index case. The occurrence of new cases in the initially affected area started to decrease a few days after vector control measures were first implemented. The infection seemed to spread both by contiguity within the initially affected villages and by jumping from place to place within and from the initial outbreak area to the other locations. A small cluster, caused by local transmission, was reported in the town of Cervia, where the infection was probably introduced through population movement from Castiglione, although passive transport of infected mosquitoes cannot be ruled out completely.

The attack rate in Castiglione di Cervia—the most affected village—was 5·4%, much lower than the 34% reported in La Réunion.[4] This difference might be due to early intervention in Italy, although the role of different background vector density or climate-dependent vector behaviour cannot be excluded. Moreover, we cannot rule out under-reporting, which could have occurred if our surveillance system had a low sensitivity in the first month or if there was an excess of asymptomatic cases compared with those found in La Réunion.[23] The attack rates by sex and age, calculated for Castiglione di Cervia and Castiglione di Ravenna, were stably low for people under 40 years of age but tended to increase for older ages, with the highest rates in the oldest group. Whether this trend was due to behavioural factors leading to differential exposure to mosquitoes or to biological factors, implying a different host response with a different proportion of asymptomatic cases, needs to be investigated further.

The clinical course of the disease was fairly mild. The case-fatality rate was less than 0·5%, consistent with the rate of one death per 1000 clinical cases reported in La Réunion.[4] Almost all patients reported joint pain, which was often severe and persistent, and which seems to be strongly indicative of CHIKV disease. Similar findings were reported in La Réunion,[24] whereas a lower proportion of cases with joint pain (78%) was found in Malaysia in 1998.[25] About half the patients presented with skin rash, similar to previous findings.[2]

85% of the cases were confirmed by either serology or PCR. No viral sequences were detected in 31 samples collected more than 7 days after the onset of symptoms, suggesting that the viraemic phase is fairly short, as found in previous reports.[26]

Measures for controlling the population of *A albopictus* were implemented in all areas where cases were reported, beginning on Aug 18. These measures included the use of fast-acting insecticides (synergised pyrethrins) for 3 days consecutively, applied with a truck-mounted atomiser in public spaces and a backpack mist blower in private spaces. Antilarval measures, using formulations of insect growth regulators and *Bacillus thuringiensis* var *israeliensis* were also implemented. House-to-house interventions were done to eliminate breeding places, and community participation was encouraged. For each suspected case of infection, these control measures were done within a radius of 100 m of the individual's residence; for clusters, the control measures were done within a 300-m radius of the most external case. Since Sept 27, 2007—the date at which the present analysis was censored—sporadic cases have continued to occur in Ravenna; two small clusters outside Ravenna (in Cesena and Rimini) have also been identified. Whether transovarial transmission of CHIKV might result in a reappearance of the infection in spring, 2008, is being considered carefully.

The occurrence of an outbreak of CHIKV infection in a country with a temperate climate emphasises that the predicted globalisation of human beings and vectors[4,7] has become a reality. To promptly identify new potential threats that were previously restricted to tropical areas, clinical and diagnostic capacities have to be developed in countries with a temperate climate and in which vectors of exotic diseases already circulate.

Contributors

GR was responsible for the clinical and epidemiological investigation and for writing the manuscript. LN was responsible for laboratory diagnosis and contributed to writing the manuscript. CF, FM, and MGC did laboratory tests on human and mosquito samples, and phylogenetic analysis; PC and MD identified viral sequences in the mosquitoes. MP developed the PCR used in this investigation and contributed to writing the manuscript. RR, GM, and PA were responsible for the entomological investigation and contributed to writing the manuscript. ACF supervised the field activities that were implemented by RA and GS, who also contributed to data analysis. SB was responsible for data management and analysis. AC supervised and coordinated all of the activities and revised the manuscript.

CHIKV study group

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Conflict of interest statement

We declare that we have no conflict of interest.

