Faculty of Medicine, University of Chile.

Diagnosis difficulties

Professor Werner Apt Baruch and **Dr Inés Zulantay Alfaro**, parasitologists based in Chile, discuss their research towards understanding and treating a parasitic infection recognised as a neglected tropical disease

Why were you initially motivated to begin your study on Chagas disease?

Chagas disease is now a public health problem in the Americas and an emerging epidemiological situation in several other countries accepting immigrants from endemic zones. In Chile, the control programme aimed at the biological vector *Triatoma infestans* has been successful; however, there are outstanding issues such as multidisciplinary congenital transmission control and care of patients in the chronic phase, especially in aspects related to therapy. One of our research lines was to establish, through a pilot project, the current situation with regard to congenital transmission in an endemic area without controls.

How can your results be projected onto familial studies of the maternal line, and how would this impact the current figures on the prevalence of Chagas disease?

When the results of our study were established, we asked ourselves about the situation in the family groups of infected women, who were considered as index cases. We then developed a study to gauge infection in the maternal line of 70 families, which presented with an average of 3.1 infected members per family group. All women of childbearing age were infected, along with 6/70 newborns, 10/57 brothers less than 15 years of age, 57/70 maternal grandmothers and 75/152 maternal siblings. Undoubtedly, as far as the maternal line is concerned, the current estimations of prevalence would increase with further research of *T. cruzi* infection.

What is Chagas cardiomyopathy?

This injury is the most serious complication of Chagas disease. The patient develops a dilated cardiopathy with extensive functional and organic alteration. Asymptomatic chagasic patients with visible electrocardiographic alteration have a 1 per cent mortality rate, and 26 per cent have clinical episodes. Cases with dilated cardiopathy, but without heart failure, have a mortality rate of 14 per cent, and 52 per cent present clinical episodes. Untreated patients with dilated cardiomyopathy, due to *T. cruzi*, with cardiac failure, have 50 per cent mortality within five years.

Which drugs are currently being used to treat Chagas disease, and what are the criteria of efficiency for treatment?

In our country, nifurtimox is currently available and the health authorities are making efforts to import benznidazole. There are no ideal drugs to treat the chronic phase because of their toxicity in adults. However, we know that treatment is capable of modifying the nature of the disease progression and preventing the development of cardiopathy – the leading cause of death. These treatments would also improve the quality of life of infected individuals and reduce the economic cost of this pathology.

It is difficult to determine the effectiveness of these therapies towards curing chronic cases because the serological tests that detect antibodies against *T. cruzi* remain positive for decades after treatment has appeared to be successful. Consequently, follow-up checks for 10-20 years are needed to determine the efficacy of the drug. The conventional parasitological tests such as xenodiagnosis and haemoculture are laborious and time-consuming, in addition to their low sensitivity – limiting their practicality and usefulness in diagnosis and the surveillance of therapeutic efficacy.

Do you think that treatment of Chagas disease is an unresolved public health problem?

Absolutely. It constitutes an important challenge for basic and clinical researchers. There are millions of people in the world who are in the indeterminate or determinate chronic period of the disease. Many have died from causes related directly to the disease without receiving any treatment. It is necessary to evaluate the presence or absence of heart or digestive damage; facilitate access to expensive procedures such as barium enema or cardiac echo-Doppler; evaluate access to surgical treatment or trypanocide drugs, according to the inclusion and exclusion criteria required by pre-determinate protocols; and generate the respective attention protocols. As a 'neglected disease', since Chagas usually affects vulnerable people from rural and low socioeconomic backgrounds, rethinking is required concerning rights to information and healthcare policies, including treatment and access to drugs. Fortunately, in recent years there has been growing concern on this matter.

