Case Report: High Mannose-Binding Lectin Serum Determined by *MBL2* Genotype and Risk for Clinical Progression to Chagasic Cardiomyopathy: A Case Report of Three Patients

Kárita Cláudia Freitas Lidani,† Fabiana Antunes Andrade,† Ronaldo Kiviatcoski Kozłowski,† Paola Rosa Luz, and Iara J. Messias-Reason*

Laboratory of Molecular Immunopathology, Clinical Hospital, Federal University of Paraná, Curitiba, Brazil

Abstract. Chagas disease (CD), caused by infection with the parasite *Trypanosoma cruzi*, leads to severe cardiomyopathy in 20–30% of patients, whereas the remainder may stay asymptomatic and never develop cardiomyopathy or other clinical manifestations. The underlying cause for this variable outcome is not fully characterized, although previous studies have found high levels of circulating mannose-binding lectin (MBL) to be associated with cardiac failure echocardiographic changes. We report three indeterminate (asymptomatic) chronic Chagas patients who were followed up for 10 years. Two of these patients developed chronic chagasic cardiomyopathy (CCC) during this follow-up period and, when genotyped, were found to be carriers of the high MBL producer *HYP A/HYP A* genotype, suggesting that genetically determined high MBL serum might be associated with the risk of CCC development. These results suggest the use of MBL quantification and *MBL2* genotyping as tools for clinical assessment in patients with chronic CD.

CASE REPORT

Three women seropositive for Chagas disease (CD) were evaluated at the Chagasic-Patient Attention Ambulatory of Hospital das Clínicas/HC-UFPR from 2005 to 2015. Seropositivity is defined as two or more positive results on different tests for anti-*Trypanosoma cruzi* antibodies. In 2005, all three patients were classified as having the indeterminate form of chronic CD, which is characterized by positive serology and a lack of cardiomyopathic or gastrointestinal symptoms, as evidenced by normal 12-lead electrocardiogram results, and normal radiological examinations of the chest, esophagus, and colon.^{1,2} Clinical and laboratory features are shown in Table 1. Because of the long-time follow-up on these patients with samples collected in the base of the study (2005), they provide an ideal opportunity to evaluate biomarkers for the prediction of early stage Chagas cardiomyopathy.

All the patients were born in Chagas-endemic regions of Brazil. Patient A, aged 61 years, European–Brazilian, born north of the state of Paraná in Wenceslau Braz, was diagnosed with CD in 1997. The last echocardiogram showed decreased relaxation in the left ventricle, with normal results in the other examinations. In 2015, Patient A was still classified as having indeterminate CD. Patient B, aged 59 years, European–Brazilian, born in Flores in the state of Pernambuco, was diagnosed with CD in 1988. In 2015, Patient B progressed to chronic chagasic cardiomyopathy (CCC) with cardiomegaly, isolated ventricular arrhythmia, and grade I diastolic dysfunction observed in radiographic, continuous ambulatory electrocardiographic (Holter) monitoring, and electrocardiographic examinations, respectively. Patient C, aged 71 years, African–Brazilian, born north of the state of Paraná in Itamaracá, was diagnosed with CD in 1997. Patient C progressed

to CCC in 2015 and presented with edema and dyspnea on exertion. An echocardiogram in 2015 showed small apical aneurysm, grade I diastolic dysfunction, and reduced left ventricular relaxation. Rare episodes of ventricular and supraventricular arrhythmia were observed in Holter monitoring.

Mannose-binding lectin (MBL) serum quantification and *MBL2* genotyping were performed in 2005. The levels of MBL were measured using a commercially available enzyme-linked immunosorbent assay (Antibody Shop; Statens Serum Institut, Copenhagen, Denmark). *MBL2* was genotyped³ by sequencing the following six single nucleotide polymorphisms (SNPs): two in the 5′ regulatory region (–550 G > C, also called –550 H/L, and –221 G > C [X/Y]), one in the 5′ untranslated region (+4 C > T [P/Q]), and three SNPs in exon 1 (223 C > T: D allele, 230 G > A: B allele, and 239 G > A: C allele).

The D, B, and C variants are collectively known as the O allele, whereas the wild-type allele is called A (Figure 1). Genetic polymorphisms in human *MBL* (*MBL2*; 10q11.2–q21), illustrated in Figure 1, can modulate both serum levels and functional activity of MBL. The *MBL2* *HYP A* haplotype is associated with increased expression of circulating MBL, whereas *LYPA* and *LYQA* are considered moderate producer haplotypes, and *LXPA*, *HYP D*, *LYPB*, and *LYQC* are associated with low levels or deficiency of this protein.⁴

Patient A, who remained in the asymptomatic form of CD, presented low levels of circulating MBL (134 ng/mL) and the *MBL2* *HYP A*/*LYPA* genotype. Both Patients B and C, who progressed to the cardiac form of CD during this study, presented with high levels of circulating MBL (2,170 and 5,400 ng/mL, respectively) and the *MBL2* genotype associated with high MBL expression (*HYP A*/*HYP A*).

