from 6 donations (MP6) was implemented in 2007 together with real-time technique (Cobas Taqscreen MPX v.1 assay on MPX). Cobas Taqsceen MPX was a significant improvement which enabled isolation on a fully automated pippeting station (Microlab STAR, Hamilton, Switzerland). It was a truly multiplex assay, where amplification and amplicon detection were performed for several viruses simultaneously in a single tube with taq-man probes on Cobas s201 system. The evaluation performed at IHTM in 2012 demonstrated high sensitivity, good performance and increased operational efficiency of the subsequent version of the assay (MPX v2 test) as compared to the previous one. There was no longer the need for viral discriminatory testing which was the most significant difference between the two versions of the assay. Characteristic for the new version was higher analytical sensitivity. The 95% limits of detection (95% confidential limits) were evaluated for 2.87 IU/mL (1.69-6.68), 13.69 IU/mL (7.65-45.30) and 28.83 IU/mL (17.57-86.91) for HBV, HCV and HIV-1 respectively. In this evaluation the Polish NAT yield cases were tested as well. The panel of such samples included most prevalent HBV (A, D and H) and HCV genotypes (1b, 3a and 4) and HIV-1 subtype B. The viral loads in individual donations ranged from 6.92 to $3.26x10^3$ IU/mL for HBV, from $3.2x10^4$ to $1.61x10^6$ IU/mL for HCV and from $1.17x10^2$ to $5.61x10^5$ IU/mL for HIV. Results were positive for all tested samples both neat and in 6-fold dilution. Invalid and false reactive pool rates were 0.05% and 0.127% respectively (4).

In Poland the first triplex assay applying TMA technology was Procleix Ultrio assay (Chiron, United States), which was launched in 2005 for simultaneous screening for HCV, HBV and HIV-1 (2). In 2013 Procleix Ultrio Elite assay (Grifols, Spain) was implemented, which is equivalent to the previous assay version (Ultrio Plus assay) with the difference in primers and probes for HIV-2 detection. Unlike the Ultrio assay - both Ultrio Plus and Ultrio Elite assays use an additional reagent with concentrated lithium hydroxide which enhances disruption of HBV particles and release of ssDNA for the target capture probe. All Ultrio versions target two regions of HIV-1 genome. The Ultrio Elite assay runs on Procleix Panther system, while the Ultrio and Ultrio Plus assays run on the Tigris instrument. In recent study the 50 and 95% limits of detection (LODs) for HBV using Ultrio Plus were 0.8 (0.6-1.0) and 4.6 (3.2-7.2) IU/mL, respectively, 2.4 (1.4-4.8)-fold more sensitive than Ultrio. The improvement factors on analytical sensitivity panels of HBV genotypes A to G ranged from 1.3 to 7.3 and 50% LODs (95% confidence interval) were increased from 12.5 (10-15) to 3.8 (3.2-4.4) copies/mL. The improvement in analytical sensitivity translated into higher clinical sensitivity (5). In 10 thousand first time donors screened in 3 Regional Blood Transfusion Centers one Ultrio Plus HBV genotype D yield sample was found which had been missed by the Ultrio assay. According to estimates it was detected in Ultrio Plus with nine-fold higher sensitivity. The specificity of individual donation nucleic acid test (ID-NAT) reached 99.41%. 100% specificity was calculated using a repeat test algorithm (comparable to the algorithm used in serology screening) (5). In a separate analysis the results for two consecutive 18-month ID-NAT screening periods using the Ultrio and Ultrio Plus assay were compared. The significantly higher analytical sensitivity of the Ultrio Plus assay was translated into a 1.9 fold higher HBV-NAT yield in the Polish donor population, despite the fact that the HBsAg prevalence had decreased 1.5 fold (6).

Further studies on TMA based assays demonstrated that LODs for HIV-1 and HCV were comparable for all TMA assay versions and addition of HIV-2 oligonuceotides to the Ultrio Elite assay does not affect the analytical sensitivity for other viruses, regardless of the genotype (7).

SCREENING TESTING AND QUALITY CONTROL ORGANIZATION

Nowadays donations from all over the country are screened in 21 Blood Transfusion Centers (BTCs) with serologic assays and NAT is performed on side or are commissioned to lab in another BTC. At the end of 2014 five labs tested individual donations with TMA and 12 performed NAT with real-time PCR in minipools of 6. All repeat reactive (RR) samples are sent to reference lab for confirmatory testing. Subsequent procedure for both serology and NAT screening repeat reactive samples includes testing of individual donations with methods alternative to screening TMA or PCR and confirmatory (HIV Western blot) or suplemental (HCV

Western blot, anti-HBc, anti-HBs) serological marker analysis. HBsAg RR donations are tested for HBV DNA and/or with neutralization test.

