MORPHOMETRIC STUDY OF THE SPLEEN IN CHRONIC CHAGAS' DISEASE

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Abstract. There is frequently an increase in spleen size in infectious systemic and chronic venous congestion. The aim of this report was to perform a comparative study of the spleen tissue of chagasic or nonchagasic autopsied patients with or without congestive heart failure. Evaluations were made of 111 cases. Connective tissue intensity, follicular density and area, and follicular arterioles wall area were determined through the morphometric study. The connective tissue was similar in all groups. The density of the lymphoid follicles was significantly less among the chagasic cases ($P = 0.032$). The follicular area was larger among the chagasic cases and in the chagasic group with congestive heart failure. The chagasic group without congestive heart failure presented a greater area of follicular arteriole walls. Therefore, the spleen modifications in chronic Chagas' disease could be a consequence not only of the heart failure but also of the Chagas infection itself.

INTRODUCTION

Chagas' disease is an infectious process caused by the blood flagellate protozoan *Trypanosoma cruzi* encountered in the Americas, one of whose basic lesions is the inflammatory response^{1–4} affecting a wide variety of mammals, including humans.

The spleen, the largest unit of the mononuclear phagocytic system, together with the lymph nodes, reacts as a component of the immune system in cases of inflammatory reaction, increasing in size; in such cases it is called activated spleen,^{5,6} hyperplastic reactional state,⁷ or acute spleen tumor.⁸ There are no alterations to the capsule or red pulp, but there is reticulum-endothelial hyperplasia of the white pulp, with the presence of germinal centers.^{5,6}

In cases of persistent or chronic venous congestion, there is an increase in spleen size, or congestive splenomegaly. Passive hyperemia of cardiac origin may occur in congestive heart failure from any cause but especially in right valve defects, chronic cor pulmonale and some cardiomyopathies, among which is Chagas' disease. The fibrous thickening and the hemosiderin deposits within the sinusoid walls that have congestion and edema produce the characteristic anatomical pattern of congestive splenomegaly.^{6,9} There is dilatation of the sinusoids, microscopic hemorrhages, and, over the long term, fibrosis of Billroth's cords and a certain degree of atrophy of the lymphoid follicles.⁷

There are few reports of spleen involvement in the chronic phase of Chagas' disease, and they generally refer to thromboembolic phenomena^{10–14} or weight alterations.¹⁵

Because Chagas' disease is an infectious disease that has an inflammatory response as one of its basic lesions, with the possibility of presenting hemodynamic repercussions, we raised the hypothesis that alterations capable of objective evaluation via morphometry could exist in these circumstances. Such alterations in the parenchyma and stroma of the spleen would be a result not only of cardiac involvement but also of the infectious process. Thus, the objective of the current article was to perform a morphometric study of the spleen using microscopy, among chagasic and nonchagasic cases with or without congestive heart failure.

MATERIALS AND METHODS

In a retrospective study, we analyzed the protocols from completed autopsies performed at the Hospital of the School of Medicine of the Triangulo Mineiro, Uberaba, Brazil, after approval by the Ethics Commission for Research of the Institution. We selected 111 cases, which were divided into four groups: (1) nonchagasic without congestive heart failure ($n = 26$); (2) nonchagasic with congestive heart failure ($n = 12$); (3) chagasic without congestive heart failure ($n = 29$); (4) chagasic with congestive heart failure ($n = 44$). So that we could make some comparisons between the Chagas' disease and non-Chagas' disease groups, we combined the chagasic cases without congestive heart failure with chagasic cases with congestive heart failure groups and the nonchagasic cases without congestive heart failure with nonchagasic cases with congestive heart failure groups, respectively. This classification was based on the autopsy protocols and reactions for Chagas' disease, Machado-Guerreiro, indirect immunofluorescence, blood agglutination, and enzyme-linked immunosorbent assay, performed in the pericardic fluid collected at autopsy. The inclusion criteria for the groups with congestive heart failure were the presence of "cardiac organs" and anasarca in addition to the characteristic heart pathological alterations. The following cases were excluded: those with neoplasia, including leukemia and lymphomas; inflammations with severe acute or chronic systemic repercussions, such as endocarditis, pneumonitis, and leptomenigitis; and diseases that course with portal hypertension, such as cirrhosis, schistosomiasis, and hepatitis.

