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Deriving a parsimonious cardiac endpoint for use in epidemiological studies of Chagas disease: results from the Retrovirus Epidemiology Donor Study-II (REDS-II) cohort

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ABSTRACT

Chagas cardiomyopathy (ChCM) is a severe consequence of *Trypanosoma cruzi* infection and has a range of electrocardiographic (ECG) and echocardiographic (ECHO) manifestations. There is a need for a standard and parsimonious research cardiac end point that does not rely on expert panel adjudication, and it is not intended to change the ChCM definition. We use data from the REDS-II cohort to propose a simplified cardiac endpoint. A total of 499 *T. cruzi*-seropositive blood donors were included. All participants underwent a 12-lead ECG, echocardiogram and clinical examination, and those with abnormal findings were reviewed by a panel of cardiologists who classified cases as having Chagas cardiomyopathy or not. We created an exhaustive set of ECG and ECHO finding combinations and compared these with the panel's classification. We selected the simplest combination that most accurately reproduced the panel's results. Individual ECG and ECHO variables had low sensitivity for panel-defined cardiomyopathy. The best performing combination was right bundle branch block and/or ECHO evidence of left ventricular hypocontractility. This combination had 98% specificity and 85% sensitivity for panel-defined ChCM. It was not possible to improve the overall accuracy by addition of any other ECG or ECHO variable. Substituting right bundle branch block for the more inclusive finding of QRS interval > 120 ms produced similar results. The combination of prolonged QRS interval and/or left ventricular hypocontractility closely reproduced the REDS-II expert panel classification of Chagas ChCM. In conclusion, the simple and reproducible research endpoint proposed here captures most of the spectrum of cardiac abnormalities in Chagas disease.

KEYWORDS: Chagas disease. *Trypanosoma cruzi*. Cardiomyopathy. Chagas cardiomyopathy.

INTRODUCTION

Chagas disease (ChD), caused by the protozoan parasite *Trypanosoma cruzi,* is a neglected tropical disease affecting 6-8 million people worldwide[1,2]. Chagas cardiomyopathy (ChCM) is the most important manifestation[3]. Although a number of electrocardiogram (ECG) and echocardiogram (ECHO) abnormalities are considered typical of ChCM[4], these findings are non-specific and occur in other common cardiomyopathies[3] that are highly prevalent, particularly among older patients with ChD[5]. For the purpose of research, there is a need for a minimum set of ECG and ECHO findings that can be used as a consistent endpoint to indicate *T. cruzi* related cardiac damage.

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There are examples of other disease processes in which simplified diagnostic criteria have been derived by comparison of clinical findings with physicians' classification (e.g. Dunkley *et al*.[6]). In a previous cohort, we used clinician adjudication of cardiac status (ChCM or not) on a case-by-case basis[7]. This required blinding to clinical status, a consensus between adjudicators, and a mechanism to resolve conflicting opinions, as well as being expensive and time consuming. Furthermore, while probably the best gold standard available, this approach makes research less reproducible, both across different groups, and at different timepoints in longitudinal studies. Herein, we use data from the well phenotyped REDS-II cohort to derive a parsimonious cardiac endpoint for use in epidemiologic studies of Chagas disease.

MATERIALS AND METHODS

We present data from the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study-II (REDS-II), previously described in detail[7,8]. This cohort is composed of three participant groups. Firstly, a group of *T. cruzi*-seropositive blood donors (SP-BD) identified between 1998 to 2002 in routine serologic screening at the Fundacao Pro-Sangue (Sao Paulo) and Hemominas (Montes Claros) blood centers. SP-BD tested positive on three (ELISA, hemaglutination, and immunofluorescence) or two (ELISA and hemagglutination) serologic assays, with repeat serologic confirmation at cohort enrollment. A negative control group was composed of 488 age- and sex-matched seronegative blood donors (SN-BD) recruited from the same blood banks. A positive control group of 101 patients with established ChCM was recruited from the specialist outpatient service at the Heart Institute (InCor) – part of the Hospital das Clinicas complex in Sao Paulo, Brazil.

Cohort phenotyping and expert panel review

The REDS-II cohort includes a wide spectrum of Chagas disease severity as represented across the SP-BD group and the established ChCM cases. Baseline phenotyping included a standard resting 12-lead ECG, 2-dimentional and tissue Doppler ECHO, as well as clinical history and physical exam. ECG and ECHO results were interpreted in a centralized study unit by investigators blind to clinical and serostatus.

