Proposal of a histopathological predictive rule for the differential diagnosis between American tegumentary leishmaniasis and sporotrichosis skin lesions

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Summary

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Conflicts of interest
None declared.

Background American tegumentary leishmaniasis (ATL) and sporotrichosis exhibit similar histopathology and low frequencies of microorganism detection.

Objectives This study seeks to identify microscopic alterations that can distinguish between these diseases.

Methods Haematoxylin and eosin stained slides of 171 ATL and 97 sporotrichosis samples from active cutaneous lesions were examined for histopathological alterations. The lesions were diagnosed by isolating the agent (which was not visible) in culture. An intuitive diagnosis was assigned to each slide. The strength of the association between the histopathological findings and the diagnosis was estimated by an odds ratio, and each finding was graded according to a regression model. A score was assigned to each sample based on the histopathological findings. A study of the interobserver reliability was performed by calculating kappa coefficients of the histopathological findings and intuitive diagnoses.

Results The markers 'macrophage concentration', 'tuberculoid granuloma' and 'extracellular matrix degeneration' were associated with ATL. 'Suppurative granuloma', 'stellate granuloma', 'different types of giant cells', 'granulomas in granulation tissue' and 'abscess outside the granuloma' were associated with a diagnosis of sporotrichosis. 'Macrophage concentration' and 'suppurative granuloma' had the highest (substantial and almost perfect, respectively) reliability. The regression model score indicated 92·0% accuracy. The intuitive diagnosis had 82·5% diagnostic accuracy and substantial reliability.

Conclusions Taking into account the clinical and epidemiological context, some histopathological alterations might be useful for the differential diagnosis between ATL and sporotrichosis cutaneous lesions in cases in which the aetiological agent is not visible.

American tegumentary leishmaniasis (ATL) and sporotrichosis exhibit similar clinical, epidemiological, laboratory and histopathological features. Therefore, the differential diagnosis for these diseases is important, particularly in Rio de Janeiro state, Brazil, where these diseases occur in the same endemic areas.[1] The isolation and identification of the aetiological agent in culture medium is the standard diagnostic method for both diseases, but the results can be negative in some cases, especially those of ATL.[2] Immunohistochemical[3–5] and molecular[6] methods have better diagnostic performance than routine histopathological examination; however, like culture-based identification, these approaches are not available at every health-care facility.

Histopathological examination is relatively quick, inexpensive, widely available and does not require special treatment of the biological material after fixation. In addition, this approach can be specific for detecting microorganisms and can help to establish other differential diagnoses, such as skin neoplasms. The histopathological characteristics of ATL and sporotrichosis correspond to diffuse granulomatous dermatitis.[7–13] The main difference between the diseases is the type of granuloma, which tends to be tuberculoid in ATL and suppurative in sporotrichosis. However, this difference is not fully specific; in some cases, one disease simulates the other. Thus, despite the fact that visualization of the infectious agent is not always possible, this visualization is

indispensable in histopathological examinations to establish the diagnosis.

Histopathological examination in ATL exhibits 14–63·7% sensitivity for the detection of amastigotes.[2,3,5,8,14–17] In sporotrichosis, the sensitivity for the detection of fungal forms can vary from 5% (one positive case out of 19)[18] to 80%[19] or more than 90%.[10] In the ongoing epidemic in Rio de Janeiro, the sensitivity of histopathological examination is approximately 30%.[1,20] Therefore, searching for morphological parameters other than visualization of the aetiological agent is important for distinguishing between ATL and sporotrichosis.

Predictive rules are tools aimed at removing some of the subjectivity in an examination by formulating a numerical score from simple isolated findings that are systematically investigated.[21] This score allows an estimation of the probability of different outcomes as a function of the actual findings. Systematic studies of the histopathological differential diagnosis between ATL and sporotrichosis do not exist in the literature.

This study aims to analyse the histopathological alterations in ATL and sporotrichosis and to determine the alterations that can form the foundation for an objective and systematic method to differentiate the diseases in cases in which the microorganism is not visible on the tissue. Furthermore, this method will be compared with intuitive histopathological diagnosis.

Materials and methods

Sample selection

The medical records of patients treated at the Evandro Chagas Clinical Research Institute between 1998 and 2009 were surveyed. The cases that were selected for inclusion in this study presented active cutaneous lesions diagnosed as ATL or sporotrichosis by isolation of the aetiological agent in culture and histopathological examination of the cutaneous lesions had been performed. A cross-sectional diagnostic study was performed with a design following the guidelines of the Standards for Reporting of Diagnostic Accuracy (STARD).[22]

The samples were retrospectively selected from the files and comprised histological slides stained with haematoxylin and eosin and paraffin blocks processed according to the standard diagnostic routine of fixation in buffered formalin and embedding in histological paraffin.

