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Emerging Infectious Threats to the Blood Supply: Seroepidemiological Studies in Iran – a Review

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Summary
The risk of transfusion-transmitted infections has been greatly reduced by improvements in donor screening and testing. However, newly recognized blood-borne infectious agents can be threats to blood safety. In order to evaluate the prevalence some of these agents in blood donors, a systematic review was conducted. Data were obtained from published papers related to HGV, Torque Teno virus (TTV), HTLV, West Nile virus (WNV) and SEN virus (SEN-V). Based on these studies, the prevalence of HGV varied from 1 to 8.6% for anti-E2 and from 0 to 4.8% for HGV RNA. The prevalence of TTV DNA and HTLV-I varied from 2.7 to 79.5% and from 0.013 to 2.3%, respectively. The WNV-specific IgM antibody and WNV RNA are negative in blood donors. Prevalence rates of SEN-V in Iranian blood donors range from 23 to 90.8%. Consequences of these infectious agents for blood safety are different. Thus, the need to perform laboratory screening as well as effectiveness and efficiency of laboratory tests depend on pathogenicity level and epidemiological conditions of emerging infections. However, being prepared based on the current level of risk and interventions to reduce the risk can be effective in reducing the potential threat for blood supply.

Introduction

Currently, blood transfusion has become a substantial part of medical practice. Every second, someone in the world needs blood for surgery, trauma, severe anemia, or complications of pregnancy [1]. In other words, without blood transfusion, life-saving medical treatments, such as surgical procedures, pregnancy-related complications, the treatment of thalassemic and other multitransfused patients, cancer treatment, organ transplants, and bone marrow transplants would not be possible. Therefore, it is necessary that sufficient blood supplies are available within a very short notice.

The safest blood donors are voluntary, non-remunerated blood donors. The number of blood donations is more than 1.7 million units annually in Iran, and 100% of our donations are voluntary and non-remunerated. In Iran 40% of all blood donations were collected from regular blood donors during the year 2007 [2].

Testing of all donated blood for hepatitis B surface antigen (HBsAg), HIV-1and -2 antigen-antibody, HCV antibody, syphilis and HTLV-I/II (being mandatory in three provinces based on the local epidemiological evidence) is one of the main strategies for protecting against serious transfusion-transmitted infections (TTIs) in blood recipients. Therefore, in recent years the risk of transfusion-transmitted infections has been greatly reduced by improvements in donor screening and testing so that today the blood supply is safer. However, given that emerging and re-emerging infections (including also infectious diseases) are considered as important factors of mortality and morbidity in different populations [3], conditions for blood centers are becoming more complex. Of the identified virulent pathogens, including viruses, bacteria, fungi, protozoa, and helminthes, approximately 175 species are considered emerging pathogens [4]. Emerging infections are defined to be those infectious diseases whose incidence has increased within the past 2 decades or threatens to increase in the near future [5].

Several factors are involved in the appearance of emerging diseases. These infections may result from ecologic changes or emanate from genetic, biological, social, and economic factors.

