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Short Commentary

Biomarkers for the Neglected Chagas Disease: How Remarkable!

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Our purpose here is to briefly comment on the current state and future trends in biomarkers for human Chagas disease. A variety of biomarkers, reflecting exposure to (asymptomatic carriers) and early effects of pathogen-related disease (clinically relevant patients), as well as individual genetic susceptibility, have become available with the intent to apply these population-based studies for Chagas disease. The data presented here reflect the lessons learned so far and the real challenges that still lie ahead to empower biomarkers to be used reliably in risk assessment for this disease.

Chagas disease or American trypanosomiasis, caused by the etiological agent *Trypanosoma cruzi*, affects at least 8 million people in Central and South America [1]. Morbidity is relatively high. The acute phase is followed by an asymptomatic phase, but roughly 30% of infected patients change to a symptomatic, chronic phase characterized by either severe cardiac or digestive forms [2,3]. Therefore, the identification of reliable indicators of Chagas disease pathology, such as biomarkers or biosignature profiles, would enable prioritization of treatment to those with the highest probability contracting this disease. In fact, those candidates may have diagnostic and prognostic power in patients with several forms of Chagas disease. Predicting factors that correlate with disease progression, morbidity and mortality to help in decision-making, follow-up and management of this complex disease is challenging. Simple, quantitative and inexpensive biomarkers, which add value to conventional approaches, are required to help in diagnosis and prognosis of patients with heart failure [4].

A recent systematic review of molecules with potential use as biomarker targets during therapeutic chronic Chagas disease, highlighted the need to develop novel biomarkers in order to evaluate early responses to treatment. It should be stressed that new diagnostic strategies for determination of cure, such as the identification of biomarkers associated with Chagas disease, is an active area of research. Data from future studies are essential to improve and identify patients for early follow-ups [5].

Several blood-derived biomarkers with clinical potential to predict the progression of early Chagas disease cardiopathy have been employed to assess the efficacy of anti-parasitic drugs and to identify early cardiac and gastrointestinal damage in asymptomatic forms of the disease. However, prospective studies with longer follow-ups are needed to evaluate biomarkers that assess clinical or parasitological cure after therapy [6].

Instructively, biomarkers can be classified based on biochemical structure and primary biological activity, such as inflammation and cellular injury biomakers, metabolic biomakers, prothrombotic biomarkers and antigenic biomarkers (specific antigens of the parasite). However, we adopted here an alternative classification according to a literature review. Therefore, the subsequent sections and the table 1 are brief compilations of recent efforts that highlight the need to

Study	Source	Biomarker Name	Result	Reference
Experimental, Parasitemia-	Antigenic	Aptamer	Increased levels	16 and 17
Chagasic cardiomyopathy	Genetic	CCL2 and MAL/TIRAP		
Chagasic cardiomyopathy	Genetic	CCR5		
Non-specific	Plasmatic	TIMP-1 and TIMP -2	Increased levels	5 and 6
Non-specific	Plasmatic	Troponin I	Increased levels	5 and 6
Non-specific	Plasmatic	TGF-β	Increased levels	5 and 6
Asymptomatic	Plasmatic	IL-10	Increased levels	9
Non-specific	Plasmatic	APOA1	Decreased levels	7
Non-specific	Plasmatic	Fibronectin	Increased levels	7
Asymptomatic	Plasmatic	MMP-2	Increased levels	12
Chagasic cardiomyopathy	Plasmatic	MMP-9	Increased levels	12
Chagasic cardiomyopathy	Plasmatic	TNF-α, IL-1β and IL-6	Increased levels	4, 8 and 9
Chagasic cardiomyopathy	Plasmatic	miRNA-208a and -208b	Decreased levels	11
Experimental, Chagasic cardiomyopathy	Plasmatic	PICP and PIIINP	Increased levels	13
Experimental, Chagasic cardiomyopathy	Plasmatic	Syndecan-4, ICAM-1 and Galectin-3	Increased levels	14
Efficacy	Management	KMP11, HSP70, PAR2 and Tgp63	Increased Ab. levels	5 and 6
Efficacy	Management	Antigen 13 and SAPA	Increased Ab. levels	5 and 6
Efficacy	Management	Tc24	Increased Ab. levels	20

Table 1. Summary of biomarkers investigation in Chagas disease.

increase relevant scientific data availability for the purpose of uncovering critical and reliable biomarker candidates for future of human Chagas disease.