The study included representative samples (containing at least intermediate reticular dermis and exhibiting diffuse dermatitis or granuloma) of satisfactory technical quality (fixation, processing and staining), which allowed the observation of microscopic details. Samples with visible amastigote or yeast forms on histopathological examination were excluded.

Histopathological analysis

The samples were observed under an optic microscope by a trained observer (L.P.Q.) who was blinded to the diagnosis.

Detailed characteristics of inflammatory infiltrates, granulomas and epidermal alterations were investigated. These previously established alterations were considered dichotomous histopathological markers (i.e. present or absent) and are defined in Table 1. An intuitive histopathological diagnosis was also attributed to each case based on subjective diagnostic impressions.

Histopathological reliability study (interobserver agreement)

The same histopathological examination was independently performed by a second trained observer (L.H.M.M.) who was also blinded to the diagnosis and detection of markers and to the intuitive diagnosis assigned by the first observer. These observations were used in an interobserver reliability study. The reliability of the dichotomous histopathological findings was estimated by calculation of a simple Cohen kappa coefficient and prevalence-adjusted bias-adjusted kappa (PABAK). These coefficients offer an appreciation of agreement beyond that expected by chance and are prerequisites to an accuracy study.[23] The values were interpreted according to Landis and Koch[24] as follows: poor (< 0·00), discrete (0·00–0·20), reasonable (0·21–0·40), moderate (0·41–0·60), substantial (0·61–0·80) and almost perfect (0·81–1·0) agreement. Considering that kappa is affected by prevalence of the characteristic studied in a sample, we also show average agreement proportion (P_{avg}) and concordance for the presence (P_{pos}) or absence (P_{neg}) of each histopathological characteristic. For histopathological markers with prevalence varying between 15% and 85%, the size of the sample sufficed to estimate kappa values higher than 0·80 with a 0·15 absolute error and a 95% confidence interval (CI).

Data collection and analysis

The data were collected on forms, inserted in databases using the software EpiData 3·1[25] and analysed by the Statistical Package for the Social Sciences (SPSS) Win version 17·0 software (SPSS Inc., Chicago, IL, U.S.A.). An exploratory analysis of the frequencies of the different histological markers was performed for both diseases. The strength of association of each marker with the diagnosis was estimated by calculating the raw odds ratio (OR) and the corresponding 95% CIs. ORs depict the ratio of the odds in favour of those with the disease presenting each particular histopathological characteristic (predictor) relative to the odds of presenting the same predictor in those without the disease. A multiple logistic regression analysis was performed using a backwards method to investigate independent associations of each histopathological marker with the diagnosis of ATL; these data are expressed as adjusted OR (OR_{adj}) and the corresponding 95% CI. The model initially tested all variables that were significant at a 0·10 level in the exploratory analysis. The criterion used to retain co-variables in the final model was a significance of < 5% in the likelihood ratio test.

Table 1 Definition of histopathological markers

Histopathological markers	Definition
Granuloma	Any aggregate of phagocytic mononuclear cells
Foreign body-like granuloma	Granuloma containing foreign body-like giant cells, macrophages and epithelioid cells with little cohesion and few or no leukocytes
Tuberculoid granuloma	Well-formed, rounded granuloma with well-differentiated cohesive epithelioid cells, Langerhans-type giant cells and associated lymphoplasmacytic infiltrate
Macrophage concentration	Loose, poorly formed and poorly delimited aggregates of epithelioid cells amid lymphoplasmacytic infiltrate
Sporotrichotic granuloma	Granuloma with central abscess, epithelioid cells and lymphoplasmacytic infiltrate organized in concentric layers
Pyogranuloma	Granuloma with abscess
Suppurative granuloma	Granuloma with neutrophils amid mononuclear cells not forming abscesses except immediately below an ulcer
Interstitial granuloma	Well-differentiated phagocytic mononuclear cells permeating or in direct contact with collagen bundles
Stellate granuloma	Spiculated granuloma with any type of central necrosis
Higher number of phagocytes compared with other cells	More macrophages, epithelioid cells and giant cells than the total of the remainder of inflammatory cells
Different types of giant cells	More than one type of multinucleated giant cell
Granulomas in the granulation tissue	Proliferation of small vessels and intense oedema associated with granulomas except for below or next to an ulcer
Plasmocyte aggregate	Collection of 10 or more plasmocytes with little or no tissue or inflammatory cells in between
Abscess outside granuloma	Collection of neutrophils and pyocytes far from granulomas, with little or no tissue or inflammatory cells in between and not associated with the bottom of an ulcer
Suppuration outside granuloma	Neutrophils in the inflammatory infiltrate not forming an abscess and not associated with a granuloma or the bottom of an ulcer
Extracellular matrix degeneration	Alteration in staining affinity and loss of definition of collagen bundles
Neutrophils in epidermis	Presence of neutrophils amid epidermal squamous cells
Transepidermal neutrophil elimination	Pseudoepitheliomatous squamous hyperplasia simulating a perforating disease with transepidermal elimination of an abscess
Transepidermal phagocyte elimination	Pseudoepitheliomatous squamous hyperplasia simulating a perforating disease with transepidermal elimination of a granuloma
Fibrosis	Proliferation of small vessels and intense deposition of extracellular matrix as in scarring. Thickening of collagen bundles was not considered

A receiver operating characteristics (ROC) curve was built with the probabilities predicted by logistic analysis, and the area under the curve (AUC) was calculated with 95% CI. The ROC curve is used to evaluate the accuracy of continuous variables. Values nearest to 1 in the AUC indicate better diagnostic performance to distinguish cases from noncases.

