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Brief Communication

Early Molecular Diagnosis of Acute Chagas Disease After Transplantation With Organs From *Trypanosoma cruzi* Infected Donors

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Organ transplantation (TX) is a novel transmission modality of Chagas disease. The results of molecular diagnosis and characterization of *Trypanosoma cruzi* acute infection in *naïve* TX recipients transplanted with organs from infected deceased donors are reported. Peripheral blood and cerebrospinal fluid samples from the TX recipients of organs from infected donors were prospectively and sequentially studied for detection of *T. cruzi* by means of kinetoplastid DNA polymerase chain reaction (kDNA-PCR). In positive blood samples, a PCR algorithm for identification of *T. cruzi* discrete typing units (DTUs) and real-time PCR (qPCR) to quantify parasitic loads were performed. Minicircle signatures of *T. cruzi* infecting populations were also analyzed using restriction fragment length polymorphism (RFLP)-PCR. Eight seronegative TX recipients from four infected donors were studied. In five, the infection was detected at 68.4 days post-TX (36–98 days). In one case, it was transmitted to two of three TX recipients. The comparison of the minicircle signatures revealed nearly identical RFLP-PCR profiles, confirming a common source of infection. The five cases were infected by DTU V. This report reveals the relevance of systematic monitoring of TX recipients using PCR strategies in order to provide an early diagnosis allowing timely anti-trypanosomal treatment.

Keywords: Extended donor criteria, genotyping, infectious diseases, PCR, transplantation

Abbreviations: bp, base pair; CNS, central nervous system; CSF, cerebrospinal fluid; DTUs, Discrete Typing Units; EB, blood samples treated with EDTA; EDTA, ethylenediamine tetraacetic acid; EIA, enzyme immunoassay; GE buffer, guanidine hydrochloride-ethylenediamine tetraacetic acid buffer; GEB, blood samples treated with GE; HN, heminested; IHA, indirect hemagglutination assay; IIF, indirect immunofluorescence assay; kDNA, kinetoplastid DNA; M, molar; PA, particle-agglutination assay; par. eq./mL, parasite equivalents in 1 mL blood; PB, peripheral blood; PCR, polymerase chain reaction; pH, hydrogen ion concentration; qPCR, real-time PCR; RFLP, restriction fragment length polymorphism; SL-IR, spliced leader intergenic region; Tc, *T. cruzi*, *Trypanosoma cruzi*; TX, organ transplantation; μg, microgram; μL, microliter; 24Sα rDNA, 24Sα-ribosomal DNA

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Introduction

Infection with the protozoan parasite *Trypanosoma cruzi*, which causes Chagas disease, remains a major public health concern in 21 endemic countries of America, with an estimated prevalence of 8 million infected people (1). The infection is most frequently acquired through vectorial transmission from triatomine bugs; however, it can also be acquired through blood transfusions, by oral transmission or congenitally from infected mother to fetus (2). From a worldwide perspective, Chagas disease represents the third-largest parasitic disease burden after malaria and schistosomiasis. Due to migrations, an important number of people infected with *T. cruzi* live in nonendemic countries (3,4). Between 1.5 and 2.0 million people are reported to have Chagas disease in Argentina (5). Two phases are described in the course of Chagas disease: acute and chronic; both can be asymptomatic or symptomatic. About 20–30% of infected people develop symptomatic chronic

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Chagas disease related to heart damage and/or digestive megasyndromes (1).

Amastigotes have been detected in various organs. Therefore, organ transplantation (TX) is an alternative route of disease transmission, possibly facilitated by immunosuppressive therapy. Organ TX in patients with chronic Chagas disease and the use of organs from infected donors have been a matter of debate in highly endemic areas. The growing number of infected individuals now living in nonendemic regions has increased the possibility that they might become transplant candidates or organ donors. Prevalence of *T. cruzi* infection among effective deceased donors in Argentina was 4.6% in 2009 (6).

The main limitation of organ TX worldwide remains access to an allograft. Unfortunately, the number of patients who can derive benefit from organ TX markedly exceeds the number of available deceased donors. According to the INCUCAI (National Institute of Procurement and Transplantation in Argentina—Ministry of Health) during the first semester of 2013, 571 organ TX were carried out from a waiting list of 7398 patients. For this reason, several TX units have been encouraged to relax the deceased donor selection criteria (7). As transmission from *T. cruzi* infected donors into uninfected recipients is to be expected but it is not a general rule, allocation of organs from infected donors could be allowed under certain circumstances (6).

Guidelines for pre-TX evaluation and for post-TX follow-up have been formulated by a consensus of the Chagas Disease Argentine Collaborative Transplant Consortium (6). In addressing the issue of TX from seropositive donors, these guidelines recommended that: (1) Infected living donors should receive trypanocidal treatment for 30 days prior to donation to allow clearance of parasitemia. (2) Infected deceased donors are unacceptable for heart transplantation. The allocation of other organs, with appropriate informed consent, could be acceptable for infected recipients, for uninfected kidney recipients and, eventually, for uninfected lung and liver recipients. (3) All uninfected recipients of organs from infected donors need to be sequentially and strictly monitored for infection transmission and promptly treated if this occurs. Active search for parasitemia with parasitological tests, such as Strout or molecular methods such as the polymerase chain reaction (PCR), allows starting specific treatment in a timely fashion (6).

