Zika virus and the never-ending story of emerging pathogens and Transfusion Medicine

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Abstract

In the last few years, the transfusion medicine community has been paying special attention to emerging vector-borne diseases transmitted by arboviruses. Zika virus is the latest of these pathogens and is responsible for major outbreaks in Africa, Asia and, more recently, in previously infection-naïve territories of the Pacific area. Many issues regarding this emerging pathogen remain unclear and require further investigation. National health authorities have adopted different prevention strategies. The aim of this review article is to discuss the currently available, though limited, information and the potential impact of this virus on transfusion medicine.

Introduction

The recognition of newly described infectious disease agents poses problems for health authorities and transfusion medicine specialists who have to ensure the safety of blood products. Surveillance, threat assessments, triggers for action, intervention development along with an ongoing revision of emerging or re-emerging infectious disease agents that pose a real or theoretical risk to transfusion safety play a key role in guaranteeing blood safety[1,2].

Recently, particular attention has been focused, also in industrialised countries, on vector-borne diseases transmitted by arboviruses (arthropod-borne viruses), such as West Nile, Dengue, and Chikungunya[3-5]. The risk of transmitting the above-mentioned arboviruses by transfusion is well known and preventive measures are well established[3-7].

Other arboviruses, which cause outbreaks in different areas of the world, are less known and there is a lack of data on their presence in blood during the donor's asymptomatic phase, the agent's survival/persistence in blood during processing/storage, as well as their role in a clinically apparent outcome in at least a proportion of recipients who become infected[1].

The last in the long list of emerging pathogens is Zika virus (ZIKAV), responsible for major outbreaks in previously naïve territories of the Pacific area; many issues regarding this pathogen remain unclear.

The aim of this review article is to discuss the currently available (limited) information on this virus focusing on the different preventive measures resulting from local risk assessments of its potential threat to public health (and transfusion) safety.

The virus

ZIKAV is an enveloped, icosahedral, arbovirus of the family *Flaviviridae*, genus *Flavivirus*[8] (Figure 1); it is phylogenetically and antigenically related to the *Spondweni virus* and is a single-stranded RNA virus with a positive-polarity RNA genome of approximately 11 kb. The virus's RNA includes its complete open reading frame (ORF) sequence. The ORF encodes a polyprotein with three structural components (capsid [C], premembrane [prM] or membrane [M], and envelope [E]) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5)[9].

ZIKAV was first isolated in 1947 from a Rhesus monkey in the Zika forest (Uganda)[10]. It was subsequently found in the *Aedes (Stegomyia) Africanus* mosquito captured in the same forest[11]. In 1954, the first three cases of human infection were reported during an epidemic of jaundice in Eastern Nigeria[12]. Interestingly, a recent study on the molecular evolution of this virus during its emergence in the 20th century showed that ZIKAV may have experienced several adaptive genetic changes (though uncommon among flaviviruses), including protein glycosylation patterns, which could be related to the lack of any clear preference for host and vector species[13]. Since its discovery, there have been ZIKAV outbreaks in African, Asian and Pacific regions[14]. In 2007-2008, a ZIKAV epidemic occurred in the island of Yap (Micronesia)[15], Gabon[16] and Senegal[17]. A major epidemic broke out in French Polynesia in October 2013, and the first autochthonous cases in New Caledonia were reported in January 2014[18]. In the same year, the European Centre for Disease Prevention and Control

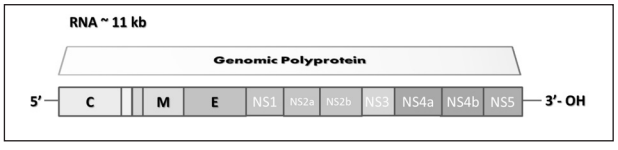

Figure 1 - Schematic representation of the Zika virus genome.

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(ECDC) confirmed one case and reported 40 suspected cases on Easter Island[18]. At the moment, virological studies, seroprevalence surveys, diagnosis of sporadic cases, and epidemics have made it possible to identify the virus in Africa (Uganda, Nigeria, Ivory Coast, Gabon, Tanzania, Egypt, Central African Republic and Sierra Leone), in Asia (Cambodia, India, Indonesia, Malaysia, Pakistan, the Philippines, Singapore, Thailand and Vietnam), and in Oceania (Micronesia/ Yap, French Polynesia, New Caledonia and the Cook islands)[14]. However, in 2014, isolated cases, most of which imported from endemic areas, were also reported in Norway, Germany, Australia, France, Canada, and Italy[14,19-23] (Figure 2).

