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BRIEF COMMUNICATION

Trypanosoma cruzi PARASITEMIA OBSERVED IN IMMUNOCOMPROMISED PATIENTS: THE IMPORTANCE OF THE ARTIFICIAL XENODIAGNOSIS

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SUMMARY

Trypanosoma cruzi parasitemia observed in immunocompromised patients (transplant or positive HIV) occurred more frequently by the artificial xenodiagnosis method (10/38) compared with hemoculture (2/38), given the same quantity of blood. Other ways of diagnosis, like mice inoculation (5/38), QBC and buffy coat (2/38), were evaluated also. This result showed the importance of the artificial xenodiagnosis. The other techniques increased only one more patient positive.

KEYWORDS: Chagas disease; *Trypanosoma cruzi*; Immunocompromised; Artificial xenodiagnosis; Hemoculture

Through xenodiagnosis – the most commonly used demonstration method of *Trypanosoma cruzi* – the feces and the triatomine intestine content, of third and fourth stages, gotten through compression or dissection are examined under optical microscopy, respectively, 30 and 60 days after feeding on patient's blood. This method demands the availability of triatomine which is free of infection, created in the lab and fed on bird's blood that is refractory to infection. A positivity of 49.3% was already reached with patients having chronic Chagas disease[2].

The *T. cruzi* is a flagellated protozoan easily cultured in acellular media that contain hemin derivates. The hemoculture technique got credibility when CHIARI & BRENER, in 1966, got 31.8% of positivity using the LIT medium, bringing new possibilities[3] . MOURÃO & MELLO, in 1975, developed a process for removing plasma and washing the cells to remove the antibodies or other factors that could apparently inhibit growth of *T. cruzi*[7].

The concomitant use of hemoculture and of artificial xenodiagnosis for Chagas disease diagnosis must be considered following those recommendations given by the aforementioned researchers, as long as the spoliation of patient's blood – many times extremely weakened when submitted to an immunosuppressive condition – does not take place. In order to investigate the xenodiagnosis sensitivity[10] against the hemoculture using lesser blood volume and removing the plasma, we decided to perform this study using an equal blood volume for each technique. Besides these two, we added the buffy coat, the QBC (Quantitative Buffy Coat) and the inoculation in mice.

The exams were performed using approximately 12 ml of blood of each of the 71 samples coming from 38 patients known to have the chronic Chagas disease and immunosuppressed related to either a transplant or to the human immunodeficiency virus (HIV). We received 1 to 4 samples from each patient. The exceptions were 2 patients who gave 7 and 10 samples.

In order to perform the artificial xenodiagnosis, we submitted 20 triatomines to feeding in 5 ml of blood, having the anticoagulant sodium citrate pre-heated to 37 °C for 45 minutes, coming from a patient immediately upon his arrival at the lab. After 30 and 60 days, respectively, we examined the feces and the intestinal content of the insects[2].

The remaining 5 ml volume collected aseptically was centrifuged at 3500 rpm, for 20 minutes, at 4 °C, immediately upon its arrival at the lab, too. After such a period, we took the plasma and seeded the sediment in 4 ml of the LIT medium. After 30 days, we examined in an optical microscope half of the media, that is approximately 3 ml, in the following way: through a direct examination of 10 µl of the media and 10 µl after centrifugation, at 3,500 rpm, for 10 minutes. The same procedure was repeated after 60 days with the remaining volume.

With the remaining blood we performed the following: 1) buffy coat: 50 µl of blood collected in a capillary tube was submitted to centrifugation at 3,500 rpm, for 15 minutes; after such a period, we cut the tube and examined a drop of the material in an optical microscope; 2) QBC (Quantitative Buffy Coat): 60 µl of blood collected in a capillary tube

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Table 1
Eleven positive cases in at least one of this five diagnosis techniques applied among 71 blood samples from 38 immunosuppressed chagasics

Patient (number of the samples collected from each patient)	Buffy coat	QBC	Hemoculture (30 and 60 days)	Inoculation in mice	Xenodiagnosis (30 and 60 days) (number of the positive sample)
BSQ (1 S)	–	–	–	–	+ (30 and 60)
CJAJ (2 S)	–	–	–	+	+ (60) (1st S)
MCS (1 S)	–	–	–	+	+ (30 and 60)
SMC (1 S)	–	–	–	–	+ (30 and 60)
ICM (1 S)	–	–	–	–	+ (30 and 60)
MTG (1 S)	–	–	+ (60)	–	–
JSS (1 S)	–	–	–	+	+ (30 and 60)
DD (2 S)	–	–	–	–	+ (30 and 60) (1st S)
JSV (3 S)	–	–	–	–	+ (30 and 60) (2nd S)
APG (10 S)	+	+	–	+	+ (30 and 60) (3rd S)
JCC (AIDS) (1 S)	+	+	+ (30 and 60)	+	+ (30 and 60)