In the same way, a consensus document has been developed in Spain with recommendations for management of Chagas disease in organ TX programs in nonendemic areas, including the exclusion criteria, post-TX monitoring and treatment in naïve TX recipients transplanted with organs from infected donors (8).

Natural parasite populations have a complex multiclonal structure (9) with evidences of genetic exchange among distantly related lineages (10). Individuals from different endemic regions are infected with distinct parasite populations, recently classified into six Discrete Typing Units (DTUs), designated as *T. cruzi* I (TcI) to *T. cruzi* VI (TcVI) (11), initially defined as ''sets of stocks that are genetically more related to each other than to any other stock and that are identifiable by common genetic, molecular or immunological markers.'' These DTUs are differently distributed in the endemic regions and in transmission cycles and are probably differently involved in the clinical manifestations and severity of the disease (12).

The development of sensitive and accurate qPCR strategies for *T. cruzi* quantification is crucial to provide a surrogate marker to assess treatment efficacy. Recently, a multiplex qPCR strategy based on TaqMan technology, aiming to quantify *T. cruzi* satellite DNA and an internal amplification control, was developed and validated (13,14).

From July 2009 to May 2013, clinical samples from 26 seronegative recipients of organs from seropositive donors (10 liver, 15 kidney and 1 lung) were referred to our laboratory for molecular diagnosis, treatment and follow-up of *T. cruzi* acute infection. This article describes the results obtained in those donor–recipient cases in which at least one of the recipients became infected and received anti-parasitic treatment.

Materials and Methods

Clinical specimens

Peripheral blood and cerebrospinal fluid (CSF) samples from eight uninfected TX recipients transplanted with organs from four infected donors from May 18, 2010 to March 20, 2011 were included in this study. The samples were prospectively and sequentially collected in different TX centers as described elsewhere (6) and referred to our Laboratory for the molecular detection of *T. cruzi* DNA. Each infected donor was named ''case'' followed by a number; a capital letter was then assigned to each TX recipient (i.e. ''Case 1A'' is ''TX recipient A'' from ''infected donor 1''). The present study only included those donor–recipient cases in which at least one of the recipients became infected and treated and whose samples were referred to our laboratory.

The study was approved by the bioethical committees of the participating institutions under informed written consent.

DNA extraction

All samples from Cases 1A, 1C, 3A, 3B, 4A and 4B (Table 1) were processed as follows: 10 mL of peripheral blood was mixed with an equal volume of GE buffer (6 M of guanidine hydrochloride and 0.2 M of EDTA, pH 8.0), boiled for 15 minutes, and 300 μL of the mixture (GEB) was extracted using High Pure PCR Template Preparation kit (Roche Diagnostics Corp., IN[Q1]) as reported in Duffy et al (14) with slight modifications (200 μL eluate).

All samples from Cases 1B and 2A (Table 1) were processed as follows: 5 mL of peripheral blood was collected in a tube with EDTA (EB), stored at 4°C for no more than 48 h, and 400 μL was processed using the High Pure PCR Template Preparation kit (Roche Diagnostics Corp.) as recommended by the manufacturer.

Table 2: Follow-up of acute infected patients after organ transplantation by means of conventional and molecular strateg

Donor–recipient case	Sample type	Interval between Tx and positive result (days post-Tx)				qPCR (par. eq./mL)		Interval of treatment[3] (days post-Tx)	PCR negativization (days post-Tx)	
		kDNA-PCR	Conventional parasitological test	Serological test[1]	*T. cruzi* DTU	T_0	Peak (days post-Tx)		kDNA-PCR	qPCR
1A	GEB	72	119	210	TcV[2]	0.1	116.5 (108)	126–186	164	129
1B	EB	98	98	97	TcV[2]	213.0	213 (98)	98–158	693	566
2A	EB	36	ND	260	TcV[2]	11.6	343.8 (56)	49–109	254	69
3A	GEB	43	ND	56	TcV[2]	88.5	890.8 (56)	53–105	91	91
4A	GEB	93 (PB), 120 (CSF)	121	121	TcV[2]	762.5	762.5 (93)	121–181	159	159

GEB, blood samples treated with GE; EB, blood samples treated with EDTA; TX, transplantation; T_0, at time of diagnosis of acute infection by means of kDNA-PCR; PB, peripheral blood; CSF, cerebrospinal fluid; ND, not done; DTU, Discrete Typing Unit; par. eq./mL, parasite equivalents in 1 mL of blood.
[1]Serological tests: reactivity by at least two different serological techniques (IIF and EIA).
[2]Coinfection by TcV + TcVI could not be excluded because the 24Sα rDNA-HN PCR showed in all cases both 125- and 140-bp bands, whereas A10 fragment showed a 525-bp fragment (see Figure 1).
[3]Benznidazole 5 mg/kg/day for 60 days.

