Evidence of Trypanosoma cruzi Infection (Chagas’ Disease) Among Patients Undergoing Cardiac Surgery

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Background—Trypanosoma cruzi, the agent of Chagas’ heart disease, is transmitted by triatomine insects and by blood transfusion. The emigration of several million people from T cruzi–endemic countries to the United States has raised concerns regarding a possible increase in cases of Chagas’ heart disease here, as well as an increased risk of transfusion-transmitted T cruzi. To investigate these 2 possible outcomes, we tested a repository of blood specimens from multiply transfused cardiac surgery patients for antibodies to T cruzi.

Methods and Results—Postoperative blood specimens from 11 430 cardiac surgery patients were tested by enzyme immunoassay, and if repeat-reactive, were confirmed by radioimmunoprecipitation. Six postoperative specimens (0.05%) were confirmed positive. Corresponding preoperative specimens, available for 4 of these patients, were also positive. The other 2 patients had undergone heart transplantations. Tissue samples from their excised hearts were tested for T cruzi by polymerase chain reaction and were positive. Despite the fact that several of these 6 patients had histories and clinical findings suggestive of Chagas’ disease, none of them were diagnosed with or tested for it. Patient demographics showed that 5 of 6 positive patients were Hispanic, and overall, 2.7% of Hispanic patients in the repository were positive.

Conclusions—No evidence for transfusion-transmitted T cruzi was found. All 6 seropositive patients apparently were infected with T cruzi before surgery; however, a diagnosis of Chagas’ disease was not known or even considered in any of these patients. Indeed, Chagas’ disease may be an underdiagnosed cause of cardiac disease in the United States, particularly among patients born in countries in which T cruzi is endemic. (Circulation. 2000;102:2978-2982.)

Key Words: Chagas’ disease ■ heart diseases ■ surgery ■ transfusion ■ Trypanosoma cruzi

Chagas’ heart disease (American trypanosomiasis) is associated with chronic infections by the protozoan parasite Trypanosoma cruzi. The parasite is endemic to Mexico, Central America, and South America, where it is transmitted primarily by triatomine insect vectors, also known as reduvid bugs. It is estimated that 16 to 18 million Latin Americans are infected with T cruzi. An estimated 10% to 30% of people harboring the parasite will develop symptomatic chronic Chagas’ disease years or decades later. Gross pathological changes, often affecting the heart, include biventricular enlargement, thinning of the ventricular walls, apical aneurysms, and mural thrombi. At a microscopic level, widespread lymphocytic infiltration, diffuse interstitial fibrosis, and atrophy of myocardial cells are often observed, but parasites are rarely seen in myocardial tissue from chronically infected patients. The conduction system also is often affected, typically resulting in right bundle-branch block, left anterior fascicular block, or complete atrioventricular block. Syncope, congestive heart failure, and symptoms of thromboembolism often occur as dysrythmias, and cardiomyopathies develop over time. Death usually results from rhythm disturbances or congestive heart failure.

Although Chagas’ heart disease remains a public health concern in T cruzi–endemic countries, it is generally thought to occur only rarely in the United States. However, during the past several decades, several million persons have emigrated to the United States from countries in which Chagas’ disease is endemic, and an estimated 50 000 to 100 000 or more of these immigrants harbor chronic, asymptomatic T cruzi infections. In view of this, it can be expected that patients with symptomatic Chagas’ heart disease will come to medical attention with increasing frequency in the United States. A
Serological Testing

The postoperative serum samples were tested for antibodies to T cruzi by enzyme immunoassay (EIA; Chagas Enzyme Immunoassay Generation 2.0, Abbott Laboratories) as described in the manufacturer’s product insert. If a sample was reactive initially, it was retested in duplicate and considered repeat-reactive if 1 or both of the 2 repeat tests were reactive. Samples that were initially nonreactive or those for which both of the repeat tests were nonreactive were considered nonreactive. All samples identified as repeat-reactive by EIA underwent confirmatory testing with a radioimmunoprecipitation assay (RIPA). Briefly, these samples were assayed in parallel with 3 negative and 3 positive control sera, the latter obtained from parasitologically confirmed cases of Chagas’ disease. Confirmation of seropositivity by RIPA was defined as the presence of immunoprecipitated bands in autoradiographs indicative of antibodies specific for the 72- and 90-kDa glycoproteins of T cruzi. Any specimen that was EIA repeat-reactive and RIPA-positive was considered a confirmed seropositive. All EIA and RIPA testing was done at the Holland Laboratory of the American Red Cross in Rockville, Md.

When a postoperative sample was confirmed as seropositive for T cruzi, the corresponding preoperative specimen was tested by EIA, and if repeat-reactive, was assayed by RIPA. Persons whose postoperative serum samples were confirmed as positive, regardless of test results for the preoperative sample, were notified via their attending physician, counseled, and referred to appropriate specialists for follow-up care whenever possible.