DISCUSSION

Chagas disease or American trypanosomiasis is a neglected tropical disease caused by infection with the protozoan parasite *T. cruzi* and affects between 7.7 and 10 million people worldwide, particularly in Latin America where 25% of people live in a high-risk environment.^{7,8} *Trypanosoma cruzi* may be

* Address correspondence to Iara J. Messias-Reason, Setor de Ciências da Saúde, Hospital de Clínicas, Universidade Federal do Paraná, Rua General Carneiro 181, Curitiba 80060-900, Brazil. E-mail: iarareason@hc.ufpr.br

† These authors contributed equally to this work.

TABLE 1
Clinical and laboratory features of chronic CD patients A, B, and C

Parameters in 2005	Patients		
	A	B	C
Erythrocyte ($\times 10^6$)	4.23	4.56	4.83
Hemoglobin (mg/dL)	13.2	14.5	14.1
Globular volume (%)	38.4	43.9	43.1
Leukocyte count	3,370	6,700	7,440
Eosinophil	2	1	3
Neutrophil	54	62	63
Lymphocyte	34	33	28
Monocyte	9	4	5
Basophil	0	0	1
Baston cell	5	0	0
Triglycerides (mg/dL)	58	146	135
Total cholesterol (mg/dL)	209	164	205
HDL-C (mg/dL)	68	59	56
LDL-C (mg/dL)	129.4	75.8	122
Creatinine (mg/dL)	0.7	0.8	1
Uric acid (mg/dL)	3.4	4.9	5
Glycemic index (mg/dL)	105	99	101
LVEF (%)	72	63.4	77
Hypertension	+	+	—
Diabetes	—	—	—
Depression	+	+	+
Obesity	—	—	—
Other comorbidities	Osteoporosis Fibromyalgia	Hiatal hernia Fibromyalgia	Artritis Migraine Fibromyalgia Antidepressant
Continuous medication*	Antihypertensive Antidepressant	Antihypertensive Antidepressant	

CD = Chagas disease; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; LVEF = left ventricular ejection fraction.

* None of the patients received antiparasitic treatment of CD.

transmitted via a hematophagous insect vector, through blood transfusions and organ transplants, vertically, or by oral ingestion of contaminated food.^{1,9} In the last decades, programs to eradicate the vector and screening for the protozoa in blood transfusion decreased considerably the number of new infections in Latin America,^{2,10} although 2–3 million patients with the chronic disease still remain in Brazil.¹¹ Chagas disease is of global concern because of international migrations from endemic to non-endemic regions as in North America and Europe.^{12–14}

Chagas disease presents as an acute infection followed by a chronic phase, during which 20–30% of patients develop CCC or other clinical symptoms, whereas the remainder may stay in an asymptomatic “indeterminate” phase.¹ The cardiac, digestive, and cardiodigestive clinical manifestations that may develop usually occur 10–30 years after infection¹ and can lead to irreversible lesions of the autonomic nervous system in the heart, esophagus, and/or colon (mega syndrome), and in the peripheral nervous system.¹¹ Chronic chagasic cardiomyopathy is characterized by cardiac complications including left ventricular dilation and dysfunction, thinning of ventricular walls, aneurysm, congestive heart failure, thromboembolism, ventricular arrhythmias, and sudden cardiac death.^{15–18} Chronic chagasic cardiomyopathy is associated with chronic inflammatory processes in heart tissue associated with cardiac insufficiency, including those moderated by the complement system. Mannose-binding lectin, a protein central to the activation of the lectin complement system pathway, may influence the severity of CCC.¹⁹ Mannose-binding lectin recognizes surface glycoconjugates of parasites, including those of *T. cruzi*,²⁰ and promotes inflammation, removal of apoptotic cells, and opsonization/phagocytosis, either directly or by

