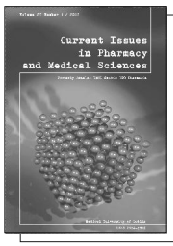

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Viral safety of blood and its components in modern blood donation – a review

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ABSTRACT

Today, we can observe that blood transfusion is carried out with greater care, which helps to prevent a number of infections in patients. Thus, emphasis is put on non-specific prophylaxis together with regular vaccinations. It is important to stress that blood transfusion is currently developing due to the following proceedings: careful selection of donors, serologic examinations, molecular investigations that check HBV, HCV, HIV, syphilis markers, providing periods for growing plasma and cryoprecipitate, leucoproduction, and common inactivation of patogens in blood components. Nowadays, higher risk of infection can be observed in many non-medical cases (body piercing, tattoos, travelling to hot and exotic countries, regular beauty treatments, *etc.*). Furthermore, newly selected microorganisms and mutations of popular strains are considered a challenge for contemporary virology, too.

Keywords: blood donation, blood treatment, blood-borne infection factors, transfusion medicine

INTRODUCTION

Despite constant progress in blood substitutes fabrication, blood and its components are still the most often used life-saving therapeutic agents [30, 27]. More than 1 million donations per year take place in Poland. [3] Blood collection is managed by 21 Blood Donation Centers with subjected affiliates [34]. National Blood Center appointed by Minister of Health in 2006 coordinates actions in blood collection organization, blood components separation, and blood supply and evaluates blood collection and haemotherapeutics. Blood is collected in unit offices or, increasingly, by blood collection mobile teams, so-called "bloodbuses" [35]. Independently of donation place the same strict procedures of donors' qualification, blood collection and its preparation, either HBV, HCV, HIV and syphilis markers diagnostics must be kept [28]. Blood donation in Poland is voluntary and free (honorary donations) [9, 33].

In contemporary haemotherapeutics indications to whole blood usage are sparse. Usually selected blood components which are deficient in patients are used. Such an approach permits to achieve better therapeutic result without burdening a recipient with unneeded blood components. It is worth mentioning that blood and its treatment components carry the risk of adverse reactions and complications at approx. 10% of recipients. They can have immunological and non-immunological background, may be induced by infectious or non-infectious factors or their manifestation can be early or delayed [16]. The development of virus diagnostics and inactivation methods had the main influence on improvement of safety of blood and its components. In addition, robust quality standards of collection procedures, preparation methods and diagnostics were introduced [33].

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BLOOD-BORNE INFECTION FACTORS

From the point of view of transfusion medicine the most important are the microorganisms which can be present in blood stream without any evident clinical symptoms [4]. Such period can be variable and depending on type of a microorganism (from days to years). The presence of microorganisms in individual blood components also varies. Microorganisms can be found in sera, lymphocytes, or red blood cells (Protista). In lymphocytes, viruses may take the form of virion particles or can be integrated into the host genome (a latent form). The viral latent form is inaccessible to host immunological system and can be reactivated causing acute infection both in donor and in recipient [4].

Although several viral species are fairly common in the population, *i.e.* HAV and CMV, no routine tests towards both of these species are performed in donors. HAVs are transmitted in fecal-oral route. Its transmission via blood is marginal and the presence of neutralizing antibodies in recipient or blood is a sufficient protection from infection. On the other hand, CMV is dangerous for immunocompromised patients but usage of leukoreduction in blood products prepared for this group of recipients protects them entirely. The most dangerous agents are those the infections of which during early stage are asymptomatic, but in further perspective mostly lethal, *i.e.* HBV, HCV and HIV viruses. Infectious

factors confirmed to be transfusion-transferable are presented in Table 1.

Table 1. Infectious factors confirmed to be transfusion-transferable [4]