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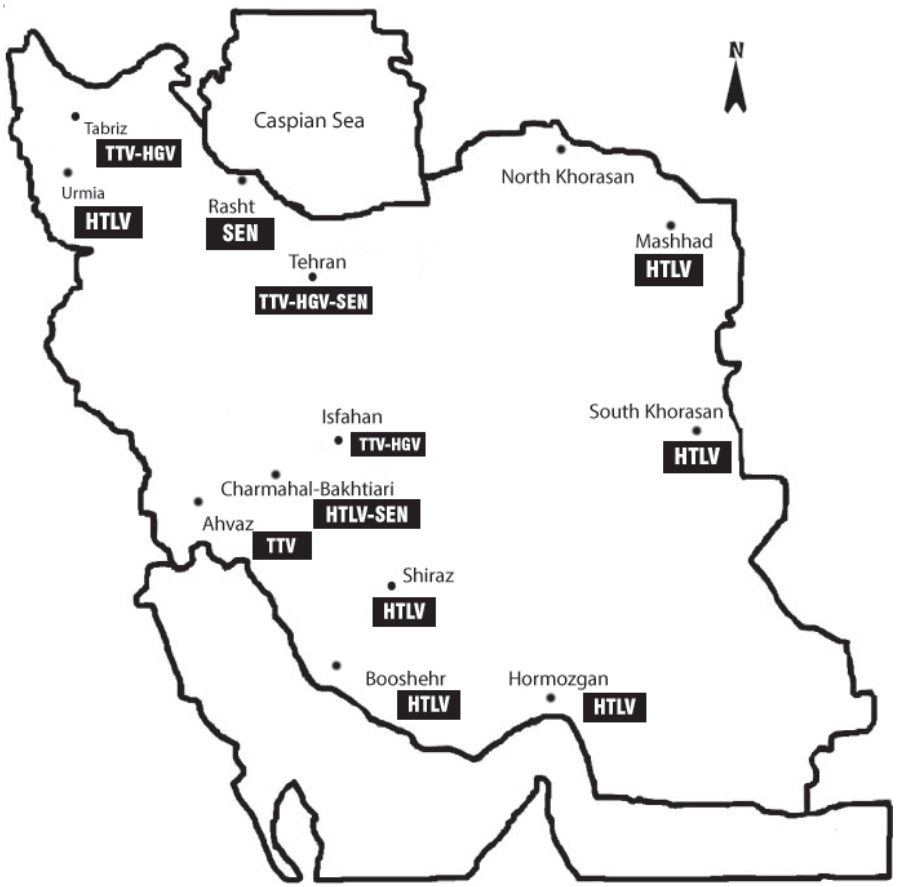

Fig. 1. Geographic distribution of some newly described viruses in Iranian blood donors.

The total effect of these factors will lead to the development of emerging diseases.

Emerging and re-emerging microorganisms, like other microbial agents, can threaten blood safety. Epidemiology of newly emerged pathogens differs according to socioeconomic, geographic, and cultural conditions. Geographically, Iran is situated in the northern temperate zone with a variety of climate types and varied in social, economic, cultural, and health aspects. Iran also neighbors the countries with various economic and health conditions. Thus, travelling across borders and vast range of commercial trading (agricultural products and livestock) adds up to the likelihood of transmission of newly emerged pathogens. In this study, the seroepidemiological status of some newly described viruses related to blood transfusion has been investigated in Iran.

Methods

A systematic review was constructed. For this review, data were obtained from published papers by a computerized search of all recorded English and Farsi literature during the years 2000 to 2011. Search in resources was performed through databases such as Medline, Scopus, Proquest, Iranmedex, and Magiran. The words used in the search were as follows: blood transfusion, Iran, emerging infections, specific viruses (HGV, TTV, HTLV-I/II, WNV, SEN-V). Furthermore, we searched for ongoing or completed studies on this issue in the documents of the Iranian Blood Transfusion Research Center.

Results

Overall, a total of 23 articles based on keywords used in connection with the emerging infectious agents in Iranian blood donors were identified. These studies were on viruses such as HGV, Torque Teno viruses (TTV), HTLV-I/II, West Nile virus (WNV), and SEN virus (SEN-V). The geographic distribution of some newly described viruses in Iranian blood donors is shown on map (fig. 1).

HGV

HGV, as non-A–E hepatitis, was discovered in 1996 by different groups of investigators. In the literature the virus was named HGV and GBV-C. Molecular specifications of these agents have shown them to be indeed identical isolates of the same virus [6, 7].

HGV is a single-stranded RNA virus belonging to the Flaviviridae family. According to striking reasons, Stapleton et al. [8] proposed to assign GBV-C/HGV as species within a new genus. HGV RNA is detected by using a reverse transcriptase polymerase chain reaction assay. The antibody to envelope

Table 1. Prevalence of HGV/GBV-C Anti E2 and RNA among Iranian voluntary blood donors

Reference	City	Total number tested	Anti E2 n (%)	HGV RNA n (%)
Ramezani et al. [13]	Tabriz	478	5 (1)	0 (0)
Gharehbaghian et al. [14]	Tehran	330	14 (14.2)	–
Amini Kafi-Abad et al. [15]	Tehran	100	–	1 (1)
Rezvan et al. [16]	Tehran	514	44 (8.6)	–
Salehi et al. [17]	Isfahan	40	2 85)	–
Amini Kafi-Abad et al. [18]	Tehran	400	–	19 (4.8)
Total		1,862	65 (4.8)	20 (2)