The β-coefficient of each variable in the regression equation was used to weigh each histopathological marker and assign a score. The performance parameters for ATL diagnosis, sensitivity (probability of a positive result cases), specificity (probability of a negative result in noncases), total accuracy (probability of a correct classification) and likelihood ratios (the magnitude of change between prior and posterior odds of disease) were calculated for the individual histopathological markers, intuitive diagnoses and scores.

Results

Studied groups

The clinical records of 455 patients treated at the Evandro Chagas Clinical Research Institute between 1998 and 2009

with a diagnosis of ATL confirmed by isolation of *Leishmania* sp. in culture medium were analysed. The clinical records of 781 patients seen during the same period with a diagnosis of sporotrichosis confirmed by isolation of *Sporothrix* sp. in culture medium were surveyed. A total of 171 samples of cutaneous lesions from 154 patients with ATL and 97 samples of cutaneous lesions from 90 patients with sporotrichosis were included, as shown by the flowchart in Figure 1.

Patients with ATL were between 2 and 90 years old (mean, 39; median, 37 years) and 94 patients (61%) were male. Patients with sporotrichosis were between 2 and 89 years old (mean, 42; median, 45) and 54 patients (60%) were female.

Data analysis

The following histopathological markers remained directly associated with a diagnosis of ATL (OR > 1) in the final logistics model (Table 2): 'macrophage concentration', 'extracellular matrix degeneration' and 'tuberculoid granuloma' (Fig. 2). The following markers remained inversely associated (OR < 1): 'suppurative granuloma', 'stellate
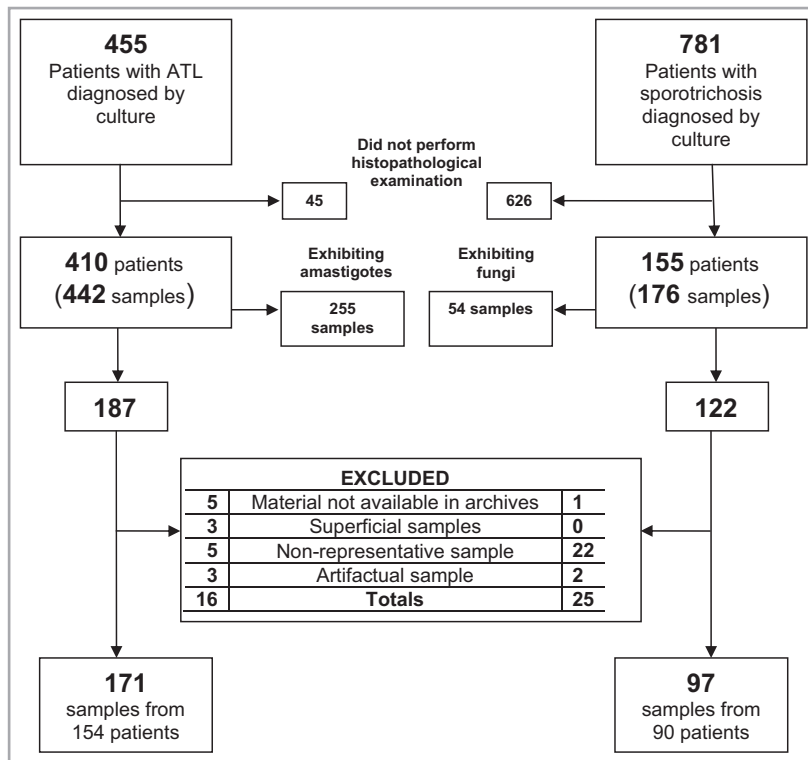
Fig 1. Flowchart for inclusion of American tegumentary leishmaniasis (ATL) and sporotrichosis active cutaneous lesion samples.