0Viruses	Hepatotropic	*Hepatitis A Virus* (HAV)
		Hepatitis B Virus (HBV)
		Hepatitis C Virus (HCV)
		Hepatitis D Virus (co-infecting with HBV)
		Hepatitis E Virus (HEV)
	Retroviruses	*human immunodeficiency viruses* (HIV1 and HIV2 and their subtypes)
		human T-cell leukemia/lymphoma virus (HTLV 1 and 2)
	Herpesviruses	*Cytomegalovirus* (CMV)
		Epstein–Barr virus (EBV)
		Kaposi's sarcoma-associated herpesvirus (KSHV)
	Erytroviruses	*parvovirus B19 with subtypes*
	Other	*GB virus C* (GBV-C)
		Transfusion Transmitted Virus or Torque teno virus TTV
		West Nile Virus (WNV)
Bacteria	Endogenous	*Treponema pallidum* (syphilis)
		Borrelia burgdorferi (Lyme disease)
		Brucella melitensis (brucellosis)
		Yersinia enterocolitica/Salmonella spp.
	Exogenous	*Staphylococus sp./Pseudomonas* spp.
	Others	*Rickettsia spp.*
Protista	*Plasmodium*	
	Trypanosoma cruzei (Chagas disease)	
	Toxoplasma gondii (toxoplasmosis)	
	Babesia microtildivergens (babesiosis)	
	Leishmania spp. (leishmaniosis), *Filaria* spp. (filariosis)	
Prions		

BLOOD-BORNE VIRUSES

In Poland, blood donors and blood donor-candidates are routinely checked towards hepatitis B and C (HBV and HCV), human immunodeficiency viruses (HIV1 and HIV2) and syphilis infection markers [21]. Blood-borne viruses can be divided into several subcategories:

- pathogenic (HBV, HCV, HIV 1/2);
- pathogenic under certain conditions (CMV, HPV, B19, EBV, HHV-8);
- pathogenic in certain regions (HTLV 1/2, WNV);
- non-pathogenic, accompanying hepatitis infections (GBV-C/HGV, TTV, SEN-V) [4].

Viruses classified as pathogenic occur quite often in the world. The number of people infected with HIV-1 is estimated at 1.1% worldwide. HCV incidence is not precisely known due to usual asymptomatic progress of the disease. It is estimated that the percentage of people infected in Western Europe is approx. 0.5-2% and up to almost 20% in Egypt. In Poland, there are approx. 750 thousand of HCV carriers. There are approx. 350 million of HBV chronic carriers worldwide. HBV infection incidence varies from 2% in Western Europe and USA, approx. 5% in Poland, and up to 8% in Asian and African countries [2].

REDUCTION OF RISKS OF BLOOD-BORNE TRANSMISSION OF INFECTIOUS AGENTS.

The incidence of detected infections is said to be higher among paid donors that honorary donors, although opinions among authors are divided. The actions decreasing blood-borne transmission of infectious agents include:

- selection of donors comprising donor questionnaire, anamnesis and physical examination,
- serological and molecular screening towards HBV, HCV, HIV and syphilis,
- grace period for blood components with long shelf life,
- lekuoreduction by filtration,
- pathogen inactivation in respective blood components.

Moreover, the increase of safety of blood and its components is achieved by automatization and computerization in order to eliminate human error from procedures. According to SHOT (Serious Hazards of Transfusion), human errors are still most causal factors in post-transfusion reactions occurrence, which constitutes 64.1% of all complications [20]. Notably important is the use of single-use blood collection kits as well as single-use containers for preparation and storage of blood components. Lastly, it is worth mentioning that National Blood Donors Registry existing in Poland gathers confirmed cases of HBV, HCV, HIV, and syphilis infections. It provides access to information about all infected donors to all blood-care organization units in Poland.

SELECTION OF DONORS

Selection of donors is the simplest way to limit microorganisms spreading via blood, i.e. not-collecting blood and its components from donors from high-risk groups. Firstly, each blood donor fills a questionnaire with questions concerning infection risk hazards [17]. Potential risks may include high-risk sexual behavior, drug use, and sexual intercourses with newly met people or with people who commit high-risk sexual behavior or have a confirmed infected status [21].

Other disqualifying factors include:

- prior infectious diseases (disqualification can be temporary or permanent),
- prolonged contact with infected individual (*i.e.* family member suffering from hepatitis),
- residing on endemic disease terrains (*i.e.* malaria),
- residing on terrains with confirmed cases of virus transmission into humans (*i.e.* WNV),
- residing in countries with high ratio of carriers of anti-HIV antibodies or AIDS-sick individuals (disqualification for 1 month up to 3 years) [21];
- exposure to blood-borne infection risk (temporary disqualification), *i.e.*: endoscopic procedures, surgery, tattooing, and acupuncture or jail sentence.

Afterwards, a donor is qualified basing on an anamnesis verifying data included in the questionnaire. Blood donor is also subjected to physical examination. It is worth mentioning that even though donor qualification procedures are more restrictive, the main basis of success in this stage is donor's high self-awareness and education level. The research results show that still donor's knowledge concerning virus transmission pathways, existence of serological window period and its length and knowledge on auto-disqualification procedure (when donor states that his/her blood is not adequate for clinical applications) is limited [33].