Polymerase Chain Reaction Testing

Preoperative samples were not available for 2 patients whose postoperative samples were confirmed as positive for T cruzi antibodies. Two patients, however, had undergone heart transplantation, and portions of their excised hearts had been formalin-fixed and embedded in paraffin blocks at the time of surgery. Paraffin was removed from microtome slices of these blocks with xylene, the remaining tissue fragments were sonicated, and DNA was extracted and amplified as described previously. Initially, 2 primers were used: TCZ1 and TCZ2, which specifically amplify a 188-nucleotide segment of the 195-nucleotide nuclear repetitive DNA sequence of T cruzi. The resulting reaction mixture was then used as template DNA in a nested polymerase chain reaction (PCR) using 2 additional primers, TCZ3 and TCZ4, which amplify a 149-nucleotide internal segment of the same repetitive sequence. As a positive control, DNA was extracted from the heart of a mouse infected with T cruzi, and DNA extracted from the lung of an uninfected rat was used as a negative control. To verify that inhibitors of the PCR were not introduced during formalin fixation or DNA extraction steps, aliquots from each sample were spiked with T cruzi DNA from cultured parasites and amplified in parallel with the other samples.

Statistical Analysis

The rate of T cruzi–positive subjects was calculated as the percentage positive by hospital and by ethnicity (ie, Hispanic patients and other patients). Because no transfusion-transmitted cases were detected, we estimated the upper 95% confidence limit for the risk of transfusion-transmitted T cruzi per unit transfused using exact binomial methods.

Results

The study was initiated and samples were collected during different time periods at the 3 study sites (Table 1). A total of 12 219 cardiac surgery patients were enrolled in the study, 9811 (80.3%) of whom received transfusions. The transfused patients received, in aggregate, a total of 127 035 blood components, which represents a mean of 13 components per patient.

Of the 12 219 patients enrolled in the study, postoperative samples from 11 430 were available for serological testing (Table 1). Twenty-three (0.20%) were repeatedly reactive by EIA, and 6 (0.05%) subsequently were confirmed as positive by RIPA; all 6 patients had received blood transfusions (Table 2). Preoperative samples were available for 4 of the 6 confirmed positive samples, and all 4 corresponding preoperative samples were confirmed as positive. As mentioned above, preoperative samples were unavailable for the 2 remaining postoperatively positive patients. However, pre-

large proportion of these cases, however, are likely to be misdiagnosed because of the generally low level of knowledge regarding Chagas’ disease among US physicians. Furthermore, immigration of T cruzi–infected Latin Americans has also raised concerns regarding the potential transmission of the parasite by blood transfusion. At present, blood screening for T cruzi has not been implemented in the United States, in part because no test for blood bank screening has been licensed by the US Food and Drug Administration. However, to date there have been only 4 published cases of transfusion-transmitted T cruzi in the United States. Despite recent seroprevalence studies demonstrating that up to 1 in 7000 blood donors from some locations have T cruzi antibodies that may be indicative of chronic infections.

In an attempt to assess the extent to which Chagas’ heart disease occurs and is recognized in the United States, we tested a repository of blood specimens from cardiac surgery patients for evidence of T cruzi infection. In addition, because cardiac surgery patients often receive multiple blood transfusions and thus are at increased risk for acquiring T cruzi by transfusion, we assessed the frequency with which blood transfusion resulted in transmission of the parasite.

Methods

Subjects and Samples

Adult patients, ≥18 years of age, undergoing cardiac surgery between 1985 and 1991 were recruited for participation in a study of infectious agents transmitted by blood transfusion at the Johns Hopkins Hospital, Baltimore, Md, and at St Luke’s Episcopal Hospital/Texas Heart Institute and the Methodist Hospital, both in Houston, Tex. Patients receiving only autologous transfusion, those having cardiac surgery not requiring transfusion, and those who were not residents of the United States were excluded from the study. Thus, eligible patients who underwent surgery that commonly required blood transfusion (eg, coronary artery bypass graft, aneurysm repair, valve replacement, or cardiac transplantation) were recruited for the study. In some cases, the surgery did not require blood transfusion as anticipated, and these otherwise eligible patients were retained as controls for the patients who did receive transfusions. For each patient, a serum sample was collected before surgery when transfusion might be required, and an abstract of medical information was completed. Data collected from the patient’s medical records included demographic data, hematology and blood chemistry results, the type and duration of the surgical procedure, the surgical outcome, and the number and type of the blood components transfused. A second serum sample was obtained from each consenting participant 6 to 9 months after surgery. Also at this latter time, patients were asked to complete a questionnaire that requested information about the interval medical history, history of rehospitalization, and interim blood transfusions. All serum samples were divided into aliquots and stored at −20°C. The repository contained samples only from cardiac surgery patients; no samples were collected from blood donors. The detailed methods used in this study have been reported previously.

