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ASSESSING RISKS OF INFECTION WITH HERPES VIRUSES DURING TRANSFUSION OF DONOR BLOOD AND ITS COMPONENTS

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A primary task transfusion medicine should solve is to provide infection safety of donor blood and its components.

Our research goal was to assess potential risks of a recipient being infected with herpes viruses during transfusion of donor blood and its components and to suggest a set of activities aimed at the risk reduction.

We examined blood samples taken from 142 donors who permanently resided in Moscow; our task was to detect markers of active infections caused by herpes simplex viruses, types 1 and 2, Epstein-Barr virus (EBV), cytomegalovirus, and human herpes type 6 virus. Immunoglobulins M and G were determined with ELISA test; antigens, via an indirect immune fluorescence reaction combined with rapid cultural technique. All the donors successfully passed all the selection procedures and were accepted for donation.

Active forms were most frequently detected for infections caused by EBV (11.97 ± 2.73 per 100 examined) and human herpes type 6 virus (9.86 ± 2.51 per 100 examined), and it was accordingly 10 and 8.96 times higher than data given by other authors. It indicates there was high epidemic activity of these infectious agents in Moscow city in November-December 2019 and higher risks of recipients being infected with EBV and human herpes type 6 virus with donor blood and its components. Frequency of detecting donors with active infections caused by herpes simples, types 1 and 2, EBV, cytomegalovirus, and human herpes type 6 virus amounted to 27.46 ± 3.76 per 100 examined. Frequency of detecting donors bearing antigens to herpes viruses in their blood amounted to 20.42 ± 3.39 per 100 examined. Risk of potential infecting with examined herpes viruses during blood transfusions amounted to 40.85 per 100 recipients.

In order to reduce this risk, we suggest wide implementation of leuko- and pathogen reduction of stored donor blood and its components.

Key words: *risk assessment, infection risk, herpes viruses, blood examination, donors, donor blood and its components, infection safety, transfusion.*

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Up-to-date medical technologies are being implemented into every sphere in health care including transfusion medicine; it allows increasing quality and efficiency of medical services rendered to population. Annually, volumes of whole donor blood that are stored up in the Russian Federation grow by 7–10 %; in 2018 they amounted to approximately 1.5 million liters and the figure didn't include plasma obtained via plasmapheresis in volumes exceeding 700 thousand liters [1, 2]. Given that, a most vital problem the domestic transfusion medicine in Russia has to tackle is providing safety of donor blood and its components; that means, among other things, minimizing risks related to recipients being infected with hemotransmissive infections [3, 4]. The existing legislative and regulatory documents stipulate that donors are to be checked in order to reveal whether they have type B hepatitis virus (BH), type C hepatitis virus (CH), HIV, or syphilis infectious agents in their blood. It allows a substantial reduction in risks of infections; however, being only a single activity, it can't provide complete biological safety of donor blood and its components as the above mentioned pathogens are not the only ones that can potentially occur in blood [5–7].

There are cases mentioned in literature when recipients got infected with herpes virus infections after transfusion of donor blood and its components [8, 9]. At present an epidemiologic situation as per these infections is considered to be rather unfavorable. According to official statistic data, in the RF over the recent years there has been a growth in morbidity with cytomegalovirus infection and infectious mononucleosis. It is rather difficult to reliably assess a situation regarding morbidity with infections caused by simple herpes viruses, types 1 and 2, and human herpes type 6 virus, due to absence of its official registration [10, 11]. Nevertheless, results obtained in research both in our country and abroad indicate that infectious pathology associated with herpes viruses is widely spread and occurs everywhere [7, 12–14]. It creates additional health risks for recipients of donor blood and its components.

Our research goal was to assess potential risks of a recipient being infected with herpes viruses during transfusion of donor blood and its components and to suggest a set of activities aimed at the risk reduction.