We collected the fragments of spleen for routine histological processing. The sections were stained with hematoxylin-eosin, periodic acid-Schiff, and Picro-Sirius. All spleen slides were evaluated by readers who were unaware of slide identification. The slides were placed under a sheet of tracing paper to obtain drawings of the sections, from which the areas were measured via an interactive image-analyzing system (MOP-Videoplan; Kontron Elektronik, Munich, Germany). The follicles were counted using a light microscope with a 5× objective. The section areas and number of follicles were obtained. From these values it was possible to determine the

TABLE 1

Density of spleen lymphoid follicles, expressed as the number of follicles per mm², from autopsies of chagasic and nonchagasic cases with and without congestive heart failure (CHF)

Group (n = 88)	Median follicles/mm ² (max-min)
Nonchagasic without CHF (n = 22)	1.07 (2.5–0.4)
Nonchagasic with CHF (n = 9)	0.64 (1.0–0.3)
Chagasic without CHF (n = 22)	0.70 (1.3–0.3)
Chagasic with CHF (n = 35)	0.70 (1.9–0.3)

Kruskal-Wallis test, $H = 14.32$; $P = 0.002$.

Mann-Whitney test, chagasic and nonchagasic, $T = 1625.0$; $P = 0.032$.

density, expressed as the number of follicles per square millimeter.

The morphometry of the follicles and the respective follicular arterioles was performed using the KS300 automatic image-analyzing system (Kontron Elektronik, Munich, Germany). The follicles were delimited using a cursor to determine the area. To determine if the arteriole wall areas in vessels sectioned transversally, the internal and external perimeters of the wall were outlined using the cursor.

The fibrous connective tissue of the spleen was evaluated in the samples stained with Picro-Sirius. We used a light microscope with a video camera coupled to a monitor. A grid containing 25 randomly distributed points was devised for morphometry, and this was placed on the monitor screen. Fifty random fields were evaluated on each slide, totaling 1,250 points per case; only the points that coincided with bundles of fibrous connective tissue were computed.

For the statistical analysis, Mann-Whitney, Kruskal-Wallis, and analysis of variance tests were performed. The differences observed were considered significant when the probability of rejection of the null hypothesis was less than 5% ($P < 0.05$).

RESULTS

The density of the lymphoid follicles, expressed as the number of follicles per square millimeter, was greater in the nonchagasic cases (median = 0.99 follicles/mm²) than in the chagasic cases (median = 0.70 follicles/mm²), with a statistically significant difference ($P = 0.032$). The density of the follicles was greater in the nonchagasic cases without congestive heart failure and less in the nonchagasic cases with congestive heart failure, with a statistically significant difference ($P = 0.002$) (Table 1).

The area of lymphoid follicles in chagasic cases (157,979.90 μm^2) was significantly greater than in nonchagasic individuals (119,964.30 μm^2) ($P < 0.005$). The follicular area was greater

TABLE 2

Area of spleen lymphoid follicles from autopsies of chagasic and nonchagasic cases with and without congestive heart failure (CHF)

Group	Median area of the follicles (μm^2) (max-min)
Nonchagasic without CHF (n = 20)	100,067.3 (197,281.7–33,841.4)
Nonchagasic with CHF (n = 8)	132,234.6 (186,379.0–67,116.8)
Chagasic without CHF (n = 19)	155,659.9 (302,423.1–67,017.2)
Chagasic with CHF (n = 36)	157,999.2 (748,365.1–23,259.7)

Kruskal-Wallis test, $H = 9.79$; $P = 0.02$.

Mann-Whitney test, among chagasic and nonchagasic cases, $T = 833.0$; $P = 0.005$.

TABLE 3

Area of follicular spleen arteriole walls from autopsies of chagasic and nonchagasic cases with and without congestive heart failure (CHF)

Group	Median area of the arterioles (μm^2) (max-min)
Nonchagasic without CHF (n = 20)	3,007.7 (7,574.8–1,487.3)
Nonchagasic with CHF (n = 8)	3,083.3 (7,740.1–1,303.5)
Chagasic without CHF (n = 18)	3,657.9 (8,621.1–960.7)
Chagasic with CHF (n = 36)	3,560.9 (8,574.9–1,028.0)

Kruskal-Wallis test, $H = 1.640$; $P = 0.650$.

