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BRIEF REPORT

Quantitative PCR-based diagnosis and follow-up of Chagas disease primary infection after solid organ transplant: a multicentre study

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Chagas disease in solid organ transplant (Tx) recipients may present as a primary infection (PI). Early detection is crucial for timely treatment. This is the largest observational multicentre study evaluating qPCR for early diagnosis and treatment monitoring of PI in seronegative recipients of organs from seropositive donors. Out of 34 patients, admitted at five health centers, PI was detected by qPCR in 8 (23.5%) within a post-Tx period of 40 days (IQR:31-50). No PI was detected by Strout or clinical symptoms/signs. All patients had favourable treatment outcome with negative qPCR 31 days (IQR:18-35) after treatment, with no post-treatment relapse episodes.

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1. BACKGROUND

Chagas disease (ChD), caused by *Trypanosoma cruzi* (*T. cruzi),* is one of the most neglected tropical diseases, affecting approximately eight million people in endemic countries of Latin America[1]. Its most common route of transmission is vector-borne and it can be transmitted by congenital and foodborne routes, transfusion, tissue and organ transplantation.

ChD in solid organ transplant (Tx) recipients may present as a primary infection (PI) in non-ChD recipients who receive an organ from *T. cruzi* infected donors.

PI has been observed in 10-20% of liver (LiTx) and kidney Tx (KTx) and up to 75% in heart Tx (HTx) recipients[2-5].

In Argentina, screening for *T. cruzi* infection is systematically performed in all candidates and potential donors. According to INCUCAI (*Instituto Nacional Central Único Coordinador de Ablación e Implante*), the incidence of ChD in organ and tissue donors decreased from 12.4% in 2010 to 6% in 2019.

In PI early detection is crucial for timely treatment. The most common scenario is the detection of parasitemia, in some cases followed by clinical manifestations that may reach high morbidity, such as panniculitis, acute myocarditis and meningoencephalitis. Implementation of etiological treatment as soon as PI is detected may avoid severe complications, so early diagnosis is essential. However, the currently used Strout method that detects bloodstream parasites lacks sensitivity and may render a positive result much later than molecular methods[7]. In this context, PCR provides a more sensitive and rapid diagnostic tool that can show positive findings days or weeks earlier than parasitological methods [7,8].

The aim of this multicentre study was to evaluate the usefulness of qPCR monitoring as a preventive tool for detection and treatment monitoring of PI-ChD among different types of organ Tx.

2. METHODS

2.1 Ethical considerations

An exempt written informed consent was requested to the Ethics Committee of each centre, considering that this is an observational and not an interventional study. Access to personal information of each centre was restricted to the study coordinator and authorized personnel when

required to verify the study data and procedures, but always maintaining their confidentiality in accordance with current legislation.

The results are reported globally, without the identification of the centre involved. The study was carried out following the principles set forth in the Declaration of Helsinki and the standards of good clinical practice. The complementary examinations were procedures usually performed by the patients' treating physicians. No new exams or treatments were requested, so no additional expenses were generated for the care of each patient.

2.2 Study sample and recruitment

Thirty four solid organ transplants at risk of PI of ChD were included..

Diagnosis of *T. cruzi* infection in the donor was made by two positive serologic tests, including indirect hemagglutination (Chagatest HAI, *Wiener Laboratorios SAIC, Rosario, Santa Fe*, Argentina), indirect immunofluorescence (Immunofluor Chagas, *Biocientífica S.A., Buenos Aires*, Argentina), or enzyme-linked immunosorbent assay (*Chagatek ELISA recombinante, Laboratorio Lemos SRL, Buenos Aires,* or *Chagatest ELISA recombinante 4.0, Wiener Laboratorios SAIC*).

2.3 Data collection

Data were collected from five health care centres from Buenos Aires between July 1, 2014 and December 31, 2018. All participating centres adhered to the recommendations for ChD in transplant follow-up of the Argentine Society of Infectious Diseases[9,10]. The Strout method[6] and qPCR[11] were performed weekly during the first three months, every other week during six months, and monthly during one year after transplantation. Monitoring was reinitiated weekly for two months when immunosuppressive therapy was increased (i.e., due to acute rejection treatment).