Table 2 Raw and adjusted logistic model OR and β-coefficients of the regression equation for a diagnosis of ATL confirmed by culture according to the presence of histopathological markers in outpatients between 1998 and 2009 (total n = 268)

Histopathological marker	ATL (N = 171), n (%)	Sporo (N = 97), n (%)	OR[a]	OR$_{adj}$[f]	95% CI	β[g]
Macrophage concentration	116 (67·8)	23 (23·7)	6·79	5·19	2·41–11·20	1·65
Extracellular matrix degeneration	49 (28·6)	10 (10·3)	3·49	4·54	1·61–12·82	1·51
Tuberculoid granuloma	68 (39·7)	21 (21·6)	2·39	3·83	1·53–9·59	1·34
Suppurative granuloma	26 (15·2)	73 (75·2)	0·06	0·08	0·04–0·18	−2·49
Abscess outside granuloma	2 (1·2)	12 (12·4)	0·08	0·15	0·02–0·94	−1·89
Granulomas in the granulation tissue	14 (8·19)	33 (34·0)	0·17	0·18	0·06–0·50	−1·71
Stellate granuloma	13 (7·6)	17 (17·5)	0·39	0·21	0·06–0·70	−1·54
Different types of giant cells	29 (16·9)	34 (35·1)	0·38	0·28	0·12–0·67	−1·27
Interstitial granuloma	99 (57·9)	36 (37·1)	2·33			
Pyogranuloma	8 (4·6)	43 (44·3)	0·06			
Sporotrichotic granuloma	4 (2·3)	21 (21·6)	0·09			
Suppuration outside granuloma	32 (18·7)	62 (63·9)	0·13			
Foreign body-like granuloma	17 (9·9)	21 (21·6)	0·40			
Fibrosis	51 (29·8)	45 (46·4)	0·49			
Higher number of phagocytes[b]	38 (22·2)	19 (19·6)	1·17			
Plasmocyte aggregate[c]	91 (53·2)	58 (59·8)	0·77			
Neutrophils in epidermis[d]	12 (7·0)	13 (13·4)	0·49			
Transepidermal neutrophil elimination	9 (5·2)	17 (17·5)	0·26			
Transepidermal phagocyte elimination[e]	30 (17·5)	26 (26·8)	0·58			

ATL, American tegumentary leishmaniasis; CI, confidence interval, OR, odds ratio; OR$_{adj}$, adjusted OR; Sporo, sporotrichosis.
[a]$P < 0.01$ except for [b]$P = 0.61$, [c]$P = 0.29$, [d]$P = 0.13$ and [e]$P = 0.07$.
[f]$P < 0.05$ (OR adjusted by logistic regression for the remainder of significant markers in bivariate).
[g]β-coefficients of logistic regression equation.

granuloma', 'granulomas in the granulation tissue', 'abscess outside granuloma' (Fig. 3) and 'different types of giant cells'. The variables 'higher number of phagocytes compared to other cells', 'plasmocyte aggregate' and 'neutrophils in epidermis' were not tested in the logistics model due to P-values above 0·10.
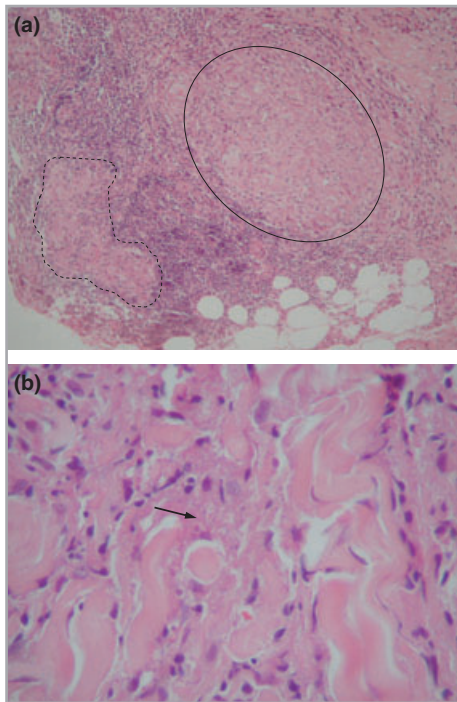
Fig 2. Histopathological markers associated with a diagnosis of American tegumentary leishmaniasis. (a) Tuberculoid granuloma (solid line) and macrophage concentration (dotted line). (b) Extracellular matrix degeneration (arrow).

The performance parameters of each investigated marker for diagnoses of ATL and sporotrichosis are described in Table 3. The best likelihood ratios for a positive test attain values not higher than 3. Intuitive diagnosis, which was not considered to be a histopathological marker, had 82·5% accuracy and a likelihood ratio of 5·6 for the positive test.

Each variable was assigned a score corresponding to its respective β-coefficient in the regression equation (Table 2), but the total score could theoretically vary between −8·9 and 4·5 in each case. The distribution of cases according to this histopathological score is represented in Figure 4; a variation between −7·01 and 4·5 in cases of sporotrichosis and between −2·69 and 4·5 in cases of ATL was observed. The probabilities predicted by the final logistic model exhibited discrimination of cases of ATL by an AUC ROC totalling 0·92 (95% CI 0·88–0·95), which is significant for $P < 0.001$ (Fig. 5). The cut-off of the score with highest accuracy was −1·2 with 92·4% sensitivity (95% CI 87·4–95·9), 77·3% specificity (95% CI 66·7–85·2), 4·07 positive test likelihood ratio (95% CI 3·6–4·6) and 0·09 negative test likelihood ratio (95% CI 0·05–0·2).