Clinical manifestations

ZIKAV disease is a relatively unknown mosquito-borne infection that is believed to be asymptomatic or mildly symptomatic in most cases and is hardly distinguishable from other, better-known diseases, caused by other arboviruses that can threaten transfusion safety, such as Dengue fever, West Nile or Chikungunya disease[4-7,18,24-27].

Although neurological complications, including Guillain-Barré syndrome, have been observed[15,28], in humans, ZIKAV infection (similarly to other arbovirus infections) is usually characterised by mild fever, arthralgia (small joints of hands and feet), myalgia, headache, asthenia, abdominal pain, oedema, lymphadenopathy, retro-orbital pain, conjunctivitis, and cutaneous maculopapular rash. It can, therefore, be misdiagnosed during the acute (viraemic) phase because of non-specific influenza-like signs and symptoms.

Diagnosis

The diagnosis of ZIKAV is primarily based on the detection of viral RNA from specimens by means of reverse transcription polymerase chain reaction (RT-PCR)[29]. The viraemic period has not been established but it is believed to be short, allowing for direct virus detection during the first 3-5 days after the onset of symptoms[29]. In addition to the RT-PCR assay focusing on the detection of Micronesian ZIKAV strains[30], specific molecular assays for Asian and African strains have been developed; these tests target the envelope gene or NS5 region, the latter being more highly conserved among flaviviruses compared to envelope genes[15,31]. Recently, the suitability of urine samples for diagnosing ZIKAV infection has been confirmed, as RNA of the virus is detectable in urine at a higher load and with a longer duration than in serum[32].

ZIKAV infection can also be diagnosed through isolation of the virus from animals or mosquitoes[31,33]. Serological tests (enzyme-linked immunosorbent assay [ELISA] or immunofluorescence) are also used. Unfortunately, there are only a few laboratories able to perform an ELISA for ZIKAV[32]. An IgM antibody response in primary ZIKAV-infected patients has been reported, but a cross-reaction with other flaviviruses, such as dengue virus or yellow fever virus, may make the diagnosis difficult[15]. Positive results should be confirmed by neutralisation assay (i.e. plaque reduction neutralisation test, PRNT) to document at least a 4-fold increase in ZIKAV neutralising antibody titres[32]. To this regard, the neutralising properties of ZIKAV antibodies have been exploited in PRNT and played a key role in the diagnosis and case classification (confirmed, probable, suspected, and no ZIKAV disease) of the 185 patients during the 2007 outbreak on Yap Island, as detailed by Duffy and colleagues[15].

However, as for other emerging microbial threats, no licensed test for ZIKAV diagnosis is currently available[34].

Routes of transmission and potential impact on blood transfusion

Most arboviruses are maintained in enzootic cycles between haematophagous arthropod vectors and susceptible primary vertebrate hosts[35]. Humans are usually dead-end hosts and do not develop a sufficient viraemia to infect vectors efficiently but West Nile, Dengue, Chikungunya, and Zika virus diseases are important exceptions as infected patients have consistent levels of viraemia and can act as the primary vertebrate hosts in urban settings[31]. However, as almost all the more than 130 arboviruses causing human disease are able to be transmitted through blood transfusion, "the association of many mosquito-borne arboviruses with large, explosive outbreaks produces the greatest transfusion safety threat"[35].

ZIKAV has been isolated from monkeys, mosquitoes, and sick persons in Africa and Southeast Asia, and although the reservoir has not yet been identified,

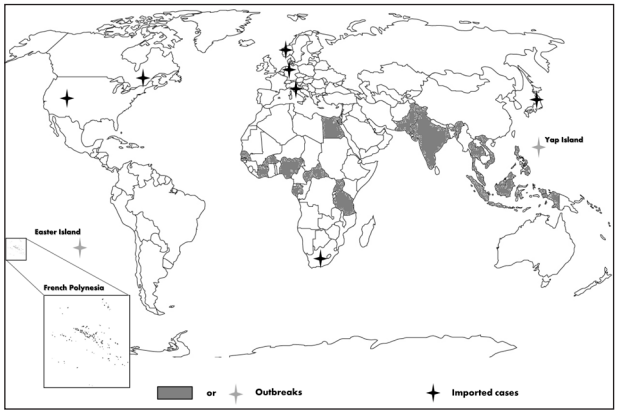

Figure 2 - Zika virus: outbreaks and imported cases.

some authors suggest that it is primates[15]. The virus is transmitted to humans mainly by the *Aedes* mosquito species[15]. Since the first description of *Aedes albopictus* as a potential vector of ZIKAV in 2007, other *Aedes* species *(Aedes aegypti, Aedes polynesiensis, Aedes dalzieli*, etc.) have been reported as competent vectors of ZIKAV, which may be explained by the virus's molecular evolution[3,13,14]. Unfortunately, this implies that the rapidly increasing presence of this vector worldwide could be responsible for the emergence of new ZIKAV epidemics, also in urban areas[3,15,28]. In addition, there are recent reports of perinatal[36], potential sexual[17,23,37], and transfusion transmission[38]. Other routes of transmission have also been reported for West Nile[39], Dengue[40] and Chikungunya viruses[41].