Table 2. Reported prevalence of HGV/GBV-C infection in healthy blood donors in some published studies

Reference	Country	Total number tested	Anti E2 n (%)	HGV RNA n (%)
Desai et al. [19]	India	120	–	0 (0)
Minton et al. [20]	UK	100	–	1 (1)
Saitoh et al. [21]	Japan	157	4 (2.5)	2 (1.3)
Roth et al. [22]	Germany	1,408	–	14 (1.34)
Alter et al. [23]	USA	500	–	7 (1.4)
Seifried et al. [24]	Germany	5,733	–	90 (1.6)
Hanci et al. [25]	Turkey	100	3 (3)	2 (2)
Blair et al. [26]	Scotland	1,020	–	23 (2.25)
Nordbo et al. [27]	Norway	1,001	105 (10.5)	25 (2.5)
Handajani et al. [28]	Indonesia	150	–	4 (2.7)
Mercier et al. [29]	France	2,739	–	93 (3.4)
Yu et al. [30]	Taiwan	500	36 (7.2)	17 (3.4)
Björkman et al. [31]	Sweden	458	62 (13.6)	18 (3.9)
Kar et al. [32]	India	50	–	2 (4)
Loiseau et al. [33]	France	500	–	21 (4.2)
Mastouri et al. [34]	Tunisia	600	–	32 (5.3)
Oliveira et al. [35]	Central Brazil	241	–	17 (7.1)
Yan et al. [36]	China	203	–	31 (15.8)
Mastouri et al.[34]	Tunisia	912	44 (4.9)	–
Mercier et al. [29]	France	2,219	210 (10)	–

protein E2 of this virus (anti-E2) is available. Anti-E2 is a marker for previous HGV infection and is useful for seroepidemiological studies. It has been demonstrated that GBV-C/HGV has worldwide distribution, and type 2 is highly prevalent in many countries [9]. The clinical significance of HGV infection with respect to acute or chronic hepatitis is not well understood. The available data suggest that HGV does not cause acute non-A–E viral hepatitis, has no pathologic effects on the liver, and is not a significant cause of fulminant hepatitis [10–12].

Epidemiology of HGV in Iranian blood donors has been investigated by several authors (table 1). All of these studies have been done on voluntary blood donors.

Laboratory studies have been conducted through anti-E2 and HGV RNA detection. Based on these studies, the prevalence of HGV anti-E2 varies from 1 to 8.6%. Overall, the average HGV antibody prevalence in voluntary blood donors is 4.8%. The lowest prevalence was reported in Tabriz and the highest in Tehran (fig. 1). All these cities are among the country's big cities. These studies have also investigated the frequency of viral RNA in apparently healthy donors. The prevalence of HGV RNA in under study donors varies from 0 to 4.8%. The average GBV-C/HGV RNA prevalence rate is 2%.

Epidemiological investigations in healthy blood donors in some other areas are shown in table 2. GBV-C/HGV RNA prevalence rates range from 0 to 4% in India, UK, Japan, USA, Germany, Turkey, Scotland, Norway, Indonesia, France, Taiwan, and Sweden. It has also been reported at varying ranges (5–15.8%) in Tunisia, Central Brazil, and China (table 2). The 2% prevalence of GBV-C/HGV RNA and the 4.8% prevalence of GBV-C/HGV antibody (anti-2) in Iranian blood donors are the same as those in healthy blood donors in many countries around the world.

HGV transmission by blood transfusion has been conclusively recognized, but its role in causing hepatitis is not well understood. Stapleton et al. [10] showed that the viral replication in peripheral blood cells occurs but replication of HGV in liver cells has not been observed.

Because GBV-C/HGV does not cause any significant diseases, currently blood products are not screened for this

virus. However, further studies need to be done to take decisions about the necessity of screening on blood and blood products.

TTV

Transfusion-transmitted virus or TTV was identified in Japanese patients (TT for the initials of the patient in whom the virus was isolated for the first time) in 1997 [37]. TTV is a non-enveloped single-stranded DNA virus. Currently, the International Committee on Taxonomy of Viruses classified TTV into genus Anellovirus [38]. The virus was first identified in humans; however, recent data showed that these viruses are prevalent in non-human primates and mammalians such as chimpanzees, cats, dogs, and pigs [39].