Subjects presenting any alteration on clinical assessment, ECG or ECHO were referred for expert panel review. The panel was composed of three cardiologists experienced in Chagas disease. Patients were classified into four groups: no ChCM, possible, probable or definite ChCM. The following criteria were used to classify a subject as having definite ChCM:

1. Typical findings (reproduced in Table 1) in at least two of the three methods: clinical examination, ECG and ECHO, or;
2. One of the following findings (independent of the other findings): right bundle branch block (RBBB) (with or without left anterior hemi-block [LAHB]) on ECG or apical aneurysm or global left ventricle (LV) hypocontractility on ECHO

However, because none of the above findings are pathognomonic for ChCM, and given the aforementioned complexities in diagnosis, the panel was allowed space for expert subjective judgement and consideration of the global clinical picture, including comorbidities, age, medications etc. Therefore, it is unknown what the relative contribution of each ECG and ECHO finding was to the final classification. We aimed to identify which of the variables available to the panel (Table 1) were most important, and which played only an ancillary role in the final classification. Ultimately, we aimed to identify the most parsimonious set of ECG and ECHO variables that closely reproduced the panel's classification.

Statistical analysis

As a simplifying assumption, and in line with the original publication[7], we considered subjects classified as "definite cardiomyopathy" to have ChCM and the other three groups (15 with probable, 53 with possible and 311 cases without) as being free of ChCM. Therefore, there were 120 SP-BD with definite ChCM and 379 without definite ChCM. An initial descriptive analysis examined each ECG and ECHO finding individually by comparing the prevalence of each across these two groups (with or without definite ChCM). As such, the proportion of panel-classified definite ChCM subjects with any given ECG or ECHO finding can be understood as the sensitivity (true positive rate) for that finding.

Conversely, the proportion of subjects without panel-defined ChCM with a given finding is the false positive rate, or 1 – specificity, associated with that finding.

We then explored different combinations of ECG and ECHO findings and determined their accuracy in reproducing the panel's classification. This analysis was limited to the 499 SP-BDs. We made an exhaustive set of two, three and four variable combinations. For example, a two-variable combination could be *ST-T abnormalities* on ECG or *apical aneurysm* or both, such that if a subject

Table 1 - ECG, echo and clinical findings according to serostatus and expert panel classification in the REDS-II Chagas disease cohort.

ECG and echo findings	Clinical groups				
	Seronegative with CM n=24	Seronegative BD without CM n=464	Seropositive BD without CM n=379	Seropositive BD with CM n=120	Patients with established CM n=101
Typical ECG findings, n(%)					
RBBB	3(12)	0(0)	0(0)	80(67)	41(41)
Other intraventricular blocks*	1(4)	2(0)	6(2)	2(2)	7(7)
Frequent premature vent beats	2(8)	3(1)	3(1)	10(8)	13(13)
Major Q-wave abnormalities	1(4)	7(2)	4(1)	8(7)	21(21)
Minor Q/ST-T abnormalities	0(0)	2(0)	1(0)	3(2)	3(3)
Major ST-T abnormalities	2(8)	15(3)	15(4)	9(8)	19(19)
Atrial fibrillation/flutter	1(4)	0(0)	1(0)	2(2)	5(5)
Sinus bradycardia (HR < 40bpm)	0(0)	0(0)	1(0)	1(1)	0(0)
2nd/3rd degree AV block	0(0)	0(0)	0(0)	1(1)	0(0)
Paced rhythm	0(0)	0(0)	0(0)	5(4)	20(20)
ECG findings possibly related to Chagas, n(%)					
Sinus bradycardia (40bpm < HR < 50 bpm)	3(12)	23(5)	22(6)	5(4)	7(7)
Frequent supraventricular premature beats	1(4)	9(2)	2(1)	7(6)	7(7)
Left anterior hemiblock	2(8)	9(2)	18(5)	56(48)	41(41)
Low QRS voltage	2(8)	8(2)	13(3)	4(3)	16(16)
First degree AV block	1(4)	4(1)	10(2)	11(9)	4(4)
Minor primary isolated ST/T abnormalities	4(17)	38(8)	46(12)	13(11)	27(27)
Number of ECG findings, n(%)					
0	11(46)	366(79)	267(71)	10(8)	2(2)
1	7(29)	78(17)	88(23)	38(32)	24(24)
2	4(17)	18(4)	19(5)	44(37)	33(33)
3 +	2(8)	2(0)	5(1)	28(23)	42(41)
Typical echocardiographic findings, n(%)					
Segmental LV contractile abnormalities	2(9)	12(3)	11(3)	20(17)	69(68)
Apical aneurysm	0(0)	1(0)	0(0)	1(1)	6(6)
Global LV hypocontractility (subjective or EF < 50%)	11(46)	2(0)	9(2)	33(28)	96(96)
Left or right ventricular dilation	4(17)	14(3)	17(4)	25(21)	87(86)
Intracavity thrombus	0(0)	0(0)	0(0)	1(1)	2(2)
Clinical criteria					
Hx of PND	3 (13)	21 (5)	35 (9)	15 (13)	58 (58)
Hx of Exertional SOB	5 (21)	50 (11)	72 (19)	36 (30)	75 (74)
JVP stasis	0 (0)	3 (1)	11 (3)	5 (4)	39 (39)
Lower limb edema	4 (17)	20 (4)	16 (4)	9 (8)	39 (39)
Lung crepitations	0 (0)	7 (2)	5 (1)	1 (1)	5 (5)
Hx of faints/LOC	0 (0)	20 (4)	31 (9)	11 (10)	40 (40)
Self-reported palpitations	6 (25)	71 (15)	108 (28)	40 (33)	54 (55)