Reliability study

The second observer analysed 211 samples. No histopathological marker exhibited almost perfect reliability by the simple kappa calculation (Table 4). The 'abscess outside granuloma' marker exhibited an average prevalence smaller than 5%, which did not justify an index calculation.

The 'macrophage concentration', 'suppurative granuloma' and 'intuitive diagnosis' markers exhibited substantial interobserver agreement with kappa ≥ 0·61. The remainder of the variables exhibited only moderate ('granulomas in the granulation tissue'), reasonable ('tuberculoid granuloma') or discrete ('extracellular matrix degeneration', 'stellate granuloma' and 'different types of giant cells') agreement.

The PABAK value for 'suppurative granuloma' attained a value of almost perfect agreement (> 0·80). However, the reliability was reasonable or discrete for the 'tuberculoid granuloma', 'stellate granuloma' and 'extracellular matrix degeneration' markers despite the presence of PABAK.
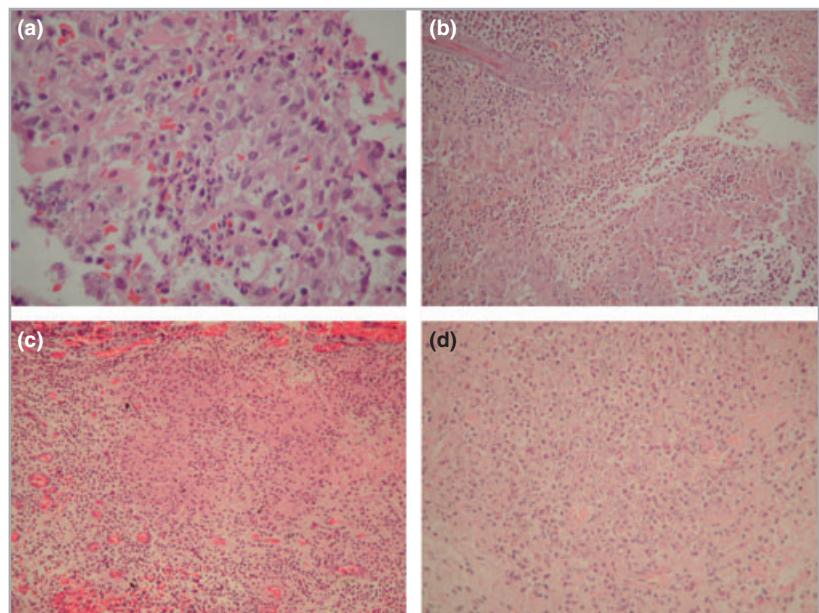
Fig 3. Histopathological markers associated with a sporotrichosis diagnosis. (a) Suppurative granuloma; (b) stellate granuloma; (c) granuloma in the granulation tissue; (d) abscess outside the granuloma.

Table 3 Diagnostic performance parameters of histopathological markers for diagnosis of ATL and sporotrichosis (total N = 268)

Histopathological marker	Sensitivity %	95% CI	Specificity %	95% CI	Accuracy %	LR+ (95% CI)	LR− (95% CI)
Macrophage concentration[a]	67·8	60·3–74·8	76·3	66·6–84·3	70·9	2·86 (1·97–4·15)	0·42 (0·33–0·54)
Tuberculoid granuloma[a]	39·8	32·4–47·5	78·4	68·8–86·8	53·7	1·84 (1·20–2·80)	0·77 (0·66–0·90)
Interstitial granuloma[a]	57·9	50·1–65·4	62·9	52·5–72·5	59·7	1·56 (1·17–2·08)	0·67 (0·53–0·85)
Extracellular matrix degeneration[a]	28·7	22·0–36·1	89·7	81·9–94·9	50·7	2·79 (1·48–5·23)	0·79 (0·71–0·89)
Suppurative granuloma[b]	75·3	65·5–83·5	84·8	78·5–89·8	81·3	4·95 (3·41–7·18)	0·29 (0·21–0·42)
Pyogranuloma[b]	44·3	34·2–54·8	95·3	91·0–98·0	76·9	9·43 (4·65–19·3)	0·58 (0·49–0·70)
Suppuration outside granuloma[b]	63·9	53·5–73·4	81·3	74·6–86·8	75·0	3·42 (2·42–4·83)	0·44 (0·34–0·58)
Granulomas in the granulation tissue[b]	34·0	24·7–44·4	91·8	86·6–95·5	70·9	4·15 (2·34–7·37)	0·71 (0·62–0·84)
Sporotrichotic granuloma[b]	21·6	14·0–31·2	97·7	94·1–99·3	71·2	9·39 (3·27–26·18)	0·80 (0·72–0·89)
Abscess outside granuloma[b]	12·5	6·6–20·6	98·8	95·8–99·9	67·6	10·33 (2·42–46·29)	0·89 (0·82–0·96)
Transepidermal neutrophil elimination[b]	17·5	11·5–26·6	94·7	90·2–97·6	66·8	3·30 (1·54–7·18)	0·87 (0·79–0·96)
Foreign body-like granuloma[b]	21·6	13·9–31·2	90·1	86·6–94·1	65·3	2·18 (1·21–3·92)	0·87 (0·78–0·98)
Different types of giant cells[b]	35·1	25·6–45·4	83·0	76·6–88·3	65·7	2·06 (1·35–3·17)	0·78 (0·67–0·92)
Stellate granuloma[b]	17·5	10·6–26·6	92·4	87·4–95·9	65·3	2·30 (1·17–4·54)	0·89 (0·81–0·99)
Neutrophils in epidermis[b]	13·4	21·8–7·3	92·8	87·7–96·2	64·2	1·86 (0·91–4·02)	0·93 (0·85–1·02)
Transepidermal phagocyte elimination[b]	26·8	18·3–36·8	82·4	78·9–87·8	62·4	1·52 (0·96–2·43)	0·88 (0·77–1·02)
Fibrosis[b]	46·4	36·2–56·7	70·1	62·7–76·9	61·6	1·56 (1·14–2·13)	0·76 (0·62–0·94)
Intuitive diagnosis[a]	80·7	74·0–86·3	85·6	77·0–91·9	82·5	5·60 (3·43–9·13)	0·23 (0·16–0·31)