Research problems to be solved:

– to detect frequency of active infections caused by herpes viruses including simple herpes types 1 and 2 virus, Epstein-Barr virus, cytomegalovirus, and human herpes type 6 virus in donors from Moscow city;

– to detect frequency of donors with active mono- and mixed herpes virus infections;

– to calculate frequency of donors with herpes viruses antigens in their blood indicating an infectious agent occurred in their circulatory system;

– to calculate potential risks of recipients being infected with herpes viruses during transfusion of donor blood and its components;

– to give recommendations on activities aimed at reducing risks.

Data and methods. To achieve our goal, over a period from November 05, 2019 to December 02, 2019 we examined blood taken from 142 donors aged from 22 to 55 (average age was 36.68 ± 9.11; 89 males and 53 females) who permanently lived in Moscow, had negative results regarding markers of syphilis infectious agent, HIV, HB and HC viruses, and negative results after a molecular-biologic examinations aimed at detecting syphilis infectious agent, HIV, HB and HC viruses in their blood. All the examined donors had alanine aminotransferase, hemoglobin, PCV, ESR, crude protein and protein fractions, and cellular formula within reference values fixed in the Order by the RF Public Healthcare Ministry issues on September 14, 2001 (last edited on June 06, 2008) No. 364 «On approving a procedure

for a medical examination for a donor of blood and its components»[1]. All the donors gave their consent in conformity with conventional procedures. After blood was taken, all the samples were encoded. No personal data were processed during the present research.

We identified immunoglobulin M (IgM) and G (IgG) to antibodies to simple herpes viruses type 1 and 2 (HHV-1 and HHV-2); Epstein-Barr virus (EBV); cytomegalovirus (CMV); human herpes type 6 virus (HHV-6); to do that, we applied ELISA test with a reagent set provided by «Vector-Best». Antigens (AG) of viruses were detected via an indirect immune fluorescence reaction (IIFR) with human hyper-immune serums, FITS conjugate and Evans blue dye test. Early antigens and herpes viruses' reproduction were detected via rapid cultural technique (RCT) on Veru, U937, and M-19 cells (human fibroblasts) with the subsequent repeated AG identification with IIRF. Virus accumulation on a cellular culture allowed achieving higher sensitivity in detecting antigens with a viral load being low.

All the results were interpreted in conformity with instructions issued for applied reagent sets and procedures. An active herpes virus infection (primary acute and reactivated one) caused by HHV-1, HHV-2, CMV, and HHV-6 was considered to be present in case AG were detected; also, IgM, including cases when it was detected together with AG and/or IgG; an infection caused by EBV was considered to be present in case AG were detected; also IgM to capsid antigen (IgMVCA), including cases when it was detected together with AG and/or IgG to capsid (IgGVCA) and/or nuclear (IgGEBNA) antigens; also IgG to an early antigen (IgGEA), including cases when it was detected together with IgMVCA, IgGVCA, IgGEBNA.

Potential risks that a recipient of donor blood and its components would be infected with herpes viruses were calculated as per the following formula (1)

$$R = M \cdot Nd/Nr, \quad (1)$$

where M is frequency of donors with herpes viruses' antigens in their blood indicating they had a pathogen in their circulatory system;

Nd is a number of blood component doses taken from one donor;

Nr is a number of blood components transfused form one donor to one recipient.

Results were treated according to conventional statistic procedures with licensed Microsoft Excel package for Microsoft Windows. We calculated frequency of herpes viruses' markers per 100 examined donors, error of the mean (m), and Student's t-test (t). Discrepancies were considered to be valid at confidence probability $p \leq 0.05$.

Results and discussion. Our research revealed that an infection caused by EBV was the most frequently detected one, both in its primary acute and reactivated forms (Table 1). The 2nd place belonged to an infection caused by HHV-6; the 3rd place, CMV; the 4th place, HVV-1. Active infections caused by HHV-2 were the most rarely detected. There were authentic discrepancies in detected frequencies of active infections caused by EBV and HVV-2 ($t = 3.64$; $p \leq 0.01$); HHV-6 and HVV-2 ($t = 3.13$; $p \leq 0.01$); CMV and HVV-2 ($t = 2.6$; $p \leq 0.05$); HHV-1 and HHV-2 ($t = 2.18$; $p \leq 0.05$). Discrepancies were not valid in all other cases ($t < 2$; $p > 0.05$).

