The results of nucleic acid testing in remunerated and non-remunerated blood donors in Lithuania

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Background. In Lithuania, governmentally covered remuneration for whole blood donations prevails. Donors may choose to accept or reject the remuneration. The purpose of this study was to compare the rate of nucleic acid testing (NAT) discriminatory-positive markers for human immunodeficiency virus-1 (HIV-1), hepatitis B virus (HBV) and hepatitis C virus (HCV) in seronegative, first-time and repeat, remunerated and non-remunerated donations at the National Blood Centre in Lithuania during the period from 2005 to 2010.

Materials and methods. All seronegative whole blood and blood component donations were individually analysed by NAT for HIV-1, HBV and HCV. Only discriminatory-positive NAT were classified. The prevalence of discriminatory-positive NAT per 100,000 donations in the donor groups and the odds ratios comparing the remunerated and non-remunerated donations were determined.

Results. Significant differences were observed for HBV NAT results: 47.42 and 26.29 per 100,000 remunerated first-time and repeat donations, respectively, compared to 10.6 and 3.58 per 100,000 non-remunerated first-time and repeat, seronegative donations, respectively. The differences were also significant for HCV NAT results: 47.42 and 51.99 for remunerated first-time and repeat donations, respectively, compared to 2.12 and 0 per 100,000 non-remunerated first-time and repeat, seronegative donations, respectively. No seronegative, discriminatory-positive NAT HIV case was found. The odds of discriminatory HBV and HCV NAT positive results were statistically significantly higher for both first-time and repeat remunerated donations compared to first-time and repeat non-remunerated donations.

Discussion. First-time and repeat remunerated seronegative donations were associated with a statistically significantly higher prevalence and odds for discriminatory-positive HBV and HCV NAT results compared to first-time and repeat non-remunerated donations at the National Blood Centre in Lithuania.

Keywords: donations, paid donations, infectious disease testing, nucleic acid testing

Introduction

The safety of blood and blood components continues to generate debates worldwide. The World Health Organization (WHO) states that safe health care with regards to preventing transmission of hepatitis B and C viruses (HBV and HCV) can be achieved by ensuring that blood supplies are safe by recruiting only voluntary, unpaid blood donors, introducing effective blood donor selection and screening all donated blood for markers of HBV and HCV infections with highly sensitive and specific assays, and by following basic, standardised procedures[1]. It is also emphasised that "the safest blood comes from unpaid donors who donate for altruistic reasons". The prevalence of human immunodeficiency virus (HIV) and hepatitis infections is lowest in this group of donors[2].

Lithuania is a country with a long history of remunerated whole blood and blood component donation and this form of donation prevailed during the period from 2005 to 2010. Table I shows the number and proportion of remunerated and non-remunerated whole blood and blood component donations in Lithuania as a whole and in the Lithuanian National Blood Centre between 2005 and 2010. Remunerated donors received compensation in money during this period. Starting from October 1, 2009, all whole blood and blood component donations in Lithuania undergo mandatory nucleic acid amplification testing (NAT) for HIV-1, HBV and HCV[3]. Although the same donor questionnaire and the same selection procedure prior to each whole blood and blood component donation are used, based on requirements of European Commission Directives 2002/98/EC and 2004/33/EC, remunerated donors are more likely to

Table I - The number and proportion of remunerated and non-remunerated whole blood and blood component donations in the National Blood Centre and in Lithuania, 2005-2010.

Year	National Blood Centre					Lithuania				
	Remunerated donations		Non-remunerated donations		All donations	Remunerated donations		Non-remunerated donations		All donations
	N.	%	N.	%	N.	N.	%	N.	%	N.
2005	40,360	82.86	8,348	17.14	48,708	75,965	84.17	14,283	15.83	90,248
2006	36,534	76.40	11,284	23.60	47,818	72,564	78.38	20,019	21.62	92,583
2007	35,417	63.68	20,198	36.32	55,615	62,633	67.87	29,651	32.13	92,284
2008	33,921	61.11	21,672	38.89	55,593	62,342	66.63	32,650	33.37	97,845
2009	31,261	57.29	23,309	42.71	54,570	58,956	65.70	32,353	34.30	94,326
2010	18,811	48.90	19,658	51.10	38,469	51,769	67.01	25,487	32.99	77,256

conceal risky behaviour and, therefore although it was not then mandatory, the National Blood Centre introduced routine NAT for HIV-1, HBV and HCV in individual donations in 2005.