CSF samples were mixed with one volume of GE buffer, boiled for 15 min, and 200 μL was processed with phenol/chloroform extraction (15).

In order to build the standard curves for quantification of parasitic loads in clinical samples, DNA from seronegative human blood samples spiked with cultured epimastigotes of TcVI (CL Brener) was prepared from both 5 mL EB and 10 mL boiled GEB in order to match the matrix of the standard curve with each sample type.

Kinetoplastid DNA-PCR

All organ recipients were monitored with conventional kinetoplastid DNA (kDNA)-PCR for detection of infection in peripheral blood samples after TX. A hot-start PCR procedure, targeted to the 330-bp variable regions of kDNA, was carried out with primers 121 and 122, as reported (16,17).

PCR identification of T. cruzi DTUs

Five microliters of DNA from positive kDNA-PCR samples were subjected to a PCR algorithm designed to genotype the six parasite DTUs targeted to a battery of nuclear genes (Figure 1) (18). Briefly, spliced leader intergenic region PCR (SL-IR PCR) was used to distinguish TcI (150 bp), TcII, TcV and TcVI (157 bp) from TcIII and TcIV (200 bp). SL-IR I was used to identify TcI (475 bp), and SL-IR II was used to identify TcII, TcV and TcVI (425 bp). Heminested (HN) PCR of 24Sα-ribosomal DNA (24Sα rDNA) was used to distinguish TcV (125 or 125 + 140 bp) from TcII and TcVI (140 bp); and HN PCR targeted to genomic fragment A10 was used to discriminate TcII (580 bp) from the rest of the DTUs (525 bp). Those samples that amplified 125 + 140 bp 24Sα rDNA and 525 bp A10 fragments were identified as TcV, although mixed populations of TcV + TcVI could not be excluded (18).

Analysis of minicircle signatures

Restriction fragment length polymorphism (RFLP)-PCR profiling was performed with 1.4 μg of purified kDNA amplicons that were digested with 5 units of *Msp*I + *Rsa*I or *Alu*I + *Hin*fI restriction enzymes for 4 h at 37°C. The digestion products were visualized after 10% polyacrylamide gel electrophoresis and SYBR Gold nucleic acid gel staining (~~Invitrogen,~~ CA[Q2]).

Monitoring of recipients

A real-time PCR (qPCR) strategy targeted to conserved motifs within the repetitive satellite sequence was used to quantify *T. cruzi* DNA in peripheral blood of recipients. Results were normalized incorporating a linearized pZERO plasmid as an internal amplification standard and *T. cruzi* standard curves were constructed as previously reported (13).

Results

Donor–recipient cases

Table 1 shows the characteristics of the donor–recipient cases. Case 1: one infected donor, with the lung, liver and kidney transplanted into three seronegative recipients, Cases 1A, 1B and 1C. Case 2: one infected donor with the liver transplanted to a seronegative recipient 2A. Case 3: one infected donor with the liver and kidney–pancreas transplanted into two seronegative recipients, Cases 3A and 3B. Case 4: Two seronegative recipients (4A and 4B) received a kidney TX from the same infected donor. Kidneys from donor 2 and liver from donor 4 were provided for TX at three different TX centers in Argentina, although no samples from their recipients were submitted for molecular diagnosis at our Laboratory and accordingly no