The classification of ECG and echo findings as "typical" or "possibly related to Chagas" were adapted from Sabino *et al.*[7]. *left bundle branch block or non-specific intraventricular block. CM = cardiomyopathy; BD = blood donor; ECG = electrocardiogram; AV = atrioventricular; HR = heart rate; LV = left ventricle; EF = ejection fraction; Hx = history; PND = paroxysmal nocturnal dyspnea; SOB = shortness of breath; JVP = jugar venous pressure; LOC = loss of consciousness. Note the percentages in the groups with CM represents the sensitivity (true positive rate for CM of that particular finding). In the groups without CM the percentage represents 1-specificity (false positive rate). Missing values: LAHB 7, first degree AV block 8, segmental LV abnormalities 4, apical aneurysm 5, diastolic dysfunction 6, intracavity thrombus 4.

had one or both of these findings they would be classified as having ChCM. If they have neither they are classified as being ChCM free. Throughout the document, when referring to variable combinations we use the terminology X and/or Y to mean X or Y or both, as described above.

As such, by adding variables the proportion of panel-defined ChCM cases would be expected to increase and more false-positives would be expected to be introduced. We used accuracy (true positives + true negatives / total) in order to compare different variable combinations against

the expert panel, and selected the best performing and most parsimonious combination.

We subsequently evaluated the best performing variable combination by comparing it with the panel's classification for the whole cohort, thus including SN-BD which represent negative controls, and patients with established cardiomyopathy representing positive controls.

Final derivation of proposed cardiac end point

Using the results of the above analysis, we consulted experienced clinicians in Chagas disease and compared their results with existing guidelines. We determined if the best performing (statistically) cardiac endpoint should be altered on theoretical or pathophysiological grounds, to be more or less inclusive.

Ethics approval and consent to participate

All participants provided informed consent at the time of cohort enrollment. The study was approved by the Brazilian National Ethics Committee (CONEP Nº 1312/2006).

RESULTS

Demographic and clinical characteristics of the entire cohort are presented in Supplementary Table S1. The full set of ECG and ECHO variables considered by the expert panel is shown in Table 1. Focusing on the seropositive blood donor group, all cases in which RBBB was present were considered to have ChCM, and 67% of seropositive donors with ChCM had this finding. LAHB was the next most prevalent finding among seropositive donors with ChCM: 48% compared to 5% among those considered free of ChCM. It is also apparent from Table 1 that the presence of at least one ECG finding identifies 92% of seropositive donors with ChCM, but would erroneously capture 29% of the seropositive donors without ChCM. Indeed, 23% (111/488) of the seronegative donors had at least one ECG alteration, highlighting the lack of specificity inherent in this definition.

Considering the ECHO findings, 28% of SP-BD with ChCM had global LV hypocontractility (subjective or EF < 50%), whereas only 2% of those considered not to have ChCM had this finding. Following this, left or right ventricular dilation was the ECHO variable that appeared to contribute the most to the diagnosis of ChCM.

