Evaluation of the agreement between clinical and laboratorial exams in the diagnosis of leprosy

Avaliação da concordância entre exames clínicos e laboratoriais no diagnóstico da hanseníase

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ABSTRACT

This study examined the correlation between the clinical and laboratory diagnosis of leprosy, using biopsy results from laboratories "A" and "B" and the ML Flow test. Clinical and histopathological diagnoses presented 67.6% agreement. The laboratories showed 73.7% agreement in the bacterial index and laboratory 'B' detected 25.4% more positives. The highest agreement was in the *LL* form and lowest, in the *I* form. The highest diagnostic discrepancy was for the *BB* form. Clinical diagnosis agreement was 41.3% for laboratory 'A' and 54% for 'B'. The ML Flow test reclassified 10.7% of the patients. The spectrum of leprosy classification is important for a clearer understanding of the disease and its proper treatment, but is not used in health services, which use the simplified WHO criteria. This could be complemented by ML Flow testing. Such simplification is unacceptable for Leprosy Reference Centers regarding patient attendance, teaching and research, for which the standardization of the Ridley-Jopling classification is recommended.

Key-words: Leprosy. Bacterial index. ML-Flow. Clinical and laboratorial agreement.

RESUMO

Este estudo avaliou a concordância entre o diagnóstico clínico e o diagnóstico laboratorial da hanseníase, utilizando os resultados de biópsias dos laboratórios A e B e o teste ML-Flow. A concordância diagnóstica clínico-histopatológica foi de 67,6%. Os laboratórios apresentaram um índice de concordância de 73,7% em relação ao índice baciloscópico, e o laboratório B detectou 25,4% a mais de casos positivos. A maior concordância foi obtida para a forma V, e a menor para a forma I. A maior discrepância diagnóstica ocorreu para a forma DD. A concordância clínico-laboratorial foi de 41,3% para o laboratório A e 54% para o B. O teste ML-Flow reclassificou 10,7% dos pacientes. A classificação espectral é importante para o melhor entendimento da doença e para seu tratamento adequado, mas não é utilizada em centros de saúde, que adotam os critérios simplificados da OMS, que poderiam ser complementados pelo teste ML-Flow. Tal simplificação é inaceitável para os Centros de Referência em assistência, ensino e pesquisa em hanseníase, de modo que é recomendada a padronização pela classificação de Ridley-Jopling.

Palavras-chaves: Hanseníase. Índice baciloscópico. ML-Flow. Concordância clínico-laboratorial.

Leprosy is insidious[25], initially affecting the peripheral nervous system[23], with patients exhibiting contrasting clinical, immunological and pathological manifestations[17]. Since bioepidemiological aspects result in several clinical manifestations and complications[25], diagnosis to confirm the disease and its correct classification are required to ensure the proper treatment. However, it is extremely difficult to detect *Mycobacterium leprae* in an individual and various clinical and laboratorial criteria are used in the absence of an exam defined as a *gold standard*[2]. For treatment purposes, the World Health Organization (WHO) recommends an operational classification (OC), whereby patients are divided into paucibacillar (PB) when they present 5 cutaneous lesions or less, or multibacillar (MB) when they have more than 5 lesions[28]. However, when bacilloscopic examination is available, patients whose skin-smear exam tests positive are classified as MB regardless of the number of lesions. For an improved operational classification, some studies have used the *Mycobacterium leprae* lateral flow test (ML Flow), which correlates the concentration of anti-PGL1 (specific antibody against *Mycobacterium leprae*) in the patient's peripheral blood with the bacillary load[6]. Serum-positive patients are classified as MB and serum-negative as PB[4].

