Chagas disease and transfusion medicine: a perspective from non-endemic countries

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Abstract

In the last decades, increasing international migration and travel from Latin America to Europe have favoured the emergence of tropical diseases outside their "historical" boundaries. Chagas disease, a zoonosis endemic in rural areas of Central and South America represents a clear example of this phenomenon. In the absence of the vector, one of the potential modes of transmission of Chagas disease in non-endemic regions is through blood and blood products. As most patients with Chagas disease are asymptomatic and unaware of their condition, in case of blood donation they can inadvertently represent a serious threat to the safety of the blood supply in non-endemic areas. Since the first cases of transfusion-transmitted Chagas disease were described in the last years, non-endemic countries began to develop ad hoc strategies to prevent and control the spread of the infection. United States, Spain, United Kingdom and France first recognised the need for *Trypanosoma cruzi* screening in at-risk blood donors. In this review, we trace an up-to-date perspective on Chagas disease, describing its peculiar features, from epidemiological, pathological, clinical and diagnostic points of view. Moreover, we describe the possible transmission of Chagas disease through blood or blood products and the current strategies for its control, focusing on non-endemic areas.

Introduction

Chagas disease (CD), also known as American trypanosomiasis, is a potentially life-threatening infection caused by the haemoflagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). Its vector cycle and clinical expression in humans were described completely in 1909 by a Brazilian doctor, Carlos Ribeiro Justiniano Chagas. CD is found mainly in endemic areas of 21 Latin American countries (Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guyana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Venezuela, and Uruguay), where it is mostly transmitted by vectors, the triatomine bugs, known as "kissing bugs"[1].

Due to the effects of CD on the productivity of people of working age and to the disability and mortality that it causes, it is estimated that 670,000 disability-adjusted life years (DALYs) are lost annually in Latin America and CD, therefore, ranks first among parasite diseases for impact on health and social systems in that area[2].

In the last decades, increasing population mobility from Latin America to Europe has determined the emergence of tropical diseases, such as CD, outside their endemic countries[3]. In the absence of the vector, one of the potential modes of transmission of CD in non-endemic regions is through blood and blood products.

With this narrative review, our aim is to provide an up-to-date overview on CD with attention to its potential impact in transfusion medicine and to describe the current strategies for its control, focusing on non-endemic areas (Table I).

Epidemiology

According to estimates of the World Health Organization (WHO), which classifies CD among the 17 "neglected tropical diseases", around 8 million people are infected worldwide, mostly in Latin America[4]. There are marked differences in CD prevalence among endemic countries. For instance, it is estimated that 18-20% of the Bolivian population is infected (approximately 1,200,000 people), while in Brazil CD affects 1.3% of the population (3-5 million people). In the last 20 years many factors have contributed to a dramatic change in the epidemiological profile of CD: the implementation of different initiatives for its control in Latin America, the sharp rise in international travels and migration, urbanisation and internal migration in endemic and recently non-endemic countries, among others[5]. As a result of the CD control programmes promoted by the National Health Systems in Latin American countries and the Panamerican Health Organisation during the last 20 years, in particular the screening coverage in blood banks, the burden of the disease has progressively

Table I - Key facts.

According to the World Health Organization about 7 to 8 million people (up to 10 million according to other sources) are estimated to be infected with *T. cruzi* worldwide, mostly in Latin America.
Chagas disease was once entirely confined to the Americas -principally Latin America- but it has now spread to other non-endemic continents.
Chagas disease is curable if treatment is initiated early after infection.
Up to 30% of chronically infected people develop cardiac alterations and up to 10% develop digestive, neurological or mixed symptoms, for which specific treatment may become necessary.
The disease can be severe and life-threatening in the acute phase, particularly in immunocompromised patients.
Chagas disease is usually asymptomatic in the chronic phase thus contributing to its under-diagnosis and silent transmission.
Vector control is the most useful method to prevent Chagas disease in Latin America followed by blood donor testing and mother-to-child transmission control programmes.
Blood screening is vital to prevent infection through transfusion and organ transplantation also in non-endemic countries.
Many blood components can transmit the infection but platelets are the most frequent cause of transfusion-related transmission.
No strategy has proven fully effective in preventing *T. cruzi* transmission, but donor/donation testing, at-risk donor exclusion or selective use of no-risk plasma derivatives have been commonly adopted in endemic and non-endemic countries.

decreased. New cases of the illness have reduced from 700,000/year in 1990 to 41,200/year in 2006, and the mortality from 50,000 deaths per year to the current 12,500.