S: Sample; +: Positive; –: Negative

internally coated by acridine orange and anticoagulant; after the centrifugation at 12,000 rpm, for 5 minutes, we examined the tube directly at the fluorescence microscope; and 3) inoculation of approximately 0.5 ml of blood in each of the two Balb/C mice, who had their blood examined weekly, for 2 months.

From the 38 patients examined, 10 (26.3%) were positive in the artificial xenodiagnosis, only 1 in the second examination (60 days). Five (13.1%) in the inoculation of the mice; the same positive in the xenodiagnosis. The hemoculture was positive only in 2 cases (5.2%); only one positive sample was found using this technique. The buffy coat and the QBC were also found positive in only 2 samples: the first one when all the other techniques were positive, and the second one only when the artificial xenodiagnosis was positive (Table 1).

The need to perfect new techniques is ever growing allowing us to appraise the reactivation of Chagas disease through observation of the parasite, in immunosuppressive situations by drug therapy in transplanted patients[1] and in patients infected by HIV[10]. SARTORI, in 1998, said the parasitemia is more frequent and higher in the amount of infected and immunosuppressed patients than those described for chronic immunocompetent patients[10], since the xenodiagnosis using 40 bugs was positive in 81.25% of the first group.

Comparing the hemoculture and the artificial xenodiagnosis using the same blood volume – that is, 5 milliliters for each, which is a reasonable volume gotten from patients already extremely debilitated – the second technique was seen to be superior (26.3%); 5 patients were positive exclusively by this technique, even when using only 20 triatomines. The use of 40 triatomines[1,2] is generally recommended. CHIARI & DIAS, in 1975, developed a pilot project in which the total blood volume for the hemoculture was increased from 10 ml to 30 ml to improve the sensitivity. They got a positivity of 30% and 50%, respectively, with the xenodiagnosis and the hemoculture in a group of 40 patients[4]. CREMA *et al.*, in 1996, and SILVA *et al.*, in 1996, have demonstrated that even with a higher blood volume used in the hemoculture, the positivity ratio was similar to the xenodiagnosis[5, 11]. In another study, using the same blood volume, the xenodiagnosis was higher: 36% of positive tests by xenodiagnosis and 9.6% by hemoculture[6]**.** Hemoculture is still quite labor intensive.

The essence of this study was that a reduced volume of blood was necessary for the two techniques and a lesser amount of insects than what is commonly professed. Also, the results point out an order of importance between the techniques; xenodiagnosis, followed by the inoculation in mice. The hemoculture, the QBC and the buffy coat presented approximate positivity figures.

After more than 80 years, xenodiagnosis is still ahead of the other classical tests, when dealing with detection of the parasitemia. The artificial xenodiagnosis has the advantage of being more comfortable for the patient with Chagas disease and under immunosuppression, eliminating eventual allergic reactions deriving from the triatomine bite, and allowing repetition of the tests with greater facility[8]. Therefore, while we wait for more concrete recommendations as for the use of a molecular technique such as the Polymerize Chain Reaction (PCR), the xenodiagnosis that depends on triatomine remains preferred as does the entire structure for the method's maintenance, costing to the Brazilian Health System known as "SUS" (Sistema Único de Saúde) $30 Brazilian Reals, with 40 triatomines[9] and which is easy to perform.

RESUMO

A importância do xenodiagnóstico artifical no diagnóstico da parasitemia pelo *Trypanosoma cruzi* em pacientes imunocomprometidos

A demonstracão da parasitemia pelo *Trypanosoma cruzi* em pacientes imunocomprometidos (transplantados ou HIV positivos) ocorreu com mais frequência por meio do xenodiagnóstico (10/38) frente à hemocultura (2/38), quando se utilizou o mesmo volume de sangue. Também foram avaliados outros métodos de diagnóstico como inoculação

em camundongos (5/38), QBC e creme leucocitário (2/38). Este resultado reitera a importância do xenodiagnóstico artificial. As outras técnicas acrescentaram apenas mais um paciente positivo.

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