PROFESSOR WERNER APT BARUCH & DR INÉS ZULANTAY ALFARO

Silent but **deadly**

A medical research group at the **University of Chile** in Santiago is implementing a tripartite approach to combatting Chagas disease, investigating the differential mechanisms of transmission

NAMED FOR THE Brazilian physician who first described it in 1909, Chagas disease is caused by infection of *Trypanosoma cruzi*. The parasite is spread primarily through insect vectors, most commonly the 'kissing bugs' of the Triatominae subfamily of Reduviidae. Since Charles Darwin was bitten by such a bug during his expeditions aboard The Beagle, it has been suggested by some that his later incapacitation and death may have been due to Chagas disease. It seems unlikely that the so-called 'Chagas hypothesis' could constitute a reasonable explanation as to Darwin's mysterious illness – but it nonetheless highlights some important features of Chagas disease, which presents with an unusual set of symptoms. Following infection with the protozoan, patients experience the acute stage of the disease – which lasts for a period of weeks or months and causes either mild symptoms or no symptoms at all. After this, the disease enters its chronic phase, but even then between 60 and 80 per cent of patients never develop symptoms. The remaining 20 to 40 per cent have determinate chronic Chagas disease, which causes life-threatening heart and digestive disorders.

CHAGAS IN CHILE

Determinate chronic Chagas disease affects mainly the heart and/or digestive tract, with cardiomyopathy being the most common cause of death of patients. Its tendency to present no symptoms, and unpredictable transmission through various sources, makes it a significant challenge to healthcare systems in South America. The disease is transmitted via insect vectors, blood transfusion and across the placenta, but it can also be contracted orally through infected food. With the rise in international emigration from the American continent, this disease is becoming an increasing problem in other parts of the world.

One team has been devoting a lot of attention to this infection. Based at the University of Chile's Biomedical Sciences Institute, the group is led by Professor Werner Apt Baruch and Dr Inés Zulantay Alfaro. Taking a three-pronged approach to understanding Chagas, they are investigating the spread of infection through the maternal line; trying to identify novel biomarkers of cardiopathy, and evaluating the efficacy and potential of the currently available drug therapies by using clinical trials. These projects are being pursued in tandem through a number of interrelated studies and reflect the three main challenges presented by Chagas disease: mechanisms of cardiac pathology, difficulties associated with diagnosis and its problematic treatment.

MATERNAL LINES

In recent years, improved education on Chagas disease, control programmes aimed at insect vectors and improvements to housing have made a big difference to the prevalence of the disease in Chile. In light of these advancements reducing vectorial transmission, vertical transmission from mother to child has become much more significant.

Researchers under the leadership of Zulantay recently conducted an investigation monitoring *T. cruzi* infection in 4,280 pregnant women with chronic Chagas disease. Using serial polymerase chain reaction (PCR), enzyme-linked immunosorbent assay and indirect immunofluorescence methods, the team confirmed that infection was present in 147 of these cases – 3.4 per cent of the mothers – and vertical transmission was confirmed in nine of their newborn children. The study also noted that applying conventional serological and molecular biological tests at birth, and as part of a regular follow-up programme, to mothers and newborn babies, was useful to confirm or discount congenital infection, estimate the parasite burden of *T. cruzi*, and the parasite lineage transmitted by the mothers. Based on the study's results, Chile should expect that 615 infected pregnant women will contribute annually to the total 18,081 births currently occurring in highly endemic areas, resulting in 25 congenital cases of Chagas disease.

ELUSIVE BIOMARKERS

The maternity study goes some way towards explaining the continuing prevalence of Chagas disease, which affects an estimated 11 million people in the Americas. Chagas' lack of symptoms means it is difficult to comprehensively estimate the size of affected populations and predict who will be seriously affected. To date, there are no useful biomarkers to distinguish a patient who will remain in the indeterminate phase of the disease for life, from one who will develop serious and fatal cardiopathies. Apt leads a team in the search for such biomarkers and it is his hope that, once found, these indicators of susceptibility will allow for targeted treatments at a greatly reduced cost.

In one study published in 2012, Apt's team investigated the genotypes of Chagas patients in an effort to discover whether mannose-binding lectin or toll-like receptor (TLR) polymorphisms could affect the contraction and presentation of Chagas disease. Most of the alleles studied were found to have no particular association with either healthy patients, determinate chronic Chagas patients or indeterminate chronic Chagas patients. However, the group did

Eggs and nymphs of *Triatoma infestans*.

