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# EVALUATION OF THE HISTOPATHOLOGICAL CLASSIFICATIONS OF AMERICAN CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS

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*In order to evaluate the reliability of histopathological classifications of cutaneous and mucocutaneous leishmaniasis the authors compared the histopathological patterns of two biopsies taken simultaneously from the same patient, and classified the material according to Ridley et al. (1980), to Magalhães et al. (1986a), and to a more simplified classification with only three patterns. Distinct histopathological aspects were observed in different lesions or even in the same lesion. The authors concluded that histopathological patterns do not represent a stage of tegumentary leishmaniasis, thus they can not be correlated with prognosis and therapeutical response as suggested in the literature.*

Key words: cutaneous leishmaniasis – mucocutaneous leishmaniasis classification – granulomatous infectious disease

Tegumentary leishmaniasis is an endemic disease in South America being a major health problem in Brazil (Grimaldi et al., 1989). The disease is caused by different leishmania species and clinically ranges from single self-healing ulcers to severe disfiguring mucosal involvement.

Bryceson (1969) classified the human tegumentary leishmaniasis on a spectrum ranging from diffuse cutaneous leishmaniasis (DCL) to cutaneous (CL) and mucocutaneous leishmaniasis (MCL) based on clinical, immunological and histopathological aspects. This classification is useful and correlates well with prognosis and treatment. DCL and borderline cutaneous leishmaniasis can easily be separated from CL and MCL in clinical, immunological and histopathological grounds (Bittencourt & Guimarães, 1968; Bryceson, 1969; Moriearty et al., 1978), but distinction between CL and MCL can not be made on histopathological and immunopathological basis (Bittencourt & Andrade, 1967; Barral et al., 1987).

The histopathological picture of CL and MCL varies from an inflammatory infiltration of mononuclear cells and neutrophils to a granulomatous reaction with or without necrosis (Montenegro, 1924; Bittencourt & Andrade, 1967; Magalhães et al., 1982). Based on these different histopathological aspects several classifications have been proposed and considered to have clinical and prognostic significance (Azulay, 1960; Ridley, 1980; Ridley et al., 1980; Magalhães et al., 1986a).

The first histopathological classification of American tegumentary leishmaniasis was proposed by Azulay (1960) who believed that acute exsudative inflammation was the initial lesion of CL and the tuberculoid granuloma the last step of the inflammatory process. In 1980, Ridley et al., classified 60 biopsies of patients with CL and MCL in five groups. Group I was almost normal except for patches of collagen degeneration. In group II, the predominant characteristic was a more severe necrotizing process than in Group I. Group III corresponded to a heavy inflammatory infiltration without necrosis and granuloma and groups IV and V were associated to epithelioid and giant cells. Patients in groups II and IV, represented only by cutaneous cases, with mean duration of disease of 0.5 and 1.3 years respectively, responded relatively well to treatment, or healed spontaneously; only few patients of group IV had relapses. Those in groups I and V had mucosal involvement and all responded poorly to treatment. Group III consisted of 68% of cutaneous cases and

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presented a variable therapeutical response with 55% of relapses; the patients of this group has a longer evolution (mean duration of 2.7 years). Based in these observations Ridley et al. (1980) considered that necrosis with reactive response observed in group II was the most favorable prognostic feature. It is important to emphasize that the patients of group II of this classification had no mucosal involvement and had a mean duration of disease of only 0.5 years.

Magalhães et al. (1986a) studing 162 biopsies of CL and MCL proposed another classification which according to them correlate well with clinical data, evolution and therapeutical response. An excelent prognosis was associated with group IV; they considered that therapy in this phase of disease merely hastened the process. Groups I, II and III were associated to a good prognosis and they believed that therapy in such patients only reduced healing time. Group V had a bad prognosis although they found a favorable response to treatment in young patients with disease of short duration. According to them the association of mucosal involvement with the patterns I and V corresponded to an unfavorable prognosis.

We have examined a large number of biopsies taken from patients in different areas of Bahia a highly endemic area for tegumentary leishmaniasis, for several years during the course of our studies on human leishmaniasis. In many cases we have tried to correlate response to treatment with the histopathological aspects of the biopsies; in many occasions we have had the opportunity of examining lesions from the same patients at different periods of the disease. These observations displayed a marked variability without being possible to correlate histopathological patterns, clinical course and response to treatment.