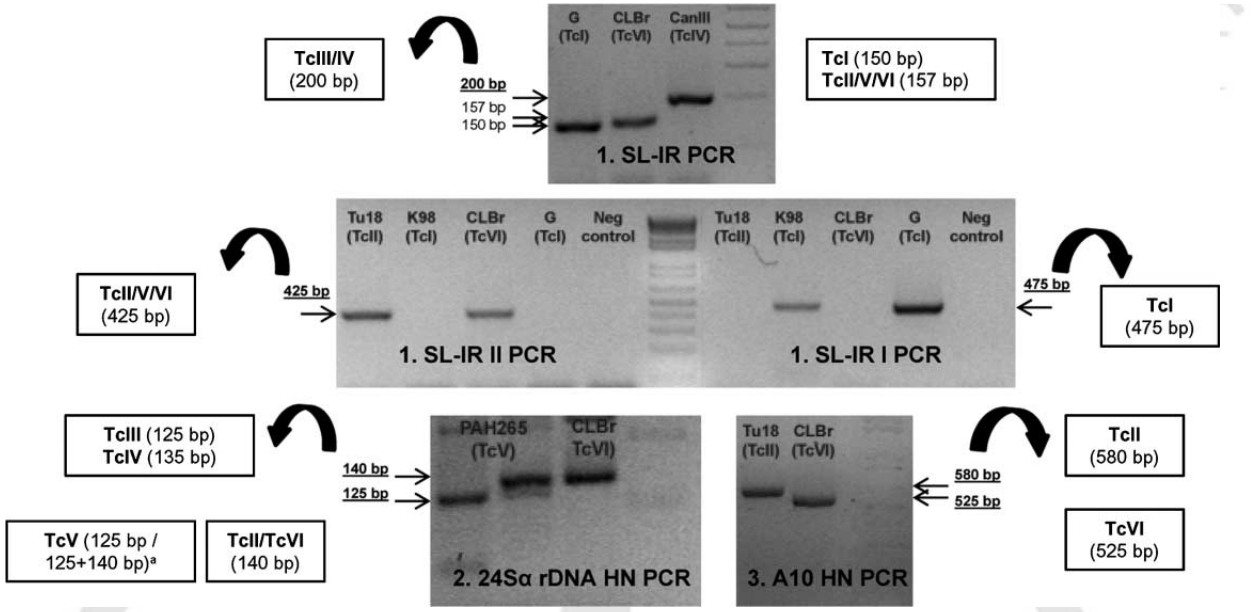


Figure 1: PCR strategies for identification of *Trypanosoma cruzi* Discrete Typing Units in human blood and tissue samples as reported in Burgos et al (18). PCR flowchart and agarose gel electrophoresis of the SL-IR (spliced-leader intergenic region), HN-24Sα rDNA (heminested amplification of the D7 domain of the 24Sα ribosomal RNA genes) and HN-A10 (heminested reaction for the A-10 fragment) amplification products. *T. cruzi* reference strains: G and K98 (TcI), Tu18 (TcII), CanIII (TcIV), PAH265 (TcV), CL Brener (TcVI). [a]In cases where both 125 and 140 bp HN-24Sα rDNA amplicons are obtained, coinfection by TcV and TcII/TcVI could not be excluded.

data regarding their parasitological and clinical evolution are reported.

Molecular diagnosis and follow-up

Case 1: Acute *T. cruzi* infection was detected in Cases 1A (lung recipient) and 1B (liver recipient) by means of kDNA-PCR performed in peripheral blood after 72 and 98 days post-TX, respectively (Table 2). Parasitic loads were determined in both infected recipients at time of kDNA-PCR detection and after treatment with Benznidazole (5 mg/kg/day for 60 days) by means of qPCR targeted to the satellite nuclear repetitive DNA sequence. The parasitic load of Case 1A was 0.1 parasite equivalents in 1 mL blood (par. eq./mL) at time of detection, reached its peak of 116.5 par. eq./mL at day 108 post-TX and turned into nondetectable 129 days post-TX (3 days after beginning of treatment). In addition, a sporadic positive kDNA-PCR result was obtained at day 157, turned negative at day 164 and remained so for at least 239 days post-TX (Tables 2 and 3 and Figure 3). In Case 1B, parasitic load was 213.0 par. eq./mL at time of detection and was nondetectable in the next follow-up sample collected 566 days post-TX (468 days posttreatment). Additionally, a sporadic positive kDNA-PCR result was obtained at day 601 and turned negative by day 693 post-TX (Tables 2 and 3 and Figure 3).

Strout test was positive only after 119 and 98 days post-TX in Cases 1A and 1B, respectively. Serology for *T. cruzi* was positive on days 210 (1A) and 97 (1B) post-TX, and remained so for at least 693 days post-TX in Case 1B (no serology results for Case 1A were obtained after 210 days after TX). Both 1A and 1B recipients were found to be infected by TcV populations (or TcV + TcVI) (Figure 1). The comparison between the minicircle signatures from kDNA-PCR products in Cases 1A and 1B revealed nearly identical RFLP-PCR profiles, confirming the common source of infection (Figure 2A).

Case 1C (kidney recipient) did not present detectable parasitemia or a positive kDNA-PCR result during at least 429 days post-TX follow-up and thus the patient was considered as not infected.

Case 2: Acute *T. cruzi* infection was detected in the liver recipient from the infected donor (Case 2A). The blood-based kDNA-PCR assay was positive 36 days post-TX (Tables 2 and 3). The parasitic load was 11.6 par. eq./mL at time of detection, reached a peak of 343.8 par. eq./mL at day 56 post-TX and turned into nondetectable parasitic load at day 69 post-TX (20 days posttreatment) (Tables 2 and 3 and Figure 3). Sporadic positive kDNA-PCR results were obtained at days 113 and 240 post-TX but turned negative at day 254 and remained so for at least 507 days post-TX. Strout test was not done; serology for *T. cruzi* was positive (only Enzyme Immunoassay—EIA—reactivity; Indirect Immunofluorescence—IIF—under the detection limit) 260 days after TX and remained so for at least 573 days

Table 2: Follow-up of acute infected patients after organ transplantation by means of conventional and molecular strategies

