of circulating CD4+CD31+ T cells (p = 0.432) or CD4+CD28+ T cells (p = 0.155). Finally, we found that CD4+, CD4+CD31null, CD4+CD28null, CD4+CD31+ and CD4+CD28+ were not correlated with FMD (r = 0.034/p = 0.794, r = −0.031/p = 0.814, r = −0.061/p = 0.645, r = −0.003/p =0.976, r = 0.045/p = 0.732 respectively) in patients with UA.

In the present study we have shown that patients with UA are characterized by a significantly higher percentage of circulating CD4+CD31null T cells and a significantly impaired FMD than healthy individuals. Our findings suggest that it is likely that the loss of the CD31 molecule from the surface of circulating CD4+ T cells characterizes the appearance of a novel subpopulation of T cells that may affect atherogenesis and potentially its complications. Moreover, we have supported further the theory of the direct involvement of CD4+CD28null T cells in plaque destabilization as the UA patients presented significantly higher frequencies of CD4+CD28null T cells. It seems that circulating CD4+CD31null and CD4+CD28null T cells can lead to a poor mechanical endothelial response in patients with unstable angina.

In conclusion, in the present study we found a higher level of T cells in patients with unstable angina and this may contribute to plaque instability in these patients. However, the exact mechanisms linking the loss of CD31 and CD28 molecules from CD4+ T cells with endothelial dysfunction in unstable angina are still unclear and many more experimental studies are required to confirm mechanistically this association.

The authors report no relationships that could be construed as a conflict of interest.

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CCR5 chemokine receptor gene variants in chronic Chagas' disease

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Chronic Chagas heart disease affects approximately 30% of patients infected by the protozoan *Trypanosoma cruzi* around 20 years after infection. The clinical manifestations of this disease are malignant arrhythmias [1], chronic systolic heart failure [2], sudden cardiac death [3], and thromboembolism [4]. Tissue damage resulting from inflammatory infiltrates and persistence of *T. cruzi* in myocardial tissue is involved in the pathogenesis of cardiomyopathy. However, the precise pathogenic mechanism of Chagas' heart disease is not completely elucidated [4,5].

The CC chemokine receptor 5 (CCR5) expressed by monocytes, macrophages and T_H1 cells is a receptor for the CCL3, CCL4 and CCL5 chemokines [6,7]. Genetic variants of the *CCR5* gene may be involved in the differential susceptibility for Chagas cardiomyopathy [8–10].

In this study, we investigated the relationship of the *CCR5*Δ32 (rs333) and *CCR5* 59029 A/G (rs1799987) polymorphisms of the *CCR5* gene in patients with chronic Chagas' disease, with and without left ventricular systolic dysfunction (LVSD); left ventricular ejection fraction (LVEF) is currently the most important predictor of all-cause mortality of patients with this condition [11].

This study was approved by the Research Ethics Committee of the Medicine School in São José do Rio Preto (# 009/2011) and informed consent was obtained from all patients. A total of 168 consecutive male and female patients were enrolled from the Cardiomyopathy Outpatient Service of Hospital de Base of the Fundação Faculdade de Medicina de São José do Rio Preto. Anti-*T. cruzi* antibodies were detected by immunosorbent assay (ELISA) according to the manufacturer's instructions (ELISAcruzi; bioMerieux S.A. Brazil).

Patients were divided into three groups according to the LVEF; patients with LVEF >60%, between 60% and 40% and those with LVEF <40% as calculated using the Teichholz' method were classified as without, mild, and severe LVSD, respectively. The severe group was defined according to the Brazilian guidelines of severe chronic heart disease [12]. For patients in which the LVEF was measured by

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Table 1

Genotype and allele frequencies of the *CCR5*Δ32 polymorphism in Chagas' disease patients with normal left ventricular systolic function, and mild to moderate, and severe left ventricular systolic dysfunction.