In order to evaluate the value of the histopathological classifications of CL and MCL we decided to make a comparative study of two biopsies taken simultaneously from the same patient classifying them according to Ridley et al. (1980) and Magalhães et al. (1986a) as well as to one simplified classification with only three patterns.

## MATERIALS AND METHODS

*Patients* – Twenty cases of CL and eight cases of MCL were included in the present study. Diagnosis of tegumentary leishmaniasis was based on epidemiological, clinical, parasitological and histopathological evidences confirmed by positivity of at least one of these procedures: skin test, serology, parasites in tissue sections or culture. Biopsies were obtained before treatment in CL patients, whereas MCL patients had a previous history of one or more series of treatment. The patients were from different areas of the state of Bahia (Brazil).

Biopsies were made in each case with a 4 mm punch. Sixteen patients of CL and one of MCL had only one skin lesion and the biopsies were taken from two diametrically opposed areas. In four patients of CL and in two of MCL (cases 25 and 26) with more than one cutaneous lesion two skin lesions were biopsied. Biopsies of mucosa were made in five patients of MCL. In three of these patients the biopsies were obtained simultaneously from the infiltrated mucosa and the skin lesion and in the other two (with healed cutaneous lesions) the biopsies were taken in different areas of the infiltrated mucosa (Table). The biopsies were fixed in buffered formalin, processed for paraffin embedding as usual and the sections were stained by hematoxilin-eosin. The sections were classified in three patterns according to one simplified criteria, and also according to Ridley et al. (1980) and Magalhães et al. (1986a).

The three patterns of the simplified classification are: A) An inflammatory infiltrate of plasma cells, lymphocytes and macrophages in the absence of epithelioid and giant cells; B) Occurrence of giant and/or epithelioid cells in a happhazard pattern associated to an inflammatory infiltrate; C) Presence of well circumscribed granulomas with epithelioid and or giant cells associated or not to the other two patterns. The five patterns of the Magalhães et al. (1986a) classification are: I – Cellular exsudative reaction, an inflammatory infiltration of plasma cells, histiocytes and lymphocytes; II – Necrotic and exsudative reaction represented by tissue necrosis associated with the same inflammatory infiltrate; III – Exsudative and necrotic granulomatous reaction: a desorganized granulomatous reaction associated to necrosis and inflammatory infiltrate; IV – Exsudative and granulomatous reaction, representing a desorganized granulomatous reaction associated with an inflammatory infiltrate

TABLE

Clinical and histopathological data of patients

| | | Lesions | | Classifications | | |
|---|---|---|---|---|---|---|
| | Age/Sex | Duration (months) | Biopsed lesions | Present study | Magalhães et al. (1986a) | Ridley et al. (1980) |
| 1 | 42/F | 6 | One ulcer(S) | C/C | V/V | V/V |
| 2 | 12/F | 2 | Ulcer; nodule(S) | A/C | I/V | III/V |
| 3 | 3/F | 3 | One ulcer(S) | B/C | III/V | IV/V |
| 4 | 24/F | 15 | One ulcer(S) | B/B | III/III | IV/IV |
| 5 | 20/M | 4 | One ulcer(S) | B/C | IV/V | IV/V |
| 6 | 20/M | 5 | One ulcer(S) | A/A | II/II | ND |
| 7 | 64/M | 5 | Two nodules(S) | A/B | II/IV | ND/IV |
| 8 | 19/M | 6 | One ulcer(S) | C/C | V/V | V/V |
| 9 | 10/M | 2 | Infiltrated plaque(S) | A/A | I/I | III/III |
| 10 | 19/M | 3 | Ulcer; nodule(S) | A/C | I/V | III/IV |
| 11 | 22/F | 7 | One ulcer(S) | B/B | III/IV | IV/IV |
| 12 | 12/F | 1 | One ulcer(S) | A/C | I/V | III/V |
| 13 | 23/M | 9 | One ulcer(S) | C/C | V/V | V/V |
| 14 | 18/M | 1 | One ulcer(S) | B/C | III/V | IV/V |
| 15 | 20/F | 3 | One ulcer(S) | A/B | I/III | III/IV |
| 16 | 33/M | 12 | Ulcer; infiltrated plaque (S) | C/C | V/V | V/V |
| 17 | 47/M | 3 | Infiltrated skin | A/A | II/II | ND |
| 18 | 70/M | 12 | One ulcer(S) | B/B | III/III | IV/IV |
| 19 | 22/M | 3 | One ulcer(S) | A/A | I/I | III/III |
| 20 | 43/M | 4 | One ulcer(S) | B/B | III/IV | IV/IV |
| 21 | 61/M | 2 | One ulcer(S) | A/B | I/III | III/IV |
| 22 | 55/M | 15 | Ulcer(S); infiltrated mucosa | A/A | I/I | III/III |
| 23 | 39/M | 7 | Infiltrated mucosa | A/B | I/III | III/IV |
| 24 | 61/M | 1 | Infiltrated mucosa | A/B | I/III | III/IV |
| 25 | 60/M | 20 | Two ulcers(S) | A/C | I/V | III/V |
| 26 | 27/M | 0.9 | Verrucous lesion(S) nodule(S) | A/C | I/V | III/V |
| 27 | 55/M | 6 | Infiltrated skin; mucosa | A/C | I/V | III/V |
| 28 | 34/M | 6 | Infiltrated skin; mucosa | A/B | I/III | III/IV |