Donor–recipient case	Sample type	Interval between Tx and positive result (days post-Tx)			*T. cruzi* DTU	qPCR (par. eq./mL)		Interval of treatment[3] (days post-Tx)	PCR negativization (days post-Tx)	
		kDNA-PCR	Conventional parasitological test	Serological test[1]		T_0	Peak (days post-Tx)		kDNA-PCR	qPCR
1A	GEB	72	119	210	TcV[2]	0.1	116.5 (108)	126–186	164	129
1B	EB	98	98	97	TcV[2]	213.0	213 (98)	98–158	693	566
2A	EB	36	ND	260	TcV[2]	11.6	343.8 (56)	49–109	254	69
3A	GEB	43	ND	56	TcV[2]	88.5	890.8 (56)	53–105	91	91
4A	GEB	93 (PB), 120 (CSF)	121	121	TcV[2]	762.5	762.5 (93)	121–181	159	159

GEB, blood samples treated with GE; EB, blood samples treated with EDTA; TX, transplantation; T_0, at time of diagnosis of acute infection by means of kDNA-PCR; PB, peripheral blood; CSF, cerebrospinal fluid; ND, not done; DTU, Discrete Typing Unit; par. eq./mL, parasite equivalents in 1 mL of blood.
[1]Serological tests: reactivity by at least two different serological techniques (IIF and EIA).
[2]Coinfection by TcV + TcVI could not be excluded because the 24Sα rDNA-HN PCR showed in all cases both 125- and 140-bp bands, whereas A10 fragment showed a 525-bp fragment (see Figure 1).
[3]Benznidazole 5 mg/kg/day for 60 days.

post-TX. DTU characterization revealed TcV (or TcV + TcVI) (Figure 1) in peripheral blood samples.

Case 3: Acute *T. cruzi* infection was detected by means of kDNA-PCR in peripheral blood samples in the liver recipient (Case 3A) 43 days post-TX. Parasitic load was 88.5 par. eq./mL at time of diagnosis and reached its peak of 890.8 par. eq./mL at day 56 after TX. The kDNA-PCR test turned nondetectable 91 days post-TX (38 days posttreatment) (Tables 2 and 3 and Figure 3).

Strout was not done, and serology for *T. cruzi* was reactive 56 days after TX by means of both IIF and EIA tests. EIA tests turned negative 91 days after TX and remained so in all posterior controls. However, the IIF maintained low titers (1/64–1/128) up to day 196 post-TX. Both serology and PCR tests turned negative from day 233 and persisted so at least until day 500 after TX, indicating favorable treatment response. DTU identification allowed detection of TcV (or TcV + TcVI) populations in peripheral blood samples.

Case 3B (kidney–pancreas recipient) did not present positive results either by kDNA-PCR or by serology (EIA, IFI) during at least 580 days of post-TX follow-up. Accordingly, this patient was considered as not infected.

Case 4: Molecular methods allowed detection of acute *T. cruzi* infection in a kidney recipient (Case 4A) 93 days post-TX in peripheral blood (Tables 2 and 3) and 120 days post-TX in a CSF sample, revealing central nervous system (CNS) involvement. At the same time, the patient had a diagnosis of meningeal cryptococcosis. The parasitic load was 762.5 par. eq./mL at time of detection and turned nondetectable 159 days post-TX (38 days posttreatment). Furthermore, Strout and kDNA-PCR tests remained negative for at least 593 days post-TX (472 days posttreatment) (Tables 2 and 3 and Figure 3).

