

Chagas Disease: Criteria of Cure and Prognosis

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Therapeutic protocols for treating the acute and recent-chronic phases of Chagas disease were unanimously agreed upon by participants of the Symposium on the Specific Treatment of Chagas Disease in 1985. However, long-term clinical trials among patients treated (cured and uncured) for the indeterminate-chronic and initial-cardiac disease forms were recommended to better evaluate the curative action of drugs and to study the clinical evolution of the disease. Clinical trials among these groups of patients were published because studies reported low efficacy of benznidazole in chronic disease (Ferreira 1970, Cançado 1980) while the evaluation of cure was defined as negative parasitologic and serologic test results (Cançado & Brener 1979). A decade later, specialists met in Brasília under the auspices of the Chagas Disease Control Program of FNS/MS. Those present were concerned about doctors abandoning the specific medication as well as the scarcity of studies on the impact of treatment in the evolution of the disease among untreated and treated patients. Therefore, the 1985 recommendations were reaffirmed.

Today, the proposal of this International Symposium of Chagas Disease to discuss the evolution of knowledge regarding chronic-phase treatment is as timely and relevant as ever.

Regarding the role of the parasite in maintaining the inflammatory process in the chronic phase, recent studies by Kalil et al. (1993) and Higushi (1995) confirm the historic studies of Chagas and Torres and the results of experimental research by Segura et al. (1994) and Andrade et al. (1991). The latter has brought new hope to clinicians because they reported the interruption of the progression of lesions in treated mice, and verified the effect of benznidazole in the regression of myocardial

inflammatory lesions and in skeletal muscle of chronically infected animals that presented parasitologic cure even though serologic tests were positive.

Even though studies of Macedo and Silveira (1987) and Manzullo et al. (1982) reported no observed differences in the clinical evolution of treated patients who had the indeterminate form of chronic disease, in the 1990s, some investigations have reported the lack of reliable parameters to determine treatment efficacy in the chronic form, which could be responsible for low levels of cure and calls into question the real benefit of treatment. Viotti et al. (1994) followed for an average of eight years, 131 chronic chagasic patients treated with benznidazole and 70 untreated subjects with an average age of less than 50 years. They reported that the treated subjects, independent of negative serologic or parasitologic results, presented fewer alterations in electrocardiograms (4.2%) compared to the untreated patients (30%), and a lower incidence of changes in the clinical stage (2.1% vs 17%). In Brazil, Fragata Filho et al. (1995) conducted a retrospective study that found that among 61% of subjects with negative xenodiagnosis, only 7% of treated patients (both cured and uncured) experienced worsened clinical states opposed to 14.3% of the placebo group, after seven years of follow-up. In 1997, Coura et al. (1997). Treated 26 chronic chagasic patients who had at least two positive results out of three xenodiagnosis tests and verified that after one year, only 1.8% continued to have positive xenodiagnosis even though serologic tests remained positive.

In 1984, Krettli et al. observed that treated chronic patients had persistently positive serologic tests for long periods of time although lytic antibodies, which recognize epitopes on the surface of living trypomastigotes disappeared early, indicating the absence of active infection. Earlier studies reported the possibility that immune memory (Cerisola et al. 1972) was responsible for persistently positive serologic tests while parasitemia was negative. Viotti (1994) reported that besides the immune memory, pauciparasitemia, low sensitiv-

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ity of xenodiagnosis and absence of short- and mid-term indicators of improved clinical state all contribute to the difficulty of evaluating etiologic treatment.

The role of the strain factor in failed treatment and even disparities in the success of treatment by were demonstrated in 1997 by Toledo et al. who verified different susceptibility levels to treatment according to the *T. cruzi* strain. Ten strains were considered sensitive (61-100% cure), one partially sensitive (50% cure) and seven resistant (40% cure). In addition, the authors reported that benznidazole totally eliminated inflammatory lesions in skeletal muscle and partially eliminated heart lesions in cured rats and even treated, uncured rats experienced a reduction in lesions.

There is consensus that adverse side effects occur in 30% of patients receiving benznidazole, but in general the side effects do not justify cessation of treatment. The alleged carcinogenic effect has been thoroughly discredited by researchers with well-known clinical experience in the management of chagasic patients.