Clearly no single ECG or ECHO variable serves as an adequate surrogate for the panel's classification. We tested all possible two-variable combinations (21 choose 2 = 210 combinations) of the ECG and ECHO parameters in Table 1 and calculated the accuracy (true positive + true negatives / total) for each possible two-variable combination (Figure 1A). The best performing combination was RBBB and/or LV hypocontractility, with an accuracy of 95%. Of the 102 SP-BD that were classified as having ChCM by this definition, 69 had RBBB alone, 22 had LV hypocontractility alone, and

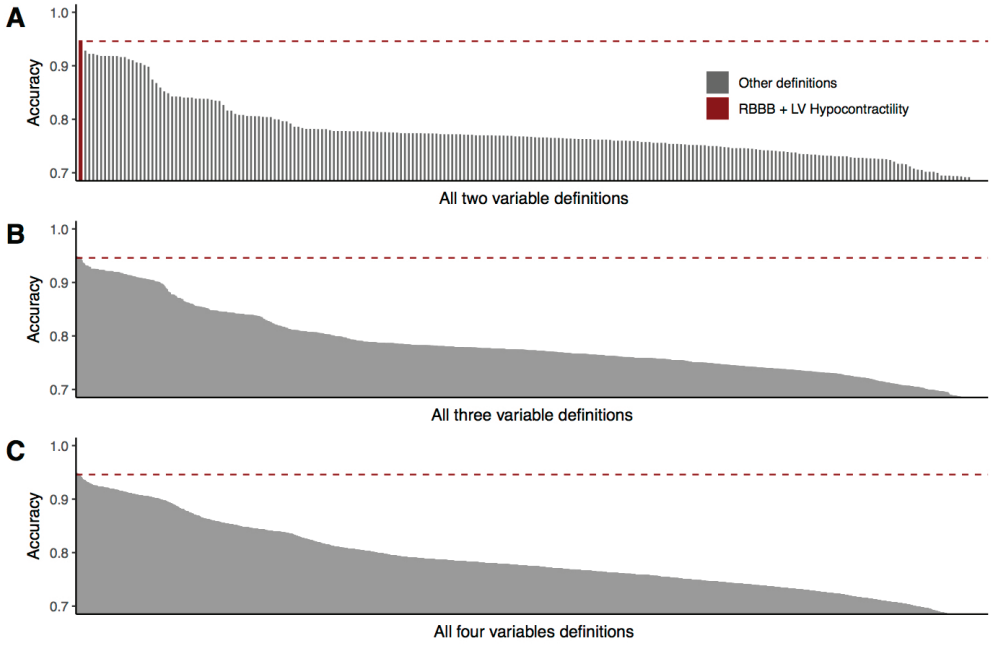

Figure 1 - Accuracy of different ECG and echo parameter combinations to reproduce the expert cardiologist panel classification. The full set of variable combinations was made by selecting all possible ways of choosing 2 (panel A), 3 (panel B) or 4 (panel C) variables from the 21 ECG and echo parameters available to the expert panel. Dashed red line is the accuracy of a two variable combination consisting of RBBB and/or echo evidence of left ventricular hypocontractility (subjective or ejection fraction < 50%).

11 had both findings. We then attempted to improve on this by constructing all possible three-variable combination (21 choose 3 = 1330) (Figure 1B) and four-variable combination (21 choose 4 = 5985) (Figure 1C). The addition of further variables did not improve the accuracy – i.e. any gain in sensitivity was offset by a reduction in specificity.

Figure 2 shows how the accuracy of the RBBB and/or LV hypocontractility definition varied when applied across the full spectrum of disease. As expected, there is an improved sensitivity (92% vs 85%) when it was applied to all subjects (including seronegative donors and patients with established cardiopathy).

The combination of LV hypocontractility and/or RBBB performs well in statistically reproducing the results of the panel's classification. Based on the expert consultation, this end point was considered to be limited from a clinical perspective, as, for example, a patient with isolated left bundle branch block would be classified as free of ChCM. Therefore, we tested the combination of

LV hypocontractility and/or QRS duration > 120 ms, as an alternative to capture all major interventricular blocks. The performance of this combination is shown in Figure 3 and was very similar to the LV hypocontractility and/or RBBB combination.