Since implementation of NAT in Poland the quality control system has been largely extended to include: validation of every new assay prior to implementation (performed at IHTM); evaluation and revalidation of all procedures used at BTC; laboratory audits (at least every two years); attendance in external quality programs (VQC Amsterdam, QCMD Glasgow, Labquality Helsinki); daily external quality control (EDC-NET); analysis of results for internal control (IC), analysis of false positive and false negative results; automatic equipment and system validations, control of storage and transportation conditions for samples and reagents (e.g. temperature monitoring); qualification of reagents and disposables and batch release.

Every day the same control samples are tested in all donation-testing laboratories with the specific screening method. Results are then introduced by on-line software and can be compared using standard deviation, mean value and other more sophisticated statistical methods.

In Poland, Parvovirus B19 (B19V) DNA testing is obligatory for donors who donate plasma for anti-D and anti-HBs production as well as cells used for immunization. B19V polymorphism is significant for the clinical sensitivity of screening assays (8, 9). It is worth to stress that genotype 2 has been identified in Poland (10) and in the neighboring countries (11, 12). It is often undetected or undetected by home made and commercial assays. Special attention has therefore been paid to evaluation of clinical sensitivity for all known virus genotypes. According to our findings the DPX test (Roche, Germany) widely used in Poland allows to accurately identify donations infected with B19V genotypes 1-3 and in consequence to prevent effectively contamination of plasma production pools with B19V DNA titers exceeding the level (>10^4 IU/mL) as recommended by the European Pharmacopeia (13). As false negative or invalid results of Parvovirus B19 DNA tests performed with real-time PCR in high viraemic samples were reported, fluorescence diagram analysis and algorithm of positive result confirmation with the purpose of excluding such phenomenon was proposed (14). Handling of such cases is monitored through regular quality control examinations performed on sample panels designed and prepared by IHTM.

RESULTS OF VIRAL SCREENING

For two decades, a decreasing tendency for HBsAg has been observed both for - first time and repeat Polish blood donors. In the former group the prevalence

Table I. NAT yields frequency in Poland: a/HBV (2005 - 2013); b/HCV (1999 - 2013) and c/HIV (2003 - 2013).

A. Method	**IDT** TMA	**MP6** R-t PCR	**MP8** TMA	**MP24** PCR	In total
Sensitivity (95% LOD, IU/mL)	U: 12 UP, UE: 4	MPXv1: 22 MPXv2: 14	UP: 24	Ampliscreen: 360	
No of donations tested	3,426,077	4,548,911	421,447	1,554,270	**9,950,705**
WP	23 1:148,960	14 1:324,922	0	2 1:777,135	**39** **1:255,146**
OBI	59 1:58,069	59 1:77,100	2 1:210,724	4 1:388,568	**124** **1:80,248**
HBV DNA	**82** **1 :41,781**	**73** **1 :62,314**	**2** **1 :210,724**	**6** **1:259,045**	**163** **1:61,047**

B. Method	**IDT** TMA	**MP6** R-t PCR	**MP8** TMA	**MP24** PCR	**MP48** PCR	Total
Sensitivity (95% LOD, IU/mL)	Prociex : 6.2 U, UP, UE: 3.0	MPXv1: 22 MPXv2: 14	UP: 24.8	Ampliescreen:504	Amplicor: 2400	
No of donations tested	3,665,048	4,548,911	421,447	1,554,270	3,403,142	13,592,818
HCV RNA frequency	**22** **1:166,593**	**27** **1:168,478**	**3** **1:140,482**	**11** **1:141,297**	**51** **1:66,728**	**114** **1:119,235**

C. Method	**IDT** TMA	**MP6** R-t PCR	**MP8** TMA	**MP24** PCR	Total
Sensitivity (95% LOD, IU/mL)	Procleix: 44.5 U: 27.6 UP,UE: 28.6	MPX1: 294 MPX2: 277	UP: 289	Ampliscreen: 2400	
No of donations tested	3,665,048	4,548,911	421,447	1,554,270	10,189,676
HIV RNA Frequency	**7** **1:523,578**	**4** **1:1,137,228**	**2** **1:210,723**	**0**	**13** **1:783,821**

Abbreviations of the tests names: U – Ultrio, UP – Ultrio Plus, UE – Ultrio Elite

approximated 1% in 1994, and was decreasing in latter years reaching about 0.3% in 2013. The improvement was mainly due to the vaccination program launched in the 80-ties (15). In 2013 a drop from 0.6% to 0.3% was reported which is most likely related to the fact that the fully vaccinated young people started to join the population of blood donors. It is noteworthy that blood donation in Poland is mostly dependant on young people. In the 90-ties HBsAg incidence slightly exceeded 0.1% of repeat blood donors, whereas now in this group of donors we register no more than several infection cases per year.