The situation at the National Blood Centre, comprising the remunerated and non-remunerated whole blood and blood component donations (not plasmapheresis) and the same methodologies for laboratory testing for both serological and NAT tests, provides the unique possibility to compare the results of NAT in seronegative remunerated and non-remunerated donations.

It is important to note that the structure of both the blood transfusion and blood collection system available in Lithuania affords the unique possibility to compare the risks among remunerated and non-remunerated donors. Firstly, there are two blood establishments: the public, non-profit blood establishment, the National Blood Centre, and a private, for-profit blood establishment, as well as two hospital-based blood centres. In almost all geographical territories of Lithuania the activities of blood establishments and hospital-based blood centres overlap, allowing the donors to choose freely in which institution they wish to donate blood. Secondly, the payment for whole blood and blood components is still organised and fully reimbursed by the government of Lithuania (not by the blood establishments or hospital-based blood centres), providing a universal payment of 40 litas (11.32 euros) throughout Lithuania. The blood or blood component donor can make the decision to take the money or not, just before donation at any blood establishment or hospital-based blood centre.

The purpose of this study was to evaluate the results of NAT in individual seronegative remunerated and non-remunerated donations. In detail, the objectives were to analyse the rate of HIV-1, HBV and HCV NAT discriminatory-positive markers per 100,000 remunerated and non-remunerated first-time and repeat donations, and to estimate the odds of HIV-1, HBV and HCV NAT-positive markers in seronegative remunerated donations in comparison with seronegative non-remunerated donations at the National Blood Centre in Lithuania during the period 2005-2010.

Materials and methods

This was a retrospective study of the results of NAT positive tests in seronegative remunerated and non-remunerated first-time and repeat donations at the National Blood Centre.

From 2005 to 2008, the following serological tests were used: HBsAg, anti-HCV and anti-HIV immunoassays, performed on an Abbott AxSym (Abbott, Wiesbaden, Germany) and syphilis passive haemagglutination test, Inno-TPHA immunoassay (Innogenetics NV, Gent, Belgium). Starting from 2008, all four serological tests (HBsAg, Anti-HCV, HIV Ag/Ab Combo, syphilis antibodies) were performed on the Abbott Architect system. All seronegative donations were tested by NAT, in individual samples, for HCV, HBV and HIV-1, using Procleix Ultrio reagents on the Procleix Tigris (Novartis, Emeryville, CA, USA) system, from 2005 to 2009, and Procleix Ultrio Plus reagents from 2010. Initially reactive NAT samples followed discriminatory testing for investigation of particular viral infections.

During the period from 2005 to 2010, a total of 300,773 whole blood and blood component donations made at the National Blood Centre were tested serologically for HBsAg, anti-HCV, anti-HIV1/2 and syphilis: 28,481 were first-time remunerated donations, 48,066 were first-time non-remunerated donations, 167,823 were repeat remunerated donations and 56,403 were repeat non-remunerated donations. Of the total 300,773 donations, 3,185 (1.06% of all donations) were initially serologically reactive: HBsAg 981 (0.33%), anti-HCV 1,610 (0.53%), anti-HIV1/2 31 (0.01%), and syphilis 563 (0.19%). These donations were excluded from the study. Thus, 297,588 seronegative whole blood and blood component donations were analysed for the purposes of this study. The majority of these donations were whole blood (n =297,291; 99.9%). From the

total 297,588 donations, 27,412 (9.21%) were from remunerated first-time donors, 167,309 (56.22%) from remunerated repeat donors, 47,130 (15.84%) from non-remunerated first-time donors and 55,737 (18.73%) from non-remunerated repeat donors.

A first-time donation is considered the donation of whole blood or a blood component by a person who has never previously given blood or blood components. A repeat donation is considered a whole blood or blood component donation from a person who has donated blood or components before[4] in the same National Blood Centre. It is important to note that donations positive for both serological tests and NAT as well as positive for NAT, but discriminatory negative donations were not analysed in this study.

Statistical analysis

The prevalence of NAT-positive markers for HIV-1, HBV and HCV was calculated per 100,000 donations. The number of discriminatory-positive donations was treated as the nominator, whereas the denominator was calculated as the sum of all NAT-negative donations and all discriminatory-positive donations for a particular infectious disease marker.