The basic criteria in the Ridley and Jopling[32] classification are the bacillary load measured by bacilloscopic exams (cutaneous biopsy and skin smear) and the cell-mediated immune response time, which is evaluated by the result of Mitsuda's intradermal test. Based on these immunopathological criteria, patients are

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divided into 6 clinical categories: indeterminate (I), tuberculoid (TT), borderline-tuberculoid (BT), borderline-borderline (BB), borderline-lepromatous (BL) and lepromatous (LL)[32]. The value of this classification is not only historical; continued application of this system is essential regarding the efforts to improve current understand of the disease and develop a strategy to combat it and, ideally, to prevent it[35].

Although classifications are important to more clearly understand the disease, they are often not standardized in health services[38], where the majority apply the simplified WHO classification.

Moreover, due to the potential neural damage and consequent disabilities and the stigma of leprosy for humans, the correct histopathological diagnosis is mandatory to assist the doctor regarding the spectral form of the patient's disease and its prognosis, favoring a therapeutic outcome during follow-up[7]. Although the prevalence of leprosy has declined worldwide, the number of new cases diagnosed annually remains stable. This paradox raises new, important, and interesting questions that will require the application of the best scientific methods available to answer them[35].

At the end of 2005, Brazil presented a detection rate of 2.23/10,000 inhabitants and a prevalence of 1.59/10,000 inhabitants, with 91% of the prevalence of leprosy cases on the American continents, thus constituting a public health problem with a prevalence coefficient of over 1 case per 10 thousand inhabitants[39].

Confirmation of the leprosy diagnosis to determine the disease load in a given population and the correct clinical classification to determine the risk of patients developing incapacities are important motives for performing the histopathological exam. The pathologist is expected to provide a definitive diagnosis; however, this exam has certain limitations, since samples do not always indicate the presence of the bacillus in patients presenting characteristic symptomatology, leading to controversies regarding the efficacy of microscopy for the identification of bacillus in smears and biopsies[18].

Studies have shown that biopsies extracted from opposite edges of the same skin lesion, or even from different lesions, do not present significant morphological discrepancies, since the individual's bacillary load and their immunological reactivity are determined systemically[9]. However, there are frequent reports of interobserver variations, proving the need for studies to evaluate these and to present suggestions to minimize them[15]. Studies that evaluate the clinical and laboratorial agreement in reference services of patient attendance, teaching and research on leprosy could underpin a proposal for more refined criteria for its diagnosis, particularly since the scientific literature contains reports of significant discrepancies of varying magnitude[22 38].

The standardization of diagnostic criteria is important for the production of knowledge concerning resistance and susceptibility to the disease and its clinical forms, as well as for high therapeutic and relapse monitoring, leading to improvement in its control and favoring the elimination of leprosy as a public health problem.

The purpose of this study was to evaluate the agreement between clinical and laboratory exams in the diagnosis of leprosy at a National Reference Center for Sanitary Dermatology and Leprosy accredited by Brazil's Ministry of Health, providing data to underpin public health policies, standardizing diagnostic resources and optimizing protocols for Reference Centers in the control of this disease.

MATERIAL AND METHODS

Cases

The study was based on an analysis of medical records covering data on clinical and laboratory exams for the diagnosis of patients with leprosy attended over the last 5 years at the National Reference Center for Sanitary Dermatology and Leprosy (CREDESH), Clinical Hospital, Faculty of Medicine, Federal University of Uberlândia (UFU), MG, Brazil.

The patients examined by the leprologist in the first consultation received an initial Operational Classification (OC) according to the number of cutaneous lesions[40] that presented a diagnosis of the clinical form, following the Ridley and Jopling criteria[32]. For classification of the clinical forms of the disease, the patients were submitted to skin-smear exams on 8 sites (right and left earlobes, right and left elbows, right and left knees and 2 cutaneous lesions) to obtain the bacterial index (BI), and to Mitsuda's intradermal test to evaluate their cell-mediated immune response. Biopsies of cutaneous lesions were also extracted for histopathological analysis by hematoxylin-eosin (HE) staining and to obtain the bacterial index of the biopsy by Ziehl-Neelsen staining[3], which was performed by two reference laboratories associated with the CREDESH/UFU, and which are referred to herein as Laboratory 'A' and Laboratory 'B'.

