Introduction and objectives. Chagas disease is the most common cause of myocarditis in Latin America, including Venezuela. Some 25% of patients progress to chronic chagasic cardiomyopathy, which is characterized by heart failure and arrhythmias. The serum levels of C-reactive protein (CRP) and interleukin-6 (IL-6) have prognostic value in non-chagasic cardiopathy. The goal of this study was to investigate the relationship between the serum levels of CRP and IL-6 and the developmental stage of Chagas disease.

Patients and methods. The study included 64 Chagas disease patients (34 female and 30 male; age 62.2 [1.7] years) and 20 healthy individuals (10 of each sex; age 50.4 [2.7] years). Clinical investigations included echocardiography and measurement of CRP and IL-6 serum levels using ELISAs. Chagas disease patients were graded according to Carrasco et al 1994 classification. Patients with ischemic cardiopathy, liver disease, autoimmune disease, a systemic inflammatory condition, immunosuppression, cancer, pericarditis, or endocarditis were excluded.

Results. Multiple regression analysis demonstrated an association between Chagas disease developmental stage and the serum IL-6 level. The serum CRP level increased during only the most advanced phase of the disease. In addition, a high left ventricular mass index was associated with a high IL-6 level and male sex.

Conclusions. IL-6 and CRP serum levels could be of prognostic value in assessing Chagas disease progression because there are significant correlations between elevated levels and the deterioration of cardiac function.

Key words: Chagasic cardiomyopathy. C-reactive protein. Interleukin-6.

Las concentraciones séricas de interleucina-6 y proteína C reactiva se incrementan a medida que la enfermedad de Chagas evoluciona hacia el deterioro de la función cardiaca

Introducción y objetivos. La enfermedad de Chagas (EC) es la causa de miocarditis más común en América Latina y Venezuela. El 25% de los pacientes evolucionó hacia una miocardiopatía chagásica crónica (MCC), caracterizada por insuficiencia cardíaca y arritmias. La proteína C reactiva (PCR) y la interleucina-6 (IL-6) tienen valor pronóstico en las cardiopatías no chagásicas. En este estudio se ha determinado la relación entre las concentraciones de PCR e IL-6 y la fase evolutiva de la EC.

Pacientes y método. Se incluyó a 64 pacientes con EC (34 mujeres y 30 varones; edad: 62.2 ± 1.7 años) y a 20 individuos sanos (10 de cada sexo; edad: 50.4 ± 2.7 años); en todos ellos se realizaron una valoración clínica, una ecocardiografía y la determinación de las concentraciones séricas de PCR e IL-6 mediante ELISA. Los pacientes fueron clasificados según Carrasco et al (1994). Se excluyó a los pacientes con cardiopatía isquémica, hepatopatías, enfermedades autoinmunitarias, procesos inflamatorios, neoplasias, inmunodepresión, pericarditis y endocarditis.

Resultados. El análisis de regresión múltiple mostró una asociación entre la fase evolutiva de la EC y las concentraciones de IL-6, mientras que los valores elevados de PCR sólo estuvieron asociados con la fase más avanzada de la EC. Adicionalmente, se observó un mayor índice de masa del ventrículo izquierdo asociado con valores elevados de IL-6 y el sexo masculino.
mediated by the release of free radicals, including therefore, weeks, accompanied by maximum parasitemia; the B-cell activation, followed by immunodepression at 6 acute phase, this response is characterized by polyclonal progression of the disease. During the first week of the acute period of Chagas disease, 10,11 but not in the chronic phase11,12 of Chagas disease. IL-6 is a key inflammatory factor, with secretion activated by CRP, and has been implicated in the pathogenesis and progression of cardiovascular diseases. The presence of elevated circulating concentrations of IL-6 in patients with heart failure, and serum levels of this marker correlate with the severity of left ventricular dysfunction. Likewise, increased IL-6 expression in cardiac tissue has been associated with progression of heart failure. T cruzi infection in experimental animal models results in elevated serum and tissue IL-6 values14 induced during the progressive phase of the parasitemia, namely, the acute period of Chagas disease;15 the transialidase enzyme of T cruzi is the highest inducer of IL-6 secretion.16 The relationship between IL-6 and the development of CCC is still not clear, and the results of animal studies are contradictory.14,16 Only 25% of patients with Chagas disease develop CCC.25 A crucial aspect in the treatment of these patients would be the availability of biochemical markers able to predict disease progression. The present research analyzes CRP and IL-6 concentrations in patients with Chagas disease in order to correlate them with the progression of cardiac involvement.