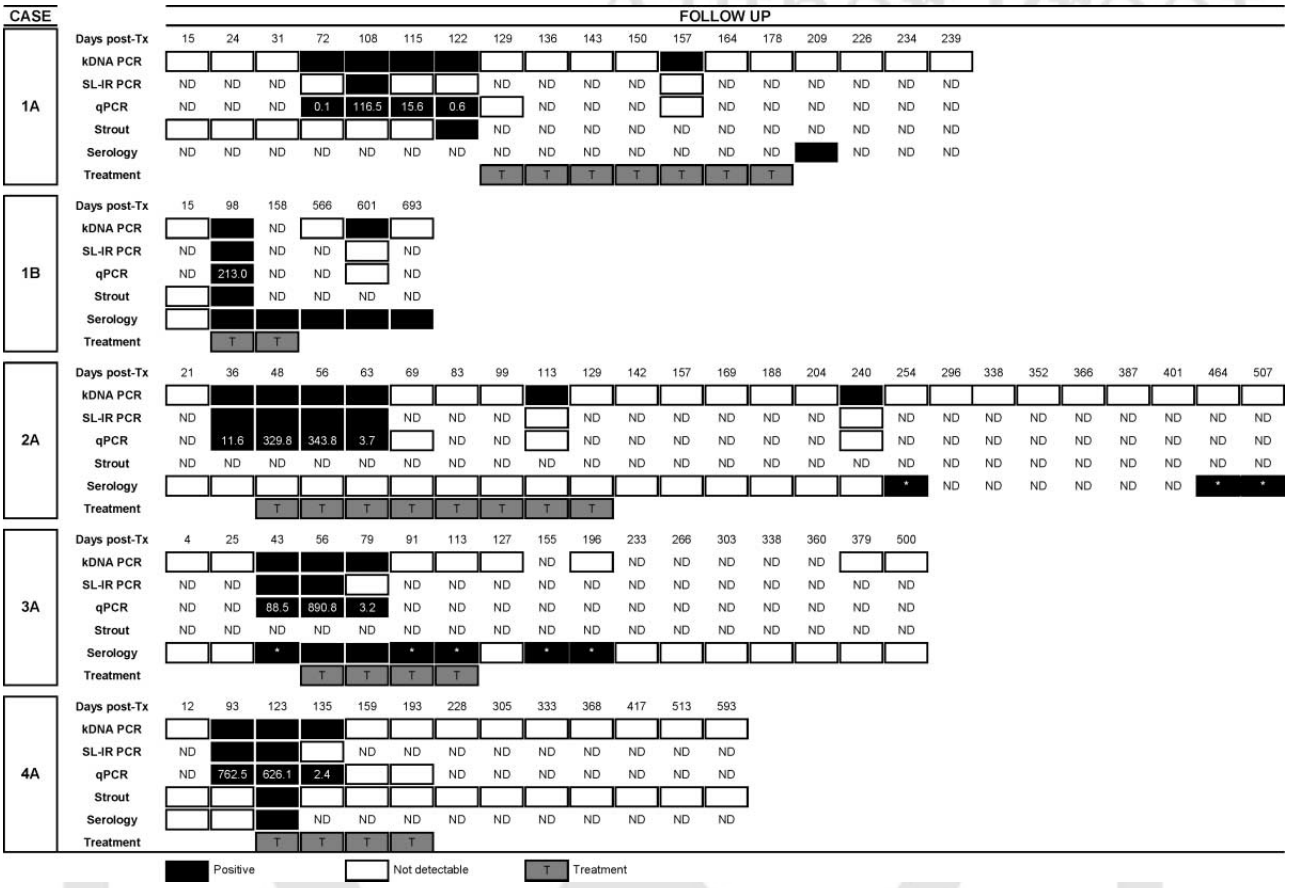
Diagnosis of acute infection by means of Strout test and serology was obtained only after 121 days post-TX. *T. cruzi* infecting populations were typed as TcV (or TcV + TcVI) (Figure 1). Comparison between the minicircle signatures from peripheral blood and CSF revealed similar RFLP-PCR profiles (Figure 2B). Interestingly, the observation of several bands present in peripheral blood and absent in the CSF sample suggested the existence of *T. cruzi* subpopulations with CNS tropism (arrows, Figure 2B).

Another kidney recipient (Case 4B) from the same infected donor did not show either detectable parasitemia or a positive kDNA-PCR result for at least 298 days post-TX and thus was diagnosed as not infected.

Discussion

T. cruzi infection was confirmed in five of the eight studied recipients. The three liver and the lung recipients became

Table 3: Follow-up of acute infected patients after organ transplantation



ND, not done.
Comparison of results obtained using molecular, parasitological and serological diagnosis.
qPCR, numbers in boxes represent parasitic load (par. eq./mL) measured in the sample obtained at this period of time.
*Reactivity observed in only one serological technique (EIA).

infected but only one of the four kidney recipients (including one pancreas–kidney TX). Although other organ recipients from Cases 1 to 4 were documented, no samples were obtained for molecular diagnosis at our Lab and, accordingly, their parasitological and clinical evolution was not recorded in this study. Furthermore, two additional cases of acute *T. cruzi* infection were detected in liver recipients. Both are currently under follow-up and have been diagnosed by means of the kDNA-PCR and qPCR prior to positivization of the Strout and serology tests (data not shown).

At the laboratory we have received samples from 26 seronegative recipients of organs from seropositive donors, from July 2009 to May 2013 (10 liver, 15 kidney and 1 lung). Although no reliable incidence rates can be obtained from these data because they do not represent the entire population of mismatch cases of seropositive donors and seronegative TX recipients, considering our sample size, 5/10 (50%) liver recipients and 1/15 (5.9%) kidney recipients became infected ($p = 0.0225$ on Fisher's exact test).

Transmission from *T. cruzi* infected donors has been reported in kidney TX recipients who were prospectively evaluated (19,20). In accordance, a 7-year follow-up study revealed that *T. cruzi* infection was transmitted to 3 (18.7%) out of 16 noninfected kidney recipients, and was detected within the first 6 months after TX by systematic search for parasitemia (21).

One case of transmission to a liver recipient in Argentina was published by Barcán et al (22) where *de novo* infection was detected by a systematic search for parasitemia with no clinical signs. McCormack et al (7) reported two out of nine (22%) cases of donor-derived *T. cruzi* transmission to uninfected liver recipients, without using prophylactic therapy. However, D'Albuquerque et al (23) reported no infection in six recipients who received liver TX from seropositive donors and post-TX prophylaxis with Benznidazole.