The clinical records of the patients were examined to collect demographics, underlying diseases, previous antiparasitic treatment, type of immunosuppression, presence of transplant rejection or cytomegalovirus (CMV) reactivation until 18 months post-transplant, data of Chagas post-transplantation (detected by the first positive Strout test or qPCR, asymptomatic or symptomatic disease, treatment, adverse effects, outcome of each episode of PI), global and attributable mortality and loss or graft dysfunction. Minimum follow-up was six months.

2.4 Definitions

Primary infection: transmission from ChD D+ to R- (PI- D+/R-): detection of parasitemia by Strout or *T. cruzi* DNA by qPCR. A PI may be symptomatic or asymptomatic.

Symptomatic PI: i) presence of non-specific febrile syndrome with patent parasitemia and/or positive qPCR, ii) presence of skin lesions (erythema nodosum or panniculitis) with amastigotes in biopsy specimens, iii) myocarditis or neurological lesions such as encephalitis or space occupying lesion (chagoma) with visualisation of parasites in cerebrospinal fluid or biopsy specimens or positive qPCR, iv) a combination of the above-mentioned settings.

2.5 Laboratory methods for diagnosis of ChD-PI

The Strout technique is based on the concentration of parasites in the blood sample by centrifugation and examination of the sediment under a microscope using 10X or 40X magnification objectives in search of mobile trypomastigote forms of *T. cruzi* [6].

The qPCR method used was based on a standardised duplex qPCR assay using TaqMan probes targeting the *T. cruzi* satellite sequence and an exogenous internal amplification standard (IAC)[11]. For quantification, a standard curve was made of serial dilutions of DNA obtained from a seronegative human blood samples spiked with known amounts of cultured *T. cruzi* cells[11]

The unit of measurement is parasite equivalents per millilitre of blood (peq/mL). It is considered "positive quantifiable" if load is ≥ 1.5 peq/mL (limit of quantification), "positive not quantifiable" (PNQ) if it <1.5 peq/mL and "undetectable" if the Cq (quantification cycle) value is >40.

2.6 Antiparasitic treatment

Treatment with benznidazole (BZN) 5 mg/kg/day was initiated for 60 days. The clinical and parasitological outcomes were recorded. In the absence of consensus guidelines, PI patients were always treated, despite their parasitic load values. Adverse effects were classified in five categories: bone marrow toxicity, liver toxicity, gastrointestinal discomfort, skin rash and others.

2.7 Statistical analysis

Data was cleaned by searching for errors and missing data. All variables were checked for extreme values, ranges and possible inconsistencies.

The statistical analysis was performed using descriptive statistics. Continuous variables were analysed with appropriate measures of centrality (mean or median) and dispersion (standard deviation or interquartile range) according to their distribution. Categorical variables were expressed as absolute frequency and percentage. The variables were compared using the chi-square test or Fisher's exact test for categorical variables, and Student's t-test or Mann-Whitney U test for continuous variables. We considered a confidence level of 95% for all the analyses. A two-tailed test was performed in all cases.

All the percentages are rounded off. If the fractional part is 0.5 or greater, the number is rounded up. If the fractional part is less than 0.5, the number is rounded down and if it is 0.5 there is no rounding.

3. RESULTS

3.1 Characteristics of the study population and qPCR findings.

Thirty-four transplants in 34 (D+/R-) patients who received organs from *T.cruzi* infected donors were included from January 2014 to December 2018. The median age was 60 years [IQR 51-66 r 29-76]. Twelve (35.3%) were women and 22 were men (64.7%).

The types of solid organ transplantation were: KTx, 17 patients (50%); LiTx, 14 (41.2) and KLTx, 3 (8.8%)(Supplemental Table). Four of them (12%) died, none with PI. The latter were followed-up for six months in three cases and eighteen months in the other one. There was no loss to follow-up in this cohort.

PI was detected in eight (23.5%) by positive qPCR findings (Table 1 and Supplemental Table).