Chagas' lack of symptoms means it is difficult to comprehensively estimate the size of affected populations and predict who will be seriously affected

Mount Chaguareche 2612 metres
Combarbalá, IV Region, Chile
Endemic zone of Chagas disease.
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Members of the Laboratory of Basic Clinical Parasitology.

uncover one particularly interesting correlation: genotypes deficient in TLR4 occurred more frequently in indeterminate Chagas patients (14.8 per cent) than in determinate ones (3.1 per cent). This indicates not only that this genotype is a potential biomarker for the disease, but also that lower levels of TLR4 activation may in some way prevent Chagas disease from becoming determinate.

DRIVING DEVELOPMENT

Both Apt and Zulantay have been involved in several clinical trials to evaluate the efficacy of various drugs for Chagas disease and to precipitate the development of new therapeutic tools. Their group has conducted important trials with itraconazole and nifurtimox, and is currently in the process of testing posaconazole as part of a multinational collaboration.

In order to effectively evaluate treatment for Chagas, it is necessary to carry out regular follow-up exams over future decades. Having treated 46 patients using itraconazole in 1992, Apt and Zulantay have had a unique opportunity to gauge the drug's efficacy over the course of their research careers. After 20 years, the study found that 21 patients had been successfully disinfected and that 15 of them showed normal electrocardiograph (ECG) readings. 32.6 per cent of the patients had therefore been cured of the disease, and only 10.86 per cent developed cardiac abnormalities. Only five of the 46 patients demonstrated a failure of therapy, suggesting that itraconazole has potential as an effective treatment for Chagas disease in most cases.

A GLOBAL GIFT

Chagas is a disease that operates over a very long timescale, and because it has been widely neglected by medical research until fairly recently, the road towards new and effective drugs would be a very long one if it were not for such well-positioned and longstanding researchers. With the threat of Chagas disease hanging not only over South America but increasingly over the US, Europe and Asia, it is sure to cease being a 'neglected' disease in the near future. The prescient and dedicated work carried out by the University of Chile researchers may well be a service not only to underprivileged endemic areas but to a much larger proportion of the global population.

Chagas fact-file

- Alternatively known as American trypanosomiasis
- Worldwide, 7-8 million people are infected with the *Trypanosoma cruzi* parasite
- Once confined to South America, Chagas is now found in the US, Canada, many European and some Western Pacific countries
- Global cost for treating Chagas patients is estimated to be US $7 billion

INTELLIGENCE

INTEGRATION OF BASIC, CLINICAL AND EPIDEMIOLOGICAL INVESTIGATIONS IN CHAGAS DISEASE WITH A TRANSLATIONAL VIEW

OBJECTIVES

The aim of these clinical epidemiological studies is to contribute to the control of Chagas disease through the implementation of a multidisciplinary approach; specifically in relation to transplacentary infection, the search for biological markers of cardiopathy, the treatment and efficiency evaluation of the drugs, the development of new tools for diagnosis and the education of rural health teams and patients.

KEY COLLABORATORS

Dr Aldo Solari; **Dr Sylvia Ortiz**; **Dr Juan Venegas**; **Professor Jorge Rodríguez**; **Dr Miguel Saavedra**; **Dr Catalina Muñoz**; **Dr Karina Cabrera**; **Dr Andrea Muñoz**; **Dr Eduardo Araya**; **Dr Gabriela Martínez**, Faculty of Medicine, Universidad de Chile, Chile

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INÉS ZULANTAY ALFARO is Associate Professor in the Biomedical Sciences Institute, University of Chile. She has a Master's degree in Parasitology and PhD in Biotechnology. During the last 25 years her principal line of research is the development and application of diverse laboratory techniques applied in the study of Chagas disease in Chile, particularly in epidemiological, diagnostic and chemotherapeutic aspects.

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