Patients from number 21 to 28 have the muco-cutaneous form of leishmaniasis ND = Not done; S = skin.

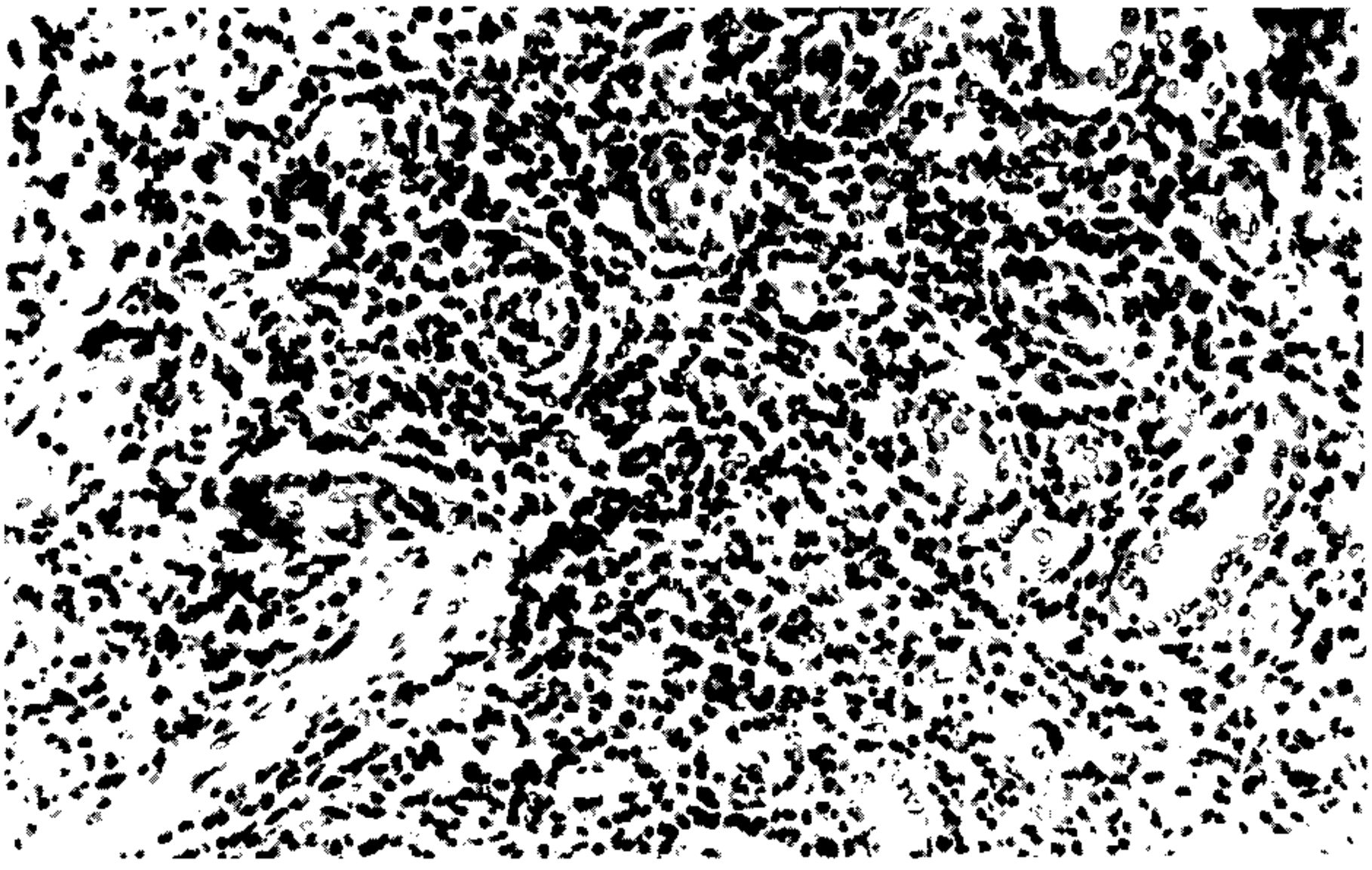

Fig. 1: pattern A. Diffuse inflammatory infiltration of mononuclear cells (group I of Magalhães' classification). Hematoxylin and Eosin, 250x.

Fig. 2: pattern B. Occurrence of giant and epithelioid cells in a haphazard pattern. See, at left, caseation necrosis (pattern III of Magalhães' classification). Hematoxylin and Eosin, 200x.