Genotypes	Normal		Mild/moderate LVSD		Severe LVSD		χ^2	DF	p[a]
*CCR5*Δ32	No.	%	No.	%	No.	%			
CCR5/CCR5	78	(91.8)	40	(93.0)	34	(85.0)	1.880	2	0.391
CCR5/CCR5Δ32	7	(8.2)	3	(7.0)	6	(15.0)			
Total	85	(100.0)	43	(100.0)	40	(100.0)			
Alleles									
CCR5	163	(95.9)	83	(96.5)	74	(92.5)	1.786	2	0.409
CCR5Δ32	7	(4.1)	3	(3.5)	6	(7.5)			

[a] Calculated by χ^2; LVSD: Left ventricular systolic dysfunction; DF: Degree of freedom.

radionuclide ventriculography, a LVEF >50% indicated normal left ventricular systolic function, LVEF between 30% and 50% indicated mild to moderate LVSD, and a LVEF <30% was consistent with severe LVSD.

Genomic DNA was attained using a commercial kit for silica column extraction (PureLink™ Genomic DNA Mini Kit, Invitrogen, Carlsbad, California/USA) and following the manufacturer's instructions. The *CCR5*Δ32 polymorphism of the *CCR5* gene was identified by polymerase chain reaction (PCR), according to the protocol of Huang et al. [13]. The polymorphism of the promoter region of the *CCR5* gene (*CCR5*-59029 A/G) was identified by PCR-restriction fragment length polymorphism (PCR-RFLP) as described by McDermott et al. [14]. Comparisons of proportions between groups were made by the chi-square test using the software GraphPad Instat (version 3.06). Differences were considered statistically significant for a p-value < 0.05.

The *CCR5*Δ32 polymorphism was investigated in 168 patients: 152 (90.5%) were wild type homozygotes, and 16 (9.5%) heterozygotes. Homozygotes for this deletion were not found. Table 1 shows data on allele and genotype frequencies. The *CCR5* 59029 A/G polymorphism was analyzed in 155 patients. The genotype frequencies were: *AA* (39.4%), *AG* (36.1%) and *GG* (24.5%). The data on allele and genotype frequencies are shown in Table 2.

The *CCR5*Δ32 deletion results in a non-functional receptor not detected on the cell surface, whereas heterozygous individuals for this polymorphism have reduced levels of CCR5 expression [15,16]. The results of this study show no association of genotypes and alleles of the *CCR5*Δ32 polymorphism between groups of patients with chronic Chagas' disease. A study suggested that the *CCR5/CCR5Δ32* genotype can protect against inflammatory cardiomyopathy, a consequence of the chronic phase of Chagas' disease [9] but this study did not confirm this due to the low frequency of the *CCR5/CCR5Δ32* genotype.

The *CCR5* 59029 A/G polymorphism of the promoter region alters the expression of CCR5 on the surface of leukocytes. It has been demonstrated that the *A* allele has higher promoter activity in vitro than the *G* allele and that the increased expression of CCR5 results from the *AA* genotype [14,17]. The genotypes and alleles of the *CCR5* 59029 A/G polymorphism did not differ between the groups of Chagas' disease patients included in this study.

Our results contrast with the results reported by other studies. Calzada et al. [8] found a higher frequency of the *AG* genotype and the presence of the *G* allele in asymptomatic patients compared to patients with cardiomyopathy. These authors suggested that the *G* allele could protect against the development of Chagas cardiomyopathy. Another study found a low frequency of the *GG* genotype in patients with arrhythmias compared to asymptomatic patients [9]. These authors suggested that this genotype may protect against the development of cardiomyopathy symptoms in individuals infected with *T. cruzi* [9].

The results of the work of Flórez et al. [10] did not show any significant association between the *CCR5* 59029 A/G polymorphism and protection against the development of Chagas' disease cardiomyopathy, however, the development of Chagas disease cardiomyopathy was associated with the HHA haplotype in which the *G* allele was present. The authors suggest that in this type of study it may be more appropriate to estimate haplotypes, as several polymorphisms in the promoter region may influence the CCR5 receptor expression levels and the cell type in which it is expressed [10,18].

In this study, the heart disease was evaluated according to the degree of LVSD. In other studies, the aim was not to investigate any possible relationship between *CCR5*Δ32 and *CCR5* 59029 A/G polymorphisms and left ventricular impairment, and so the criteria to classify patients were different [8–10]. These distinct strategies may have contributed to the discrepant results reported in this study compared to other research.