TTV DNA has been detected in blood and body fluids such as semen, saliva, vaginal secretion, breast milk, and tears, and it can be transmitted through parenteral, sexual, and mother-to-child routes [40–42]. Transmission by transfusion of blood and blood products is an identified way of transmission.

TTV DNA is detected by using a reverse transcriptase polymerase chain reaction assay. However, because of genetic variability of the virus and technical aspects, the results of PCR assays can be different [43].

Epidemiology of TTV in Iranian voluntary healthy blood donors has been investigated by several authors. Based on these studies, the prevalence of TTV DNA varies from 2.7 to 79.5% (table 3). All, except two, of these studies used the primers NG059, NG063, and NG061. Bouzari et al. [48, 49] used the primers from the untranslated region (T801, T935) in their studies. The average TTV DNA prevalence rate in voluntary blood donors is 28.5%. Prevalence of TTV in healthy blood donors in some other areas are shown in table 4. The reported prevalence rates of TTV infection in healthy blood donors in different countries range from 1 to 75%.

TTV infection in blood donors is common around the world. On the other hand, the cases of transmission through blood transfusion have been proven. However, the ability of TTV to induce primary hepatitis, its effect on severity of hepatitis due to other viruses, and its pathogenicity for acute or chronic liver disease are controversial [51, 60, 61]. Due to the above-mentioned reasons, currently blood products are not screened for this virus.

HTLV

HTLV-I/II is a single-stranded RNA virus belonging to Retroviridae family. HTLV-I was identified in 1979 and was reported on in 1980 [62]. This virus is endemic in many geographic regions around the world such as parts of Africa, South America, and southwestern Japan. Currently, approximately 10–20 million people around the world are infected [63]. The virus is also endemic in some geographical regions of Iran such as Khorasan provinces (fig. 1). The first report of a serological survey of the virus in Iran showed a prevalence rate of 2.3% in Mashhad blood donors [64].

Diagnostic methods have relied upon serological screening by using an enzyme-linked immunoassay. If the result is positive, as a confirmatory testing a Western blot analysis or a PCR assay may be carried out. Since the available diagnostic kits cannot clearly distinguish these two types of viruses, they may be reported as HTLV-I/II.

There are several known modes of transmission, including mother to child, mainly through breastfeeding, sexual contact, and parenteral transmission by blood transfusion, or by sharing of needles and syringes.

HTLV-I is associated with several diseases such as adult T-cell leukemia/lymphoma and HTLV-I-associated myelopathy/tropical spastic paraparesis. Moreover; this virus is likely

Table 3. Prevalence of TTV among Iranian voluntary blood donors

Reference	City	Total number tested	TTV DNA n (%)
Khoshbaten et al. [44]	Tabriz	407	11 (2.7)
Pourshams et al. [45]	Tehran	312	70 (22.4)
Jalali Far et al. [46]	Ahwaz	253	60 (23.7)
Zandie et al. [47]	Tehran	250	103 (41)
Bouzari et al. [48]	Tabriz	100	65 (65)
Bouzari et al. [49]	Isfahan	132	105 (79.5)
Total		1,454	414 (28.5)

Table 4. Reported prevalence of TTV infection in healthy blood donors in some published studies

Reference	Country	Total number tested	TTV DNA n (%)
Charlton et al. [50]	USA	100	1 (1)
Werno et al. [51]	New Zealand	413	13 (3.15)
Handa et al. [52]	USA	250	21 (8. 4)
Ho et al. [53]	Taiwan	244	29 (11.9)
de Castro Amarante et al. [54]	Brazil	270	32 (11.9)
Nakano et al.[55]	Korea	100	14 (14)
Kalkan et al. [56]	Turkey	125	21 (16.8)
Urwijitaroon et al. [57]	Northeast Thailand	101	28 (28)
Hashish et al. [58]	Egypt	95	46 (48.4)
Alfaresi et al. [59]	United Arab Emirates	100	75 (75)

Table 5. Prevalence of HTLV-1 among Iranian voluntary blood donors

Reference	City	Total number tested	HTLV-1 n (%)
Ghafouri et al. [65]	South Khorasan	42,652	18 (0,042)
Rezvan et al. [66]	multicenter	15,866	47 (0.29)
Khameneh et al. [67]	Urmia	2,046	7 (0.34)
Tarhini et al. [68]	Mashhad	232,648	1,054 (0.45)
Karimi et al. [69]	Charmahal-Bakhtiari	800	5 (0.62)
Abbaszadegan et al. [70]	Mashhad	28,487	219 (0.77)
Farid et al. [64]	Mashhad	1,511	35 (2.3)

linked to diseases such as uveitis, infective dermatitis, synovitis, and thyroiditis.

