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Editorial

What is the role of real time PCR in the follow up of patients with chronic Chagas' disease?

¿Cuál es el papel de la PCR en tiempo real en el seguimiento de pacientes con enfermedad de Chagas crónica?

Chagas disease is a tropical parasitic disease caused by the protozoan parasite *Trypanosoma cruzi.* This disease is endemic in 21 countries on the American continent, from the southern states of the USA to the north of Argentina and Chile. Over the past several decades, migration of infected Latin American people to countries outside Latin America as well as congenital transmission and blood donation, has made Chagas disease a global epidemic.[1] In Europe, about 120,000 immigrants are estimated to be infected with *T. cruzi* and around 50,000 of them are living in Spain.[2] This situation poses a challenge for health systems of both endemic and non-endemic countries.

Chagas disease has two phases, acute and chronic. The acute phase is characterized by high levels of parasitaemia while the chronic phase of disease is intermittent and is characterized by the presence of antibodies against *T. cruzi.* Although the difference between both phases is not always clear, patients in the chronic phase of the disease can suffer immunosuppressive conditions and return to a high level of parasite replication similar to that seen in the acute phase. Therefore, the usefulness of different diagnostic techniques depends on the phase of the disease.

Although severe disease can occur, clinically, acute infection is typically asymptomatic or with nonspecific manifestations. Afterwards people enter the indeterminate phase that is characterized by chronic asymptomatic infection and they can remain in this state throughout their life. Trypanocidal treatment in this phase have the disadvantage of being less effective and worse tolerated.[1] Nevertheless, treatment in the chronic phase has shown clearance of the parasite in blood, decreases in the specific titer of antibodies against *T. cruzi* and, although this point is controversial, some studies have shown a reduction in disease progression toward symptomatic form (the cardiac form, the digestive form, or both).[3] In addition, the treatment of infected women of childbearing age has shown that the transmission of congenital Chagas disease can be prevented.[4]

The classic cure criterion requires that patients have two consecutively negative conventional serology tests.[5] In this context, the assessment of therapeutic efficacy after treatment in patients with chronic Chagas disease is difficult to carry out due to the presence of antibodies that may persist in these patients for many years, even a lifetime. This situation makes it difficult to evaluate therapeutic efficacy in a short period of time. Non-conventional serology tests and biomarkers have been developed in order to achieve this objective.[6,7] Despite efforts, obtaining a biomarker with optimal accuracy to confirm the effectiveness of treatment in this phase of the disease remains a challenge. In a recent years, one of the most widely used techniques in the follow-up of parasitological treatment are molecular techniques, such as Polymerase Chain Reaction (PCR).

The development of PCR has entailed a great advance in the diagnosis and follow-up of Chagas disease in recent years. This technique uses sequences known as primers that detect and amplify nucleic acid target sequences in the parasite, satellite DNA and the variable region of kinetoplast DNA *(kDNA)* minicircles from *T. cruzi*, has been the most widely used for parasite detection.[8] This technology has been improved with the development of real time PCR, a technique that is easier to automate and standardize and also allows for the quantification of parasitic load.[9] Given the wide variety of PCR techniques and protocols that has been used for *T. cruzi* detection, it became necessary to carry out an international multicenter study for the clinical validation and standardization of this technique where it was corroborated that the analytic sensitivity of PCR is high.[8,10] This was mainly found in the acute phase of infection, as in congenital cases, where PCR has a sensitivity around 100%.[11]

In the chronic phase of the disease, PCR has a less performance than in the acute phase due to low and fluctuating parasitaemia as described by Sulleiro and colleagues in a recent study.[12] In this study they detected the presence of *T. cruzi* by real time PCR in 42% of a cohort of 495 untreated chronic Chagas disease patients and around 55% of subgroup of them had intermittent parasitaemia. Moreover, the rate of PCR positivity decreased in patients with 5 years or more of residence in Spain, which suggests that the influence of external factors in the parasite presence in peripheral blood must also be taken into account. Therefore, although PCR is not suitable for diagnosis in the chronic phase, it is useful in determining the initial parasitological status of patients in order to assess their response after treatment.