PATIENTS AND METHODS
A cross-sectional analytical study was conducted in patients previously diagnosed with Chagas disease who came to the Chagas outpatient clinic at the Centro
Cardiovascular Regional ASCARDIO and the Escuela de Medicina Pablo Acosta Ortiz (Universidad Centrocircular Lisandro Alvarado) in Barquisimeto, Estado Lara, Venezuela.

The nonprobabilistic sample was composed of 64 nonconsecutive patients of both sexes. Chagas disease was confirmed by 3 serological tests: direct agglutination, immunofluorescence, and enzyme-linked immunoassay (ELISA), according to previously established protocols, and patients with 2 or more positive assays were accepted as positive.

The patients were classified according to the 3 phases proposed by Carrasco et al (1994): Phase I (n=24), asymptomatic patients with no electrocardiographic or echocardiographic evidence of cardiac involvement; Phase II (n=20), asymptomatic patients with electrocardiographic or echocardiographic evidence of cardiac involvement; and Phase III (n=20), patients with heart failure.

The exclusion criteria were: a) patients with acute or chronic ischemic heart disease defined as a confirmed history of anterior or recent myocardial infarction, history of angina pectoris and/or positive stress test or stress echocardiogram for ischemia, or cardiac catheterization indicative of coronary artery disease; b) patients with acute or chronic liver disease; c) patients with acute or chronic inflammatory processes (e.g., rheumatoid arthritis, collagen disease, vasculitis, or cancer) and acute or chronic infections (e.g., endocarditis, pneumonia, and/or tuberculosis); d) patients who are immunosuppressed or receiving corticoid therapy; e) patients with non-Chagas acute or recurrent pericarditis; and f) patients with primary valve disease due to disorders that are congenital or secondary to infectious processes (e.g., rheumatic fever and/or endocarditis).

The study also included a control group of 20 individuals over age 18 with no history or serological evidence of Chagas disease or other heart condition.

In keeping with the standards of the Centro Cardiovascular Regional ASCARDIO Institutional Review Board, which are based on the Declaration of Helsinki amended in 1996, all patients underwent a clinical history, chest x-ray, echocardiogram, hematological testing, and blood biochemistry. Serum CRP and IL-6 concentrations were determined using commercial ELISA kits (VITRO 250, Johnson & Johnson, for CRP and Diaclone Research, for IL-6).

**Statistical Analysis**

After determining the respective descriptive statistics for characterizing the final sample, the multiple linear regression models used to relate the IL-6 and CRP values to the phase of Chagas disease were considered. The Kolmogorov-Smirnov test showed that the distribution of the levels of the mediators cited was not Gaussian and therefore, logarithmic transformation of the levels of these mediators prior to inclusion in the respective models was performed. The following variables were included in the regression analysis: Chagas disease phase, sex, age, heart failure, hypertension, diabetes mellitus, and dyslipidemia.

The following equation was used for each model:

\[ Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\ldots+\beta_nX_n \]

in which \( Y \) (IL-6 or CRP values) was correlated to each variable combination; \( \beta \) represents the regression coefficients, subindices 1 to n, each respective variable being analyzed; subscript 0, the independent term or baseline value (intercept) and \( X_n \), the value of each variable. The variable values (\( X_n \)) were determined as follows: dichotomous variables, such as sex or presence of the diseases mentioned above, were considered dummy variables and assigned values of 1 (male sex, presence of each particular disease) or 0 (female sex, absence of each disease). Since age is a numeric value with approximately normal distribution, it was used without transformations. The progressive phases of Chagas heart disease were also included in the regression models as dummy variables, with 0 and 1 used to represent the absence or presence of a particular phase. The control group included individuals without Chagas disease. Backward elimination was used to exclude variables with no significant effect.

Results are expressed as mean ± standard error or 95% confidence interval (95% CI). Statistical significance was set at \( P=0.05 \). Statistix 1.0 and Prism 3.0 were used for the statistical analysis.

**RESULTS**

The average age of the participants was 50.4±2.7 years for the population of healthy volunteers and 62.2±1.7 years for seropositive patients. The average age of patients according to phase of Chagas disease was as follows: Phase I, 56.9±3.0; Phase II, 62.7±2.7, and Phase III, 68.3±2.2 years, respectively, with a significant difference observed in Phase II and III patients versus the control group and Phase I patients (Table 1). The multiple regression analysis (see below) found no correlation between age and status of Chagas disease progression.

Distribution according to sex was similar in both study groups, with a significant decrease in the proportion of women as the disease progressed and a higher proportion of men in Chagas Phases II and III (Table 1). However, the multiple regression analysis (see below) did not disclose any correlation between sex and the phase of Chagas disease.