The odds ratio was estimated in order to compare the odds of HIV-1, HBV and HCV NAT-positive tests for remunerated and non-remunerated donations, during the period 2005-2010. An *ad/bc* cross-tabulation was used to estimate the odds ratio. Differences were considered statistically significant when the P-value was <0.05[5]. The data were analysed using Statistica software, version 9.0 (StatSoft Inc., Tulsa, OK, USA).

Results

Out of the 297,588 seronegative donations, 186 (0.062% of all seronegative donations) were initially reactive NAT-positive donations: 21 initially reactive, but not discriminated donations and 165 (0.05% of all seronegative donations) initially reactive and discriminated donations. Of the 165 NAT-positive donations, only eight were from non-remunerated donors (4.8% of all NAT positive donations): six were first-time and two were repeat donors. The 157 NAT-positive, seronegative donations from remunerated donors comprised 26 donations from first-time donors and 131 from repeat donors. Of the 165 NAT-positive donations, 101 were positive for HBV and the other 64 for positive for HCV. Of the 21 initially reactive, but not discriminated NAT-positive donations there were 17 repeat remunerated donations, one first-time remunerated donation, one first-time non-remunerated donation and two repeat non-remunerated donations.

Both first-time and repeat remunerated donations had a statistically significant higher prevalence per 100,000 donations of positive HBV NAT and HCV NAT results, as compared to non-remunerated first-time and repeat donations. The overall results of NAT among the seronegative remunerated and non-remunerated, first-time and repeat donations during 2005-2010 are presented in Table II.

Statistically significant higher odds were estimated for discriminatory-positive HBV NAT and HCV NAT results in seronegative first-time remunerated and repeat remunerated whole blood and blood component donations, compared with first-time non-remunerated and repeat non-remunerated donations. Table III summarises the odds ratios of NAT positive results for remunerated whole blood and platelet seronegative donations compared with non-remunerated ones at the National Blood Centre during 2005-2010.

Table II - The results of NAT of seronegative whole blood and platelet donations at the National Blood Centre, 2005-2010.

Donation type	N. of tested donations	Hepatitis B		Hepatitis C		HIV-1		Initially NAT reactive, not discriminated
		N. of NAT positive donations	Prevalence per 100,000 seronegative donations	N. of NAT positive donations	Prevalence per 100,000 seronegative donations	N. of NAT positive donations	Prevalence per 100,000 seronegative donations	
Remunerated, first-time	27,412	13	47.42*	13	47.42*	0	0.00	2
Non-remunerated, first-time	47,130	5	10.61	1	2.12	0	0.00	1
Remunerated, repeat	167,309	44	26.29**.***	87	51.99**	0	0.00	17
Non-remunerated, repeat	55,737	2	3.58	0	0.00	0	0.00	2
All	297,588	101	33.94	64	21.51	0	0.00	21

*P <0.001, compared to non-remunerated first-time donations during the same period; **P <0.001, compared to non-remunerated repeat donations during the same period; ***P <0.05, compared to the prevalence of HCV NAT in remunerated repeat donations during the same period.

Table III - The odds ratio of NAT positive results in seronegative remunerated whole blood and platelet donations compared to non-remunerated donations at the National Blood Centre, 2005-2010.

NAT positive marker	Donation type	Odds ratio*	95% CI	P value
Discriminatory positive HBV NAT	First-time	4.47	1.59-12.54	<0.05
	Repeat	7.33	1.77-30.24	<0.05
Discriminatory positive HCV NAT	First-time	22.35	2.92-170.95	<0.05
	Repeat	58.33	3.62-940.02	<0.001
Initially NAT reactive, not discriminated	First-time	3.44	0.31-37.92	>0.05
	Repeat	2.83	0.65-12.25	>0.05
All	First-time	6.88	3.0-15.76	<0.001
	Repeat	12.33	4.57-33.30	<0.001

*Odds ratio value, estimating the odds of NAT positive marker in seronegative remunerated donations compared to seronegative non-remunerated donations, 2005-2010.