DISCUSSION

In this re-analysis of the REDS-II cohort we show that the diagnosis of Chagas cardiomyopathy by a panel of cardiologists was reproduced with 95% accuracy considering only the presence of QRS prolongation and/or left ventricular hypocontractility. Accuracy was not improved by the addition of other ECG or ECHO variables. This finding could be used to operationalize and standardize a cardiac endpoint in epidemiologic studies of ChD. This would improve comparability across studies, but in particular this approach is more feasible than an expert panel, as clinician adjudication is expensive, time-consuming and prone to inconsistencies (subjectivity).

There are three classical manifestations of Chagas cardiomyopathy: arrhythmias, cardiac failure and thromboembolic phenomena[2,4]. Arrhythmias arise because Chagas-related fibrosis has a predilection for the conduction system and produce reentry circuits in the ventricular myocardium[9]. Indeed, a wide range of ECG alterations are more prevalent in *T. cruzi*-seropositive patients when compared with seronegative populations[10]. These abnormalities increase with age as a result of the continuous process of cardiac damage over the years[5,11]. In the REDS-II cohort, RBBB was the most discriminatory finding between seropositive and seronegative individuals and this is consistent with other studies in populations with comparable age structure[5,10,12,13]. Other ECG findings, although common in Chagas, are more non-specific, such as left anterior hemi-block, or frequent extra ventricular contractions. It is appropriate, therefore, that RBBB would be a sufficient condition to diagnose Chagas cardiomyopathy in seropositive individuals.

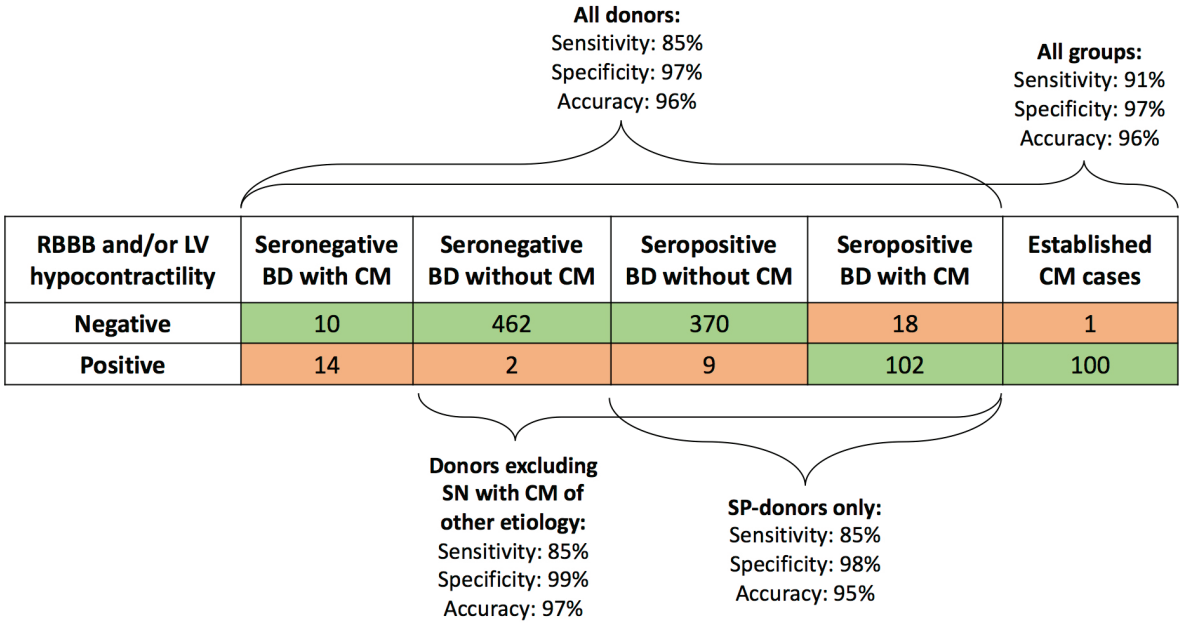

Figure 2 - Sensitivity, specificity and accuracy of right bundle branch block and/or left ventricular hypocontractility to identify ChCM among different clinical groups of the REDS-II cohort. CM = cardiomyopathy; BD = blood donor; RBBB = right bundle branch block; LV = left ventricle; SN = seronegative; SP = seropositive. Among the 102 SP-BD with CM that correctly met the RBBB and/or LV hypocontractility definition, 69 had RBBB alone, 22 had LV hypocontractility alone, and 11 had both findings.

