

## LONGITUDINAL STUDY OF THE INDIRECT IMMUNOFLUORESCENCE AND COMPLEMENT FIXATION TESTS FOR DIAGNOSIS OF CHAGAS' DISEASE IN IMMUNOSUPPRESSED PATIENTS SUBMITTED TO RENAL TRANSPLANTATION

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*Clinical and serological follow-up of 7 patients submitted to renal transplantation and presenting positive serological reactions to Chagas' disease before immunosuppression did not show significant changes in indirect immunofluorescence and complement fixation titres for Chagas' disease, or signs and symptoms indicating exacerbation of the disease during follow-up. In addition, 18 of 66 recipients of renal transplants considered to be non-chagasic before immunosuppression showed at least one positive result to the indirect immunofluorescence test for Chagas' disease during the study period. The results suggest that the immunosuppression state induced in chagasic patients submitted to renal transplant did not promoted exacerbation of the chronic infection in these patients and not interfere with the serological response of chronic chagasics, thus permitting the use of these serologic reactions for diagnostic purposes in these cases. However, the positive results of the indirect immunofluorescence test in non-chagasic patients indicate the need for judicious interpretation of the indirect immunofluorescence test for the diagnosis of Chagas' disease in renal transplanted patients.*

*Key-words: Chagas' disease. Immunosuppressed patients. Serologic diagnosis. Renal transplantation.*

The influence of immunosuppressive drugs on the natural history of chagasic infection has been studied both clinically and experimentally. Acute infection is usually more severe in immunosuppressed animals, with high mortality rates and more elevated parasitemia<sup>9, 10</sup>, whereas immunosuppression induced immediately after the acute phase<sup>1</sup> or during the chronic phase of the experimental disease<sup>3</sup> does not necessarily modify the course of the disease in animals.

As to human chagasic infection among immunosuppressed patients, the severity of the acute disease was not changed when accidental transmission occurred through blood transfusion<sup>13</sup> or renal transplant<sup>5</sup>. Similarly, chronic chagasic

patients submitted to immunosuppression do not appear to evolve in a more serious manner than usual<sup>2</sup> though they may show exacerbation of parasitemia<sup>11</sup>.

The objective of the present investigation was to study the repercussions of renal transplantation and medical immunosuppression on the behavior of serological tests used for the diagnosis of Chagas' disease.

### MATERIAL AND METHODS

#### Clinical follow-up

The following groups of patients were followed by anamnesis and clinical examination at 3 month intervals:

**group I:** Eighteen patients with terminal renal failure chronically treated by hemodialysis while waiting for a renal transplant, with negative indirect immunofluorescence (IIF) and complement fixation (CF) tests for Chagas' disease at the beginning of the study. This group was used as control for groups II and III.

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**group II:** Sixty-six patients submitted to renal transplant from 1980 to 1984 at the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo. All had negative IIF and CF tests for Chagas' disease before the renal transplant and were submitted to the following immunosuppression schedule: azathioprine: 5mg/kg per day for the first 5 days after surgery; the dose was then reduced to 3mg/kg per day up to the 12<sup>th</sup> month when a maintenance dose of 2mg/kg per day was established. Corticosteroids; 2.0mg/kg per day prednisolone for the first 3 days after surgery, followed by 1.5mg/kg prednisone per day up to the 10<sup>th</sup> day; the dose was then reduced by 10mg 3/3 d until a maintenance dose of 50mg/d, when the dose was reduced by 5mg per week until the maintenance dose of 0,4mg/kg per day was reached.

**group III:** Seven patients submitted to renal transplant from 1980 to 1984 at the University Hospital using the same immunosuppression schedule as described above. All were from an area in which Chagas' disease is endemic and had positive IIF and CF tests for the disease before transplant. None of the patients received drugs considered to have a trypanosomicidal action before immunosuppression.

### Serological follow-up

Serum samples were obtained from fasted patients on the days of clinical evaluation and stored frozen at -20°C until analysis. All sera were tested at the same time at the end of clinical follow-up by IIF<sup>4</sup> and CF<sup>7</sup>.

IIF titres were determined on the basis of the greatest serum dilution that showed perfectly discernible fluorescence, starting from a 1:30 dilution. Slides were read independently by two observers and the results were later compared, when there was disagreement between readings, the lowest serum dilution was considered for analysis. Sera with titre  $\geq 1:30$  were considered positive.

CF titres were determined by considering reactions with titres  $\geq 1.9$  as positive, and reactions with titres between 1.5 and 1.8 as doubtful. Anticomplementary reactions were also considered to be doubtful for analysis purposes.

### RESULTS

Comparative data for the three groups of patients are summarised in Table 1. When the three groups are considered as a whole, clinical follow-up ranged from 3 to 65 months and no patients showed signs or symptoms that would permit the diagnosis of chagasic infection during follow-up.

Serological follow-up showed different IIF behavior in the three groups (Table 2). Thirty-eight of the 631 serum samples (6%) obtained from group II patients were considered to be positive to IIF, resulting in at least 1 positive sample in 18 patients in this group (27.2%). All of these patients showed fluctuations in IIF results, which were considered to be positive at times and negative at others throughout follow-up. In contrast, none of the 168 serum samples obtained from group I patients gave positive IIF results, whereas all the sera from group III patients were IIF positive (Table 2).