In December 2013 and February 2014, Besnard and colleagues[36] described clinical and laboratory evidence of two cases of perinatal transmission of ZIKAV in French Polynesia.

The first hypothesis of the sexual transmission of ZIKAV (by semen) was advanced by Foy and colleagues[17] who reported that the wife of a ZIKAV-positive patient had been infected in south-eastern Senegal in 2008. In addition, Musso and colleagues demonstrated a high ZIKAV RNA load and replicative ZIKAV in semen samples of a patient (the virus was not detected by PCR in the blood sample collected at the same time)[37], supporting the hypothesis that ZIKAV can be transmitted by sexual intercourse.

As far as transfusion transmission is concerned, considering the high rate of asymptomatic subjects during outbreaks, the potential blood-borne transmission of other arboviruses, the wide spread of the vectors also in countries with temperate weather[3], as well as the recently hypothesised new routes of transmission[17,36-38], the potential role of ZIKAV in transfusion medicine is plausible but still under evaluation. However, should autochthonous transmission of ZIKAV occur in countries able to supply blood components to the affected areas, the unavailability of a licensed laboratory test for routine biological qualification of blood components through systematic screening for ZIKAV genome by nucleic acid amplification tests (NAT) would probably lead to the adoption of blood safety (i.e. blood collection suspension) and self-sufficiency measures similar to those adopted to deal with 2007 Italian Chikungunya outbreak, which had considerable repercussions on the blood system[6].

Unfortunately, to date, there is a lack of data on the prevalence of ZIKAV in blood donors. The first molecular analyses were performed during the outbreak in French Polynesia and are the principal source of data[38]. RT-PCR for ZIKAV was performed in 1,505 blood donors and, surprisingly, an unexpectedly high number of positive asymptomatic blood donors (42/1,505; 3%) was detected. Of the blood donors positive for ZIKAV, 11 (26.2 %) declared that they had had a "Zika fever-like syndrome" 3 to 10 days after donating.

Further studies are, therefore, needed to assess the real seroprevalence of ZIKAV in endemic regions, to define the efficacy of its transmission through blood products and to determine its capacity to generate a disease in the recipient.

Different preventive strategies for different countries

The tools to pinpoint the actual threat posed by arboviruses to blood supply safety include NAT (if available) of blood donors during outbreaks, implementing surveillance systems and improving cooperation between public health, virology, entomology, and transfusion medicine specialists to intercept possible transfusion-transmitted infections, as well as look-back studies of recipients of blood components from infected donors[35].

Furthermore, experience gained from dealing with Chikungunya outbreak[6,42] showed that preparedness plans should encompass both: (i) preventive measures aimed at enhancing the vigilance towards imported cases of arbovirus infection to reduce the risk of autochthonous transmission, such as monitoring vector species and human cases; and (ii) blood safety measures such as deferral of potential donors living in or who have travelled to outbreak areas, NAT, risk algorithms to curtail blood collection if suitable NAT is not available, along with plans to sustain the blood supply and definition of an acceptable, evidence-based level of risk to restart blood collection. An additional and underestimated issue is the occurrence of co-infections with other arboviruses because, given the similar clinical features and lack of concurrent testing, they might not identified[43]. During the 2014 outbreak of ZIKAV infection in New Caledonia, the local arbovirus sentinel network enabled the diagnosis of two co-infections with ZIKAV and Dengue virus in patients tested with RT-PCR for Dengue virus, Chikungunya virus, and ZIKAV[43]. Therefore, infection with multiple pathogens should be kept in mind when making the differential diagnosis of Dengue-like illness, especially in travellers returning from tropical regions where the co-circulation of multiple arboviruses is common; in this regard, multiplex RT-PCR could play a crucial role in improving the diagnostic process in preparedness plans.

Pathogen reduction technologies could be an additional efficient strategy to prevent blood-borne transmission of infectious pathogens, particularly unknown ones. Unfortunately, at the moment, no specific data are available on the rate of reduction ($\log_{10}$

reduction) of ZIKAV with available pathogen reduction technologies and we can only infer their efficacy from their capacity to inactivate similar pathogens[44].