Due to complications attributed to this virus, its transmission through blood transfusion, especially in patients undergoing repeated blood transfusions, is an important issue. In this study 9 authoritative articles were identified related to the prevalence of HTLV-I/II in Iranian blood donors. There are 2 articles in Farsi language about healthy blood donors that are related to prevalence of HTLV-I which are searchable by using Farsi keywords only. These studies have been conducted in regions such as Bushehr and Hormozgan. In these areas, the frequency rate was 0.013% (3/22,740) and 0.18% (2/1,100), respectively. In addition, we found 7 articles in English language related to the frequency of virus in blood donors (table 5). In these studies (Farsi and English), laboratory screening tests have been conducted on 347,850 healthy blood donors, with HTLV-I/II antibody being confirmed in 2,784 donors. Based on these studies, the prevalence rate of HTLV-I/II varies from 0.013 to 2.3%. The lowest prevalence rate was reported in Bushehr and the highest in Mashhad. Differences in diagnostic kits, geographical conditions, and demographic status of the subjects can affect the results of studies in different parts of the country. The seroprevalence rates of HTLV infections in blood donors in some countries are as follows: 0% in Turkey (Izmir) [71] and Lebanon [72], 0.005% in the UK [73] , 0.002% in Sweden [74], 0.01–0.03% in the USA and Canada, 0.002% in Norway, 0.0056% in Greece [75], 1.9% in India [76], and 0.08–8% in Japan [77]. Blood banks of some countries, such as those of the UK, the USA, Canada, and Japan, routinely screen all blood donations for HTLV-I/II.

Khorasan provinces (Khorasan Razavi, Southern Khorasan, and Northern Khorasan) located in the north-east region of Iran are known as endemic areas (fig. 1). The town of Mashad as a religious center hosts people both as residents and pilgrims with different economic, social, cultural, and health backgrounds. For this reason laboratory screening has started routinely since 1995 in these regions [78]. Dispersion of the virus has been reported in other areas among donors. But given the fact that data from blood donors should not be generalized to other groups for making decision to perform routine screening tests in other parts of the country, there is a need for more evidence with regard to the prevalence of the virus in the general population.

WNV

WNV is a single-stranded RNA virus belonging to the family Flaviviridae. The virus was first isolated in the West Nile district of Uganda in 1937. Distribution of WNV has been reported from many geographic locations in Africa, Europe, Asia, and America [79].

The virus is transmitted to humans by mosquitoes. Different species of mosquitoes and different species of birds as a reservoir of the virus are involved in the transmission of the virus [80, 81].

Presence of birds as the reservoir and of mosquitoes capable of transmitting the virus has been reported in various parts of Iran [82]. Studies also indicated that there is seropositivity in some parts of the country in the general population. A seroepidemiological survey in preschool children from the north of Iran (Caspian Sea) showed a 10% prevalence rate [83]. In another study, a seroprevalence rate of 26.6% (186/698) has been reported in the general population in some provinces, with a higher rate in central and southwestern parts [84]. Clinical manifestation of infection with this virus ranges from an asymptomatic infection to severe central nervous system involvement. In mild and moderate forms, sign and symptoms such as fever, headaches, malaise, muscle and joint pains, lymphadenopathy, and skin rash occur. Meningoencephalitis as a lethal complication is located at the end of the clinical manifestation spectrum [85].

Subsequent to the clinical findings related to WNV, including fever and meningoencephalitis in four cases of organ transplantation, investigations related to the possibility of virus transmission through blood transfusion were done. Finally, 21 cases of posttransfusion WNV was confirmed by the Centers of Disease Control and Prevention in 2002, and WNV was recognized as an emerging pathogen transmitted through blood transfusion [85].

Different prevalence rates of the virus in blood donors from different countries have been reported.