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served tissues from hearts excised at surgery were tested by PCR for the presence of parasite DNA and in both cases were positive for a 149-bp product (Figure). Therefore, the observed incidence per unit transfused of transfusion-transmitted *T. cruzi* was 0%; however, the upper 95% confidence limit of the observed incidence was 0.0029%. The 6 confirmed positive patients underwent surgery for a variety of cardiac complications (Table 2), but a review of their medical abstracts did not indicate that a diagnosis of Chagas’ disease was considered in any of these patients.

Available demographic information indicated that 5 of the 6 confirmed positive patients were Hispanic, and at least 4 of these 5 positive patients had been born in and spent extensive periods of time in *T. cruzi*–endemic countries. Only patient 4, who was born in and lived in Texas, was non-Hispanic. Overall, 184 (1.5%) of the 12 219 patients enrolled in the study were Hispanic, and 2.7% of subjects in this group were seropositive for *T. cruzi*.

### Discussion

Chagas’ disease in the United States remains largely an issue of immigration, although rare reports of autochthonous and congenital transmission of the etiological agent *T. cruzi* have appeared. As mentioned previously, estimates indicate that 50 000 to 100 000 Latin American immigrants in the United States may be infected with *T. cruzi*. Indeed, the flow of immigrants from *T. cruzi*–endemic countries to the United States continues and may have increased recently following natural disasters such as Hurricane Mitch. The lifelong nature of *T. cruzi* infection increases the likelihood that infected persons may transmit the parasite to others via transfusion, and for pregnant women, by congenital transmission. Recent seroprevalence studies of US blood donors have determined that up to 1 in 7000 donors in selected locations is infected with *T. cruzi*. In almost all cases, the seropositive donors had been born in and spent extensive time in *T. cruzi*–endemic countries. One of these studies also identified several US-born seropositive donors who may have acquired the infection congenitally. As demonstrated in several earlier studies, people infected with *T. cruzi* can be found throughout the United States, and prevalence rates are directly related to the relative number of people with *T. cruzi* risk factors in a given area. One of the original goals of our study was to identify cases of transfusion-transmitted *T. cruzi*. Cardiac surgery patients generally receive multiple blood transfusions and thus have an increased likelihood of receiving *T. cruzi*–tainted blood or blood products. This pattern was borne out in the present study, because the cardiac patients who were transfused received on average 13 U of blood. All 6 patients identified as seropositive for *T. cruzi* had received multiple transfusions.
TABLE 2. Clinical Characteristics of Cardiac Surgery Patients Identified as Seropositive for Antibodies to T cruzi

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hospital</th>
<th>Preoperative Test Result</th>
<th>No. of Units Transfused</th>
<th>Diagnosis</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>JH</td>
<td>Positive</td>
<td>2</td>
<td>Severe 3-vessel disease, exertional angina</td>
<td>Triple coronary artery bypass graft</td>
</tr>
<tr>
<td>2*</td>
<td>M</td>
<td>Positive</td>
<td>64</td>
<td>Symptomatic thoracic aortic aneurysm Abdominal aortic aneurysm</td>
<td>Resection and graft replacement of aneurysms</td>
</tr>
<tr>
<td>3</td>
<td>SL</td>
<td>NA†</td>
<td>18</td>
<td>Congestive heart failure secondary to congestive cardiomyopathy</td>
<td>Heart transplant</td>
</tr>
<tr>
<td>4</td>
<td>SL</td>
<td>NA†</td>
<td>9</td>
<td>Ischemic cardiomyopathy, coronary heart disease with thrombus in apex of left ventricle</td>
<td>Heart transplant</td>
</tr>
<tr>
<td>5</td>
<td>SL</td>
<td>Positive</td>
<td>59</td>
<td>Aortic aneurysms</td>
<td>Aortic valve replacement</td>
</tr>
<tr>
<td>6</td>
<td>SL</td>
<td>Positive</td>
<td>6</td>
<td>3-Vessel disease with congestive heart failure</td>
<td>Triple coronary artery bypass graft</td>
</tr>
</tbody>
</table>

JH indicates Johns Hopkins; M, Methodist; SL, St Luke’s Episcopal; and NA, no sample available.

*Patient underwent 2 separate surgeries within 2 months.
†Identified as positive preoperatively by PCR (see text).

ranging from 2 to 64 U per recipient. Nonetheless, each of these 6 patients apparently had been infected with T cruzi before surgery and blood transfusion. Thus, although a high proportion of our study subjects received multiple transfusions, many presumably from donations collected in areas (ie, Houston, Baltimore) in which T cruzi–seropositive donors have been identified previously, we did not observe transmission of T cruzi by blood transfusion.

