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LETTER TO THE EDITOR

REACTIVATION OF *Trypanosoma cruzi* INFECTION IN IMMUNOSUPPRESSED PATIENTS: CONTRIBUTIONS FOR THE LABORATORIAL DIAGNOSIS STANDARDIZATION

São Paulo, October 2, 2007

Dear Sir,

Diagnosis of parasitemia associated with reactivation of Chagas disease remains a challenge in clinical practice. Since 1990, criteria to establish the differential diagnosis of chronic Chagas disease sporadic parasitemia, and reactivation have been proposed in heart transplant recipients including magnitude of parasitemia, severity of clinical manifestations, evidence of miocarditis, and the finding of *Trypanosoma cruzi* in tissues[1]. LUQUETTI & RASSI[12] recommended searching for parasites in fresh blood by means of QBC - Quantitative Buffy Coat, Strout or micro-hematocrit whenever Chagas disease reactivation is under suspicion, and in negative cases, investigation should continue by means of xenodiagnosis with anticipation of microscopic examination. More recently, SARTORI *et al*.[17] have defined reactivation as the presence of clinical manifestations that are not observed in immunocompetent individuals with chronic *T. cruzi* infection: detection of parasites by microscopy examination of blood or cerebrospinal fluid (CSF); and high burdens of *T. cruzi* amastigotes in tissue biopsies or biological samples collected during autopsies. The same authors classified parasitemia in three categories according to microscopic detection: very high parasitemia, high parasitemia and low parasitemia, when *T. cruzi* was detected by direct examination of blood and/or CSF, when ≥20% of triatomines fed on the patient's blood were positive, or < 20% of triatomines were positive, respectively (alternatively, in the last case, when only blood culture gave a positive result).

The gold standard of Chagas disease laboratory diagnosis remains xenodiagnosis and/or hemoculture[10,14] even though the former still lacks standardization after 90 years of existence, and the latter has got limited sensitivity[7,13]. More recently, a number of studies have reported higher sensitivities of the Polymerase Chain Reaction (PCR) in comparison with xenodiagnosis and hemoculture[5,6,8]. However, none of the studies was able to perform a metanalysis due to the heterogeneity of laboratory techniques. Besides, there is still no standardization on the amount of blood volume to be analyzed from each patient, either by xenodiagnosis or hemocultures, and how many blood samplings have to be performed in order to ensure a negative result[10,14]. In addition, there is also no consensus on the superiority of minicircles (kDNA), or the genomic DNA (TCZ sequence) as the best PCR target[4,9,11,16,18]. Both systems appear to be specific and highly sensitive (detection of one parasite or fractions), thus being suitable for PCR.

In 2006, our laboratory used a murine model of Chagas disease presenting with low parasitemia at the time of triatomines feeding[3]. To analyze samples, PCR was performed with primers chosen on the TCZ sequence coupled to microscopy examination performed in the context of xenodiagnosis. The aim was to detect *T. cruzi* DNA in *Triatoma infestans* digestive tract samples as soon as possible after bugs feeding on infected mice, in an attempt to mimic the situation found during reactivation of Chagas disease in immunosuppressed patients. In each of the time-points considered starting on day one until day 60 after blood meal, bugs were more likely to be found PCR-positive than positive by microscopy and, on day one post-feeding, infections were only detected by PCR in 40% of insects. On the following days tested within the first week after blood meal (2^{nd}, 3^{rd}, 4^{th} and 5^{th} days), superiority of PCR was sustained, and detection occurred in 55%, 40%, 70% and 63% of insects, respectively. RUSSOMANDO *et al.*[16], detected positive-PCR (TCZ sequence), studying feces of triatomines fed with infected monkeys blood, as soon as day two after bugs' blood meal. ROMAÑA & BRIONES[15] succeeded in detecting *T. cruzi* in a human model of acute Chagas disease beginning the investigation on day two up to day ten after the insects' blood meal. It is likely that the very early PCR detection has occurred before parasites multiplication took place in the insect organisms, thus representing forms that had been ingested during feeding in infected mice. Nevertheless, earlier detection of parasites might be of clinical relevance irrespective of the parasite DNA origin (either from the blood meal itself, or originated from replication of parasites that had been ingested during the blood meal), because Chagas disease reactivation in immunosuppressed patients constitutes a life threatening event, and should be promptly diagnosed to increase survival rates.

Therefore, aside from previously defined conventional laboratorial methods already used to diagnose Chagas disease reactivation (Consenso Brasileiro em Doença de Chagas, 2005)[2], we propose that PCR should be performed directly from the patient's fresh blood, coupled to microscopy examination in the context of xenodiagnosis and/or to hemoculture (depending on the laboratory infrastructure and personnel expertise), in order to investigate reactivation in immunosuppressed patients. We also propose to anticipate *T. cruzi* detection by means of earlier tests beginning on day one after triatomines feeding with the patient's blood, followed by at least one test (preferentially two other tests) performed within the first five days after the patient's blood sampling. It is possible that two or three tests performed during the first week might impact the laboratory diagnosis of Chagas disease. We also recommend analysis on intermediate days, e.g., day 10 and/ or 15 (in our murine model corresponding to 60 and 65% of positive-PCR detection), as well as the maintenance of the traditional xenodiagnosis microscopic examination performed on days 30 and 60 post-feeding (in our study corresponding to 78.9% and 68.4% of positive-PCR detection), to enable comparison with previously reported studies (Fig. 1).

It seems judicious that a considerable number of cases should be analyzed simultaneously by qualitative PCR (kDNA and TCZ), and conventional diagnostic methods to allow a better understanding of the advantages and limitations of each of the techniques before starting

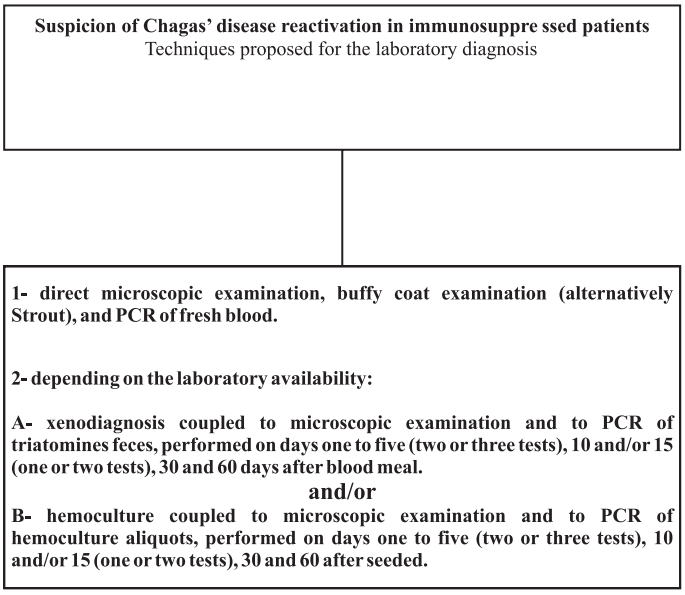

Fig. 1 - Model for the investigation of Chagas disease reactivation in immunosuppressed patients

T. cruzi quantification. Evaluation of parasite loads might, in a near future, become the technique of choice to diagnose Chagas disease, perform the follow-up of patients, and establish cure criteria.

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