[1] On approving a procedure for a medical examination for a donor of blood and its components: The Order by the RF Public Healthcare Ministry issued on September 14, 2001 No. 364 (last edited on June 06, 2008). *Garant: information and legal database*. Available at: http://base.garant.ru/4177987/ (08.02.2020) (in Russian).

Table 1

Frequency of active infections (primary acute and reactivated) caused by EBV, HHV-1, HHV-2, CMV and HVV-6 in donors (per 100 examined)

Markers of active infections	HHV-1	HHV-2	EBV	CMV	HVV-6
$M \pm m$	6.34 ± 2.05	1.4 ± 0.99	11.97 ± 2.73	7.74 ± 2.24	9.86 ± 2.51

Table 2

Frequency of active mono- and mixed infections caused by HHV-1, HHV-2, CMV, HVV-6 and EBV in donors (per 100 examined)

Overall 142 people examined		Number of donors with active infections	Per 100 examined ($M \pm m$)
Active mono-infection	HHV-1	6	4.23 ± 1.69
	HHV-2	1	0.7 ± 0.7
	CMV	7	4.93 ± 1.82
	HHV-6	8	5.64 ± 1.94
	EBV	7	4.93 ± 1.82
Active mixed infection	HHV-1 + EBV	1	0.7 ± 0.7
	HHV-1 + HHV-2 + EBV	1	0.7 ± 0.7
	HHV-1 + EBV + CMV + HHV-6	1	0.7 ± 0.7
	CMV + HHV-6	1	0.7 ± 0.7
	EBV + CMV	2	1.41 ± 0.99
	EBV + HHV-6	4	2.82 ± 1.39
Total		39	27.46 ± 3,76

As the same donor can simultaneously have several active herpes virus infections, we made an additional assessment aimed at detecting frequency of active mono- and mixed infections (Table 2).

Markers of active mono-infection were detected for all the examined infectious agents; they were most frequently detected for an infection caused by HHV-6; and the least frequently, for an infection caused by HHV-2. Authentic discrepancies were detected for frequency of active mono-infection caused by HHV-6 and HHV-2 ($t = 2.4$; $p \leq 0.05$); CMV and HHV-2 ($t = 2.17$; $p \leq 0.05$); EBV and HHV-2 ($t = 2.17$; $p \leq 0.05$).

Apart from detecting markers of an active mono-infection, we revealed that examined donors had active mixed infections. In one case a donor was infected with three viruses (HHV-1 + HHV-2 + EBV); and in another one, with four (HHV-1 + EBV + CMV + HHV-6). A combination of two viruses was authentically more frequent (5.64 ± 1.94 per 100 examined donors), $t = 2.4$; $p \leq 0.05$.

There were totally 39 donors out of 142 examined with various combinations of active herpes virus infections detected in their blood (27.46 ± 3.76 per 100 examined). Virus antigens including virus replication in a cellular culture were detected in 29 donors (20.42 ± 3.39 per 100 examined).

To calculate risks related to recipients being infected, we applied frequency of donors with herpes viruses' antigens in their blood that indicated they had a pathogen in

their circulatory system. Given that one donor on average usually gives blood for two doses of blood components per one donation, and one recipient on average gets one dose, a potential risk that a recipient would be infected with the examined pathogens amounted to 40.85 per 100 recipients.