In the means calculated for the echocardiographic parameters (Table 2), in particular, left ventricular end-diastolic diameter, left ventricular end-systolic...
were only significant in the most advanced phase. Changes according to the phase of the disease, which involve left ventricular mass (LVM), showed similar alterations, with direct impacts on ventricular mass and left ventricular mass index (LVMI), measuring dilation and cardiac remodeling (e.g., left ventricular end-diastolic diameter, left atrial diastolic diameter, right ventricular diastolic diameter, LVEDD, LVEDV, LVESD, LVESV, LA, RV, LVMI, LVM, RV, EF, Shortening, %). The ejection fraction was inversely proportionate to the degree of the disease (P<.005) as the disease progressed. An assessment of left ventricular end-diastolic volume and left ventricular end-systolic volume showed a significant increase (P<.05) as the disease progressed. The ejection fraction was inversely proportionate to the phase of disease progression; the parameters yielded an intercept with a coefficient of –0.4 (95% CI, –0.9 to –1.3) and P<.0001. C-reactive protein (CRP) values, taking into consideration the intervening variables, show a significant correlation to disease phase (Table 3). Backward elimination of nonsignificant variables yielded an intercept with a coefficient of –0.4 (95% CI, –0.9 to –1.3) and P<.0001. C-reactive protein correlated only to Chagas Phase III (Table 4), obtaining intercept values of –1.2 (95% CI, –1.5 to –1.1) and P<.0001, for Phase III of 1 (95% CI, 0.5-1.2) and P=0.0001.

Finally, a multiple regression analysis was performed between values for the functional variables obtained from echocardiographic studies and serum IL-6 and CRP values, taking into consideration the intervening variables. The results showed that LVMi was associated with male sex, Phase III disease, and IL-6 values (Table 5 and Figure). Although the simple correlation analyses showed a positive correlation between IL-6 or CRP and LVMi, a negative correlation between IL-6 or CRP and the ejection fraction, as well as between IL-6 and BMI, the multiple regression analysis did not confirm these results.

**DISCUSSION**

In the control groups and in patients with Chagas disease, a similarity was observed with regard to the sex of the individuals, thus supporting the validity of the sample; nevertheless, there was a predominance of men in the more advanced phases of the disease (Table 1). However, the multiple regression analysis found no
correlation between Chagas phases and either age or sex. Male sex in Chagas cardiomyopathy is a factor of poor prognosis, with a higher overall mortality among men between age 30 and 59, a finding that has been related to a higher frequency of electrocardiographic abnormalities.19

The controls and the patients with Phase I disease had basically the same characteristics in terms of age, whereas the patients with Phases II and III showed significant differences when compared to both the control group and Phase I group. Studies show that the onset of the clinical symptoms of Chagas disease occurs around age 40, with an estimated average of 6 to 12 years elapsing before the patient reaches Phase II and an identical period occurring in most cases until Phase III is reached.20

Various authors support the theory that the chronic inflammatory mechanisms of Chagas cardiomyopathy are due to autoimmune processes in the host. The cells involved in the autoimmune process are modulators of CRP and interleukin production, which could trigger the cascade of focal or generalized inflammatory responses.21

TABLE 3. Relationship Between the Phase of Chagas Disease and Interleukin-6 Values. Multiple Linear Regression Analysis*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.409</td>
<td>-0.924 to 0.106</td>
<td>.124</td>
</tr>
<tr>
<td>Age, years</td>
<td>0.001</td>
<td>-0.008 to 0.0105</td>
<td>.764</td>
</tr>
<tr>
<td>Male</td>
<td>-0.128</td>
<td>-0.366 to 0.110</td>
<td>.295</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.136</td>
<td>-0.549 to 0.278</td>
<td>.523</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.118</td>
<td>-0.348 to 0.113</td>
<td>.321</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>0.111</td>
<td>-0.420 to 0.842</td>
<td>.884</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.284</td>
<td>-0.003 to 0.570</td>
<td>.056</td>
</tr>
<tr>
<td>Phase I</td>
<td>0.690</td>
<td>0.358 to 1.024</td>
<td>.001</td>
</tr>
<tr>
<td>Phase II</td>
<td>0.761</td>
<td>0.421 to 1.101</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Phase III</td>
<td>1.114</td>
<td>0.562 to 1.665</td>
<td>.002</td>
</tr>
</tbody>
</table>

*Logarithmic transformation was used to normalize the data; only significant variables remaining after backwards elimination are shown.