Plasmatic-related candidates: Of note, biomarker patterns in the circulation strongly associated with Chagas disease can be used to identify successfully treated patients [7]. Recent studies denoted that serum markers, such as A- and B-type natriuretic peptides (ANP and BNP, respectively), N-terminal pro BNP, troponin I, TGF-β, MMP-2, and TIMP-1 and -2, were elevated during severe stages of Chagas disease, denoting cardiac damage and inflammation. However, these markers are not specific for Chagas disease. Thus, both 2 formers natriuretic peptides were higher in patients with Chagasic cardiomyopathy than in those with dilated forms or functional class of other etiologies. Yet, those peptide levels were also high in asymptomatic patients with Chagas disease, meaning no evidence of ventricular dysfunction, and had a high predictive value for the outcomes analyzed [4,8]. According to these results, BNP would be equivalent to echocardiograms in regards to the evaluation of cardiological patients. Nevertheless, BNP use is simple and quick, which makes this biomarker a useful tool to perform field studies in endemic zones of Chagas disease with limited access to echocardiographic-housing facilities. In another setting, Sousa and colleagues [9] found that plasma cytokine expression is associated with cardiac morbidity in Chagas disease. Asymptomatic patients had higher IL-10 expression, which is associated with improved cardiac function. By contrast, IFN-γ, TNF-α, IL-1β and IL-6 achieved their highest levels in patients with Chagasic cardiomyopathy. Altogether, these findings reinforce the concept that the fine-tune balance between regulatory and inflammatory cytokines represents a key element in the establishment of distinct forms of chronic Chagas disease [9]. Furthermore, patients with moderate and severe cardiomyopathies produced higher levels of TNF-α and IFN-γ, and lower levels of IL-10 and IL-17 compared to mild cardiomyopathy or cardiomyopathy-free patients in a previous study [10]. Micro(mi)RNAs have been recently described as small non-coding RNAs with gene regulation properties and specific expression profiles. Some miRNAs, such as miRNA-1, miRNA-133a and -133b, and miRNA-208a and -208b are dysregulated in Chagasic cardiomyopathy [11]. Very recently, though, Santamaria and colleagues [7] sought to identify serum biomarkers that could be used as surrogates of therapeutic responses after treatment of Chagas disease. In order to achieve this aim, human sera were compared using a range of proteomic and immunologic techniques. APOA1 and specific fragments thereof and one fragment of fibronectin were identified. In Chagasic samples, all biomarkers, except for the full-length APOA1, were upregulated. These biomarkers returned to normal in 43% of treated patients. Most importantly, whenever there is a predominance of serum MMP-9 levels, cardiac remodeling is intensified and favors the development of the cardiac form of Chagas disease. Conversely, when serum MMP-2 levels prevail, patients remain clinically asymptomatic. These processes may be IL-1β and TNF-α dependent [12]. During guinea pig infection, the cardiac levels of collagen I, III and IV increase progressively, achieving their highest levels in the chronic phase of Chagas disease. High serum levels of procollagen type I carboxy-terminal propeptide (PICP) and procollagen type III amino-terminal propeptide (PIIINP) are also observed throughout the infection.Increased levels of both biomarkers are associated with cardiac fibrosis, confirming the role of apoptosis in cell loss mainly during the chronic phase, and the utility of PICP and PIIINP as fibrosis biomarkers during cardiac remodeling associated with *T. cruzi* infection [13]. Finally, galectin-3, syndecan-4 and ICAM-1 were overexpressed in hearts of mice chronically infected with *T. cruzi* [14]. The same group also described high expression of galectin-3 in inflammatory cells, and concluded that galectin-3 levels were correlated with a decrease in inflammation. In fact, a decrease in syndecan-4 (which is TNFα-regulated) and ICAM-1 may contribute to reduce cell migration into the myocardium, leading to reduced inflammation [15].