Outside endemic areas, CD cases (mainly imported) have been increasingly detected in North America (where, excluding Mexico, some autochthonous cases have been recorded and a relevant 300,000 to 1 million cases are estimated, while in Canada there are fewer than 100,000 cases)[6], many European countries (where more than 100,000 cases are estimated)[3] and some Western Pacific countries[4]. Europe is heavily involved: the majority of cases are recorded in Spain and Italy, followed by United Kingdom, Portugal, Switzerland, France and Sweden[3,7].

Transmission

In Latin America, *T. cruzi* parasites are mainly transmitted by the infected faeces of blood-sucking triatomine bugs. These bugs typically live in the cracks of poorly-constructed homes in rural or suburban areas. Normally they hide during the day and become active at night when they feed on humans. They usually bite an exposed area of skin, and defecate close to the bite. The parasites enter the body when the person instinctively smears the bug faeces into the skin bite, the eyes and the mouth[1].

T. cruzi can also be transmitted by:

- food contaminated by infected triatomine faeces[1,8];
- blood transfusions[1,9];
- transplacental passage from an infected mother to her neonate during pregnancy or childbirth[1,10];
- transplantation of organs/cells/tissues[1,11];
- laboratory accidents[1].

Transmission of *T. cruzi* in countries in which the vector does not exist occurs mainly through congenital transmission and blood transfusion[1].

Clinical features and natural history of the disease

Acute phase

The incubation period varies from 7 to 15 days in the case of vector transmission and from 30 to 40 days in the case of transfusion transmission. The initial, acute phase lasts for about two months. During this phase, a high number of parasites circulate in the blood. Most patients are asymptomatic or have mild symptoms (95%). When (rarely) the disease is clinically evident, the main symptom is moderate fever, which can be accompanied by headache, pallor, myalgia, dyspnoea, generalised or local oedema (lower limbs or face), abdominal pain, cough, hepatomegaly, rash, splenomegaly, diarrhoea, multiple lymphoadenopathies, myocarditis and more rarely meningo-encephalitis or neuropathy. In vector transmission, depending on the inoculation site, the first (pathognomonic) sign can be a skin chancre (chagoma) or unilateral purplish orbital oedema (Romaña sign) with local lymphoadenopathies lasting over several weeks. Acute disease has higher morbidity in children under 5 years old, the elderly, immunocompromised patients or in cases with possible high parasite inoculum, such as in oral outbreaks. In the immunocompromised host, the chronic form of the disease can evolve into an acute phase with particular features. For instance, in patients with acquired immunodeficiency syndrome the meningo-encephalitis is the more frequent manifestation with high mortality (not less than 70%)[12].

Indeterminate phase

After the 2- to 4-month long acute phase, the infection usually progresses to a latent phase, called the "chronic indeterminate phase". This phase is characterised by the absence of symptoms and apparent organ injuries, low parasitaemia and positive serology. It can either last lifelong (in about 70-80% of patients), or progress to the clinically evident disease after decades[1].

Chronic phase

Approximately 20% to 30% of patients will progress towards a clinically evident disease. Up to 30% of the patients suffer from cardiac disorders, such as conduction abnormalities, arrhythmias, cardiomyopathy, heart failure and secondary thromboembolism. Up to 15% have involvement of the oesophagus (mega-

oesophagus), 15-20% of the colon (dolicho/megacolon), and less than 5% suffer from neurological manifestations (CD is also an independent risk factor for stroke)[13]. Mixed forms are also possible. As a consequence, the infection can lead to sudden death, heart failure, achalasia, bowel complications and neurological disability[1].

Diagnosis

The diagnosis of CD relies on different approaches, depending on the phase of the infection.