Pursuant to the results of the exams, the patients were given a final classification of the clinical form, based on the Ridley and Jopling criteria, of I, TT, BT, BB, BL or LL[32].

For treatment purposes, the OC based on the WHO criteria (OC-WHO) was conferred, considering the number of lesions and the skin-smear BI.

For the final operational classification (OC-final), the results of the skin-smear BI and ML-Flow serum test were adopted, as follows: PB, patients with a negative BI and ML Flow; MB, patients with a positive BI and/or positive ML Flow, with the BI being decisive for this classification.

Statistical methods

The agreement between the clinical and laboratory diagnoses was calculated by dividing the number of congruent cases by the total number of patients. The Kappa test was applied to evaluate the agreement results. The Kappa values and their interpretations varied as follows: <0 (no agreement), 0-0.19 (poor agreement), 0.20-0.39 (fair agreement), 0.40-0.59 (moderate agreement), 0.60-0.79 (substantial agreement), 0.80-1.00 (almost perfect agreement)[24].

RESULTS

Studies conducted by other researchers in various countries have shown agreement between the clinical diagnosis of leprosy and the histopathological classification based on the Ridley and Jopling[32] criteria, which vary from 29.7% to 89%, as shown in **Table 1**[2 13 20 22 23 27 36 37]. The present work found an overall agreement of 67.7%, which is similar to that found in previous works[2 20].

It is important to emphasize that, in the present study, a different number (N) of patients were involved in each set of parameters analyzed.

Regarding the agreement between the OC based on the number of cutaneous lesions, the skin-smear BI, and the serum ML Flow test result, 4.1% (7/173) of the patients with ≤ 5 skin lesions presented a positive skin-smear BI and 8.1% (14/173) tested serum-positive for the ML Flow test and were therefore reclassified as MB (**Table 2**).

Among the patients with more than 5 skin lesions who were given an OC of MB, 9.8% (17/173) presented a negative ML Flow and were reclassified as PB. However, among those with more than 5 lesions and classified as MB, 71.2% (74/104) presented a positive BI with moderate agreement (Kappa = 0.5777; P < 0.001) and 83.7% (87/104) showed a positive ML Flow with substantial agreement (Kappa = 0.6290; P < 0.001), which was congruent with this OC (**Table 2**).

Although the overall agreement between the OC-WHO (number of skin lesions + skin-smear BI) and the OC-final (WHO + ML Flow) was 89.3%, which is considered almost perfect agreement (209/234; Kappa = 0.7521 / P < 0.001), 6.0% of the PB (14/234) and 4.7% (11/234) of the MB patients were reclassified as MB and PB, respectively, by the OC-final, which also considered the result of the ML Flow test. Thus, the ML Flow test alone, reclassified 10.7% (25/234) of the patients (**Table 3**).

TABLE 1

Comparison of the indices of concordance (%) of the clinical diagnosis and the histopathological diagnosis of leprosy obtained in the present study and in previous studies conducted by other researchers, (CREDESH-HC/UFU, 2008).

	Researchers									
Clinical Form	Sehgal VN et al (1977)	Dubey GK et al (1981)	Jerath VE, Desai SR (1982)	McDougall AC (1987)	Bathia AS et al (1993)	Kumar SK et al (1996)	Singh PA et al (2000)	Kalla G et al (2000)	Vargas-Ocampo R (2004)	Teixeira et al. (present study) (2008)
I	—	—	88.8	0.0	36.0	77.8	60.0	—	19.9	33.3
TT	30.0	76.9	74.5	30.9	50.0	7.2	52.9	76.7	27.0	75.0
BT	26.3	100.0	64.7	68.4	77.0	57.7	66.7	44.2	—	77.2
BB	66.7	71.7	53.8	16.7	26.0	—	30.8	37.0	—	68.6
BL	42.9	100.0	28.5	37.5	26.0	—	30.8	43.7	—	58.8
LL	66.7	93.6	61.5	100.0	91.0	—	90.0	75.6	63.9	92.5
TT + BT	49.4	96.6	—	55.8	80.0	60.0	83.0	—	—	93.5
BT + BB+ BL	59.4	100.0	—	59.5	80.0	75.4	83.0	—	52.4	89.0
BL + LL	59.4	100.0	—	50.0	93.0	—	65.4	—	—	91.2
General	29.7	89.0	68.5	40.4	69.0	51.7	58.6	64.6	42.9	67.6