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Regarding post-treatment monitoring, PCR has been widely used for the post-treatment parasitological follow-up of Chagas disease. These techniques, conventional PCR and real time PCR, provide support for the efficacy of benznidazole to clear parasites after treatment, and almost 100% of the patients have negative PCR results 90 days post-treatment. Therefore, PCR has shown to be a sensitive and specific tool for the early detection of the effectiveness of benznidazole.[7,13] What is more, real-time PCR detected the reduction of parasite load in the follow-up of patients with chronic Chagas cardiomyopathy, making it possible to assess the effectiveness of treatment after its administration in these patients.[14] Moreover, PCR is capable of detecting treatment failure in patients whose PCR shifts to positive in a short-term follow-up, enabling early therapy modification in cases of reactivation of the infection.[13]

There is a broad consensus for considering PCR as a marker of parasitological cure, however its negativity does not guarantee the cure of the infection. The treatment showed excellent efficacy in eliminating blood stage parasites, but it is not at all clear if it is capable of acting on the parasitic tissue forms. Nonetheless, it has recently been demonstrated that post-treatment sustained PCR-negative results are associated with a significant decrease in *T. cruzi*-specific antibodies, which highlights the effectiveness of the treatment.[7,15]

Another important application of real-time PCR is in the follow-up of immunosuppressed patients with Chagas disease, as occurs in patients coinfected with human immunodeficiency virus (HIV), solid organ or bone marrow transplant recipients who receive an organ from a donor infected with *T. cruzi;* and patients with cancer or other diseases that weaken the immune system. The immunosuppressive conditions that these patients suffer could imply the reactivation of the disease and the consequent increase of parasite load. Hence, PCR is a useful tool to follow-up these patients and it provides an early and sensitive indicator of reactivations.[16] In the case of recipients of transplants from infected donors, PCR allows for the early detection of infections transmitted from an organ donor to recipient. Concretely, the study of Diez and colleagues[17] reported that real-time PCR became positive 38–85 days before the onset of symptoms in transplant recipients from infected donors, allowing this technique to provide a rapid detection of transplant rejection.

Studies focused on evaluating the role of real-time PCR as a predictive marker of disease progression, or risk of infection, have also been carried out. In this regard, the relationship between the detection of *T. cruzi* DNA in blood by PCR in patients and the risk of developing advanced Chagas has been studied but there is no consensus about this association. The study of Sulleiro and colleagues[12] showed that a positive real-time PCR result is not related to the presence of visceral abnormalities, whereas Sabino and colleagues[18] described that positive real-time PCR is associated with Chagas cardiomyopathy and disease severity; therefore future studies are necessary to clarify this point. With regard to PCR as a predictive marker of the risk of infection, it has been demonstrated that this technique is useful for predicting the risk of congenital transmission. Mothers with parasitaemia detectable by PCR during the third trimester of pregnancy have a higher risk of *T. cruzi* transmission to their newborns which implies that they and their newborns are going to require an exhaustive medical follow-up.[19] Likewise, sustained PCR-negative results have been observed in the majority of women who were treated before they became pregnant, so the treatment of infected women of childbearing age reduces parasitaemia detectable by PCR.[4] Therefore, PCR is a useful tool for identifying a priori those chronically infected mothers who have a high probability of transmitting the infection to their infants.

Real-time PCR is not exempt from limitations such as a higher cost, requiring a thermal cycler coupled with an optical reading system to allow for the interpretation and a high level of technical skill. Despite these limitations this technique is the most widely used due to its automation and high sensitivity for *T. cruzi* detection. Commercial PCR diagnostic kits have recently been developed for the detection of *T. cruzi*,[20] which have enabled the standardization and implementation of PCR in clinical diagnostic laboratories.

In conclusion, PCR makes it possible to detect parasite load after treatment confirming its efficacy in the short term and is also able to detect therapeutic failure. At the same time, sustained negative PCR results are indicative of the long-term effectiveness of treatment. Real-time PCR has also shown its utility in immunosuppressed patients and recipients of transplants from infected donors, where it acts as an early marker of reactivations. Another important application of real time PCR is in pregnant women chronically infected with Chagas disease where it is used as a predictive marker of the risk of congenital transmission. Therefore, real-time PCR has become a highly useful tool in the parasitological follow-up and clinical management of patients with chronic Chagas disease in recent years.

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Marina Simón [a,*], M. Asunción Iborra [a,b], Bartolomé Carrilero [a,b], Manuel Segovia [a,b]
[a] *Unidad Regional de Medicina Tropical, Servicio de Microbiología, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain*
[b] *Departamento de Genética y Microbiología, Universidad de Murcia, Spain*

* Corresponding author.
E-mail address: marina.simon.paez@gmail.com (M. Simón).