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No. 8

医薬品 研究報告 調査報告書

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研究報告の概要

○輸血によるシャーガス病:感染受血者および供血者の寄生虫学的、血清学的モニタリング

シャーガス病はラテンアメリカの風土病であるが、人の移動により分布が拡大している。スペインでは、2005年からラテンアメリカ出身の供血者に対して*T. cruzi*抗体検査を実施している。

本報は、血液製剤の輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査の報告である。患者は25歳男性で白血病の既往があり、少なくとも176名の供血者由来の血液製剤を輸血されていた。2005年1月(輸血後45日)に原因不明の発熱を発症し、抗菌薬による治療を行った。臍帯血移植後も発熱と神経障害を発症し、多臓器不全で7月上旬に死亡した(輸血後212日)。患者血清中に*T. cruzi* DNAがPCRで確認された。過去の検体を調べたところ、輸血後48日にはDNAが検出されていた。抗体はIFATとELISAで輸血後159日で陽性になり、204日で陰性化していた。輸血された製剤の供血者の血清学検査では、58歳のブラジル出身の女性供血者が抗体陽性であったことが判明した。彼女は2004年12月上旬に供血を行い、血小板製剤が患者に輸血されていた。追加調査時のPCRでは、血中に寄生虫は検出されなかったが、1ヵ月後シャーガス病の精密検査を行った際の血液からはPCRで検出された。

抗体価の動態から、患者はシャーガス病の急性期であったことが示唆された。移植のための免疫抑制状態で、寄生虫が血液脳関門を通過して神経系に感染したことが、CSF検体中の*T. cruzi* DNAから確認された。供血者は無症候の状態であったことから、患者の免疫状態が発症に関連したことが考えられる。複数回輸血患者は、免疫抑制剤治療実施前に、抗*T. cruzi*抗体のスクリーニングを受けるべきである。

報告企業の意見	今後の対応
輸血によりシャーガス病に感染し、死亡したスペイン人患者の寄生虫学的、血清学的疾患経過、ならびに供血者の調査についての報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、国と協議しつつ対応を検討中である。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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BRIEF REPORT

Transfusional Chagas Disease: Parasitological and Serological Monitoring of an Infected Recipient and Blood Donor

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Chagas disease is endemic to Latin America, but human migration is extending its distribution. This report describes the parasitological and serological course of disease in a Spanish patient fatally infected via a blood product transfusion, as well as the monitoring of the donor. Before undergoing immunosuppression, multitransfused patients should be screened for anti–*Trypanosoma cruzi* antibodies.

Chagas disease, or American trypanosomiasis, is endemic to Latin America. However, the recent changes in human patterns of migration have prompted the appearance of cases in areas where the vector of the disease is not found [1, 2]. The natural progress of infection involves an acute and a chronic phase. In areas of endemicity both forms are seen, whereas in nonendemic areas, the great majority of infections are diagnosed in the chronic phase, although 70% of infected persons remain asymptomatic. Despite technological advances, there is no reference standard laboratory technique for diagnosing Chagas disease [3]. In the acute phase, parasitological diagnostic methods are the most reliable. However, in the chronic, phase there may be little or no parasitemia, and diagnosis is made mainly on the basis of results of tests for anti–*Trypanosoma cruzi* antibodies. In recent years, Spain has become one of the favorite destination countries for South American emigrants. These citizens achieve a good degree of social integration in Spain, and they often voluntarily and altruistically support blood donation programs. Thus, since 2005, Spanish blood donation legislation has required donors from Latin America to be serologically screened for anti–*T. cruzi* antibodies (Royal Decree 1088/2005) [4]. The present work describes the retrospective laboratory evaluation of a Spanish patient with leukemia who died of Chagas disease contracted via a transfusion with contaminated blood, the retrospective study to identify the source of infection, and the monitoring of the donor.

Methods and materials. Anti–*T. cruzi* antibodies were sought in serum samples collected at different times before the patient's death; these samples were stored at −80°C in the serum library of the Centro Nacional de Microbiología (National Microbiology Center [Madrid]). Parasite DNA was also sought in these samples, in CSF (also collected before death), and in lung, kidney, and liver necropsy samples.

For the retrospective study, serum samples of 176 donors whose blood derivatives had been transfused into the patient were examined. Of these, 168 lived in Madrid (159 were of Spanish origin, 1 was Brazilian, 1 was Ecuadorian, 2 were Colombian, 3 were French, 1 was Polish, and 1 was German), 5 lived in Albacete (southeastern Spain), and 3 lived in Jaén (southern Spain). Samples belonging to all of the Madrid donors were preserved at the serum library of the Centro de Transfusión de Madrid (Madrid Transfusion Center); new samples were collected from the donors living in Albacete and Jaén once they had been traced. Serum and blood samples were collected from the infected blood donor to confirm the results of the retrospective study and to monitor the development of the infection after treatment.

