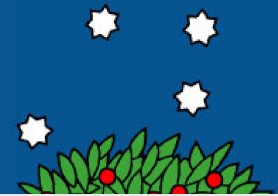




STUDY FOR THE EARLY DETECTION OF CARDIOVASCULAR DAMAGE IN CHAGAS DISEASE (AMERICAN TRYPANOSOMIASIS)

Development of a New Non-invasive Diagnostic Test
Based on Micro RNA-specific Pattern Identification

CHAGAS DISEASE: A Disease of the Poor



Latin America:

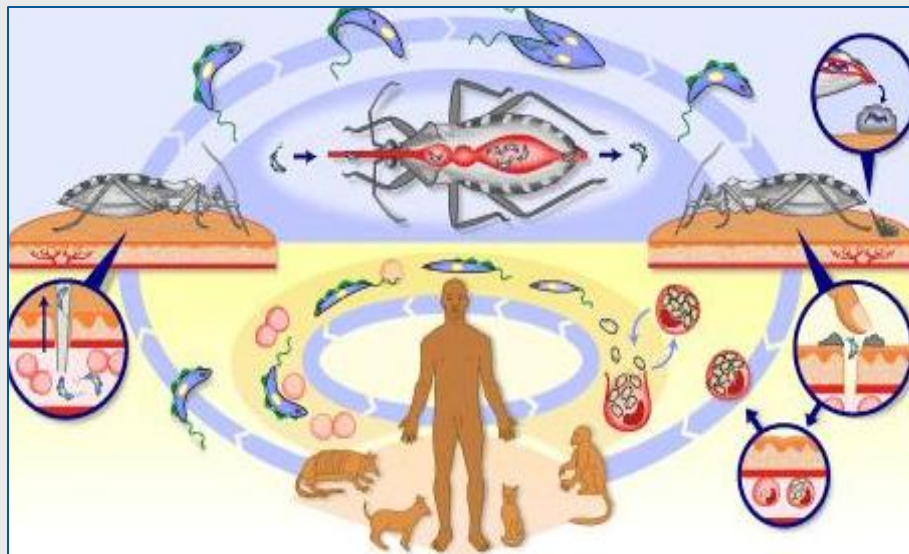
16-18 million infected individuals
4th leading cause of mortality

Disease transmission:

- Vectorial (*Triatoma infestans*)
- Blood transfusion
- Organ transplant
- Maternal