TABLE 4. Relationship Between the Phase of Chagas Disease and CRP Values. Multiple Linear Regression Analysis*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.141</td>
<td>-2.107 to -0.174</td>
<td>.024</td>
</tr>
<tr>
<td>Age, years</td>
<td>-0.002</td>
<td>-0.002 to -0.01</td>
<td>.782</td>
</tr>
<tr>
<td>Male</td>
<td>0.093</td>
<td>-0.35 to 0.54</td>
<td>.684</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.037</td>
<td>-0.81 to 0.74</td>
<td>.925</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.002</td>
<td>-0.44 to 0.43</td>
<td>.994</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>-0.063</td>
<td>-0.60 to 0.48</td>
<td>.820</td>
</tr>
<tr>
<td>Phase I</td>
<td>-0.100</td>
<td>-0.73 to 0.53</td>
<td>.756</td>
</tr>
<tr>
<td>Phase II</td>
<td>-0.014</td>
<td>-0.65 to 0.62</td>
<td>.966</td>
</tr>
<tr>
<td>Phase III</td>
<td>1.620</td>
<td>0.59 to 2.66</td>
<td>.003</td>
</tr>
</tbody>
</table>

*Logarithmic transformation was used to normalize the data; only significant variables remaining after backwards elimination are shown.

An assessment of plasma CRP concentrations in patients with Chagas disease according to phase and in the controls showed a clear, significant increase among Phase III patients. This difference suggests that inflammatory changes are present and active during the more advanced stage of the disease. The presence of inflammatory foci and myocyte necrosis due to lymphocyte migration has been described in Chagas disease, even in the presence of a low degree of parasitism.22 The inflammatory foci may be the result of microcirculation changes, which cause ischemic alterations followed by fibrosis and myocardial remodeling.23

Serum CRP concentrations increase during the acute phase of Chagas disease10,11; however, elevated values in
the chronic stage\textsuperscript{11,12} have not been reported, something apparently not consistent with the findings observed in this research. Nevertheless, the investigations cited were conducted with individuals in an indeterminate stage of Chagas disease, which would correspond to Phases I and II of our study, in which we were unable to find statistically significant CRP increases.

\textit{T. cruzi} infection in experimental animal models leads to elevated serum and tissue IL-6,\textsuperscript{14} which is induced during the increasing stage of parasitemia in the acute period of Chagas disease.\textsuperscript{15} It has been postulated that the main inducer of IL-6 in \textit{T. cruzi} infection is the enzyme transialidase of the parasite itself.\textsuperscript{16} The relationship between IL-6 and the development of CCC is still unclear and contradictory; for instance, transgenic mice that do not express IL-6 present greater parasitemia and die earlier than wild strains.\textsuperscript{14} On the other hand, animals sensitized with \textit{T. cruzi} transialidase and therefore, with elevated IL-6 concentrations are also more susceptible to invasion by the parasite.\textsuperscript{16} In the present study, multiple regression analysis showed that the IL-6 values were associated with the progressive phases of Chagas disease, indicating that the values of this cytokine might increase as the disease progresses, thus contributing to the progression of the myocardial damage.

Sato et al (1999)\textsuperscript{24} showed that IL-6 has a negative inotropic effect which induces a hypotrophic state in the myocardium. These investigators have also shown that plasma IL-6 concentrations are higher in the decompensated stage of heart failure syndrome compared to the recovery stage.

Petretta et al (2000)\textsuperscript{25} correlated IL-6 concentrations to the functional class of patients with heart failure syndrome and found that IL-6 concentrations were progressively higher with a higher functional class (NYHA). This study concludes that IL-6 determination provides a more accurate indication of hemodynamic deterioration among patients with heart failure.

Lastly, the multiple regression analysis revealed that plasma IL-6 concentrations are related to the echocardiographic parameter LVMI and to male sex. Left ventricular mass index reflects the cellular remodeling that leads to chamber dilation and, secondarily, to ejection alterations. The relationship between IL-6 and higher LVMI in male patients could help explain the worse prognosis of these patients in Chagas disease.

CONCLUSIONS

Elevated IL-6 concentrations were related to the phase of Chagas disease, indicating that once these patients have progressed beyond the acute phase, they experience a chronic inflammatory process, which becomes more severe with progression to Phase III status. This suggests an increase in the cell injury that leads to deterioration of cardiac function, with men being more prone to cell injury mediated by this pathophysiological mechanism. C-reactive protein elevation appears to occur later and be related to progression toward Phase III and functionally to heart failure, which would reflect recurrence of the acute inflammatory process. According to the data obtained from the statistical analyses regarding the concentrations of these plasma proteins and the phase of the disease, IL-6 could be used in the future as a prognostic marker in patients with this disease. However, the type of design used does not allow us to state that the markers precede disease progression; a long-term prospective cohort study with a consecutive sample would, therefore, be needed. The implications of these findings may suggest that new guidelines should be established for the stratification of patients with Chagas disease and guide therapeutic strategies that might change the prognosis and survival of these patients.

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