The observed absence of transfusion cases in this study may in part be attributed to the number and type of blood products transfused. To estimate the greatest potential number of infectious units transfused in this study, we made the assumption that the transfused components came from about 127,000 different donors. We previously estimated that 2.5% of donors nationwide are at risk for T cruzi infection (ie, were born or resided in an endemic country). Thus, the components transfused in this study may have come from 3175 at-risk donors. On the basis of a confirmed seropositive rate of 1 in 725 for at-risk donors, one could reasonably estimate that about 4 components transfused in the present study came from T cruzi–infected donors. Published reports suggest that 12% to 53% of donations from seropositive donors transmit infection, but because of the relatively low number of infectious blood components calculated to be involved in this study, the absence of demonstrable transmission was not surprising. Moreover, the observation that study subjects were transfused with fewer platelet units than red cells is also significant, because platelet transfusions have been implicated as a risk factor for transfusion-acquired T cruzi.

The observation that a diagnosis of Chagas’ disease was not established or even considered in any of the 6 patients found to be seropositive in our study was unexpected. Two of the patients (patients 3 and 4) underwent cardiac transplantation, and both were diagnosed with congestive heart failure accompanied by arrhythmias and cardiomyopathy, findings frequently associated with Chagas’ heart disease. Moreover, 1 of the patients (patient 3) was a 55-year-old Hispanic male born in Honduras, a country in which T cruzi is endemic. In patients such as these, a diagnosis of Chagas’ disease before surgery can be invaluable for planning prudent preoperative and postoperative care, including prophylaxis for potential recrudescence of T cruzi infection. Although prophylactic antiparasitic therapy is often beneficial, several reports have described reactivation of T cruzi infections in patients receiving immunosuppressive drugs to prevent rejection of transplanted hearts. Indeed, several months after surgery, 1 of the heart transplant recipients in the present study (patient 3) who was receiving long-term cyclosporine treatment developed a cutaneous T cruzi infection that was consistent with reactivated Chagas’ disease. Although it has been routinely suggested that the long-term viability of transplanted hearts may be compromised by the presence of an existing T cruzi infection, recent clinical approaches, including reduced levels of immunosuppressive drugs, may enhance the survival of the transplanted heart and the patient. In contrast, others have raised ethical questions, including whether, in light of persistent shortages of organs available for transplantation, transplanting hearts into patients infected with T cruzi should be avoided. Obviously, implementation of such a policy would require that 2-stage serological testing, such as that we performed, be done before surgery.

In addition to the heart transplantation patients, ≈2 of the other patients (patients 1 and 6) had medical histories that may have been suggestive of Chagas’ heart disease. Patient 1 had a strong family history of heart disease, with his father and 2 brothers dying of sudden death in their 50s. Patient 6 presented with coronary artery disease and congestive heart failure that required bypass surgery. Again, testing for Chagas’ disease before surgery would have aided the physicians in planning preoperative and postoperative care. Recent recommendations by a Latin American panel of experts convened by the WHO suggest that all T cruzi–positive persons be treated for Chagas’ disease regardless of the duration of infection and clinical status. Testing all patients undergoing cardiac surgery, however, may not be the most practical approach, given the infrequency of T cruzi infection...
in the United States. A better method would be to test all cardiac patients with risk factors for \textit{T. cruzi} infection. It has generally been thought that blood donors at risk for \textit{T. cruzi} infection are those who were born in or have resided in an endemic country who also give histories of exposure to insect vectors or residence in substandard housing in rural areas. A recent study of US blood donors, however, revealed that the only risk factor that reliably identified \textit{T. cruzi}–infected people was birth in and/or extended time spent in an endemic country. In the present study, 4 seropositive patients fulfilled this criterion for risk, and 5 identified themselves as Hispanic. The only non-Hispanic, seropositive patient (patient 4) resided in Corpus Christi, Tex, where he worked as a self-employed, consulting geologist. Because several cases of autochthonous \textit{T. cruzi} have been reported in that part of Texas, it is possible that this patient may have been infected during one of his frequent field trips. Overall, 2.7% of the Hispanics enrolled in this cardiac surgery study were infected with \textit{T. cruzi}. This figure, however, should be considered a conservative estimate, because the subjects in the present study were limited to US residents. Thus, in view of this and given the usefulness of knowing that a person is infected with \textit{T. cruzi}, we recommend that all Hispanics with cardiac disease who are at risk for \textit{T. cruzi} infection by birth or residence in \textit{T. cruzi}–endemic countries be tested for specific antibodies to the parasite.

Acknowledgments

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