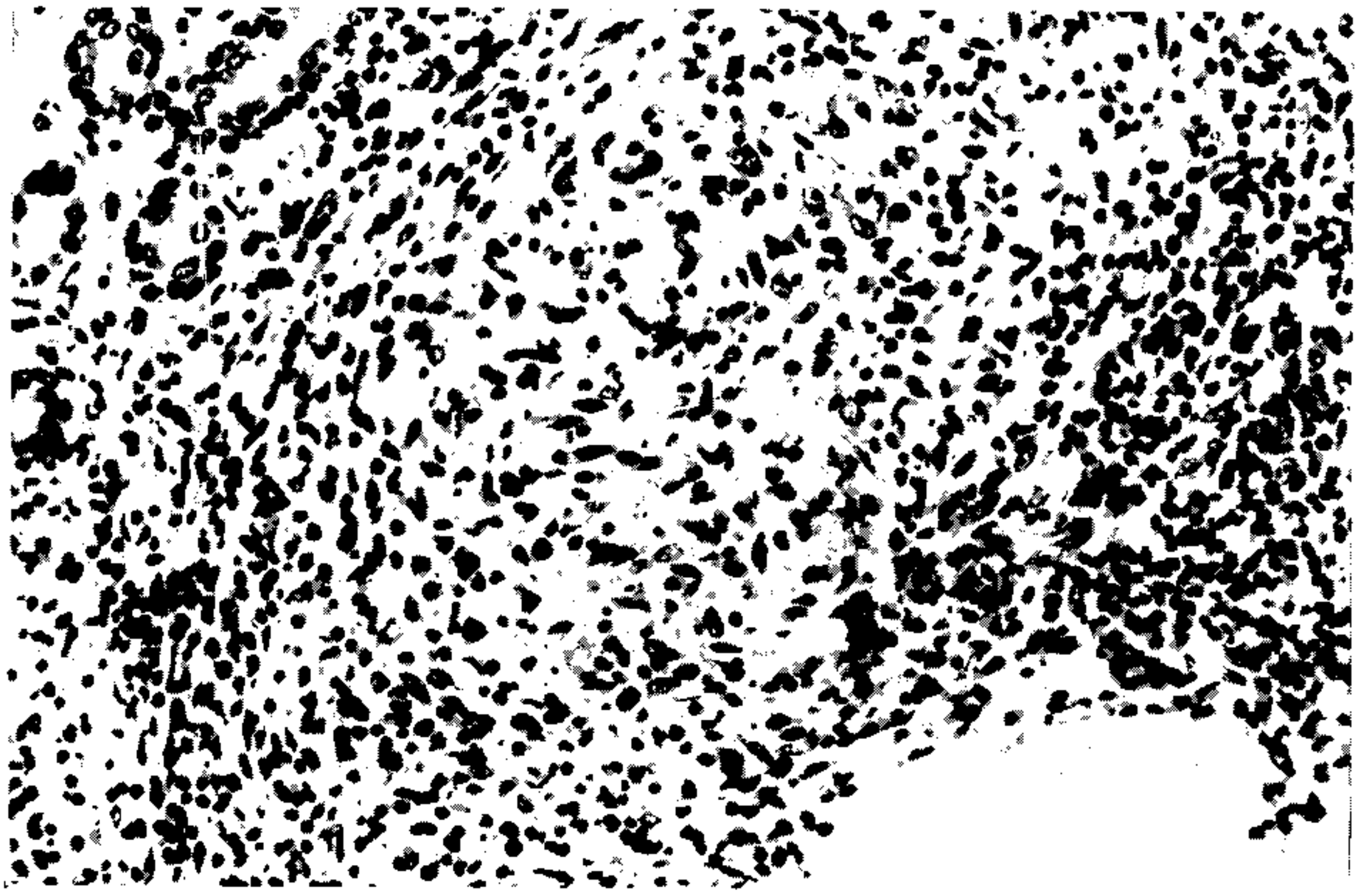

Fig. 3: pattern C. See a well circumscribed granuloma with epithelioid cells (pattern V of Magalhães' classification) Hematoxylin and Eosin, 250x.

without necrosis; V – Tuberculoid and exsudative reaction, consisting of well organized granulomas plus the infiltration of plasma cells, lymphocytes and histiocytes. The other classification consists of: I – Unreactive pattern, with scant or absent inflammatory cells; II – Reactive pattern, with a slight inflammatory reaction and severe necrotizing process; III – Infiltrative pattern with a heavy infiltration of the dermis but without granulomas and necrosis; IV – Tuberculoid pattern, with scattered giant cells and small foci of epithelioid cells that may be associated with necrosis; V – Hypersensitive pattern, with granuloma composed

of well developed epithelioid cells associated or not to giant cells (Ridley, 1980; Ridley et al., 1980).

## RESULTS

The patterns seen more frequently in the same patient were A and B and A and C (Figs 1-3). It is important to refer that in some biopsies all these patterns were found simultaneously.

The histopathological patterns according to the classifications used are listed in the Table. Considering the three patterns of the simplified classification in 54% of the patients different histopathological patterns were observed simultaneously. If classified according to Magalhães et al. (1986a) 61% of the patients exhibited two different histopathological patterns simultaneously. In all biopsies there was a moderate to heavy inflammatory infiltrate thus no one of them could be included in groups I and II of the other classification (Ridley et al., 1980). Besides, it was not possible to classify five biopsies according to Ridley et al. (1980) because there was no item to include a moderate to heavy inflammatory infiltrate associated with necrosis in this classification.

Thirty-four biopsies of the 56 performed (60%) showed granulomatous reaction. Otherwise in only five patients granulomatous reaction was not observed.