HBV DNA is relatively frequent in blood donors (Table I). Most cases are occult hepatitis B (OBI) – 1/80,248 donations. This is the echo of one of the highest HBV incidence rates in Europe (45 per 100,000) observed in Poland in the mid-80thies (15). Window period (WP) cases are less frequent - 1/255,146. As stated previously the effectiveness of screening HBV DNA NAT depends on the sensitivity of the system used (16). Present analysis on a larger number of donations confirmed the frequency to be significantly ($p<0.05$, several fold) higher for both WP and OBI as compared to MP of 24 donations with IDT and MP of 6. This can be explained by a relatively slow doubling time for HBV resulting relatively long window period. Infection detection in this phase greatly depends on the sensitivity of the screening system (17).

It is worth noting that HBV genotype A2 (80%) is predominant in HBsAg positive donors in Poland whereas genotype D is in minority (20%) (18). However a different distribution of genotypes in OBI donors (60% genotype D, 35% genotype A, and occasional genotype H infection cases) was observed (19). Genotype D strains were significantly more substituted than genotype A2 strains potentially affecting the course of infection (18).

HCV epidemiology changes based on donors screening in Poland seems to be less optimistic as compared to HBV. Prevalence of repeat reactives for the period 1993 – 2013 oscillated around 0.8 and 0.2% for first time and repeat blood donors respectively and did not reflect significant improvement. HCV RNA is detected in one per 119,235 donations on average and there is no significant overall difference related to the higher or lower analytical sensitivity of the system used. The explanation lies in the shorter doubling time as compared to HBV and thus lower significance of the screening systems for NAT yields detection efficiency. During the first several years following implementation of HCV RNA screening there appeared records of an unexpectedly high frequency of genotype 4 and subtype 3a and low frequency of subtype 1b in window period (WP) donors as compared to anti-HCV-positive persons (1). Thirty six percent (36%) of HCV infected seronegative donors exhibited subtype 1b, whereas subtypes 3a and 4c/d were identified in 40% and 14 % respectively. The distribution of genotypes was different than in anti-HCV-positive donors and CHC patients with chronic hepatitis where the frequency of subtype 1b was significantly higher (75.7 and 85.3%, respectively). The differences in distribution of genotypes in early-phase and chronically-infected donors are most likely due to different natural history of HCV polymorphic forms and recent changes in HCV infection routes related to genotypes. Previously transfusion of infected donations and other hospital procedures were believed to be main infection routs of transmission. But our last statistical analysis of epidemiologic factors independently associated ($p<0.05$) with recent HCV infection revealed: accidental exposure to blood, tattooing, injection and/ or non-injection drugs, two and more sexual partners within 6 months before donation and sharing shaving razor/ toothbrush (20).

Since NAT implementation for HCV RNA up to the end of 2014 one transfusion transmitted hepatitis C case was described in Poland. It was reported in 2007 for a recipient of red blood cell concentrate from a regular blood donor who was HCV RNA negative in routine mini-pool (48 donations) screening (21).

Although Poland is a low endemic area for HIV, a slight elevation of HIV infection frequency has been observed since 2009 and the recent level is 7-9 per 100,000 donors. A similar rule of distribution in first time and repeat blood donors was observed for seropositive HIV cases as for anti-HCV and HBsAg. Prevalence of the markers was significantly higher in the former category of blood donors as compared to the latter. Nowadays however a similar frequency for anti-HIV confirmed donors is observed in both donor groups. This may be explained by the phenomenon of test seekers in the population of blood donors. In spite of comparing to other European countries good epidemiological situation relatively high number of HIV NAT yields have been registrated. Up to the end of 2014 thirteen (13) such cases have been identified in 10 million donations. In four cases genetical polymorphism was analyzed and subtype B was identified (22).

Acknowledgments

We thank our colleagues from the Institute of Hematology and Transfusion Medicine and from the Regional Blood Transfusion Centers for excellent cooperation in routine collection of the data. We appreciate Ms Krystyna Dudziak for support in manuscript preparation and Ms Natalia Parda for translation.

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Received: 09.01.2015
Accepted for publication: 11.05.2015

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