Table 1 - Median values of blood transfusions, immunosuppression time and number of serum samples obtained for the three groups of patients followed clinically and by IIF and CF tests for Chagas' disease.

Group	N° of blood transfusions	Follow-up (months)	Immunosuppression (months)	Number of serum samples per patient
I (N = 18)	4.5 (1-38) <sup>a</sup>	36.5 (10-64) <sup>a</sup>	-	10.5 (6-18) <sup>a</sup>
II (N = 66)	8.0 (1-82)	25.5 (3-65)	29.5 (3-68)	9.0 (2-20)
III (N = 7)	8.0 (2-17)	38.0 (19-40)	44.0 (19-58)	9.0 (5-15)

<sup>a</sup> Ranges.

Table 2 - IIF titres for Chagas' disease observed during serological follow-up of the three groups of patients.

IIF titres	group I	group II	group III
negative	168 (100.0) <sup>a</sup>	593 (94.0) <sup>a</sup>	-
1:30	-	27 (4.4)	-
1:60	-	11 (1.6)	-
1:120	-	-	8 (13.1) <sup>a</sup>
> 1:120	-	-	53 (86.9)
Total n°			
of samples	168 (100.0)	631 (100.0)	61 (100.0)

<sup>a</sup> percentages.

Table 3 - Results of the CF tests for Chagas' disease obtained during serological follow-up of the three groups of patients.

CF	group I	group II	group III
Negative	168 (100.0) <sup>a</sup>	627 (99.4) <sup>a</sup>	1 (1.6) <sup>a</sup>
Doubtful	-	4 (0.6)	8* (13.1)
Positive	-	-	52 (85.3)
Total	-	631 (100.0)	61 (100.0)

<sup>a</sup> percentages; \* Six anticomplementary sera.

The results of the CF test for three groups of patients differed from the IIF results, i.e., none of the patient sera in groups I and II gave positive results. In group II, 4 of 631 serum samples were doubtful (0.6%) and in group III 52 samples (85.3%) were considered positive.

## DISCUSSION

Clinical and serological follow-up of 7 patients who had shown positive tests for Chagas' disease before renal transplant revealed constant positive results with high titres for both tests, as well as the absence of signs and symptoms indicating reactivation of the disease during the follow-up. These data agree with those reported by Barousse et al<sup>2</sup> who demonstrated that immunosuppression started during the chronic phase of Chagas' disease does not have a marked effect on the natural evolution of the disease. We may also conclude that immunosuppression did not interfere with the serological response of chronic chagasics, thus

permitting the use of the IIF and CF tests for diagnostic purposes in these cases.

However, the positive IIF results obtained after renal transplant for a significant number (18/66) of patients considered not to have Chagas' disease before immunosuppression deserves comment. An initial analysis may lead to the assumption that the test became positive following chagasic infection acquired after surgery.

However, the clinical and serological behavior of our patients differed from that reported in the series studied by Chocair<sup>5</sup>. Whereas these investigators reported the occurrence of fever, hepatosplenomegaly and elevated titres of circulating antibodies in all of the patients who acquired acute chagasic infection after renal transplant, none of the present patients showed signs or symptoms indicative of this condition. All of our patients were serologically positive to the IIF test only, with low titres (< 1:60), and their results fluctuated between positivity and negativity throughout follow-up.

These considerations suggest the possibility that the present results were due to false-positive IIF tests in group II patients. This may have been a consequence of renal transplantation itself, associated with immunosuppression. In this case, the positive IIF results for Chagas' disease may be consequence of cross-reactions between antigen determinants shared by *Trypanosoma cruzi* and cells of the human host<sup>6 8 12 14 15</sup>.

On this basis, we suggest that positive IIF tests for Chagas' disease in patients submitted to renal transplantation be considered with caution, especially when titres are low and when positivity is detected in only one serum sample.

## RESUMO

O seguimento clínico e sorológico de pacientes submetidos ao transplante renal e que apresentavam reações sorológicas positivas para doença de Chagas antes do transplante não evidenciou alterações significativas nos resultados das reações sorológicas nem sinais ou sintomas indicativos de reagudização da infecção chagásica nesses pacientes. Por outro lado, reações de imunofluorescência indireta com resultado positivo em baixos títulos foram ocasionalmente observadas durante o seguimento de 18/66 pacientes considerados não-chagásicos antes do transplante renal. Com base nesses achados concluímos que o estado de

imunossupressão induzida após o transplante renal não interfere significativamente nos resultados das reações de imunofluorescência indireta e fixação do complemento para o diagnóstico da doença de Chagas. Por outro lado, a presença de resultados falso-positivos na reação de imunofluorescência indireta para doença de Chagas verificada após o transplante renal recomenda cautela na interpretação dos resultados dessa reação, principalmente se verificados em títulos baixos e em uma única amostra de soro.

**Palavras-chaves:** Doença de Chagas. Imunossupressão. Diagnóstico sorológico. Transplante renal.

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