Antigenic-related candidates: A correlated ELISA approach to detect circulating parasite excreted-secreted antigens (TESA) in mice plasma by means of specific ligands called aptamers highly specific for those biomarkers of *T. cruzi* infection was developed [16,17]. Thus, in one study a given aptamer showed significant and specific binding to TESA, as well as to trypomastigote extract, but not to host proteins nor *Leishmania donovani* proteins. Infected mice showed a significant higher level of binding compared to non-infected mice, suggesting that the aptamer can detect a biomarker of *T. cruzi* infection. Additionally, the candidate could detect circulating biomarkers in both acute and chronic phases of Chagas disease [16]. In a recent study, the same group corroborated that chagasic infected mice had significantly higher biomarker levels than their non-infected counterparts. They also observed that biomarker levels reduced upon treatment [17]. However, biomarker levels in the infected, treated group did not reduce completely and remained above the assay cutoff point, suggesting that parasitemia was reduced but cure was not achieved. The assay was capable of detecting circulating biomarkers in mice infected with various strains of *T. cruzi*. Therefore, it could also detect residual parasitemia in treated mice by providing an overall picture of the infection in the host.

Genetic-related candidates: Ideally, the identification of genetic markers will provide information for pathogenesis, as well as therapeutic targets. Frade and colleagues [18] studied genetic susceptibility to the left ventricular ejection fraction in a Brazilian cohort. They found that CCL2 and MAL/TIRAP, but not CCR5, were associated to an increased susceptibility to that Chagasic cardiomyopathy.

Management-related candidates: Despite recent progress in the development of better drugs, there is no consensus among different research groups regarding the use of therapeutic response markers to evaluate efficacy of newly proposed drugs early after treatment. For the 2 main classes of recombinant proteins that are effective at different ages and stages of Chagas disease, a combination of KMP11, HSP70, PAR2 and Tgp63 seems promising. Also, antibodies against

the antigen 13, among 5 others including SAPA, were shown to be good markers of treatment efficacy (Reviewed by 5). Moreover, a complement-mediated lysis test and an ELISA approach based on Tc24 were both developed and found to be reliable candidates as helpful parasite biomarkers as well [19,20].

A main hurdle for the development of new drugs for Chagas disease has been the lack of clear and early biomarkers that can indicate parasitological outcome status and definite cure. Researchers agree that the use of biomarkers in human Chagas disease will improve clinical assessment, help to establish reliable diagnostic tools to diminish the time gap between evolution and detection of disease-relevant events, and allow the identification of genetic (primary immunodeficiencies) and acquired factors (secondary immune deficiencies) modulating individual susceptibility. There is a major need to develop a reliable method to evaluate the cure for Chagas disease, particularly for clinical trial purposes. Some biomarkers are based on the detection of parasite proteins in biological samples and provide a global picture of parasitemia in the host. Therefore, this type of biomarker has the potential to yield continuous longitudinal data on Chagas disease therapy. Furthermore, commonly used protocols to detect biomarkers cannot be employed as endpoint assays for human clinical trials due to ethical reasons. Some of the current studies aim to establish alternative applications based on the results of chagasic biomarker detection research. In addition, knowledge from biomarker detection research could be used in vaccine development, such as TESA presence. Actually, in parasite-challenged vaccinated animals, the TESA positivity could be an indication that the immune response was not sufficient to control the infection [16,17]. Despite struggles, the success of biomarker research in Chagas disease has not yet allowed for a better understanding of the disease risk from the clinical point of view. A range of aspects may explain this partial frustration, one of which being the incomplete field of validation of many biomarker candidates. Before conducting human trials, it is necessary to identify and validate biomarkers that indicate that patients have been cured. Hence, there is a clear need for biomarker validation, especially for specific biomarkers of clinical forms of Chagas disease. There is also a need to use carefully designed studies to assess risks associated with the use of these new agents in ever larger cohorts, which imposes a need for high-throughput biomarker methodologies. It should be stated that the elimination of transmission of Chagas disease by the year 2010 as proposed by WHO has not been achieved yet, and in fact the disease is spreading beyond the initial areas in which it was endemic [1]. This in turn creates urgency in solving this validation issue. Unless lessons are drawn from the partial corroboration of simpler biomarker technologies that have been used so far, there is a tangible risk of drowning in an ocean of data which will be created as the application of novel technologies in population studies becomes more widespread. A wide variety of biomarker-based clinical trials designed to assess the clinical utility of a biomarker, or a new treatment with a companion biomarker should be the focus of future studies.

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