Anti–*T. cruzi* antibodies were detected by the indirect immunofluorescent antibody test (IFAT) and by ELISA with modifications introduced by the Department of Parasitology at the Centro Nacional de Microbiología [5, 6]. *T. cruzi* DNA was detected by PCR with use of oligonucleotides 121–122 and Tcz1–Tcz2, which amplify the variable region of the kinetoplast DNA minicircle (330 bp) and a repetitive sequence of satellite DNA (195 bp), respectively [7, 8]. All assays were performed in duplicate with negative and positive controls.

Results. The Spanish patient was a 25–year-old man who had a history of leukemia [9] that eventually required a cord blood transplant; he received blood derivatives from at least 176 persons who donated blood at different transfusion centers. In January 2005, 45 days after infection onset, the patient was examined for fever of unknown origin. None of the infectious agents that commonly cause this problem in this kind of patient

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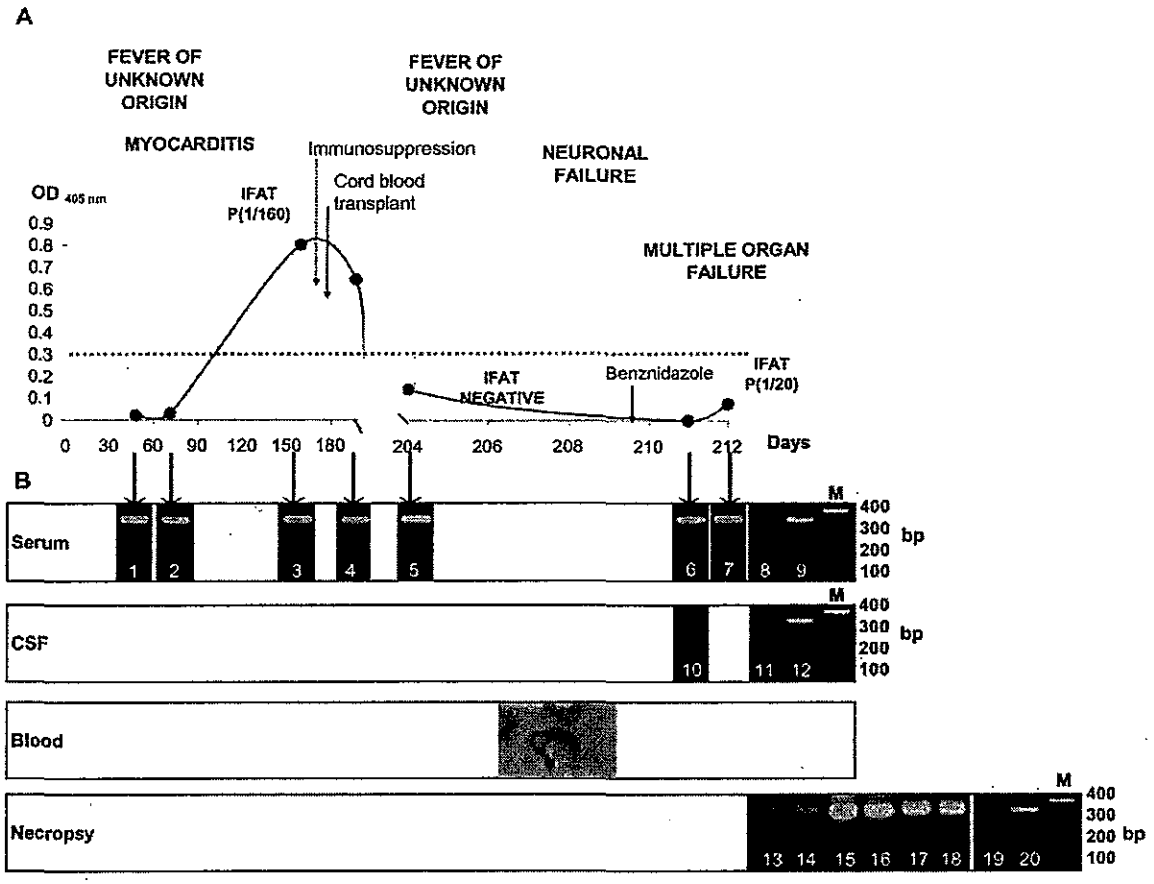

Figure 1. Parasitological and serological changes according to the clinical status of the patient. The day the patient received the platelet concentrate was defined as day 0. *A,* Changes in anti–*Trypanosoma cruzi* antibody levels according to indirect immunofluorescent antibody test (IFAT) and ELISA. *The last serum dilution with a positive (P) reaction is shown. B, Presence of parasites as determined by microscopy and PCR. Lanes 8, 11, and 19,* Negative controls. *Lanes 9, 12,* and *20,* Positive controls. *Lanes 13–14, 15–16,*and *17–18,* Duplicate samples of *T. cruzi* DNA amplified from kidney, liver, and lung tissues, respectively. The PCR results obtained using the oligonucleotides 121–122 confirmed those obtained with Tcz1–Tcz2. *Dotted line,* Threshold; OD, optical density.