At present, as far as ZIKAV preparedness plans are concerned, different approaches have been exploited by different national health authorities taking into account the specific local situations.

In general, in countries with sporadic imported cases of ZIKAV infection or in ZIKAV-free countries, no particular action, except notification, has been taken.

In Australia, although ZIKAV is not endemic and imported cases of the disease along with the presence of the *Aedes aegypti* mosquito have been reported only in some coastal towns, the Queensland Government issued a guideline for early detection and response to imported and/or locally acquired cases of ZIKAV and to monitor the epidemiology of ZIKAV and its vector mosquitoes in this region[45].

Using a different strategy, in New Zealand, where the mosquitoes that transmit ZIKAV are not found, the New Zealand Ministry of Health recommended laboratory testing for people who have recently travelled overseas and have a clinical history of ZIKAV infection[46].

In Europe, according to the ECDC, blood authorities need to be vigilant regarding the epidemiological situation and should consider deferral of donors with travel history in line with measures defined for West Nile virus. Blood safety procedures are already in place in the Pacific region in the context of the ongoing outbreak of Dengue and Chikungunya and, in early January 2014, NAT for ZIKAV was introduced in French Polynesia[38]. Given its geographically isolated location, it was impossible to import fresh blood products into the country, so blood authorities decided to implement three-sample minipool ZIKAV NAT as soon as possible, using a modified RT-PCR, in order to prevent virus transmission through blood transfusion without discontinuing blood donations.

In France (ZIKAV is endemic in French Overseas Departments and mosquitoes of the *Aedes* species are present in 18 continental departments), the Ministry of Social Affairs and Health established increased monitoring of vector species and human cases. The purpose of this strategy is to identify cases of imported and indigenous arbovirus disease as soon as possible in order to take a series of measures aimed at reducing the risk of transmission during the season when the vector is present[47].

In Italy, where arbovirus vectors are also present, the Ministry of Health, in addition to active surveillance for the early identification of imported cases, recently decided to start seasonal epidemiological, entomological, and clinical surveillance in the territories in which the presence of the vectors is more widespread in order to intercept possible autochthonous cases promptly[48]. In this regard, the first two cases of ZIKAV infection imported into Italy have recently been reported, highlighting the importance of quickly implementing adequate public health preventive measures, such as public education and mosquito bite prevention[23].

Unfortunately, at present, there are no ongoing entomological surveillance systems targeting vectors of arboviruses established in the aforementioned endemic (African and Pacific) regions (except in New Caledonia, Fiji, and French Polynesia)[49].

On the other hand, in the endemic areas, where the capacity to control the vector is often limited or insufficient, strategies to limit viral circulation should be implemented and the possible production of a specific vaccine could be a valid option[14].

Discussion

Prior to 2007, only a few cases of ZIKAV infection had ever been described in medical literature. It was only in April 2007, during an outbreak on Yap Island, that the discovery of ZIKAV in the serum of sick patients clearly showed that this virus was capable of causing much more than isolated cases and that it had spread outside its usual geographic boundaries in Africa[33,49]. We consider that the potential threat of ZIKAV to transfusion safety should not be overlooked because "as West Nile virus aptly demonstrated, the unexpected should be expected"[35]. In fact, as with all arboviruses, the main problem in the prevention of transfusion transmission is the high rate of asymptomatic infections and the mild disease that can go unnoticed. Furthermore, as there is a viraemic period (albeit short) after the onset of symptoms, professionals charged with donor selection should very carefully evaluate the donation suitability after the manifestation of ZIKAV infection-like symptoms. In fact, arbovirus transfusion risk models and evaluations of viraemia prevalence in blood donations indicate significant transfusion transmission of viruses in epidemic areas.

In conclusion, at the moment, the prevention strategies adopted by national health authorities are mainly based on active surveillance of imported cases and national blood authorities should consider the deferral of donors coming from outbreak areas. However, in countries that have a stable presence of arbovirus vectors, seasonal entomological surveillance should also be implemented because, as for West Nile virus, it could play a key role in ensuring blood safety[5]. The implementation, when possible, of systematic screening for ZIKAV genome through highly sensitive NAT would be an additional safety tool, although not yet available.

In the event of a possible global increasing emergence of arbovirus-related diseases, all the above strategies,

including pathogen reduction technologies if and when effective, are valid tools to tackle both endemic and exotic pathogens that put blood safety at risk[35].

Keywords: Zika virus, arboviruses, *Flaviviridae* infections, blood transfusion, preventive health services.

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