During the acute phase, parasitaemia is usually high and direct parasitological methods are, therefore, preferred. The diagnosis is based on parasite detection through microscopic examination of fresh anticoagulated blood or through quantitative buffy coat (QBC™), or preferably through the identification of motile trypomastigotes in multiple micro-haematocrit tubes (following Strout's concentration technique)[14]. Parasites can also be seen in Giemsa-stained thin and thick blood smears. Although not yet standardised, genomic techniques (polymerase chain reaction, PCR) are beginning to be used routinely in suspected acute and congenital infections[15].

During the chronic phase, parasitaemia is usually undetectable and inconstant. Direct parasitological methods or PCR are not, therefore, helpful in routine diagnosis[16], while serology is considered the best option[17]. Enzyme-linked immunosorbent assays (ELISA), indirect immunofluorescence tests and indirect hemagglutination are commonly used.

The USA Food and Drug Administration (FDA) licensed two different serological assays, one based on crude antigen (2006), the other on a recombinant antigen (2010), to be used for the screening of blood donors.

The WHO criteria for the serological diagnosis of chronic CD recommend that a patient should have two positive serological tests based on different antigens and techniques; however, a single serological test is acceptable to certify the suitability of a blood unit for transfusion[18].

In the case of discordance of two tests used to diagnose CD, a confirmatory one should be available. Although there is not a diagnostic gold standard for chronic CD, some methods deserve attention in this regard: (i) a radioimmunoprecipitation assay (RIPA) has been used to screen sera for IgG antibodies and classify a sample as confirmed sero-reactive, indeterminate, or non-reactive[19]. The method is only available at the Centers for Disease Control (Atlanta, USA); and (ii) other confirmatory methods, such as western blots[20,21] and PCR[22,23] have also been examined. Currently, only trypomastigote excreted-secreted antigen (TESA)-blot[24] is commercially available in Latin America, but is not available in Europe for clinical use because it lacks the European CE mark.

Treatment

Treatment of CD is divided in aetiological and non-aetiological.

Non-aetiological treatment includes all the therapies which are necessary in case of organ involvement (pace-maker implantation, supportive inotropic drugs for heart failure, symptomatic drugs for constipation and so on arriving at heart transplantation or surgical intervention for megaviscera).

Anti-trypanosomal treatment is based on only two drugs, nifurtimox and benznidazole, and aims to reduce *T. cruzi* burden and the possible evolution of the disease[1,25,26]. Benznidazole has been more extensively investigated in clinical studies and has the better (although unsatisfactory) safety and efficacy profile and is, therefore, used as first-line treatment[27]. However, an ongoing, large, multicentre, randomised trial ("BENEFIT") is assessing definitely the parasitological and clinical efficacy of benznidazole in cases of chronic cardiac CD[28].

Other drugs have been used but their efficacy was not demonstrated (itraconazole, allopurinol…)[29] or they were ineffective (posaconazole)[30]. This fact, in addition to the bad tolerability of the two available drugs which is an important obstacle to completion of treatment (for 5.6% to 29.7% of patients in series from non-endemic countries do not complete treatment)[31,32] urges the development of new additional drugs[33].

Generally, treatment is offered to patients in the chronic indeterminate or early chronic cardiac phase of CD who are younger than 50-55 years. Aetiological treatment is also considered mandatory for all patients with acute or reactivated disease if immuno-compromised (including patients with acquired immunodeficiency syndrome).

The treatment is contraindicated in case of pregnancy, advanced renal or hepatic failure or chronic advanced cardiomyopathy.

Transfusion-transmitted Chagas disease

Transmission of CD via blood transfusion has been recognised since 1952[34], although the possibility of this transmission mode was first raised by Mazza in 1936[35]. The total number of transfusion-transmitted (TT)-CD cases has been estimated to be between 300 and 800 in the last decades[36,37]. However, it was only with the advent of the human immunodeficiency virus pandemic in the 1980s that blood control programmes were implemented in most Latin American countries, paving the way to prevent other widespread infectious diseases such as CD.

The relevance of this route of transmission is related to the disease prevalence in the population. The existence of an asymptomatic, parasitaemic, chronic phase puts

blood donations at risk, particularly because affected donors are frequently unaware of their status[38].