(lymphotropic viruses, exanthema-causing viruses, adenoviruses, influenza virus, *Mycoplasma pneumoniae,* or *Toxoplasma gondii,* among others) were detected. After treatment with itraconazole, the symptoms receded, and the patient was assessed and treated in preparation for cord blood transplantation as described by Forés et al. [9]. In the first week of July 2005 (day 211 after infection onset), the Department of Parasitology at the Centro Nacional de Microbiología received several serum and CSF samples obtained from the patient, as well as the supernatants of cell cultures used in the identification of flagellates by microscopy and in diagnostic tests. Microscopy revealed the presence of trypomastigotes, and PCR identified DNA of *T. cruzi,* indicating infection by this pathogen. Tests for anti–*T. cruzi* antibodies, however, yielded negative results.

The patient died of multiorgan failure (day 212 after infection onset), and a retrospective evaluation was undertaken to determine the source of infection. Patient serum samples that were sent to the Centro Nacional de Microbiología for the diagnosis of problems other than Chagas disease and that were preserved at our center's serum library were analyzed by IFAT, ELISA, and PCR (figure 1). PCR showed *T. cruzi* to have first appeared in the patient's serum 48 days after he received a transfusion of platelets. IFAT and ELISA confirmed positive seroconversion on day 159 after infection onset, followed by a negative seroconversion on day 204 after infection onset.

At the same time, the donors whose blood products had been given to the patient were screened for anti–*T. cruzi* antibodies (figure 2*A*). This analysis ruled out all of the donors from Albacete and Jaén and 167 of the donors from Madrid as potential sources of infection. IFAT and ELISA yielded positive results for the remaining Madrid-based donor. This person made a blood donation at the beginning of December 2004 (figure 2*B*); the patient received a concentrate of platelets prepared from this blood (day 0).

The donor was a 58–year-old woman originally from Alto Parnaiba, in the Brazilian state of Maranhão. She was asked to

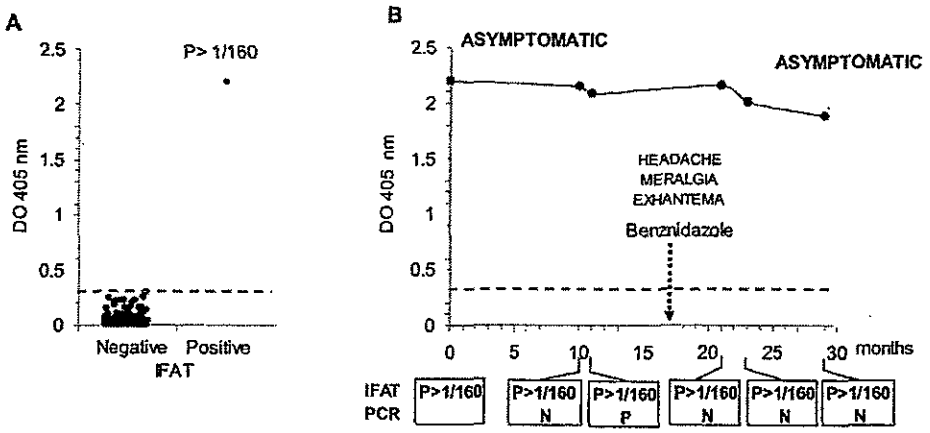

Figure 2. *A,* Determination of anti–*Trypanosoma cruzi* antibodies by indirect immunofluorescent antibody test (IFAT) and ELISA in serum of the different blood donors. The last serum dilution with a positive (P) reaction is shown. *B,* Serological and PCR monitoring of the infected donor. The month in which the infected donor made a blood donation was defined as month 0. *Dotted line,* Threshold. N, negative; OD, optical density.

