doi: 10.5216/rpt.v50i4.71556

SHORT REPORT

CHAGAS DISEASE. THE USE OF SEROLOGY TO ESTABLISH DRUG EFFICACY. VALUE AND LIMITATIONS*

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Chagas disease presents two different phases: acute and chronic. The acute phase is defined by the presence of easily demonstrable parasites, while the chronic phase is characterized by the presence of circulating antibodies against *Trypanosoma cruzi.* These antibodies are normally present and may be the only evidence of infection, since parasites are not often detected in the chronic phase (Luquetti & Rassi, 2000).

After specific treatment, if successful, the antibodies disappear (Rassi & Luquetti, 1992). The kinetics of this clearing up, differ according to several variables, as follows.

Considering that before specific treatment, patients present specific antibodies and sometimes parasites, in order to verify a cure, both should disappear. Once the cause (parasite) is no longer present, the effect (antibodies) ceases. If one or both are present, the cure has not been attained; the cause (parasite) will be there, and treatment failed, so the drug was not effective at the doses and period of time employed (Cançado, 1999).

Presence and levels of anti T. cruzi *antibodies*

Specific antibodies are present in a constant concentration in a given patient. Differences found in the past, should be attributed to technical problems, including different reagents and kit lots. We will show evidences of follow-ups in untreated patients, followed for decades, presenting a constant level of antibodies, measured by different techniques. Concentration measured by

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*Presented at the "Consultative Meeting on the Strategic and Operational Aspects for the Clinical Development of Trypanocidal Drugs for Chagas Disease, 23-24 April 2007, Buenos Aires, Argentina.", sponsored by TDR, WHO.

This publication is dedicated to one of the authors (deceased) due to his life dedicated to Chagas disease and particularly to this subject. After 14 years, this material is still valid.

**Deceased

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indirect immunofluorescence (IIF) is the same (plus/minus 1-2 titers) in the same patient, but different from other individuals (Luquetti & Rassi, 1998).

This is a key concept for understanding that if a shift occurred after drug intervention, this was due to the drug and not to chance. (Rassi & Luquetti, 2003)

How to measure antibody concentration

Conventional tests (ELISA, IIF and indirect hemagglutination) have been used since 1975. There is vast experience of their use in all endemic countries. A number of trademarks are available on the market, which are frequently tested and subjected to different studies to assess their performance. All three types of test give the concentration of antibodies present, comparable in the same patient, at different time intervals.

Rapid tests are on sale, some of them have been tested on large scale (Luquetti et al. 2003), but are not useful for the follow up of a treated patient, since the result is only qualitative (yes or no). In order to follow up a treated patient, differences in titers must be noted from the start and over time.

Other quantitative tests that employ chemiluminiscence, FACS scan, and others, are not available on the market, and the necessary equipment to run them is usually not at hand in the field (Martins-Filho et al,, 2002).

For the required follow up, it is essential to store serum samples from the beginning. These could be run in parallel with the available tests. This storage also allows the use of future techniques, which have not yet been developed. Storing serum in 50% glycerin is very useful as it preserves the antibodies for decades.

When the serological shift is achieved

This is another critical point. It depends mainly on the phase in which the treatment is performed. In fact, it depends on how long the parasite has been in contact with the patient. In the acute phase, *T. cruzi* has been in the individual for some weeks and, if destroyed by the drug, antibodies disappear in around one to five years. We emphasize that even the immunological memory has been lost.

The situation is diverse if children are treated (coined as recent chronic phase), with an estimated presence of the parasite for a few years. In our region, it takes from five to ten years, in order to have no more (or a negligible amounts of) antibodies. Evidences from other regions (see below) did show shorter periods.

Finally, in adults (late chronic phase in terms of treatment), who hold the parasite for decades, we need to wait the same amount of time (decades) in order to see a decline and finally absence of antibodies which were formerly present (Rassi & Luquetti, 2000).

Geographical differences

As known, *T. cruzi* is very heterogeneous and currently divided into at least two groups: *T. cruzi* I and II. The first mainly in humans north of the Amazonian River and *T. cruzi* II, prevalent in humans in the South Cone. The latter is associated with mega viscera and severe heart disease (Luquetti et al, 1986). Specific treatment has been performed recently in children from Colombia and Honduras, regions with *T cruzi* I. (Yun et al. 2009). Reports from both regions show a serology conversion in a very short time (one year).

Conclusions

We believe that the quantitative follow up of specific antibody concentrations is the cheapest, fastest and easiest tool to verify if a given drug has been fully successful in killing all the parasites and is, therefore, very valuable. The limiting factor is the time for this verification, measured in years.

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