Discussion

Analysis of the prevalence of NAT-positive markers per 100,000 seronegative donations at the National Blood Centre revealed statistically significant higher prevalences of HBV NAT and HCV NAT both for first-time and repeat remunerated donations compared to first-time and repeat non-remunerated donations in the period 2005-2010. Moreover, the odds of HBV and HCV NAT-positive results in seronegative first-time remunerated and repeat remunerated donations were statistically significantly higher than in first-time non-remunerated and repeat remunerated donations. These results confirm previous findings that the prevalence of infectious disease markers is higher in remunerated donations than in non-remunerated ones, in Lithuania[6,7]. The results showing that repeat remunerated donations had the highest prevalence of NAT-positive HBV and HCV markers per 100,000 seronegative donations and, contrariwise, that the repeat non-remunerated donations had the lowest prevalence of NAT-positive HBV and HCV markers per 100,000 seronegative donations fully support the statement of Van der Poel *et al*. who emphasised that "paid donors are more likely to donate blood during the window period", when blood-borne viruses may not be detectable in screening tests[8]. Thus, non-remunerated whole blood and blood component donors are safer than remunerated ones. Although Farrugia *et al*. stated that "purist arguments against compensated donation have little basis in evidence..."[9], the findings in Lithuania provide support for the opposite arguments, emphasising the evidence of higher risk for remunerated donations compared to non-remunerated ones from the same population and same geographical area and for the same donations (whole blood or platelets).

Comparing the data on all whole blood and blood component donations (remunerated and non-remunerated) with the data obtained in Eastern Europe countries for NAT-only positive donations since the introduction of NAT[10], Lithuania shows the highest prevalence of HBV (339.4 cases/million donations in Lithuania versus 118.57 cases/million donations in Greece) and HCV (215.1 cases/million donations in Lithuania versus 23.91 cases/million donations in Estonia), and the lowest prevalence of HIV-1 (0 cases/million donations in Lithuania versus 11.95 cases/million donations in Estonia and 4.26 cases/million donations in Greece). The situation is different when comparing non-remunerated donation (both first-time and repeat): the prevalence rates of HBV and HCV (68.04 cases/million donations and 9.72 cases/million donations, respectively) are lower than in other Eastern European countries. These results emphatically prove the importance of achieving only voluntary, non-remunerated whole blood and blood component donations in order to obtain the safest blood components.

As throughout the world, the results of NAT in remunerated and non-remunerated blood donors in Lithuania also indicate the remarkable contribution of NAT techniques to the safety of blood, since some of the viraemic donations would have been missed by only serological screening methods. Obviously, the introduction of NAT for HIV and especially for HBV and HCV has improved blood safety. The results of the study show that the safest blood and blood component donations are obtained from repeat non-remunerated donors. Therefore the recruitment and retention of the voluntary, non-remunerated donors should remain a major task for Lithuanian health policy makers. Similar studies in other countries also conclude on the importance of encouraging non-remunerated donors for the safety of blood and its components[11-13], and the positive impact of introducing NAT as a routine test for minimising the risk of transfusion-transmitted infections[10,14]. Overall, the findings of the analysis show that NAT in individual donor samples is essential in those areas in which remunerated donations prevail, since the rate of NAT-positive cases remains high in regular remunerated donors.

Although this analysis was based on data from Lithuania's largest blood establishment, which collects more than 50% of all the country's whole blood and blood component (platelets only) donations, ensuring that the same standard procedures were applied for both remunerated and non-remunerated donors, the study does have some limitations. The first issue concerns the standardised follow-up procedure for donors with positive NAT results, which was introduced a few years later than NAT of the individual donors' samples at the National Blood Centre. Secondly, after the introduction of the standardised follow-up procedure, only 11 (24.4%) of the 45 donors returned for additional testing. Therefore, the prevalence of NAT-positive markers per 100,000 seronegative donations and the odds ratio of NAT-positive results for seronegative donations have been calculated based only on the results of the discriminatory NAT-positive donation. Since the donors were not followed up, the high rate of HBV and HCV NAT positive results could be explained by some false-positive results.

Conclusions

Remunerated first time and repeat whole blood and platelet seronegative donations have a statistically significant higher prevalence of NAT-positive HBV and HCV markers compared with voluntary, non-remunerated first-time and repeat donations. There was a statistically significant difference in the prevalence of HCV NAT-positive markers compared to HBV NAT-positive markers in repeat remunerated donations. No seronegative, NAT HIV-1 positive marker was found. There were statistically significant higher odds ratios of NAT-positive HBV and HCV markers in seronegative, first-time remunerated and repeat remunerated donations than in seronegative first-time non-remunerated and repeat non-remunerated donations at the National Blood Centre during 2005-2010. In order to ensure blood safety, the recruitment and retention of voluntary, non-remunerated donors should be a major commitment for Lithuanian healthcare policy-makers.

The authors declare that they have no conflicts of interest relevant to the manuscript submitted.

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