SCREENING TOWARDS HBV, HCV, HIV AND SYPHILIS MARKERS

In Poland, screening is performed using serological and molecular biology methods (NAT). The tests are performed after every donation of blood or its component at every donor. The result is a basis for qualification or disqualification of a respective donor.

- HBV carriers are determined by detection of HBsAg and/or HBV DNA,
- HCV carriers are determined by detection of anti-HCV antibodies and/or HCV RNA,
- HIV carriers are determined by detection of anti-HIV antibodies and/or HIV RNA,
- syphilis-infected individuals are identified by detection of antibodies directed against *Trypanoma pallidum* epitopes.

A reactive test results must be reconfirmed using the same test twice. If a result of at least one repeated test is reactive, the whole donation is considered reactive. In such a case, a verification test must be performed to confirm a positive result. Confirmation tests should have a higher specificity that screening tests. When verification test result is positive, a second aliquot must be collected from a donor and the test should be repeated in order to exclude a human error. Regional Blood Donation Centers and/or Institute of Hematology and Transfusion Medicine in Warsaw [21] perform verification tests.

Up to 2010, also alanine transaminase (ALT) levels were determined in donors of blood samples. This test was ceased to be performed because ALT activity is non-specific indication of hepatitis [8, 9, 10, 11]. Many countries desisted from performing this test during blood collection procedure after introduction of sensitive serological techniques and molecular biology-based tests detecting HBV and HCV markers. Over many years in Poland no case was confirmed when increase in ALT activity preceded appearance of HBV DNA or serological markers or HCV RNA [7].

In case of suspected recipient of post-transfusion HBV, HCV and HIV infection by whole blood or its component, a look-back procedure is implemented. This procedure applies to all repeated donors, who had positive verification test towards one of detected viruses. Look-back procedure consists of monitoring the course of all blood components prepared during 6 months since the last donation with negative screening test results. In case of positive test result at donor, a recipient of potentially invective blood or its components must be determined, and in the event of fractioned plasma, proceed according to fractionators specification [21].

It is worth mentioning that in several countries where different blood-borne viruses exist, tests towards their detection are performed. For example West Nile Virus (WNV) has been detected in USA and Canada (since 2002) and human T-cell leukemia/lymphoma virus type 1 and 2 (HTLV 1 and 2) being detected in USA, Japan and several European countries. In Poland, procedures preventing spreading of the mentioned above viruses are limited to adequate questions in donor questionnaire and the anamnesis [4].

PLASMA AND CRYOPRECIPITATE GRACE PERIOD

In Poland, grace period for blood components was introduced in 1994 [1]. At the beginning, grace period was 2 months, and in the late 90s, the period was extended to 16 weeks [32]. From obvious reasons grace period encompasses only components with long shelf life *i.e.* plasma and cryoprecipitate (shelf life - 3 years if stored in temperature -25^{o}C or lower [29]). Grace period consists in storage of a blood component for at least 112 days and assessment after this period of virus markers at donor, from whom this component was obtained. "Grace perioded" component comes from a donor, in whose blood samples at least two consecutive tests towards HBV, HCV, HIV and syphilis markers were negative. The first test is performed on a day of grace-perioded donation, and the latter at least 112 days after donation. The main goal of the grace period is elimination of serological window in donors, i.e. early stages of infection [22]. For clinical application, only components after grace period may be used. In case of their shortage, inactivated plasma may be used instead.

LEUKOREDUCTION

Leukoreduction was introduced in the 80s as potential protection from spreading of a variant of Creutzfeldt-Jakob Disease (vCJD) [4]. Low leukocyte agents are obtained with the use of anti-leukocyte filters. Filters designed for packed red blood cells, PRBCs, hold leukocytes and platelets; in turn, filters designed for PRP – platelet-rich plasma – selectively filter out only leukocytes. Leukocytes turnover is led by adsorption and/or adhesion [21, 23]. By removing leukocytes all intracellular infectious agents i.e. viruses, bacteria, parasites, and prions are removed as well [4, 15]. For the latter special filters were designed, which apart from removing leukocytes, demonstrate high-prion affinity [6]. It is worth mentioning that bacteria and some parasite forms may be removed by direct adhesion to filter membrane [4]. Usage of filtration effectively prevents CMV infection, reducing infection risk to approx. 1% [14] and HTLV respectively [15]. Leukoreduction can also reduce the risk of spreading: HLTV, Rikettsia and several parasites *e.g. Leishmania* or *Trypanosoma cruzi*. Leukoreduction, apart from lowering the risk of infectious agents spreading, can also contribute to preventing HLA autoimmunization, non-hemolytic fever reactions, and Transfusion-Associated Graft Versus Host Disease – TA-GVHD [24]. In some European countries, leukoreduction is commonly used; in others like in Poland - to a limited extent. This is due to high leukoreduction cost and not sufficient evidence in limiting leukocyte-induced post-transfusion complications [4].