I: indeterminate, TT: tuberculoid, BT: borderline-tuberculoid, BB: borderline-borderline, BL: borderline-lepromatous, LL: lepromatous.

TABLE 2

Concordance between the initial operational classification based on the number of cutaneous lesions of leprosy patients indicated by the skin-smear bacterial index and the result of the ML-Flow test, (CREDESH - HC/UFU, 2008).

OC	BI nº (%)			ML-Flow nº (%)		
Lesions nº	negative	positive	total	negative	positive	total
PB	62	7	69	55	14	69
≤ 5	(35.8)	(4.1)	(39.9)	(31.8)	(8.1)	(39.9)
	30	74	104	17	87	104
MB	(17.3)	(42.8)	(60.1)	(9.8)	(50.3)	(60.1)
> 5						
Total	92	81	136/173	72	101	142/173
	(53.2)	(46.8)	(78.6)	(41.6)	(58.4)	(82.1)

Kappa MIB = 0.5777 / P < 0.001 / Z = 7.8745

Kappa ML-Flow = 0.6290 / P < 0.001 / Z = 8.2790

OC: operational classification, BI: bacterial index, MB: multibacillary, PB: paucibacillary.

The overall agreement between the OC-WHO, considering the number of skin lesions and the skin-smear BI, and the OC-final of the clinical form according to Ridley and Jopling[32], for 226 patients was 89.8% (203/226). The lowest agreement was obtained for patients presenting the clinical form BT, with a variation of 72.5% (29/40) for BT MB to 73.3% (22/30) for BT PB (**Table 4**).

As for the biopsy BI from the histopathological exams, an overall agreement of 73.7% (171/232) was obtained between Laboratories 'A' and 'B', with Laboratory 'B' confirming the presence of the bacillus in the biopsies of skin lesions of 25.4% (59/232) of the cases that Laboratory 'A' did not observe, presenting a moderate agreement (Kappa = 0.5040; P < 0.001) (**Table 5**).

The histopathological diagnoses of the two Laboratories were congruent in 50.9% (89/175) of cases, most of them involving the LL form (92.3% - 36/39) and the fewest involving the I form (28.1% - 9/32). Laboratory 'A' diagnosed 18.3% (32/175) of the patients as I, which was not the case with Laboratory 'B', which classified half of these patients (71.9% - 23/32) as TT and borderline. Laboratory 'B' diagnosed 5.7% (10/175) of the cases as I (**Table 6**).

Laboratory 'B' also diagnosed a larger number of patients as TT compared to 'A', which in turn classified 58.7% (27/46) of the patients as TT/BT (24/46 - 52.2%) or *BT* (3/46 - 6.5%) (**Table 6**).

Another difference observed was in the diagnosis of the clinical form BB, which Laboratory 'B' diagnosed in 14.3% (25/175) of the cases, while Laboratory 'A' diagnosed 3.4% (6/175) (**Table 6**).