ATL, American tegumentary leishmaniasis; CI, confidence interval; LR+, likelihood ratio for positive test; LR−, likelihood ratio for negative test. [a]For ATL diagnosis. [b]For sporotrichosis diagnosis.
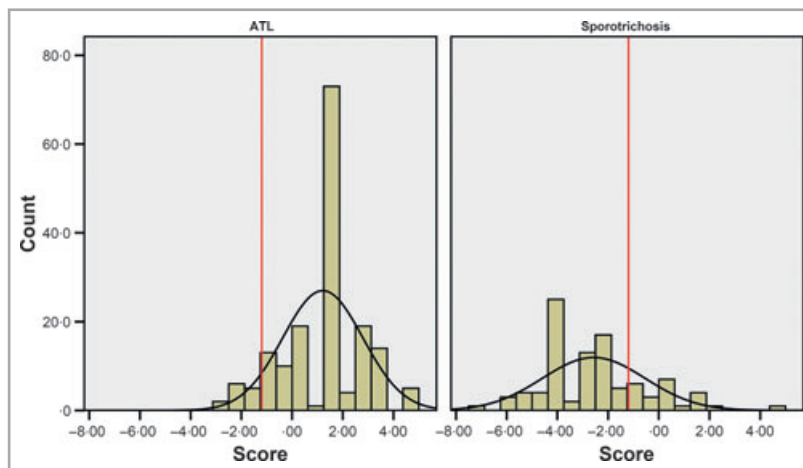

Fig 4. Distribution of cases (n = 268) according to the histopathological score in 171 cases of American tegumentary leishmaniasis (ATL) and 97 cases of sporotrichosis and the highest accuracy cut-off (−1·2; red line).

Discussion

A study of the histopathological differential diagnosis between ATL and sporotrichosis active cutaneous lesions in humans was performed, and a predictive rule was proposed for this diagnosis. Interobserver agreement regarding the investigated histopathological alterations was evaluated. Most of the analysed alterations were selected from the histopathological descriptions of ATL and sporotrichosis found in the literature.

Both Magalhães *et al.*[8] and Ridley *et al.*[26] have reported tuberculoid granulomas in ATL. In turn, De Beurmann and Gougerot[13,27] have elaborated extensively on the differential diagnosis between sporotrichosis and cutaneous tuberculosis, stating that 'there is no other disease with a more tuberculoid form than sporotrichosis'. Reports exist of caseous necrosis in granulomas of sporotrichosis.[9,20] In this study, granulomas defined as tuberculoid were significantly associated with ATL.