The disparity in the trypanocidal action of available drugs as well as their negative side effects have prompted a search for new compounds to treat Chagas disease. Nitromidasolics, like metronidazol, and antifungal azolic derivatives, like ketoconazol, have been studied *in vitro* and in animal models but their efficacy has not been demonstrated in humans. Some authors have raised the possibility of using allopurinol and itraconazole but Rassi et al. (1996) conducted a randomized double-blind study in 35 patients and concluded that a daily dose of 900 mg of allopurinol was ineffective, having results similar to those of a placebo. Recently, Urbina et al. (1998) investigated the antiproliferative effect of a new antifungal triazole compound and found that it was better than ketoconazole or itraconazole. In a murine model of chronic Chagas disease, the drug was more effective than ketoconazole, providing 75 to 85% protection from death, with 60 to 75% parasitologic cures in the surviving animals, while no parasitologic cures were observed with ketoconazole.

The search continues for new compounds against *T. cruzi* that have low toxicity and increased efficacy during the chronic phase. Furthermore, there is a lack of a reliable technique to evaluate cure. Therefore, identifying an antigen that can fulfill the role of evaluating the efficacy of Chagas disease treatment is of the utmost importance. Criteria in treated patients are problematic due to the absence of routine methods to discriminate between those who have been cured and those who have not. Indirect parasitologic tests like xenodiagnosis and hemoculture detect circulating para-

sites in 50-70% of cases (Cerisola et al. 1972, Castro et al. 1983, Chiari et al. 1989, Galvão et al. 1993, Luz et al. 1994). Conventional serology (CS) tests detect antibodies directed against internal or surface components of intact *T. cruzi* or its extracts. Such tests include indirect immunofluorescence, indirect hemagglutination and ELISA, all of which are sufficiently sensitive while having a low percentage of false positives, which normally occur in sera of individuals infected with *Leishmania* sp. e *T. rangeli* (Araújo 1986, Chiller et al. 1990). Nevertheless, evaluation of treated chagasic patients presents a certain degree of difficulty since CS remain positive for many years post-treatment in a large percentage of patients, including those who have repeatedly negative parasitologic results (Galvão et al. 1993). CS used to monitor cure (Cançado 1981) slowly and progressively become negative in a small number of treated patients (Krettli et al. 1984, Galvão et al. 1993). Antibodies present in the sera of chagasic patients induce lysis in trypomastigote forms in the presence of a complement (lytic antibodies), indicating the presence of active infection - CoML (Krettli & Brener 1982). Following specific treatment, lytic antibodies disappear from the sera of some patients while CS remains positive. This tendency has created new perspectives regarding the evaluation of cure in chagasic patients and shows clear discordance between CS antibodies and lytic antibodies in both human patients and experimental animals, the former being classified as dissociated (Krettli et al. 1982). In 82 chronic patients, 9% had negative results in all tests and 26% had persistently negative CoML and hemoculture tests even though CS remained positive, confirming the suspicion that dissociated patients were in fact free of infection. Therefore, the absence of lytic antibodies as measured by repeatedly negative CoML tests post-treatment indicates cure and is a promising result that increases the possibility of detecting successful treatment (Galvão et al. 1993).

Several *T. cruzi* antigens have been used in ELISA and Western Blot tests to substitute living parasites in the evaluation of cure among chagasic patients who have received specific treatment. The soluble excretory antigens of supernatant trypomastigotes in cell culture (Krautz et al. 1994) as well as GP57/51, purified cystein proteinase of epimastigote forms (Murta et al. 1990) are capable of identifying 70% of the dissociated treated patients (Gazzinelli et al. 1993). *T. cruzi* antigens like the F2 fraction glycoconjugate (Almeida et al. 1993) and the 24 kDa-Tc24 recombinant protein (Ouaissi et al. 1992, Taibi et al. 1993, Krautz et al. 1995) as well as the new approach of CoML test using flow cytometry (Martins-Filho et al. 1995)

have been used to detect anti-living trypomastigote antibodies in sera of treated chagasic patients and have opened new perspectives for evaluating cure.

Another promising tool is polymerase chain reaction (PCR), which has been used to detect *T. cruzi* in the blood of chronic chagasic patients. Initial data reveal positive PCR results in 83-100% of patients (Ávila et al. 1993, Brito et al. 1995, Gomes et al. 1998). Recently, a strong correlation was reported between positive PCR and CoML results among chagasic patients, suggesting that PCR could be effective in evaluating parasitologic cure in patients who received specific treatment (Gomes et al. 1999).