Disease vector: *Triatoma infestans*

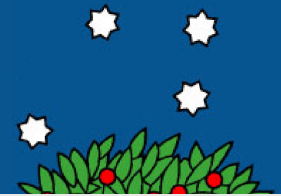


Disease agent: *Trypanosoma cruzi*



The infected insects hide in crevices inside human dwellings

CHAGAS DISEASE: A Disease of the Poor



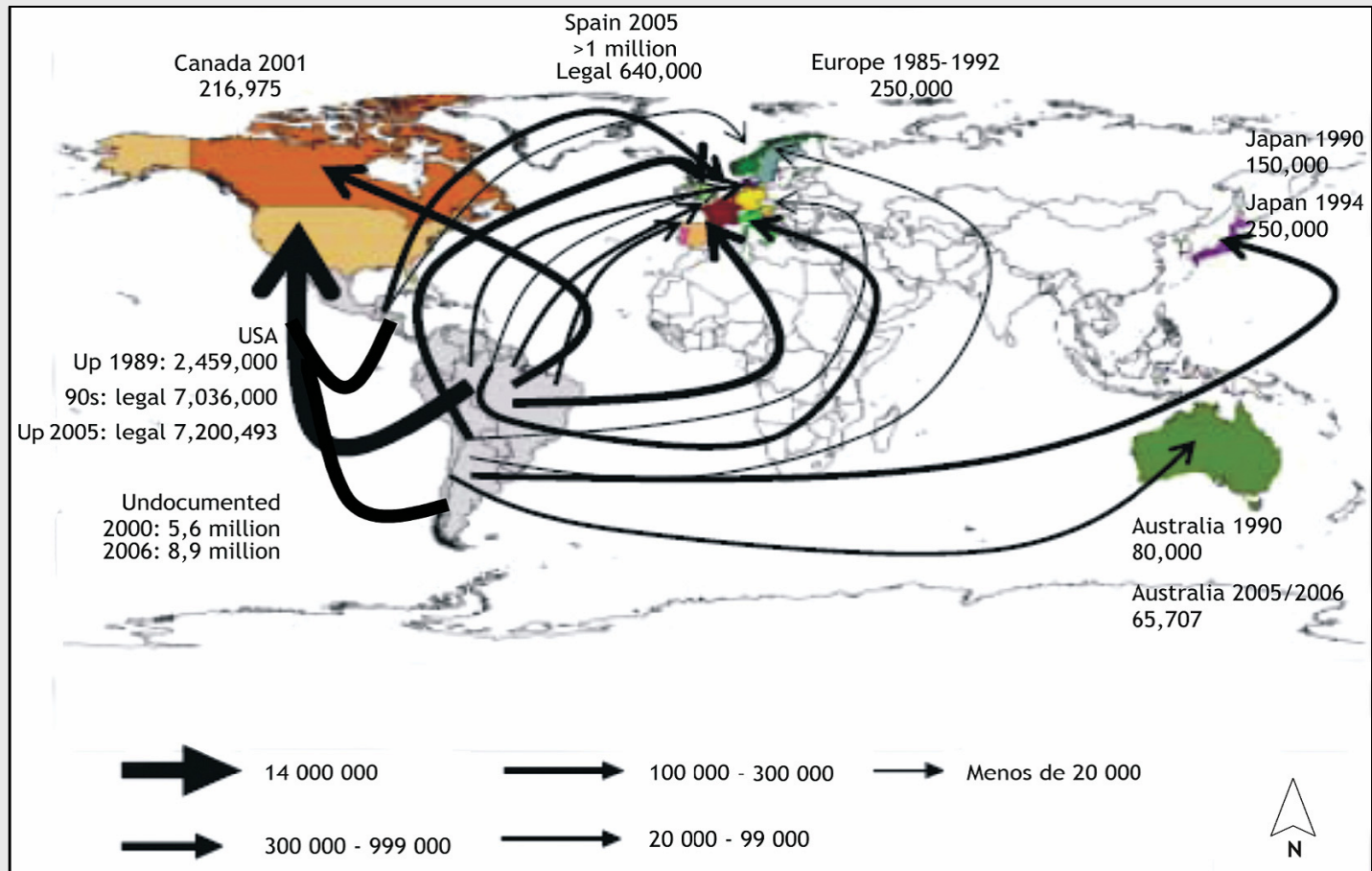
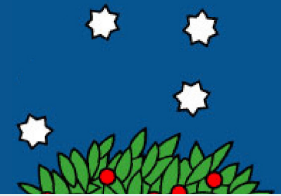
The primary infection largely occurs at a very young age



Migration
in search
of new
opportunities

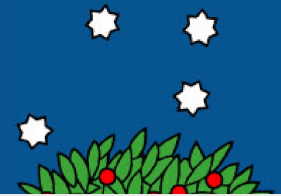


CHAGAS DISEASE: Effects of Migration



Due to migration the disease has spread to the **United States, Canada, Australia, and Spain**.
In **Latin America** 100 million people are at risk of contracting the disease in 21 countries.

CHAGAS DISEASE



Latin America: 16-18 Million Infected Individuals

ACUTE PHASE

- Circulating and tisular *T cruzi* (myocardium and other tissues)
- Generally antibodies appear
- Inflammation, myocarditis
- Mortality: ~ 10% due to myocarditis or meningoencefalitis.