'Macrophage concentration' was described in ATL in 1924.[12] Later, a similar pattern (named 'exudative granulomatous reaction') was also reported.[8] 'Granulomatoid areas', which are similar to 'macrophage concentration' and are usually also associated with suppuration,[10] have been described in sporotrichosis. In this study, the presence of neutrophils defined these granulomas as suppurative.
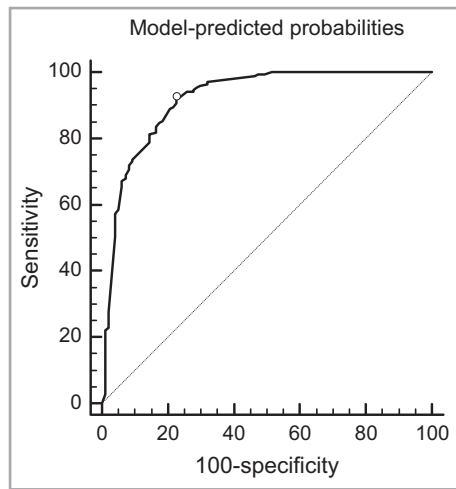
Fig 5. Receiver operating characteristic curve for the histopathological model of a diagnosis of American tegumentary leishmaniasis (ATL) and the highest accuracy cut-off ($-1·2$); sensitivity, 92·4% (87·4–95·9); specificity, 77·3 (66·7–85·2); likelihood ratio for positive test, 4·07 (3·6–4·6); likelihood ratio for negative test, 0·09 (0·05–0·2); area under curve, 0·92 (0·88–0·95).

The presence of suppurative granulomas often attended with a central abscess represents the classic histopathological description of sporotrichosis.[9–11,13,27,28] Also, 'diffuse granulomas'[9] associated with suppuration, without abscess, and neutrophil permeation of the epithelioid cell zone in pyogranulomas have been described.[27] The tissue reaction in ATL is mainly characterized as nonsuppurative.[12] However, neutrophils associated with a vasculitis-like reaction,[29] necrosis[8,30] or even suppurative granulomas[31,32] have been described. 'Suppurative granuloma' was the investigated marker with the highest strength of association in sporotrichosis.

Stellate granulomas are described as 'branching' in sporotrichosis and might arise from the coalescence of several pyogranulomas.[11] We could not find references to stellate or 'branching' granulomas in ATL, and they were very rare in the ATL cases reported here.

Langerhans-type giant and foreign body-like cells have been reported in ATL.[8,12,26] Among our ATL cases, we found one Touton giant cell (data not shown), which has not previously been reported in the literature. Some authors have described giant cells in sporotrichosis but have not specified their types.[13,27,33] Other authors have stated that giant cells are mostly foreign body-like cells.[9,34]

Reports of granulomas associated with granulation tissue could not be found in the literature. The presence of these granulomas in sporotrichosis is an original observation made by our group. One report has described intense oedema associated with 'post-necrotic granulomas' in ATL[26] and fibroblast proliferation around granulomas in sporotrichosis,[28] findings that do not completely coincide with the alteration searched for in the present study.

The suppurative nature of the tissue reaction in sporotrichosis may be manifested as abscesses[11] or 'suppurative foci'[34] outside granulomas. In ATL, the presence of neutrophils has been reported in nongranulomatous lesions.[26] A recent study has shown by histochemistry the participation of neutrophils even in old ATL lesions.[35] In spite of its infrequency, the presence of abscesses outside granulomas was significantly associated with a diagnosis of sporotrichosis.

In ATL, collagen degeneration or necrosis was reported in Montenegro's descriptive study[12] and in a later study that included approximately 400 cases.[26] This collagen degeneration or necrosis has been attributed to the deposit of immune complexes[29] and is related to a nonreactive parasite-rich form.[36] This finding had not been previously described or given any particular value in sporotrichosis.

Histopathological markers examined individually are simple and may be widely used. Regarding differential diagnosis, 'macrophage concentration' and 'suppurative granuloma' exhibited high accuracy and substantial or higher interobserver agreement.

The intuitive histopathological differential diagnosis between these two diseases exhibited higher performance parameters than any isolated histopathological marker and substantial interobserver agreement. Nevertheless, this histopathological impression is quite subjective and dependent on

Table 4 Positive, negative and total agreement and simple kappa with 95% CI for histopathological markers relevant to the differential diagnosis between ATL and sporotrichosis and intuitive diagnosis (N = 211)

Histopathological marker	N	P_{avg} (%)	P_{pos}	P_{neg}	P_{total}	Kappa (95% CI)	PABAK
Macrophage concentration	103	61·0	0·84	0·78	0·82	0·62 (0·52–0·73)	0·63
Suppurative granuloma	63	31·3	0·88	0·94	0·92	0·62 (0·54–0·90)	0·84
Granuloma in the granulation tissue	25	22·3	0·61	0·91	0·85	0·52 (0·37–0·66)	0·70
Tuberculoid granuloma	20	10·0	0·40	0·81	0·72	0·27 (0·15–0·39)	0·44
Different types of giant cells	14	13·7	0·34	0·21	0·75	0·20 (0·05–0·34)	0·49
Stellate granuloma	20	12·0	0·29	0·18	0·16	0·13 (0·05–0·21)	0·11
Extracellular matrix degeneration	43	22·7	0·39	0·34	0·37	0·06 (0·00–0·11)	0·27
Intuitive diagnosis	115	64·9	0·87	0·78	0·83	0·65 (0·54–0·75)	0·67

ATL, American tegumentary leishmaniasis; CI, confidence interval; P_{avg}, average prevalence; P_{pos} and P_{neg}, proportion of positive and negative agreement; PABAK, prevalence-adjusted bias-adjusted kappa; P_{total}, proportion of total agreement observed.

the observer's experience. The good performance of the intuitive diagnosis might be based on qualitative histopathological alterations or quantitative features that were not taken into account in this study. Compared with the low agreement regarding most histopathological markers, the high agreement observed in the intuitive diagnosis might indicate that the histopathological markers with the highest agreement combined with quantitative or qualitative nonanalysed features more decisively influence the intuitive diagnosis.