activating the antibody-independent lectin pathway of complement.²¹

Each year, about 2–5% of chronic asymptomatic patients progress to symptomatic forms of CD, developing chronic and potentially fatal cardiac, digestive, or cardiodigestive clinical manifestations.^{10,22} Currently, there are no available markers to indicate progression from asymptomatic to symptomatic CD or to determine disease severity. Proteins of the complement system such as ficolin-2,²³ mannan-binding lectin associated serine protease-2 (MASP-2),²⁴ Pentraxin-related protein (PTX3),²⁵ and MBL¹⁹ have been suggested to contribute to cardiac tissue damage due to chronic excessive activation of complement. Mannose-binding lectin is a particularly strong candidate as it plays a central role in the initial interaction between pathogens and phagocytes and because recent studies report that high MBL levels are associated with the presence of echocardiographic alterations and cardiac insufficiency in patients with chronic CD from Brazil, and with high levels of some pro-inflammatory cytokines involved in CCC (platelet-derived growth factor and regulated on activation, normal T cell expressed and secreted).³ Mannose-binding lectin may bind to ligands on the myocardium expressing *T. cruzi* antigens and/or neoantigens exposed after tissue injury, exacerbating complement activation and leading to persistent inflammation and cardiac tissue damage during the chronic stage of CD.¹⁹ Conversely, it has been proposed that there is a protective effect of genetically determined MBL deficiency against the development and progression of CCC, possibly by protecting against cellular invasion by *T. cruzi* and by minimizing the exacerbated damage caused by unwarranted activation of complement.³

FIGURE 1. *MBL2* gene, and monomeric and tetrameric structure of MBL protein. *MBL2* is a polymorphic gene with variants associated with alterations in MBL levels in serum and functional activity, including two single SNPs in the 5' regulatory region at the nucleotide position -550 G > C (also called *MBL2*^H and *L* alleles) and -221 G > C (X/Y), one in the 5' untranslated sequence at position +4 C > T (P/Q) pointed by red arrows, and three non-synonymous polymorphisms in the coding sequence of exon 1 at the position 223 C > T (CGT to TGT causing an amino acid change at position 52 of arginine to cysteine - p.Arg52Cys: D allele), 230 G > A (GGC to GAC - p.Gly54Asp: B allele), and 239 G > A (GGA to GAA - p.Gly57Glu: C allele), pointed by the purple arrow.^{4,5} Mannose-binding lectin protein possesses an N-terminal domain (indicated as a blue box in the monomeric structure), a collagen-like domain (blue zig zag), a neck region (green zig zag), and a carbohydrate-recognition domain (light red circle) responsible for ligand binding. Mannose-binding lectin monomers form homotrimeric subunits that oligomerize into trimeric to hexameric structures, able to activate the complement cascade.⁶ MBL = mannose-binding lectin; SNP = single nucleotide polymorphism. This figure appears in color at www.ajtmh.org.

Genetic polymorphisms in human *MBL* (*MBL2*; 10q11.2-q21) (Figure 1) can modulate both serum levels and functional activity of MBL. The *MBL2* *HYPA* haplotype is associated with increased expression of circulating MBL, whereas *LYPA* and *LYQA* are considered moderate producer haplotypes, and *LXPA*, *HYPD*, *LYPB*, and *LYQC* are associated with low levels or deficiency of this protein.⁴ In our cases, the patients with high levels of MBL and *MBL2* *HYPA*/*HYPA* genotype developed CCC, whereas the patient presenting low levels of MBL and *MBL2* *HYPA*/*LYPA* genotype remained asymptomatic.

Our results demonstrated that the two patients who progressed from indeterminate CD to CCC during this study (Patients B and C) were carriers of the high MBL producer *HYPA*/*HYPA* genotype. Patient A, who remained indeterminate during the same time of follow-up, had low levels of MBL and a different *MBL2* genotype (*HYPA*/*LYPA*). Taking into consideration other recent studies, our case report suggests that high circulating MBL due to the *HYPA*/*HYPA* *MBL2* genotype may be associated with the risk of developing CCC and that circulating MBL quantification and *MBL2* genotyping could be useful prognostic markers in the follow-up of chronic CD.

Received September 6, 2017. Accepted for publication April 13, 2018.

Published online December 3, 2018.

Acknowledgments: We thank Indira Chakravarti, from Johns Hopkins Bloomberg School of Public Health, for critically reading the manuscript and suggesting substantial improvements.

Financial support: This work was supported by research grants from Coordenação de Aperfeiçoamento de Pessoal Superior (CAPES

Edital Parasitologia Básica 32/2010) and Fundação Araucária (PPSUS-01/2016).

Conflict of interest: All authors read and approved the final version of the manuscript and declare that they have no conflicts of interest. Written consent was obtained from the patients to publish their cases.

Authors' addresses: Kárita Cláudia Freitas Lidani, Fabiana Antunes Andrade, Ronaldo Kiviatcoski Kozłowski, and Paola Rosa Luz, Department of Medical Pathology, Universidade Federal do Paraná Hospital de Clínicas, Curitiba, Brazil, E-mails: kari.lidani@gmail.com, fabiana.una@gmail.com, ronaldo.koki@gmail.com, and pazinha.bio@gmail.com. Iara J. Messias-Reason, Department of Immunopathology, Hospital de Clínicas, Curitiba, Brazil, E-mail: iaramessias@yahoo.com.br.