All patients were asymptomatic and the Strout test was negative. They were all treated and no further episodes occurred. Out of 14 D+/R- LiTx patients, five had PI (38%). All patients completed treatment with BZN with a favourable response, except one who stopped treatment at day 40 because of bone marrow toxicity but did not relapse. Two of 17 D+/R- KTx presented PI (12%). Both patients completed treatment without adverse events. One of three D+/R- KLTx recipients had PI and was treated with BZN based on a PNQ qPCR result. The difference in the rate of transmission between LiTx and KTx was not statistically significant (p = 0.17).

Parasitic loads at time of diagnosis and treatment initiation are indicated in Table 1. Figure 1A shows the distribution of parasitic loads at time of diagnosis of PI and Figure 1B shows their distribution according to organ type transplant.

3.2 Immunosuppressive regimen, rejection episodes and Cytomegalovirus Reactivation.

Thirty three patients (97%) received mycophenolate, associated with tacrolimus and meprednisone and the remaining patients received combination treatment with everolimus.

Sixteen patients received induction with thymoglobulin, fifteen were KTx and one was a liver-kidney Tx. Three of them (all KTx) presented PI (p = ns).

Out of eight patients with PI, only one had a rejection episode, whereas out of the 26 patients. without PI eight presented acute rejection (p=ns).

Eight patients presented CMV reactivation, three belonged to the PI group (N=8) and five were within the non-PI group (5/26) (p=ns).

4. DISCUSSION

Donors born or residing in endemic regions or whose mothers were born in endemic areas are at risk for potential *T.cruzi* infection and transmission of ChD. Even in the absence of disease, some people could be chronically infected, undergoing the so-called asymptomatic or indeterminate phase of ChD, and thus they can transmit infections. The scarcity of donors, with the consequent death increase of candidates on the waiting list, leads to the decision of using marginal donors, including those with ChD.

Thus, the decision to accept an organ from an infected donor had been based on the emergency of performing the Tx, despite the risk of a de novo infection in the recipient. Nevertheless, the feasibility of qPCR monitoring after Tx allows accepting organs from any infected donor and not only those in emergency status, since PI can be detected early with high sensitivity, allowing for rapid treatment.

The risk of transmission using kidneys and livers from ChD donors varies from 10 to 20% in different series; after HTx, transmission is 75% or greater with poor outcomes and high mortality rates. There is limited experience with LuTx from infected donors [12,13]. Current guidelines support the use of kidneys and livers from ChD donors but not of small bowel and hearts because they are ChD target organs [9,10]. In our series, the use of kidneys and livers from ChD donors monitored by qPCR was safe (asymptomatic, no further ep, good treatment response and no mortality).

We observed a similar frequency of PI as in previous studies. (Table 1). The median period of 40 days between Tx and detection of PI was also in line with other studies [8].

In a previous series of Tx patients, conventional PCR enabled bloodstream detection of *T. cruzi* DNA between 28 and 47 days earlier than Strout[7]. In this cohort Strout was always negative.

Previous publications described clinical manifestations in PI, but none of our cases was symptomatic[12-14] This could be explained due to prompt treatment after detecting the first qPCR positive sample. Treatment with BZN was effective, as expected for acute *T.cruzi* infections. Indeed, persistent negative qPCR findings were observed after treatment without subsequent episodes of *T.cruzi* infection.

Although no randomised clinical studies have evaluated the association between immunosuppressive treatment and PI, mycophenolate-based immunosuppression has been associated with increased risk of reactivation in HTx patients, compared with azathioprine [15]. Most of our patients received mycophenolate, so we could not compare different regimens. We did not find any correlation between the use of thymoglobulin and increased risk of PI.

In our study, ChD prophylaxis was not indicated; we preferred pre-emptive therapy with qPCR monitoring. Indeed, only 23.5% of patients experienced PI and qPCR allows early detection of infection before complications may occur. Besides, prophylactic therapy can mask signs of infection, and the risk of drug toxicity is high.