## DISCUSSION

It seems to us that the better prognosis observed in some groups of the classifications of Ridley et al. (1980) and Magalhães et al. (1986a) is related to absence of mucous involvement and to a shorter time of evolution but the strict association with histopathological findings seems to be fortuitous in such series. The relation between MCL and poor therapeutical response is a well known fact (Marsden, 1985). The unreactive pattern of Ridley's classification probably includes biopsies performed outside the area of activity of lesion. If a biopsy of leishmaniasis has only few or no inflammatory cells we must consider that this procedure was not correctly made.

Magalhães et al. (1986c) observed 50% of granulomatous reaction examining only one biopsy of each patient. In the present paper, 82% of the patients presented granulomatous reaction. Probably if more than two biopsies were performed in each patient the frequency of granulomatous reaction would be greater.

Even using the simplified criteria for histopathological classification adopted in this study diverse histopathological aspects were observed in biopsies taken simultaneously from the same patient evidencing the impossibility in classifying CL and MCL in a histopathological basis. Furthermore, these distinct aspects have been observed even in biopsies taken from different areas of the same lesion. If only one biopsy had been performed in most of our patients large diverging prognosis could have erroneouly been drawn based on histopathological classification. One the other hand Magalhaes et al. (1986b) studying successive biopsies in patients of CL and MCL observed changes in the histopathological aspects and based in this study concluded that the exsudative cellular reaction represents both the initial and final patterns of leishmaniasis. Considering the diversity of aspects in the lesions of a same patient there is no sense in considering that one pattern represents the initial and final aspects of CL and MCL.