Despite the fact that donors are often used in epidemiologic research as a reference group, at present there are very few available works on prevalence of active herpes virus infections in them. Experts from Russia and Belarus give some data that frequency of active infections caused by simple herpes viruses varied from 0.0 to 30.2 per 100 examined donors; CMV, from 2.2 to 16.5; EBV and HVV-6, 1.1 per 100 examined donors [15, 16]. Hence, our data on frequency of active infections caused by simple HVV and CMV were quite comparable with data taken from literature. We detected active EBV and HHv-6 infections accordingly 10 and 8.96 times more frequently in our research and it indicates that these infectious agents were highly epidemically active on the examined territory in November and December 2019; consequently, a risk that a recipient would be infected with the said viruses during blood transfusion was higher than calculated basing on data given by other authors.

Our research revealed a possibility that a recipient would be infected simultaneously with several herpes viruses with transfused donor blood and its components; it is a real challenge for clinical medicine [17–19] that can't be faced without applying a wider set of preventive and anti-epidemic activities.

An existing system that ensures infectious safety of donor blood and its components in the RF is based on proper selection of donors including medical examinations and laboratory instrumental research. Additional procedures include leuko- and pathogen reduction of blood components stipulated by the Rules for taking, storing up, transporting and clinical use of donor blood and its components (approved by the RF Government Order issued on June 22, 2019 No. 797[2]). However, the procedures are not obligatory and are applied to discretion of an organization that stores up donor blood and its components. At the same time, these procedures are widely used in other countries and they play an important role in providing donor blood safety as there are a lot of pathogens that potentially circulate in blood including those that are not indentified with up-to-date tools for laboratory diagnostics [5]. Leuko-reduction is especially relevant when it comes to cellular-associated pathogens, and herpes viruses belong exactly to this category. Several research works revealed that a risk related to CMV infection is significantly reduced after leuko-reduction performed with leuko-filters [5, 8, 9]; EBV is not at all detected after leuko-filtration has been performed on knowingly infected doses of thrombocytes concentrations [20]. Pathogen reduction is also a significant preventive procedure as it is efficient against most bacteria, protozoa, and viruses with lipid membranes [21–23]. The procedure is aimed at inhibiting infectious agents' reproduction; however, it can be insufficient for protecting a recipient from being infected in case viruses are already present in blood [24].

Our results indicate that recipients run a considerable risk of being infected with herpes viruses; to reduce it, it is necessary to widely implement both leuko- and pathogen reduction into storing up donor blood and its components. Another fact that

[2] On Approval of the Rules for taking, storing up, transporting and clinic use of donor blood and its components and on certain RF Government Orders being no longer valid: The RF Government Order issued on June 22, 2019 No. 797. *Garant: information and legal database*. Available at: https://www.garant.ru/products/ipo/prime/doc/72184110/ (08.02.2020) (in Russian).

proves the necessity of these procedures is that all the donors who took part in our research had been selected according to all the existing legal and regulatory documents and had been accepted for donation. They didn't have any clinical signs of infections and their clinical and biochemical parameters revealed via laboratory research were within reference values.

Conclusions.

1. Active infections (both in their primary acute and reactivated forms) caused by EBV and HHV-6 were the most frequently detected (11.97 ± 2.73 per 100 examined and 9.86 ± 2.51 per 100 examined accordingly), and it was respectively 10 and 8.96 times more frequent than according to data given by other authors. It indicates that those infectious agents were highly epidemically active in Moscow in November and December 2019 and recipients ran high risks of being infected with EBV and HHV-6 with donor blood and its components.

2. Frequency of donors with active infections caused by simple herpes type 1 and 2 viruses, Epstein-Barr virus, cytomegalovirus, and human herpes type 6 virus amounted to 27.46 ± 3.76 per 100 examined.

3. Frequency of donors with herpes virus antigens detected in their blood amounted to 20.42 ± 3.39 per 100 examined.

4. Risk of a recipient being infected with the examined herpes viruses during blood transfusions amounted to 40.85 per 100 recipients.

5. To reduce risks of a recipient being infected with the examined infectious agents, it is necessary to widely implement leuko- and pathogen reduction and perform these procedures on stored donor blood and its components.

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