In conclusion, the *CCR5*Δ32 (rs333) and *CCR5* 59029 A/G (rs1799987) polymorphisms had no relationship with LVSD in patients with chronic Chagas' heart disease in this study population. Thus, these polymorphisms do not seem to influence left ventricular impairment.

The authors declare no conflicts of interest.

Table 2

Genotype and allele frequencies of the *CCR5* 59029 A/G polymorphism in Chagas' disease patients with normal left ventricular systolic function, and mild to moderate, and severe left ventricular systolic dysfunction.

Genotypes	Normal		Mild/moderate LVSD		Severe LVSD		χ^2	DF	p[a]
CCR5 59029A/G	No.	%	No.	%	No.	%			
AA	30	(37.5)	16	(40.0)	15	(42.9)	0.302	2	0.860
AG	29	(36.3)	14	(35.0)	13	(37.1)	0.038	2	0.981
GG	21	(26.2)	10	(25.0)	7	(20.0)	0.521	2	0.771
Total	80	(100.0)	40	(100.0)	35	(100.0)			
Alleles									
A	89	(55.6)	46	(57.5)	43	(61.4)	0.671	2	0.715
G	71	(44.4)	34	(42.5)	27	(38.6)			

[a] Calculated by χ^2; LVSD: Left ventricular systolic dysfunction; DF: Degree of freedom.

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Reduction of myocardial inflammation with steroid is not necessarily associated with improvement in left ventricular function in patients with cardiac sarcoidosis: Predictors of functional improvement

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ARTICLE INFO

Cardiac sarcoidosis is rare but being increasingly recognized because of poor prognosis [1]. Steroids are the mainstay of treatment for cardiac sarcoidosis to resolve active myocardial inflammation [2,3]. Gallium-67 citrate (^{67}Ga) scintigraphy and ^{18}F-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) are used to evaluate the response to steroid treatment [4,5]. However, it remains unknown whether steroid treatment can completely resolve active myocardial inflammation evaluated by these imaging modalities and whether the resolution of inflammation is associated with an improvement in left ventricular (LV) function. The aim of this study was to determine the efficacy of steroid treatment for resolving inflammation and improving LV function and to elucidate predictors of the functional responder to steroid treatment in patients with cardiac sarcoidosis.

The study population consisted of 30 consecutive patients with cardiac sarcoidosis who had positive myocardial uptake of ^{67}Ga or ^{18}F-FDG at baseline between December 1994 and November 2012. ^{18}F-FDG PET was performed in 5 patients who had no positive

myocardial uptake of ^{67}Ga, after patients were instructed to fast for at least 12 h, blood glucose level was determined to ensure a level of <150 mg/dl, and unfractionated heparin was administrated. Cardiac sarcoidosis was diagnosed on the basis of guidelines of the Japanese Ministry of Health and Welfare [6], and guidelines revised in 2006 by the Japan Society of Sarcoidosis and Other

Table 1
Clinical characteristics.

	All patients (n = 30)
Age (year)	61 ± 12
Female	20 (67)
Extracardiac organ involvement	
Lung	18 (60)
Skin	5 (17)
Eye	12 (40)
Other	6 (20)
Number of organ involvement	
1 site	6 (20)
2 sites	13 (43)
≥3 sites	11 (37)
NYHA functional class III or IV	10 (33)
LV end-diastolic volume (ml/m^2)	83 ± 19
LV end-systolic volume (ml/m^2)	49 ± 23
LV ejection fraction (%)	43 ± 15
High-degree heart block	13 (43)
Sustained ventricular tachycardia	12 (40)
Angiotensin-converting enzyme (IU/l)	14.9 ± 6.7
B-type natriuretic peptide (pg/ml)	361 ± 455
Initial dose of prednisone 30 mg/40 mg	27 (90)/3 (10)
Period of initial dose of prednisone (week)	5.6 ± 3.5
Maintenance dose of prednisone 5 to 7.5 mg/7.6 to 10 mg	16 (53)/14 (47)
Beta-blocker use	20 (67)
Cardiac resynchronization therapy	7 (23)

Data are presented as means ± standard deviation or numbers (%) of patients.
High-degree heart block includes complete atrioventricular block and Mobitz II block.
NYHA, New York Heart Association; LV, left ventricular.

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