Besides the use of parasitic and serologic markers to evaluate the effectiveness of trypanocidal drugs, another approach is to identify immunologic markers that are correlated with the presence of the parasite. It is known that *T. cruzi* lives freely within the cytoplasm of infected host cells, interfering with the host immune response, which allows for the establishment of a persistent infection, indicating its success at evading immune clearance.

Immunologic events that occur in Chagas disease are multiple and include diverse effector and regulatory processes that result in a balance between resistance and pathogenesis. Concerning the last mentioned, when a pathologic consequence of the host response develops, severe morbidity secondary to the inflammatory process is seen. Among the implicated immune events, we highlight the involvement of T cells, which is evidenced by an increase in the proliferative response to parasite-related stimuli of peripheral T cells, as well as by immunohistochemical studies in heart tissue that show predominantly CD8+ T cell infiltrates and increased HLA class I and II molecule expression in histiocytes and endothelium cells (Reis et al. 1993, Reed 1998). Moreover, immunophenotypic analyses consistently reveal findings of peripheral T cell activation, like the increase in HLA-DR expression consonant to the increase of CD5+ B cell populations (Dutra et al. 1994). Similarly, analyses of cytokines demonstrate type 1 immune response, especially preferential IFN- γ production (Gomes 1998).

The hypothesis of active participation of autoimmunity in disease pathogenesis is illustrated by experimental models showing polyclonal activation of B and T cells, during the acute phase (Minoprio et al. 1991) and, in human disease, an increase of peripheral CD5+ B cells in chronic infection was observed (Dutra et al. 1994). It has also been demonstrated that infiltrate cells recognize cardiac myosin rather than primary *T. cruzi* antigens in Chagas patient heart tissue (Cunha-Neto et al. 1996). In the

other hand, recent studies have revealed a positive association between tissue parasitism and severity of inflammation. Furthermore, some researchers consider parasitization of heart tissue to be both necessary and sufficient to cause tissue damage, thus creating a case against a major autoimmune etiology of the disease (Tarleton et al. 1997).

The long-term effect of etiological treatment on immune response has been investigated in studies in which patients were treated during the acute phase and evaluated years later. It has been reported that, years after treatment and independent of therapy success, a return to normal percentages of T cells subsets and activated T cells population is observed (Gomes 1998). Nevertheless, a longitudinal follow up had never been elaborated to elucidate the pathways of immune reorganization.

In conclusion, it is necessary to implement studies that establish the effect of specific therapy on the immune state and on the evolution of Chagas disease. Evaluating cell populations in peripheral blood of patients who received specific chemotherapy is extremely important in determining the role of the parasite and the immune system in the pathological development of Chagas disease. This type of research will allow for the identification of immunologic markers that indicate cure.

Since 1997, researchers from the Chagas Disease Clinic of the Hospital das Clínicas of the Federal University of Minas Gerais, together with those from the Biological Sciences Institute and Fiocruz, have been conducting a prospective study of 140 patients to test the hypothesis that specific treatment can impact the evolution of disease, preventing or retarding the development of defined clinical forms, and that treated chagasic patients differ from untreated ones, independent of cure. In addition to titer serology and hemoculture, lytic antibody test, PCR and parasite antigens were included to improve evaluation of cure. An immunologic evaluation has been made to determine predictive markers of improved clinical outcome. The evaluation focussed on activated cell populations, which could be involved in the immunopathologic mechanisms of disease.

Using flow cytometric immunofluorescence staining for cell surface markers, our preliminary data show a clear tendency of decreased mean and individual percentages of activated T cells (CD3+ HLA DR+) and CD5+ B cells, and an increase in the population of CD3+ cells as early as 6 months after complete treatment. At the same time, we observed an increase in the expression of CD28 in the CD8 T cell subpopulations. These findings support the hypothesis that cell activation is correlated to parasite burden.

Among 129 subjects between the ages of 20 and 50, we conducted a single hemoculture for each patient and found that 51% of them had positive parasitemia. A year later, during annual check-ups, 88% of treated patients had negative test results.

Whether these results have an impact on preventing disease progression will be determined through extended clinical follow-up. If a positive correlation is observed, we will be able to indicate the use of any of these criteria as predictive indicators of good therapeutic response and improved outcome.

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