In endemic countries, blood transfusion was considered the second most common way to acquire CD. Therefore, screening programs have been set up in endemic countries and screening coverage in blood banks has progressively reached 100% in many countries in the last 20 years. This has dramatically reduced the risk of transmitting the infection by transfusion[39]. In endemic countries with fully implemented screening strategies, the residual risk of infection was calculated to be around 1:200,000 units[9,39].

Nevertheless, there are varying degrees of success in implementing these control programmes[40]. In Mexico, a country with the lowest level of screening coverage in Latin America, cases of TT-CD have been described in the last decade and great efforts have been made to pass from a donor screening coverage of 36.5% in 2005 to 92% in 2012[41].

The migration of affected and asymptomatic individuals from endemic to non-endemic areas may lead to transmission of CD by transfusion anywhere. Some TT-CD cases have already been described in the USA, Canada and Spain[42-48].

Benjamin *et al.*[49] reviewed reported TT-CD cases in North America and Spain: seven were described in the USA, five in Spain, two in Canada and one in Mexico. Implicated donors were born in Bolivia, Argentina, Brazil, Chile and Paraguay. All definite cases involved platelets, from either a whole blood or an apheresis donation. Irradiation and leucoreduction did not provide any protection in these cases.

In non-endemic countries, CD is considered an emerging infection because of the increasing number of immigrants coming from Latin America (Spain hosts approximately 4 million immigrants, and 1.5 million of them were born in a country in which CD is endemic).

The number of *T. cruzi* carriers in the USA, Australia, Spain, and other countries was previously estimated on the basis of the prevalence of CD in their countries of origin[50]. Guerri-Guttenberg and Colleagues[51] extended these data to include France, Italy, and countries of Northern Europe. Their estimates are based on the number of legal immigrants: 7,200,493 in the USA, 922,294 in Spain, 76,841 in France, and 59,189 in the United Kingdom. This suggests that the number of CD carriers would be between 38,777 and 339,954 in the USA[51], 12,533 and 25,728 in Spain[51], 1311 and 1712 in France[51] and 1,006 and 1,324 in the United Kingdom[51]. Strasen *et al.*[3] recently published a comprehensive estimation of affected people in Europe, indicating that a minimum of about 14,000 to a maximum of about 180,000 cases would be present in Europe. The general prevalence was estimated to be 35 cases per 100,000 inhabitants, although varying greatly across Europe from a substantial absence of the disease in Eastern countries to 307 cases/100,000 inhabitants in Spain, 28 cases/100,000 inhabitants in Italy, 25 in Sweden and Portugal and 22 in Switzerland and the Netherlands.

Jackson *et al.* evaluated the attitude/willingness to donate of a group of immigrants who participated in a serological survey in 2010, finding that a discrete proportion of immigrants considered donating their blood in countries of residence[52].

Low level parasitaemia may be detected several years after the infection in up to 50% of those infected[53]. The parasite is able to survive in labile blood component storage conditions (4 °C-22 °C) and can also withstand freezing and thawing. Whole blood, packed red blood cells, granulocytes, cryoprecipitate and platelets are, therefore, all capable of transmitting the disease, whereas plasma derivatives are not[53].

The infective capacity of each type of labile blood component is different, with platelets being the most frequently reported means of transfusion transmission[42-46,54,55].

The possibility of TT-CD depends on several factors: amount of transfused blood, infective capacity of the parasite present in each blood component, parasite strain, presence of parasitaemia at the time of donation, recipient immune status and screening tests[39,56,57]. Data from the 1960s and 1970s demonstrated that the real infectivity rate derived from one infected whole blood unit is around 12-25%[58]. However, to our knowledge, these data have not been verified with the current manufacturing practices. In the USA, despite a not negligible prevalence of CD in donors only sparse cases of TT-CD have been described[49] and look-back studies have identified only few cases (mainly related to platelet transfusion)[59].

Laboratory methods for Chagas disease testing in transfusion medicine

As previously stated, the diagnosis of CD is complex.