REFERENCES

1. Rassi A, Marin-Neto JA, 2010. Chagas disease. *Lancet* 375: 1388–1402.
2. Moncayo A, 2003. Chagas disease: current epidemiological trends after the interruption of vectorial and transfusional transmission in the Southern Cone countries. *Mem Inst Oswaldo Cruz* 98: 577–591.
3. Luz PR, Miyazaki MI, Chiminacio Neto N, Padeski MC, Barros ACM, Boldt ABW, Messias-Reason IJ, 2016. Genetically determined MBL deficiency is associated with protection against chronic cardiomyopathy in Chagas disease. *PLoS Negl Trop Dis* 10: e0004257.
4. Turner M, 2003. The role of mannose-binding lectin in health and disease. *Mol Immunol* 40: 423–429.
5. Jack DL, Klein NJ, Turner MW, 2001. Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis. *Immunol Rev* 180: 86–99.
6. Jensenius H, Klein DCG, van Hecke M, Oosterkamp TH, Schmidt T, Jensenius JC, 2009. Mannan-binding lectin: structure,

- oligomerization, and flexibility studied by atomic force microscopy. *J Mol Biol* 391: 246–259.
7. Rassi A, Marcondes de Rezende J, 2012. American trypanosomiasis (Chagas disease). *Infect Dis Clin North Am* 26: 275–291.
8. WHO. *Chagas Disease (American trypanosomiasis)*. Geneva, Switzerland: World Health Organization. Available at: <http://www.who.int/mediacentre/factsheets/fs340/en/>. Accessed June 13, 2015.
9. Pereira KS, Schmidt FL, Guaraldo AMA, Franco RMB, Dias VL, Passos LAC, 2009. Chagas' disease as a foodborne illness. *J Food Prot* 72: 441–446.
10. Malik LH, Singh GD, Amsterdam EA, 2015. Chagas heart disease: an update. *Am J Med* 128: 1251.e7–1251.e9.
11. Moncayo A, Silveira AC, 2009. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. *Mem Inst Oswaldo Cruz* 104 (Suppl): 17–30.
12. Bern C, Montgomery SP, 2009. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis* 49: e52–e54.
13. Benziger CP, do Carmo GAL, Ribeiro ALP, 2017. Chagas cardiomyopathy. *Cardiol Clin* 35: 31–47.
14. Cantey PT et al., 2012. The United States *Trypanosoma cruzi* Infection Study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. *Transfusion* 52: 1922–1930.
15. Ribeiro AL, Nunes MP, Teixeira MM, Rocha MOC, 2012. Diagnosis and management of Chagas disease and cardiomyopathy. *Nat Rev Cardiol* 9: 576–589.
16. Rassi A, Little WC, 2000. Chagas' heart disease. *Clin Cardiol* 23: 883–889.
17. Malik LH, Singh GD, Amsterdam EA, 2015. The epidemiology, clinical manifestations, and management of Chagas heart disease. *Clin Cardiol* 38: 565–569.
18. Roberts A, Kemp C, 2005. Chagas' disease (American trypanosomiasis). *J Am Acad Nurse Pract* 13: 152–153.
19. Luz PR, Miyazaki MI, Neto NC, Nisihara RM, Messias-Reason IJ, 2010. High levels of mannose-binding lectin are associated with the risk of severe cardiomyopathy in chronic Chagas Disease. *Int J Cardiol* 143: 448–450.
20. Cestari I dos S, Krarup A, Sim RB, Inal JM, Ramirez MI, 2009. Role of early lectin pathway activation in the complement-mediated killing of *Trypanosoma cruzi*. *Mol Immunol* 47: 426–437.
21. Beltrame MH, Boldt ABW, Catarino SJ, Mendes HC, Boschmann SE, Goeldner I, Messias-Reason I, 2015. MBL-associated serine proteases (MASPs) and infectious diseases. *Mol Immunol* 67: 85–100.
22. Dutra WO, Rocha MOC, Teixeira MM, 2005. The clinical immunology of human Chagas disease. *Trends Parasitol* 21: 581–587.
23. Luz PR, Boldt ABW, Grisbach C, Kun JFJ, Velavan TP, Messias-Reason IJT, 2013. Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease. *PLoS One* 8: e60237.
24. Boldt ABW, Luz PR, Messias-Reason IJT, 2011. MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease. *Clin Immunol* 140: 63–70.
25. Lidani KCF, Beltrame MH, Luz PR, Sandri TL, Nisihara RM, de Messias-Reaso IJ, 2015. Is pentraxin 3 a cardiovascular marker in patients with chronic Chagas disease? *Int J Cardiol* 190: 233–235.