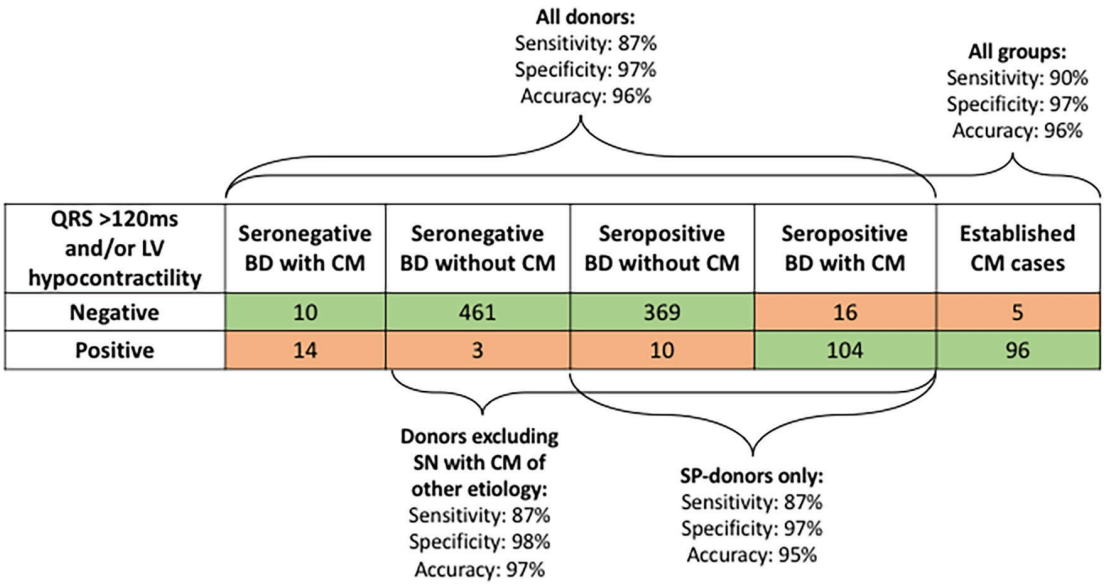

Figure 3 - Sensitivity, specificity and accuracy of QRS duration >120ms and/or left ventricular hypocontractility to identify ChCM among different clinical groups of the REDS-II cohort. CM = cardiomyopathy; BD = blood donor; RBBB = right bundle branch block; LV = left ventricle; SN = seronegative; SP = seropositive. Among the 104 SP-BD with CM that correctly met the QRS duration >120ms and/or LV hypocontractility definition, 71 had QRS duration >120ms alone, 22 had LV hypocontractility alone, and 11 had both findings.

There is an important cohort effect among patients with Chagas disease. This has occurred due to effective vector control strategies limiting new infections, and as such most patients enrolled into studies are older adults[5,7,14]. ECG findings that may be typical of Chagas disease in younger patients, such as left anterior hemi-block, have poor specificity in an older age group. This is consistent with our results that ECG alterations other than RBBB played a secondary role in the expert panel's classification.

Addressing the other manifestations of ChCM, heart failure arises in the context of myocardial fibrosis resulting in a dilated cardiomyopathy[2,4]. Both systolic and diastolic impairment are typical[15]. Thromboembolism results from the formation of mural thrombi that occur in the context of extensive cardiac damage. Therefore, it makes sense that a definition that incorporates interventricular conduction abnormalities (the most typical ECG findings) and left ventricular dysfunction would capture most of the spectrum of cardiac manifestations, and therefore perform well as a pragmatic cardiac endpoint for research purposes.

The improvement in sensitivity for ChCM when the definition is applied to the whole cohort (SN-BD, SP-BD, and established ChCM cases) is expected. Sensitivity varies with the spectrum of disease in the population to which the test/diagnostic procedure is being applied[16]. By including the established cases – 96% of which have left heart failure, and 98% an abnormal ECG – the sensitivity inevitably increases as the disease is well-established with clear clinical signs. Conversely, as previously reported[7], 5% (24/488) of seronegative donors were incorrectly classified as having definite ChCM, and half (14/24) met the QRS >120 ms and/or LV failure criterion. This highlights the lack of specificity among ECG and ECHO findings for ChCM. Indeed, a proportion of *T. cruzi*-infected individuals will have cardiomyopathies of other etiologies that are indistinguishable from ChCM.