INACTIVATION

Formerly, inactivation methods were used only during sera fractionation, from which blood components were produced (albumin, coagulation factors, immunoglobulins). Nowadays much more popular are methods of pathogen inactivation in blood components especially PRP and *FFP. Clinical trials on pathogen inactivation in* PRBCs are on course. [25]. From among several inactivation techniques, a few are used as routine practice in several countries blood banks [19]. These methods are as follows:

– inactivation methods – destroying viral envelope, capside or genetic material, *i.e.* solvent/detergent (S/D) method, heating, methods using beta-propiolactone with UV radiation, method using caprylic acid;
– separation methods – infectious agent turnover (fractionation, Cohn's method, chromatography, usage of neutralizing antibodies, nanofiltration) [22].

It is necessary to mention that there is no universal method. S/D method is effective against enveloped viruses (HIV, HBV, HCV, HTLV, EBV, CMV, WNV). Nanofiltration is efficient against nonenveloped species i.e. parvovirus B19, HAV. In turn, heating-pasteurization is effective against both enveloped and nonenveloped species. Therefore to maximize action spectrum during blood components production at least two inactivation techniques must be used simultaneously [4, 22]. However, inactivation methods are not flawless. All of the mentioned methods, to some extent cause lowering of coagulation factors and albumin levels [26]. It is also necessary to remember about emerging communications about new infectious agents, *e.g.* QNV, bird flu virus, Denga virus, coronavarus SARS or retrovirus XMRV (*xenotropic murine leukemia related virus*) [12].

Increasingly the applied methods of blood components inactivation increase their safety by inactivation of pathogens not detected routinely at blood donors. These methods utilize visible light and UV (Table 2, 3). Moreover, single-use pathogen inactivation kits in sera (using methylene blue method) possess anti-leukocyte filter providing even more safe preparation [18].

Table 2. Pathogen inactivation methods in blood components [4]

Procedure	Packed Red Blood Cell's (PRBCs)	Platelet Concentrates (PC)	Fresh Frozen Plasma (FFP)
Solvent/Detergent	–	–	+
Methylene blue	–	–	+
Psoralen (S-59)	–	+	+
Riboflavin	–	+	+
Inactin	+	–	+
S-303	+	–	–

In recent years, a notable progress has been observed in prevention of infections spreading during blood transfusions. The main emphasis is put on broadly defined non-specific prophylaxis and vaccinations. However in blood donation a rapid development of techniques and procedures serving improvement of blood healing safety recently emerged, *i.e.* donors selection, serological and molecular screening tests towards HBV, HCV, HIV and syphilis markers, plasma and cryoprecipitate grace periods, leukoreduction and (increasingly popular) pathogen inactivation in blood components.

Table 3. Comparison of pathogen inactivation methods in blood components [14]

	Riboflavin	Amotosalen hydrochloride (S-59)	Methylene blue (BM)	Solvent/ Detergent (SD)
Active compound	Riboflavin and UV	Poralen (S-59) and UVA	Methylene blue and visible light	Solvent and detergent
Primary target	Nucleic acids	Nucleic acids	Nucleic acids	Lipid membranes
Secondary target	Proteins and lipids?	Proteins and lipids?	Proteins and lipids?	None
Compound toxicity	None	High	Low	Low
Compound turnover	Not removed	YES (CAD, compound absorbing device)	YES (Special filters)	YES (oil extraction and hydrophoblic chromatography)
Amount of active compound	Low amount of riboflavin and its photoproducts – physiologically present in blood	Low amount of free S-59* and its photoproducts	Low amount of free BM* and its photoproducts	Non-determinable or under toxic level
Stable linking to lipids and/ or proteins in sera	Not ascertained	13% linking with lipids, 1-2% linking with proteins	Proteins links with phenotizine dyes	Not linking with proteins or lipids
Formation of new antigens or autoantibodies	Not ascertained	Not ascertained	Not ascertained	Not ascertained

* Mutagen activity, low probable, but not excludable

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