A clinical predictive rule for the diagnosis of ATL has already been suggested[37] and validated.[38] Anatomopathological scoring systems for the classification and prognosis of neoplasms are well known and have been used for several decades.[39,40] However, scores for differential diagnosis are not very frequent in the literature. Complex histopathological diagnostic issues, such as reflux esophagitis in children[41] and the differentiation between melanocytic naevi and malignant melanoma,[42] and between benign and malignant tumours of the adrenal gland[43] have already been addressed using scoring systems. We could not find histopathology-based predictive rules for the differential diagnosis among infectious diseases in the literature.

Using logistic multiple analysis, we observed that a given set of histopathological alterations was more accurate in discriminating between the two investigated diseases than any isolated alteration or intuitive diagnosis. The model based on the included variables allowed the proposal of a score to classify cases as a function of the presence or absence of these alterations. Other authors have used arbitrary values[42] or values based on bivariate analysis, such as prevalence ratios[43] to weight histological markers when developing scoring systems. In the present study, weighting histopathological markers by a multivariate analysis may have provided better diagnostic performance of the proposed score. This score has potential use in diagnostic practice. A likelihood ratio for positive test of 4·07 means that a positive result (i.e. a score higher than −1·2) is four times more frequent in ATL than in cases of sporotrichosis. Although a cut-off of −1·2 exhibited the highest accuracy, a higher or a lower cut-off may be stipulated to increase the sensitivity or specificity as a function of the clinical context.

Despite good diagnostic performance of the rule, the 'tuberculoid granuloma', 'stellate granuloma' and 'extracellular matrix degeneration' markers exhibited merely reasonable or discrete reliability even when PABAK was taken into account, which indicates that these results might not be reproducible. A more strict and detailed definition of the histopathological markers and training in the performance of the examination might increase their reliability and allow these markers to be used. Thus, one or more studies to validate this score and analyse the impact of its application are needed before this method can be adopted.

The proposed method of histopathological analysis has proved useful in the differential diagnosis between ATL and sporotrichosis. The possibility of making this distinction by simple and systematic histopathological analysis would represent a significant contribution in areas where both diseases occur (mostly in health-care facilities lacking sophisticated laboratory resources) and would establish histopathological examination as an important diagnostic tool that adds good performance to convenience and low cost. Although a precise diagnosis might not be possible in a fraction of cases, the combination of statistical analyses and subjective and qualitative analyses of histopathological data allows the quantification of uncertainty and might be useful in decision-making.[44]

We emphasize that this study did not include other granulomatous skin diseases. So, before applying the predictive rule, such diseases must be ruled out. Of special concern are those diseases in which no microorganisms are found on histopathological examination, such as sarcoidosis, tuberculosis and other mycobacterioses. Sarcoidosis usually presents characteristic naked granulomas and, we believe, can be ruled out based on granuloma morphology alone in most cases. To rule out tuberculosis and other mycobacterioses, clinical and epidemiological data are essential. In skin mycoses other than sporotrichosis (such as paracoccidioidomycosis and chromomycosis, for example), the aetiological agent, as a rule, is visible on histopathological examination. Thus, the diagnostic possibility of these mycoses should not affect the usefulness of the proposed predictive rule once they are considered and a search for these microorganisms in histological sections is carried out.

Finally, we stress that this study was performed on samples in which the microorganisms were not detected by standard histopathology despite positive detection of the infectious agents in cultures. Because parasite-rich cases may exhibit tissue responses different from those in which parasites are not present,[20,30,45] the histopathological score must be applied only after a negative careful search for the infectious agent.

What's already known about this topic?

- American tegumentary leishmaniasis (ATL) and sporotrichosis are important clinical and histopathological differential diagnoses.

What does this study add?

- Even when the aetiological agent is not seen in the histopathological examination, a set of histopathological changes can be useful in distinguishing ATL from sporotrichosis.

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Supporting Information

Additional Supporting Information may be found in the online version of the article:

Figure S1. Interstitial granuloma.

Figure S2. Different types of giant cells.

Figure S3. Plasmocyte aggregate.

Figure S4. Neutrophils in the epidermis.

Figure S5. Transepidermal neutrophil elimination.

Figure S6. Transepidermal phagocyte elimination.

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