>95% non-treated acute patients

- Presence of infection (serologic and parasitologic tests)
- Absence of cardiac abnormalities

10–20 year (33.8%)

30 years (49.3%)

40 years (58.1%)

*Persistence
of T cruzi in
myocardium?
Autoimmunity?*

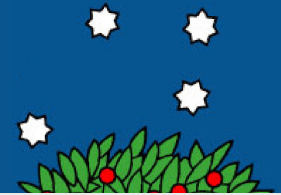
Infected Individuals would be clinically
asymptomatic until the accumulation of
a critical mass of damaged heart tissue:
Clinical Sintomatology



CHRONIC PHASE

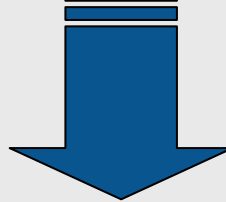
- Chronic Myocarditis (30% of patients)
- Slow progression to chagasic heart damage
- Discapacity
- ↑ risk sudden death

CHAGAS DISEASE: Discapacity



The primary infection occurs at a very young age

Slow evolution to
cardiovascular
damage



By the age of **30 to 40** years, Chagas' disease patients become **handicapped** and/or **unable to work** due to the complications and consequences of the disease

CHAGAS DISEASE: Patients and Argentinian Public Hospitals

CHAGAS´DISEASE PATIENTS

- belong to low income groups that for the most part have no access to private medical care
- mostly detected when they attend public hospitals once they already present cardiac symptoms of the disease

PUBLIC HOSPITALS

Lack the economic resources and complex diagnostic image infrastructure that would allow the early detection of subclinical cardiac symptoms in indeterminate patients

NEED FOR IMPROVEMENT

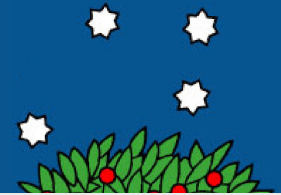
Availability in public hospitals of low cost screening methods to detect incipient cardiac damage in individuals at risk of being indeterminate Chagas´disease patients (on the basis of their geographical region of origin)

Organism	Trypanosoma cruzi	MSGPP, University of Washington, Dept Biochemistry
At Risk	100 million - Central and South America	
Humans Infected	16-18 million	
Disease Outcome	10-30% die of complications after decades of infection	
Vaccine Prospects	Poor due to antigenic variation	
Available Drugs	Nifurtimox and benznidazole (reduce parasitic load): ▪ 60-70 % effective: acute stage, recent chronic stage, congenital infection; ▪ ~60% effective: indeterminate phase in children ▪ 8%-26% effective: chronic stage	
Drug Resistance	Suspected	

In the majority of **indeterminate** chagasic patients, parasite clearance or negativization of serum markers with **nifurtimox and benznidazole** **does not protect** from the development of those **cardiological complications** that result from **immunological changes** initiated in the **acute phase** of the disease.

This underscores the importance of early diagnosis of subclinical myocardial damage in asymptomatic Chagas patients, in order to submit them to cardiovascular protection treatment

CHAGAS DISEASE: Goals and Objectives



MAIN GOAL

To reduce morbidity and sudden death in early phase of Chagas' disease .

SPECIFIC OBJECTIVES

1. To assess the prevalence of early minimal (clinically undetectable) cardiovascular damage in early (indeterminate) chagasic patients
2. To identify a minimally invasive biochemical indicator as a surrogate marker of early minimal (clinically undetectable) cardiovascular damage in indeterminate Chagas' patients

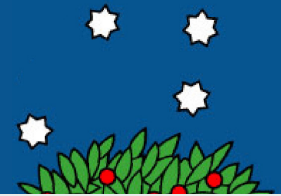
JUSTIFICATION OF THE METHODOLOGY

- MicroRNAs negatively regulate specific gene expression, and modulate physiological or pathological processes by acting on entire functional networks
- Certain disease are associated with specific microRNA tissue patterns
- Specific microRNA patterns of expression serve as diagnostic molecular signatures to identify different forms of cardiovascular disease

HYPOTHESIS

Subclinical alterations in cardiovascular structure –detectable only with advanced medical imaging systems- are associated with specific plasma microRNA patterns that can be easily obtained from patient plasma samples

CHAGAS DISEASE: Methodology



INCLUSION CRITERIA

Male or female, ages 18-45 years, with *T. cruzi* infection confirmed by indirect immunofluorescence, indirect hemagglutination, and/or enzyme-linked immunosorbent assay. Those patients positive by at least two tests will be identified as seropositive according to criteria of the Instituto Nacional de Parasitología Dr. Mario Fatała Chabén, Argentina.