Quantitative PCR provides parasitic load measurements that could serve as prognostic markers of infection and reactivation. Its availability of anticipating PI has encouraged Tx centres to accept ChD donors for non-ChD recipients (except in cases of heart or small bowel Tx), considering it a safe clinical practice. In sum, this study highlights the benefits of qPCR for early diagnosis of PI to initiate pre-emptive therapy and monitoring treatment response.

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As qPCR monitoring is a standard of care in transplant patients at risk of ChD, the test has been also paid by the Social Security of the affected patients.

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Conflicts of interest: None declared.

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Table Parasitic Loads detected in Study patients

Transplant D+/R- (1)	**Transmission of *T.cruzi* Positive/Total (%)**	**Time to positive test**	**Parasitic load at time of diagnosis.**	**Parasitic load at start of treatment**	**Parasitic load clearance after treatment**
Total	8/34(23%)	Median:40 days (IQR: 31-50)	Median:4 peq/ mL (IQR<1.5-24)	Median:24 peq/ mL (IQR 3-33)	Median: 31 days (IQR 18-35)
Liver	5/14(36%)	Median:46 days (IQR 21-69).	Median: 6 IQR (<1.5-79.5)	Median: 25. IQR (25-87.5)	Median: 21 days (IQR 11-34)
Kidney	2/17(12%).	Median: 39 days (30 & 48 days)	Median : 11.7 peq/mL .(22.4 peq/mL & PNQ)	Median: 11.7 peq/mL.(22.4 peq/mL & PNQ)	Median: 54.5 days (36 & 73 days)
Liver-kidney	1/ 3 (33%)	36 days	PNQ	PNQ	29 days

D: Donor; R: Recipient; IQR: Interquartile; PNQ; positive non quantifiable.

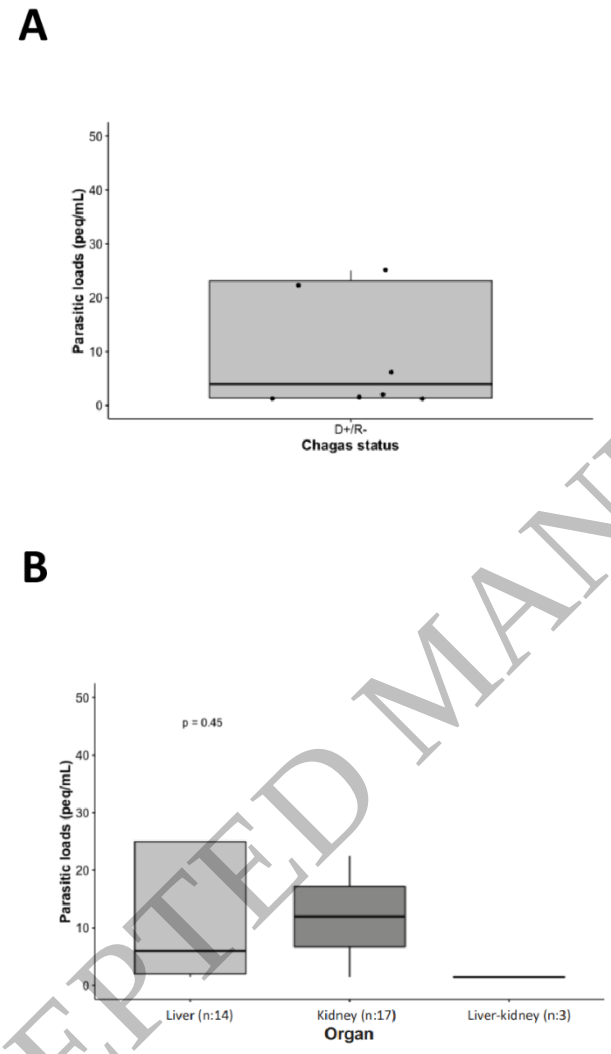

Fig 1: Box-plots showing the distribution of parasitic loads expressed in parasite equivalents per milliliter of body fluid (peq/ml) at time of diagnosis.
A: All cases ; B: Distribution according to organ type.