Frequencies of WNV RNA in the USA were 1.49 and 0.44 per 10,000 donations in 2003 and 2004, respectively [86]. Prevalence of anti-WNV antibodies was 6.8 per 1,000 sera and for WNV RNA 17.5 per 100,000 donations among blood donors in north-east Italy [87]. Moreover, 0.56% of blood donors in Central Anatolia (Turkey) were WNV-seropositive [88], but 0% (0/500) in the United Arab Emirates [89].

Because of the clinical importance of this virus and reported cases of WNV transmission through blood transfusion, in some countries, routine screening is done at present [90].

Only one study was performed in Iran to evaluate the frequency of WNV in blood donors. This study, which was conducted on 500 healthy blood donors in Tehran, showed that all donors were negative for WNV-specific IgM antibody and WNV RNA. 5% (25\500) were positive for WNV-IgG antibody [91]. It is necessary to emphasize that this study was performed on Tehran blood donors. Therefore, it could not be generalized to all geographic areas, and more comprehensive studies should be conducted.

SEN-V

SEN-V (SEN for the initials of the patient in whom the virus was isolated for the first time) is a non-enveloped and single-stranded circular DNA virus belonging to the family of TTV-related viruses. To date, phylogenetic analysis shows nine SEN-V isolates, named SEN-V-A–I [92]. SEN-V is endemic throughout the world, but its prevalence differs by geographic region. The prevalence rates in healthy persons in the USA, Taiwan, Thailand, Greece and Italy have been reported to be 1.8%, 15%, 5%, 24% and 13%, respectively; moreover, prevalence rates of 8–17% and of 10–22% have been reported in Germany and Japan, respectively [93].

SEN-V is usually detected by using a reverse transcriptase PCR assay. With respect to primers used, the sensitivity of this test can be different. This difference in sensitivity might be an explanation for the differences in the reported prevalences of the SEN-V [94].

SEN-V transmission occurs through parenteral and non-parenteral routes. In fact, transmission in intravenous drug users as well as via iatrogenic, vertical transmission and blood transfusion has been reported [94].

In order to investigate the epidemiology of this virus, three different studies have been conducted on blood donors in Iran. Prevalence rates of SEN-V in Iranian blood donors range from 23% (60/260) [95] over 23.33% (14/60) [96] to 90.8% (109/120) [97]. These studies have been conducted in three different cities (fig. 1). The higher frequency belongs to Rasht in the northern Iran. This significant difference might be related to laboratory methods used or geographical differences.

Umemura et al. [98] strongly suggest that SEN-V is transmitted through blood transfusions, and they showed that acute infection with SEN-V transmitted by blood transfusion can lead to hepatitis. In addition, there is a relation between the number of blood units transfused and SEN-V infection [98]. On the other hand, Shibata et al. [99] did not find any significant association with SEN-V as a causative agent of non A–C hepatitis or liver cirrhosis.

In conclusion, despite the high prevalence of SEN-V in healthy blood donors, and given these controversies and the lack of clinical significance of infection with the virus, screening tests are not performed in blood centers.

Conclusion

The safety of blood supply is a multifactorial process. Donor screening and testing are two mainstays in blood processing centers. With respect to all measures taken, the blood supply is safer than any time, and in comparison to other medical interventions the risks associated with blood transfusion are extremely low. Due to the nature of the factors associated with emerging infections, identification of the epidemic and pandemic status of newly recognized or emerging blood-borne infectious agents in a community is an important issue.

Out of the viruses studied in the present research, HTLV and WNV are known factors threatening health of blood recipients. Considering the epidemiological map of the viruses in Iran, HTLV is currently the greatest concern. Routine screening tests are being conducted in a few provinces with high prevalence. But if a prospective comprehensive epidemiological study suggests a countrywide screening test, test sensitivity and specificity might be challenging and also government funds will be necessary.

As far as other emerging or re-emerging pathogens are concerned, it is imperative for another comprehensive epidemiological study to be conducted on the basis of newly formulated strategies.

It is worthwhile to apply pathogen inactivation methods, use leukocyte reduction techniques, establish hemovigilance system, and implement look-back programs to aim at ensuring blood safety and reducing the risk of transmission of emerging infections down to near zero.

Disclosure Statement

The authors declared no conflict of interest.

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