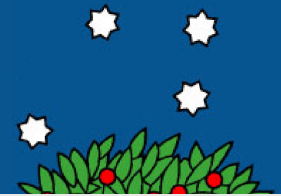
EXCLUSION CRITERIA

Presence of cardiovascular risk factors, including hypertension, diabetes, dyslipemia, obesity, smoking habits, alcoholism, and clinically significant renal disease; endocrinopathy, or any other disease with cardiac involvement

METHODOLOGY

To investigate the potential presence of subliminal cardiovascular alterations, 60 to 100 consecutive indeterminate chagasic patients will be submitted to cardiovascular studies in order to identify 20 patients with, and 20 patients without, subclinical cardiovascular damage (see definition below). Twenty age and sex matched healthy individuals will be submitted to the same studies to confirm the absence of subclinical cardiovascular damage.

CHAGAS DISEASE: Methodology



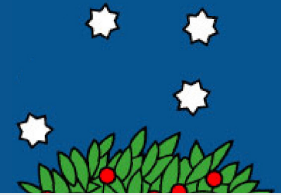
IDENTIFICATION OF SUBCLINICAL CARDIOVASCULAR DAMAGE

a) myocardial fibrosis will be detected by gadolinium-enhanced cardiovascular magnetic resonance and backscattering tisular echocardiogram ; b) functional and structural vascular damage will be evaluated by assessing endothelial function (endothelial-dependent flow-mediated vasodilation of the brachial artery), carotid artery intima-media thickness, and pulse wave velocity by using a non invasive Vivid 5 echodoppler high resolution vascular scanner (GE Health Care, Milwaukee, Wisconsin) with a linear array 10 mHz transducer, with images being processed with an Hemodyn 4 (Hemodyn 4 ®, DINAP SRL, Argentina), c) 64-slice carotid tomography

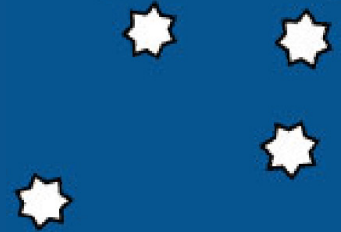
IDENTIFICATION OF A DIAGNOSTIC SET OF PLASMA MICRORNAs

Blood samples will be collected, and plasma used for microarray analysis of the expression of 875 human microRNAs. Real time quantitative reverse transcription polymerase chain reaction (qRT-PCR) will be used as the gold standard for validation of microarray expression data. Full data analysis will be conducted by using the services of LC Sciences. We expect that an ideal marker will be expressed in the heart and/or vascular cells at moderate or high levels and will be present at very low or undetectable levels in plasma from healthy individuals. We created a list of likely blood-based miRNA biomarker candidates for subclinical heart damage by compiling a list of miRNAs expressed in human/rodent cardiovascular pathology specimens based on published miRNA expression profiling data and by filtering out miRNAs detected in healthy donor-derived plasma in published work. Detection of blood plasma microRNAs by Northern blot or PCR will be conducted by optimization of the standard technologies. Receiver Operating Characteristic analysis will be used to assess the sensitivity and specificity of Northern blot- or PCR-based identification of the putative microRNA subset for the detection of subclinical cardiovascular damage.

CHAGAS DISEASE: Long Term Goals



- i) To set the bases for the development of safe, effective and economically feasible drug or biotechnology (miRNA mimics or antagonists) treatments for cardiovascular protection in chagasic patients
- ii) to raise awareness of public health authorities on the need to take measures to prevent/attenuate the progression to disability in chagasic patients
- iii) to advocate for public health support to deal with diseases that do not receive enough attention in this early phase.



STUDY PARTICIPANTS

Cardiology Department
Arterial Hypertension Center, Austral University Hospital

Cardiology Department
Hospital General de Agudos Dr. Teodoro Alvarez