Parasitological tests (thick film microscopic observation, QBC™, Strout's or micro-haematocrit method) are useful in the acute phase and in the reactivation of the disease, with detectable parasitaemia. However, parasite concentration in blood decreases progressively and it is usually low in the chronic phase, so that direct methods lose sensitivity.

PCR is not yet standardised or sensitive enough to be considered a screening method for at-risk individuals and selection of blood donors/donations.

The most sensitive methods in chronic phase CD are immunological ones, based on detection of specific anti-*T. cruzi* antibodies. They are, therefore, applicable to blood banks.

In this regard, indirect hemagglutination tests are rarely used in endemic countries because of their low specificity and sensitivity profile. Immunofluorescence testing is an operator-dependent technique, with disadvantages in traceability and interpretation, and is therefore used only in centres with a lot of experience. An ELISA remains the ideal screening tool, particularly in blood transfusion centres. Two types of antigens are used: native ones from a parasite lysate or recombinant antigens. Many ELISA for CD are available on the market but the majority of manufacturers do not clearly declare on which antigens their tests are based. Moreover, few studies using reference serum panels are available to guide test selection[60,61].

Based on this considerations and a WHO statement that a single (highly sensitive) test is acceptable for determining the suitability of a blood unit for transfusion, ELISA are commonly used in transfusion medicine[62].

Current situation in non-endemic countries

As previously stated, issues related to CD transmission through blood and blood derivatives are not restricted only to Latin America as a result of international mobility and migration. Immigrants currently represent a growing part of the population in European and North American countries, and a proportion of them come from countries in which "neglected tropical diseases" are prevalent. They can, therefore, host diseases which can be inadvertently transmitted or developed out of endemic countries. A spectrum of diseases can somehow emerge in migrant populations and partially reflect the epidemiological situation in the countries of origin. CD constitutes a paradigm in this regard, because of the sustained increase of foreign residents from Latin America in Europe and North America. Imported CD is a new threat[38] and non-endemic countries have to face the challenge of providing health care for a not well-known disease, without proper diagnostic and therapeutic means, and with low public perception[50].

As stated at the beginning of this review, in non-endemic countries, imported CD is an emerging public health problem because of the potential complications associated with its chronic evolution, as well as of the risk of transmission. Additionally, data on prevalence in non-endemic areas are unsatisfactory, given the asymptomatic nature of chronic CD, the lack of familiarity of local physicians with it and, therefore, the high index of under-diagnosis[63]. The undocumented status of some infected patients also contributes to this worrisome scenario. Transfusion-transmitted cases may be even more difficult to detect as a result of these factors[64,65].

Consequently, in the last decade, various strategies have been developed in non-endemic countries to control TT-CD.

Preventive strategies

Policies to protect the blood supply are different in endemic and non-endemic countries. Currently, in endemic countries all donations should be analysed for *T. cruzi* antibodies[50]. In non-endemic countries, in which the number of at-risk donors is lower, blood supply protection is based on different interventions (Table II):

- deferral of donors who acknowledge that they have had the disease, or are at-risk of being carriers. These individuals are detected mainly through questionnaires that include questions about birth/residence/transfusion in endemic countries. Unfortunately, various studies have shown that this type of approach is not completely effective[38,66-68] and moreover there is a loss of donors;
- selection through donor/donation screening: donations from at-risk individuals are accepted, provided a negative result is obtained in a validated antibody test. Strategies for donor selection can rely on universal testing of all blood donations or on selected donor screening. These strategies have been adopted in countries in which numerous Latin Americans have settled, such as in the USA[49], Spain[38] and France[69] and have been suggested in Italy.

Selective *T. cruzi* screening is nearly as effective as universal screening, but costs less[70]. This seems to be applicable to both high-risk and low-risk scenarios and is reasonable: there are few at-risk donors and they can be identified through a questionnaire assessing potential exposure.

Pathogen reduction systems

In addition to the strategies based on donor selection, certain interventions to blood components could contribute to improve donation safety.

Blood component leucoreduction by filtering could contribute to reduce the amount of parasites present. Some studies have demonstrated a certain degree of reduction in *T. cruzi* burden[71]; however, the levels achieved are not sufficient to avoid transmission[72]. In fact, Benjamin *et al.*[49] report two cases of transmission of CD through a platelet product previously leucoreduced and irradiated.