Two reports of transmission of acute *T. cruzi* infection by TX from unscreened deceased donors were published in the

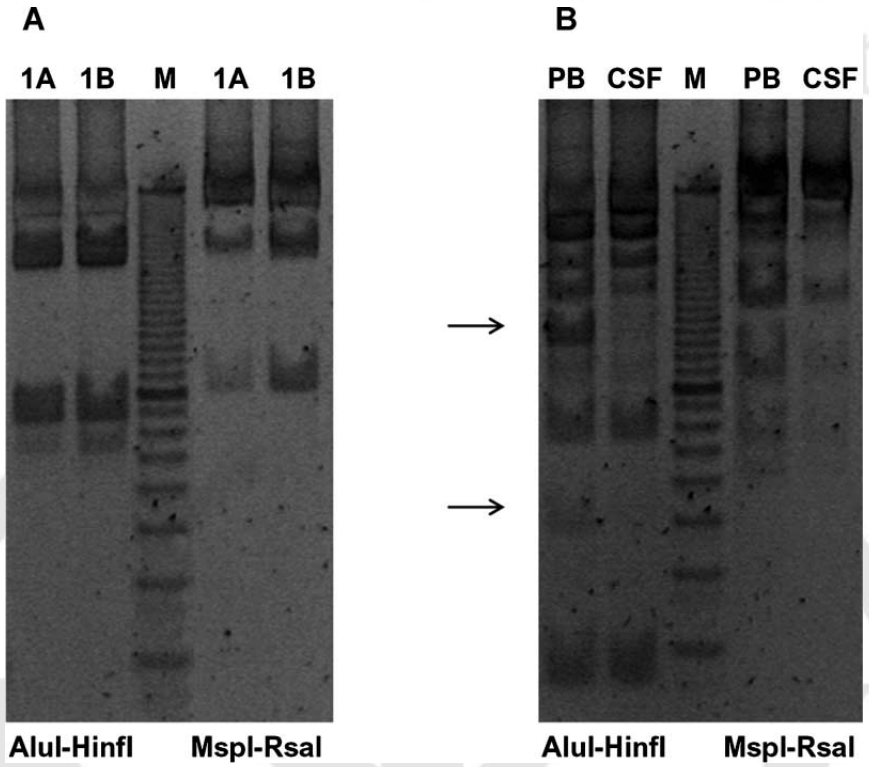


Figure 2: Minicircle signatures of blood and tissue samples from infected recipients. (A) Minicircle signatures obtained from blood samples of Cases 1A and 1B. (B) Minicircle signatures from peripheral blood (PB) and cerebrospinal fluid (CSF) of Case 4A. Arrows indicate subpopulations present in PB and not detectable in CSF, suggesting tissue tropism. Minicircle RFLP-PCR was performed by *AluI* + *Hin*fI and *Msp*I + *Rsa*I enzyme digestion and revealed in 10% polyacrylamide gels stained with SYBR green.

United States. In these reports, three TX recipients (kidney, kidney–pancreas and liver) from the same infected donor and two cardiac TX recipients became infected (24,25).

This study shows that molecular tools allow earlier diagnosis of acute *T. cruzi* infection in comparison with conventional parasitological and serological tests (Table 3). Indeed, in four out of five acute cases, kDNA-PCR was positive between 13 and 224 days (mean 100.8 days) prior to positivization of the serology tests. In two out of three acute cases that were followed up with conventional parasitological methods, the kDNA-PCR showed a positive result 28–47 days (mean 37.5 days) earlier with respect to the positivization of the Strout test. In the remaining case (1B), the three mentioned tests were found positive at about the same date (98 days post-TX), probably because only one previous sample (15 days post-TX) was referred for molecular studies.

Serology in a previous experience in patients with kidney transplantation (21) was an unreliable tool for diagnostic purposes and misleading for the monitoring of response to treatment in this setting. Blocking of synthesis of IL-2 or T cell proliferation by the immunosuppressive drugs can induce total or partial abrogation of IgG and its isotypes, making the search for parasites in blood, fluids and tissues the method of choice for diagnosis of reactivation and infection during immunosupression (21). In our series, Case 2A is an example of this.

Early molecular detection of parasite infection was previously reported in a cohort of seropositive recipients subjected to organ TX that suffered episodes of Chagas disease reactivation where kDNA-PCR and SL-IR PCR were positive between 38 and 85 days (mean 59 days) and 31–78 days (mean 46 days), respectively, prior to detection of clinical signs of reactivation and positivization of the Strout test (26).