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## REFERENCES

AZULAY, R. D., 1960. Histopatologia da leishmaniose tegumentar. *Dermatol. Iber. Latin. Amer., 2:*7-15.

BARRAL, A.; ALMEIDA, A. R. J.; CARVALHO, E. M.; BARRAL-NETTO, M.; COSTA, J. M.; BADARÓ, R.; ROCHA, H. & JOHNSON, W. D., 1987. Evaluation of T-cell subsets in the lesion infiltrates of human cutaneous and mucocutaneous leishmaniasis. *Parasite Immunol., 9:*487-497.

BITTENCOURT, A. L. & ANDRADE, A., 1967. Aspectos imunopatologicos na leishmaniose cutaneo-mucosa. *Hospital, 71:*975-984.

BITTENCOURT, A. L. & GUIMARÃES, N., 1968. Imunopatologia da leishmaniose tegumentar difusa. *Med. Cut. ILA, 2:*395-402.

BRYCESON, A. D. M., 1969. Diffuse cutaneous leishmaniasis in Ethiopia. I The clinical and histopathological features of the disease. *Trans. R. Soc. Trop. Med. Hyg., 63:*708-737.

GRIMALDI, Jr. G.; TESH, R. B. & MCMAHON-PRATT, D., 1989. A review of the geographic distribution and epidemiology of leishmaniasis in the new world. *Am. J. Trop. Med. Hyg., 41:*687-725.

MAGALHÃES, A. V.; CHIARINI, L. H. & RAICK, A. N., 1982. Histopatologia da leishmaniose tegu-

mentar. *Rev. Inst. Med. Trop. São Paulo, 24:* 267-276.

MAGALHÃES, A. V.; MORAES, M. A. P.; RAICK, A. N.; LLÃNOS-CUENTAS, A.; COSTA, J. M. L.; CUBA, C. C. & MARSDEN, P. D., 1986a. Histopatologia da leishmaniose tegumentar por *Leishmania braziliensis braziliensis* 4. Classificação histopatológica 1. *Rev. Inst. Med. Trop. São Paulo, 28:* 421-430.

MAGALHÃES, A. V.; MORAES, M. A. P.; RAICK, A. N.; LLÃNOS-CUENTAS, A.; COSTA, J. M. L.; CUBA, C. C. & MARSDEN, P. D., 1986b. Histopatologia da leishmaniose tegumentar por *Leishmania braziliensis braziliensis* 1. Padrões histopatologicos e estudo evolutivo das lesões. *Rev. Inst. Med. Trop. São Paulo, 28:* 253-262.

MAGALHÃES, A. V.; MORAES, M. A. P.; RAICK, A. N.; LLÃNOS-CUENTAS, A.; COSTA, J. M. L.; CUBA, C. C. & MARSDEN, P. D., 1986c. Histopatologia da leishmaniose tegumentar por *Leishmania braziliensis braziliensis.* 3. Reação celular nos tecidos. *Rev. Inst. Med. Trop. São Paulo, 28:* 300-311.

MARSDEN, P. D., 1985. Pentavalent antimonials: old drugs for new diseases. Revisão. *Rev. Soc. Bras. Med. Trop., 18:* 187-198.

MONTENEGRO, J., 1924. Anatomo-pathologia da leishmaniose cutanea. *An. Paul. Med. Cir., 15:* 5-11.

MORIEARTY, P. L.; BITTENCOURT, A. L.; PEREIRA, C.; TEIXEIRA, R.; BARRETO, E. & GUIMARÃES, N., 1978. Borderline cutaneous leishmaniasis. Clinical, immunological and histological differences from mucocutaneous leishmaniasis. *Rev. Inst. Med. Trop. São Paulo, 20:* 15-21.

RIDLEY, D. S., 1980. A histological classification of cutaneous leishmaniasis and its geographical expression. *Trans. R. Soc. Trop. Med. Hyg., 74:* 515-521.

RIDLEY, D. S.; MARSDEN, P. D.; CUBA, C. C. & BARRETO, A. C. A., 1980. Histological classification of mucocutaneous leishmaniasis in Brazil and its clinical evaluation. *Trans. R. Soc. Trop. Med. Hyg., 74:* 508-514.