Our study has some limitations. Firstly, our results require external validation. Secondly, the end point proposed here requires echocardiography that may not always be available in all settings. Finally, and most fundamentally, we have used the terminology of *sensitivity, specificity* and *accuracy* for convenience, but we emphasize that this is not a diagnostic accuracy study *per se*. We are not presenting an independent comparison between a gold-standard and a potential novel diagnostic procedure. In fact, the ECG and ECHO variables we evaluated as possible components of a research endpoint, are the same as those used by the expert panel to define ChCM. It is therefore unsurprising that, for example, RBBB has a 100% specificity among SP-BD, as this ECG finding was a hard criterion for ChCM used by the panel (See Methods and the online appendix of Sabino *et al.*[7]). As such, our results are correctly interpreted as follows: the diagnosis of definite ChCM among *T. cruzi*-seropositive blood donors, as performed by an expert panel of cardiologists, can be reduced to only two variables – prolonged QRS complex and/or LV hypocontractility – with 95% of subjects remaining classified in the same way.

A prolonged QRS complex or LV failure represents a hard endpoint: subjects meeting this criterion have definite ChCM, but milder/borderline cases may not meet this

definition. Indeed, the remaining 16 (13%) SP-BD with panel-defined ChCM that were not identified by our proposed endpoint do not share a single ECG or ECHO finding that is also not present in the panel-defined ChCM-free group. Therefore, these subjects were presumably classified as having ChCM based on a combination of findings that, when considered together, were strongly suggestive of ChCM, but individually were not. Whether the exclusion of this group with normal QRS duration and LV function is epidemiologically important will depend on whether these patients have increased mortality compared to patients with no ECG or ECHO abnormalities. More generally, our proposed cardiac endpoint requires further validation by determining its association with mortality or other important outcomes.

CONCLUSIONS

The combination of a prolonged QRS complex and/or impaired left ventricular function can serve a simple cardiac endpoint in studies of ChD, avoiding the need for an expert panel adjudication. Future studies should validate this cardiac endpoint in other cohorts and its association with mortality.

AUTHORS' CONTRIBUTIONS

LFB analyzed the data, interpreted the results, and drafted the manuscript; TB analyzed the data, interpretation the results and drafted the manuscript; AP conceived of the study and interpreted the results; LN curated and analyzed the data; CDO conceived the study, interpretation the results and revised the manuscript; ALPR conceived the study, analyzed the data, interpreted the results, revised the manuscript, and provided supervision; ECS conceived the study, advised on the analysis of the data, interpreted the results, revised the manuscript and provided supervision. All authors read and approved the final version.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1 - Clinical and demographic characteristics of participants in the REDS-II Chagas disease cohort.

ECG and echo findings	Clinical groups				
	Seronegative with CM n=24 n(%) or median(IQR)	**Seronegative BD without CM** n=464 n(%) or median(IQR)	**Seropositive BD without CM** n=379 n(%) or median(IQR)	**Seropositive BD with CM** n=120 n(%) or median(IQR)	**Patients with established CM** n=101 n(%) or median(IQR)
Age (years)	50 (44-61)	49 (42-58)	49 (42-58)	50 (44-61)	48 (42-54)
Sex					
Male	15 (63)	226 (49)	186 (49)	75 (63)	60 (59)
Female	9 (37)	238 (51)	193 (51)	45 (38)	41 (41)
Smoking status					
Never	12 (50)	243 (42)	222 (59)	61 (51)	47 (47)
Past	7 (29)	151 (32)	114 (30)	47 (39)	46 (46)
Current	5 (21)	70 (15)	43 (11)	12 (10)	8 (8)
Comorbidities					
Diabetes	2 (8)	22 (5)	20 (5)	7 (6)	6 (6)
Hypertension	7 (29)	112 (24)	89 (24)	24 (20)	36 (36)
Myocardial infarction	3 (13)	2 (4)	1 (3)	2 (2)	12 (12)
Renal disease	1 (4)	14 (3)	10 (3)	5 (4)	10 (10)
BMI, (kg/m^2)					
<25	5 (21)	127 (27)	127 (34)	44 (37)	44 (44)
25-29.9	10 (42)	219 (47)	172 (45)	62 (52)	43 (43)
30+	9 (38)	118 (25)	80 (21)	14 (12)	14 (14)

CM = cardiomyopathy; BD = blood donor; BMI = body mass index.