In 2009, Castro listed various pathogen inactivation systems (crystal violet[73,74], methylene blue[72,75], amotosalen[76-78], S-303[79], riboflavin[80-82], thiopyrylium[83]) that are applicable to labile blood components, such as platelets or plasma, and have demonstrated high efficacy (reaching a parasite level reduction greater than 5 log in culture)[72,76,77,82]. After 2009, novel compounds have been tested with promising results, such as arylimidamides[84] and the aminoquinolone WR6026[85].

Some of these systems are currently available on the European market and constitute an interesting option

Table II - Different control strategies for the prevention of transfusion-transmitted Chagas disease (TT-CD) in non-endemic countries.

Non -endemic country	Estimated n. of people affected by CD	Strategy for TT-CD control	Implemented since year	Infected donations/ donors	Transfusion-acquired cases
United States of America	38,777-339,954[1]	Universal donor screening/selective one time testing of donors	1989-2009/ 2010 →	1/27,500	Yes
Canada	Fewer than 100,000[2]	Selective donor screening (questionnaire)	2010	3/1,000	Yes
Spain	12,533-25,728[1]	Selective donor screening (questionnaire)	2005	1/218	Yes
France	1,311-1,712[1]	Selective donor screening (questionnaire)	2007	1/32,800	No
United Kingdom	1,006-1,324[1]	Selective donor screening (questionnaire)	1998-2005 (donors), 2005 → (donations)	1/12,861	No
Italy	6,000-12,000[3]	Deferral period after exposure (no testing): under revision	2005	3.9/100	No
Sweden	1,118[3]	Permanent deferral of at-risk donors	-	-	No
Switzerland	3,000[3]	Selective donor screening (questionnaire)	2013	-	No
Australia	1,928[4]	Selective donor screening (questionnaire)	-	-	Yes
China	-	No strategies	-	-	No
Japan	3,000[4]	Permanent deferral of affected donors	-	-	No

-: data not known;
Other European countries are currently following the European Commission's 35 directives, 2004/33/CE and 2006/17/CE.

1) Guerri-Guttenberg RA, Grana DR, Ambrosio G, Milei J. Chagas cardiomyopathy: Europe is not spared! Eur Heart J 2008; **29**: 2587-91.
2) Hotez PJ, Dumonteil E, Betancourt Cravioto M, et al. An unfolding tragedy of Chagas disease in North America. PLoS Negl Trop Dis 2013; **7**: e2300.
3) World Health Organization. Control and prevention of Chagas disease in Europe, Report of a WHO Informal Consultation (jointly organized by WHO headquarters and the WHO Regional Office for Europe) 2009;WHO/HTM/NTD/IDM/2010.1.
4) Jackson Y, Pinto A, Pett S. Chagas disease in Australia and New Zealand: risks and needs for public health interventions. Trop Med Int Health 2014; **19:** 212-8.

that should be investigated[38]. It is noteworthy that no commercial pathogen reduction methods for red cells are commercially available at present.

Situation in the United States of America

In 1989, the USA Blood Products Advisory Committee recommended universal screening for CD once a suitable assay became available. Only in 2006 did the FDA license a first ELISA for detection of antibodies to *T. cruzi*, and in early 2007, universal serological testing of blood donors for *T. cruzi* infection was initiated in the USA by the two largest blood collecting systems, the American Red Cross and Blood Systems, Inc.[86]

FDA draft guidance recommending universal blood donation screening was released in March 2009. After 16 months of testing, serological evidence of infection was confirmed in approximately 1:27,500 donations overall, but was specially concentrated in areas with large Latin America immigrant communities[87] (Table II). With an observed low rate of transfusion transmission and apparent absence of infections in the USA donor pool, many blood centres moved thereafter to selective one-time testing of all allogeneic donors[88,89]. A FDA guidance released in December 2010 finally approved this approach[49].

Situation in Canada

Up to 2008 there were two reported cases of TT-CD in Canada[68] (Table II). A questionnaire was, therefore, introduced in February 2009 and donations were not used from at-risk donors for the production of platelets or transfusable plasma. Since May 2010, Canadian blood providers implemented a selecting testing model[90]. The following risk factors are assessed: being born in Latin America; having a mother or maternal grandmother born in Latin America; and having a history of 6 months or more of travel or residence in endemic countries.