attend an appointment to confirm the results obtained in the retrospective investigation. At that time, no parasites were detected in her blood by PCR. She was then referred to the Tropical Diseases Unit at the Hospital Carlos III in Madrid, where she underwent a clinical examination, chest radiography, electrocardiography, and echocardiography, all of which yielded normal results. No other signs or symptoms of interest were noted except for constipation, which the donor had experienced for some 8 years (defecation once every 2–3 days). On this occasion (1 month after the first appointment), however, PCR did detect parasites in the blood. In March 2006, treatment with benznidazole (6 mg/kg/day) was begun, but this was suspended after 24 days because of the appearance of intense headaches, meralgia paresthesica of the femorocutaneous nerve, and generalized macular exanthema. No hematologic toxicity was recorded. Following this treatment, test results for blood parasites remained negative, although anti–*T. cruzi* antibodies remained detectable (figure 2*B*).

Discussion. Figure 1 shows that anti–*T. cruzi* antibodies were detectable in the patient only before the start of the immunosuppressive protocol associated with the cord blood transplant (day 159 after infection onset). In the absence of an immune response, the parasites crossed the blood-brain barrier and infected the nervous system. This was confirmed by the presence of *T. cruzi* DNA in the CSF sample. Given the general condition of the patient, treatment with benznidazole had no immediate effect on the parasite load, although IFAT detected a slight increase in the antibody titer (1/20).

The detection of *T. cruzi* in the necropsy samples agrees with the systemic distribution of the parasite and the multiorgan failure that caused the patient's death. The kinetics of the antibody titer can be explained in terms of an acute, recently acquired infection. The detection of *T. cruzi* by PCR since January 2005 (day 48 after infection onset) agrees with the date when the patient received the infected blood products. Thus, the results of the parasitological and serological investigations agree with the patient's clinical signs and symptoms and suggest that he was in the acute phase of Chagas disease. Acute transfusional Chagas disease can last from 1 to 6 months after the entry of the parasite [3].

The discrepant PCR results (1 positive and 1 negative) obtained for the infected donor before benznidazole treatment was begun agree with the low-level parasitemia typical of the chronic phase of *T. cruzi* infection [10]. These results could also be because the first analysis involved a 5-mL blood sample and the second a 10-mL sample. When blood parasite concentrations are low, detection is more likely in larger blood volumes [11]. Similarly, at blood donation units, collecting as much as 450 mL of blood from donors increases the risk of contamination with small numbers of parasites.

Although, for successful blood culturing and artificial xenodiagnosis, it is recommended that blood samples be processed within 4 h of collection to ensure parasite viability [12]; in the present case, the parasites remained viable over the entire platelet conservation period, because the maintenance temperature (22°C; range, 20°C–24°C) is close to that used for culturing *T. cruzi* (25°C–27°C). The recipient's immunodepression caused by his leukemia and the immunosuppression induced before cord blood transplantation appear to have been of maximum importance in the development of the infection, because the parasite caused no appreciable symptoms in the donor. This highlights the role of the host immune system in protection from and the development of infection. In immunodepressed patients, infection may be severe and have fatal consequences. It is therefore recommended that higher-risk organ donors be screened for anti–*T. cruzi* antibodies, as should

multitransfused candidates for transplantation—irrespective of their origin—if they are to undergo immunosuppression protocols.

It should be stressed that before October 2005, Spanish blood donation legislation permanently excluded donors with Chagas disease. It did not, however, contemplate the use of a reliable screening test for the detection of healthy *T. cruzi* carriers. In the present case, the donor did not know of her trypanosome infection status, and no risks were detected during the predonation assessment interview. Her blood donation was therefore accepted in December 2004. In contrast, the current legislation (October 2005) outlines new technical requirements for blood donation [4] and establishes the use of a *T. cruzi* diagnostic assay to assess the eligibility of donors from areas where Chagas disease is endemic, as well as those with risk factors for infection. Under this legislation, the present donor would have been excluded.

In Spain, the supply of blood is a permanent problem, and the Latin American population—~1.5 million residents—has already became an important source of potential donors. A preliminary *T. cruzi* seroprevalence survey of immigrants from areas of endemicity returned positive estimates of close to 1% [13]. Because blood transfusion is the main route for *T. cruzi* transmission in Spain, the new legislation guarantees the quality of blood and blood component transfusions for recipients and allows the inclusion of immigrants from the Americas in the pool of potential blood donors.

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Potential conflicts of interest. All authors: no conflicts.

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