Mann-Whitney test, among chagasic and nonchagasic cases, $T = 1038$; $P = 0.227$.

in the chagasic cases with congestive heart failure and less in the nonchagasic cases without congestive heart failure, with a statistically significant difference ($P = 0.02$) (Table 2).

Although no statistically significant difference was found between the different groups ($P = 0.65$), the chagasic cases without congestive heart failure presented a greater follicular arteriole wall area and the nonchagasic cases without congestive heart failure a lesser area of these spleen vessels (Table 3).

There was no significant difference in the quantification of fibrous connective tissue of the spleen between the four groups analyzed ($F = 2.32$; $P = 0.083$) (Table 4).

DISCUSSION

In this study of autopsies, we verified that the area of lymphoid follicles was greater and the density of spleen follicles was less among chagasic cases. The increase in the area of lymphoid follicles may have occurred as a reflex of the systemic response from Chagas infection. The presence of chronic inflammation could be the explanation for this response, particularly in the myocardium, or possibly the persistence of *T. cruzi* or its antigens in the spleen, as has already been proven in other organs.^{16–19} This phenomenon would presumably imply the formation and recirculation of leukocytes, which would contribute to the increase in area of the lymphoid follicles.

The density of the lymphoid follicles among the chagasic cases was significantly less than among nonchagasic cases. Nevertheless, the areas of these follicles, as well as the congestion of the sinusoids, were significantly greater among the chagasic cases. These data apparently indicate that, because of the increase in spleen weight¹⁵ and possibly its size, there would be greater dispersion of the follicles, thus justifying the lesser density. On the other hand, although "a certain degree of atrophy of the follicles" has previously been described in cases of chronic passive hyperemia,⁷ hyperplasia of the white

TABLE 4

Number of morphometry grid points coinciding with fibrous connective tissue of the spleen from autopsies of chagasic and nonchagasic cases with and without congestive heart failure (CHF)

Group	No. grid points
Nonchagasic without CHF (n = 19)	12.1 $\pm$ 1.5
Nonchagasic with CHF (n = 7)	10.3 $\pm$ 1.0
Chagasic without CHF (n = 17)	11.1 $\pm$ 1.8
Chagasic with CHF (n = 32)	11.3 $\pm$ 1.8

Values represent means $\pm$ standard deviations. Analysis of variance, $F = 2.32$; $P = 0.083$.

pulp has been described in reactional spleen hyperplasia.^{6,9} Although the reactions found in the spleens of mice at the acute experimental phase of infection by *T. cruzi* represent a severe response to the parasitism, they were shown to be ineffective in the control of the infection in spite of the hyperplasia of the white pulp (Maria TA, unpublished data).

In an earlier report,¹⁵ we described a significant increase in spleen weight in chagasic cases without congestive heart failure in comparison to nonchagasic cases without congestive heart failure, in groups of Chagas' disease or non-Chagas' disease individuals, with or without congestive heart failure, and homogenized relation to age, sex, color, and body mass index. We concluded that the inflammatory component in Chagas' disease plays an important role in the increase of spleen weight together with hemodynamic alterations arising from congestive heart failure.

In the chronic human phase of Chagas' disease, a chronic inflammatory response mechanism apparently exists, with the blood circulation being hemodynamically compromised at the same time. Data from the literature prove that in chronic Chagas' disease, independent of the anatomical-clinical form of the disease, the weight of the heart is greater^{20,21} and arrhythmia more frequent,²²⁻²⁴ compared with controls. These events may indicate varying degrees to which myocardial function is compromised, with possible systemic hemodynamic repercussions. Thus, we found simultaneous characteristics of reactional hyperplasia in the spleens of these patients, with an increase in follicle area associated with the characteristics of congestive splenomegaly and a reduction in follicle density and congestion in the sinusoids, reflecting a possible association with compromised cardiac function.

In conclusion, we verified that the area of the lymphoid follicles in the spleen was greater among chagasic cases, which leads us to conclude that the inflammatory component of chronic Chagas' disease performs an important role in this increase in area and, in association with the hemodynamic alterations resulting from the congestive heart failure, may also be contributing toward the increase in spleen weight, as described by us previously.¹⁵

Financial support: This work was supported by research grants from the Fundação de Ensino e Pesquisa de Uberaba (FUNEP), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Universidade de Uberaba (UNIUBE).

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