In 1997, in Toronto, among 1,337 (1.6% of all surveyed) at-risk donors none was positive for CD[68]. A more recent survey showed that among 421,979 donors, 7,255 (1,72%) were selected by questionnaire and 13 resulted positive for *T. cruzi* antibodies. A lookback enquiry on 148 previous donations permitted

identification of 28% of the recipients, who all resulted negative for CD[90]. In 2009, O'Brien *et al.* decided to determine the seroprevalence of donors who answered "no" to risk questions finding only one positive donor who answered the questionnaire correctly[91].

Consistent with estimations, selective testing in Canada has identified few donations confirmed positive for CD. Thus, given the immigration patterns and low seroprevalence seen in previous studies and the good performance of the selecting testing model, donor assessment through a questionnaire is considered the best strategy in Canada[91].

Situation in Spain

The first TT-CD case occurred in Spain in 1984, followed by two other cases in 1995 and 2004[92]. These reports and the identification of positive donors[49] contributed to the introduction of blood screening applied to selected donors since September 2005. Spanish regulatory law requires all at-risk donors to be screened for CD or, otherwise, excluded from donation. Among 17 Autonomous Communities, just two (Castilla La Mancha and Extremadura) follow the donor deferral strategy[92].

Donors considered at-risk by the Spanish Ministry of Health regulations include people born in an endemic area, those born from a mother native to an endemic area, having been resident or having received a blood transfusion in an endemic country[93].

Since 2005, five other TT-CD cases have been notified in Spain[92]. According to the 2009 report of the Spanish Ministry of Health[60], the 0.46% of tested donations were confirmed positive for *T. cruzi* antibodies (Table II). Moreover, an estimated 53,000 donors could be positive for CD with an index of potential infectious donations between 0.02 and 2.35 per million[61].

Situation in France

In May 2007, the National French Blood Service (EFS) introduced systematic screening of at-risk blood donors for anti-*T. cruzi* antibodies. The concerned donors are people originating from an endemic area, donors with mothers originating from such areas and individuals who have lived in or travelled to endemic areas, irrespective of the duration of stay. Donors are generally screened with two ELISA simultaneously: one based on purified parasite lysate (crude antigens) and the second based on recombinant antigens. Positive results and discrepant results are further assayed with an immunofluorescence assay. A donor is eligible to donate if both ELISA are negative. In the case of discordance, irrespectively of the immunofluorescence assay result, all the donor's blood products are destroyed and donor is invited to repeat testing after 1 month. Depending on the new results, he/she can be re-admitted to donation[69].

A sero-prevalence survey was performed in the 17 French blood centres from May 2007 to December 2008. During this period 4,637,479 donations were collected. Out of these, 163,740 donations were tested for anti-*T. cruzi* antibodies (3.5%). The prevalence of anti-*T. cruzi* antibodies was one in 32,800 donations[69], similar to the magnitude in the United States.

Situation in Italy

In Italy, blood banks are currently following EU directives 2004/33/CE and 2006/17/CE which specifically mention CD as an exclusion criterion to donation for affected donors. Unfortunately, these documents do not recommend measures to be adopted when a donor has been exposed to CD and not yet screened. Moreover, a deferral period recommended after staying in tropical-subtropical countries does not add any protection to prevent CD transmission because after the acute phase, the disease enters an asymptomatic period with low and intermittent parasitaemia[17]. National recommendations on blood donor selection are going to be reviewed. The recommendations will include specific measures to be taken regarding CD. In particular, at-risk donors, i.e. donors coming from CD endemic countries, born from Latin American mothers or who have been transfused in CD endemic countries, will be admitted to donate only with negative sensitive *T. cruzi* serology. Positive donors will be addressed to tropical disease units for confirmatory diagnosis and appropriate treatment/follow-up.