In Case 1, in which more than one organ from the same donor was transplanted, the route of transmission could be confirmed by fingerprinting of minicircle signatures of the detected *T. cruzi* populations, directly in peripheral blood from the TX recipients. Another interesting finding of this study was the differential minicircle signature detected in CSF and peripheral blood samples from the same patient (Case 4), revealing a possible tissue tropism of the infecting parasite populations. This genetic divergence between *T. cruzi* populations in different body locations of a same patient was also observed in cases of Chagas disease

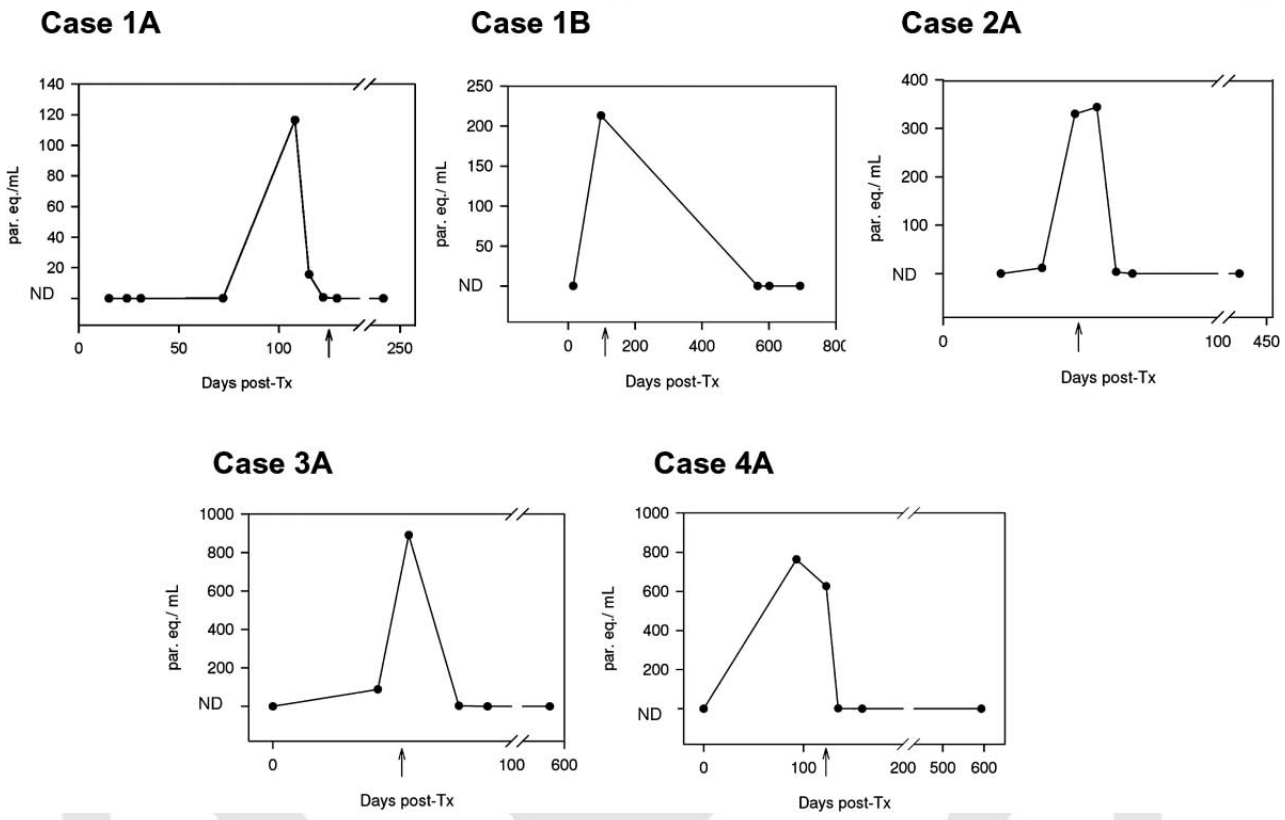


Figure 3: Follow-up of parasitic loads in the infected organ recipients using qPCR. Progression of parasitic load after transplantation is shown as well as posttreatment follow-up. A qPCR strategy targeted to conserved motifs within the repetitive satellite sequence was used to quantify *T. cruzi* DNA in peripheral blood of recipients using a CL-Brener (TcVI) calibration curve. Days posttransplantation (TX) are represented on the x-axis. The number of par. eq./mL of blood is represented on the y-axis. Arrow marks initiation of Benznidazole treatment. ND, not detectable.

reactivation after heart transplantation (18,27) or due to human immunodeficiency virus coinfection (15,28).

Our findings are in agreement with previous reports showing TcV as the prevalent DTU in Chagas disease patients from Argentina (27,29) and Bolivia (30). In the analyzed samples, two amplicons (125 + 140 bp) were obtained using the 24sα rDNA-PCR, impeding distinction between pure TcV and mixed TcV and TcVI infections. Microsatellite loci polymorphism analysis of kDNA-PCR positive blood samples from Cases 1B, 2A and 4A showed three allelic peaks for at least one tested locus, indicating that the infecting TcV populations were polyclonal ((31), Corrêa V., personal communication).

Finally, this report reveals qPCR as a promising tool for early diagnosis and quantitative monitoring of bloodstream *T. cruzi* loads in recipients of organs from seropositive donors, allowing implementation of preemptive treatment. Once qPCR-based systematic monitoring of recipients is established in transplantation units, the use of organs from seropositive donors might be expanded, thus reducing mortality by shortening the waiting period on the organ transplant lists in the Americas.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of*

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