Few data are available in Italy regarding the issue of CD transmission. Angheben *et al.* described a prevalence of 0% among Latin American donors affiliated to two blood banks (one in the Region of Veneto and one in the Region of Tuscany)[94] while a study conducted in Rome[95] raised major concern on blood safety because of a high rate of seropositivity (3.9%) and one case of active parasitaemia in a donor returning from Brazil (Table II).

Two unaware donors with positive *T. cruzi* serology tests have been identified through routine screening in the Province of Bergamo (northern Italy) during a 5-year period (2009-2014), but the local blood bank verified that one of them was admitted only to plasma donation and the other was temporarily excluded waiting the result of *T. cruzi* serology and thereafter permanently excluded from donation (Giussani B, Bergamo AVIS blood bank, personal communication).

Situation in the United Kingdom

In the United Kingdom, screening for at-risk donors began in 1998. Through 2005, donors selected based on residence or travel to rural endemic areas or exposure

to primitive living conditions in *T. cruzi* endemic areas were accepted for donation only if they were seronegative 6 months after returning. Before 2005 at-risk donors provided blood samples that were tested and therefore were allowed to donate if seronegative. After 2005 donors provided a full donation that was tested subsequently. Only three donors of the 38,583 tested since 1998 had confirmed positive results (Table II). In 2005, that strategy permitted collection of more than 15,000 seronegative donations that would have wasted before[96].

Situation in other non-endemic countries (Table II)

Switzerland has changed its directives regarding donor selection for CD in January 2013 and is in line with Spanish and French recommendations[97].

In Portugal, a blood safety protocol is under approval by the Instituto Português do Sangue e da Transplantação and is oriented to the exclusion of all at-risk donors. Similarly in Sweden all individuals who have lived more than 5 years in CD endemic countries are definitively excluded from donation[97].

Other European countries, not cited before, are currently following the European Commission's directives, 2004/33/CE and 2006/17/CE[97], which state that all donors affected by CD are permanently excluded from donation; however, nothing is suggested regarding which measures must be undertaken for those donors potentially exposed to *T. cruzi*[97].

In Australia, the Blood Service complies with the requirements of the Council of Europe, "Guide to the preparation of blood components". A donor questionnaire permits identification of at-risk donors and, therefore, exclusion from donation of affected individuals or their restriction to plasma donation (for fractionation only). The first case of TT-CD was detected in Australia in 2008[98].

China does not apply any policy to control TT-CD. In Japan, donors with a history of CD are permanently deferred[98].

Conclusions

T. cruzi can be transmitted through blood transfusions by individuals who are chronically infected and mainly asymptomatic. In countries with an indigenous or immigrant population at-risk of being infected, the blood supply should, therefore, be protected by effective strategies. These can be based either on screening blood donations/at-risk donors or through at-risk donor exclusion or addressing at-risk donors only to plasma donation for plasma-derived products.

The "Safety Tripod" concept[99] is based on the selection of appropriate and "low-risk" donors, usage of screening tests for the relevant infection marker and elimination of residual pathogens. A first step to be taken is the recognition, by both regulatory agencies and transfusion medicine experts, that *T. cruzi* has been threatening the blood supply (albeit to a lesser extent than other diseases) for decades[9] and is currently a threat in non-endemic countries.

Generally, screening of blood donors allows a balance between the number of eligible donors and transfusion safety and seems to be a good strategy in countries with an increasing Latin American population. New approaches, using labile blood component pathogen reduction techniques, can also contribute to deal with parasitic infections such as CD[72,100] but must be improved.

Following United States, Canada, Spain, the United Kingdom, France, Sweden and Switzerland, new recommendations on donor selection are going to be adopted in Italy for the prevention of TT-CD: they seem to stay in step with international good practice, already applied in the aforementioned countries. These measures should not only improve the safety of blood donations but also avoid exclusion of immigrant donors who can provide rare blood phenotypes for selected patients and contribute to the society in which they take part after immigration.

However, the introduction of blood donor screening for CD in Italy and other countries opens new challenges that must be addressed:

- defining standards for blood testing methods,
- implementing donor selection questionnaires,
- identifying and enforcing a network of reference centres for the management of positive cases.

Keywords: blood transfusion, Chagas disease, *T. cruzi*, non-endemic countries.

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