Among the 150 patients with leprosy submitted to histopathological exams, 41.3% (62/150) of the cases in Laboratory 'A' and 54% (81//150) in Laboratory 'B' showed complete agreement between the clinical diagnosis (clinical exam + skin-smear bacilloscopy + Mitsuda's test) and the histopathological diagnosis. Among the patients classified clinically as *BT*, 31.11% (14/45) and 24.44% (11/45) were classified, respectively, as TT/BT and *I* by Laboratory 'A', while among the BB patients, 36% (9/25) were classified as BL and 28% (7/25) as I. The fact that 37.8% (17/45) of the patients classified clinically as BT were classified as TT by Laboratory 'B' explains the low agreement for this form at this laboratory (**Table 7**).

TABLE 3

Operational classification of the WHO (number of skin lesions and skin-smear bacterial index) *versus* the final operational classification (WHO associated with the ML-Flow test), (CREDESH - HC/UFU, 2008).

	OC-final		
	PB	MB	Total
OC-WHO	nº (%)	nº (%)	nº (%)
	61	14	75
PB	(26.1)	(6.0)	(32.1)
	11	148	159
MB	(4.7)	(63.2)	(67.9)
	72	162	209/234
Total	(30.8)	(69.2)	(89.3)

Kappa = 0.7521 / P < 0.001 / Z = 11.5100

MB: multibacillary, PB: paucibacillary, OC: operational classification.

TABLE 4

Concordance between the WHO operational classification (number of skin lesions + skin-smear bacterial index), the final operational classification (WHO + ML-Flow), and the final clinical form by Ridley and Jopling's classification for leprosy patients, (CREDESH - HC/UFU, 2008).

		OC WHO		Agreement	
OC Final	Clinical form	PB	MB	nº (%)	Total
	I	1	—	1/1 (100.0)	1
PB	T	36	3	36/39 (92.3)	39
	DT	22	8	22/30 (73.3)	30
	DT	11	29	29/40 (72.5)	40
MB	DD	1	40	40/41 (97.6)	41
	DV	—	26	26/26 (100.0)	26
	V	—	49	49/49 (100.0)	49
Total		71	155	203/226 (89.8)	226

OC: operational classification, MB: multibacillary, PB: paucibacillary.

TABLE 5

Concordance between the BI of skin lesion biopsies obtained by Laboratory 'A' and the biopsy bacterial index obtained by Laboratory 'B', (CREDESH - HC/UFU, 2008).

	BI Lab 'B'		
	negative	positive	Total
BI Lab 'A'	nº(%)	nº(%)	nº(%)
Negative	84	59	143
	(36.2)	(25.4)	(61.6)
	2	87	89
Positive	(0.9)	(37.5)	(38.4)
	86	146	171/232
Total	(37.1)	(62.9)	(73.7)

Kappa = 0.5040 / P < 0.001 / Z = 8.663.

BI: bacterial index.

TABLE 6

Agreement between the histopathological diagnoses of Laboratories 'A' and 'B' (interobservers), according to Ridley and Jopling's classification for leprosy patients, (CREDESH - HC/UFU, 2008).

	Laboratory 'B'									
Laboratory 'A'	I	TT	TT/BT	BT	BB	BL	LL	Inconclusive	Agreement nº(%)	Total (nº)
I	9	5	3	4	9	1	1	—	9/32 32 (28.1)	
TT	1	14	1	2	1	—	—	—	14/19 (73.7)	19
TT/BT	—	24	3	4	1	—	—	—	3/32 (9.4)	32
BT	—	3	1	10	—	2	—	—	10/16 (62.5)	16
BB	—	—	—	1	4	—	1	—	4/6 6 (66.7)	
BL	—	—	—	2	8	12	7	—	12/29 (41.4)	29
LL	—	—	—	1	1	1	36	—	36/39 (92.3)	39
Inconclusive	—	—	—	—	1	—	—	1	1/2 (50.0)	2
Total	10	46	8	24	25	16	45	1	89/175 (50.9)	175

I: indeterminate, TT: tuberculoid, BT: borderline-tuberculoid, BB: borderline-borderline, BL: borderline-lepromatous, LL: lepromatous.

TABLE 7

Agreement between the clinical diagnosis and the histopathological diagnosis of Laboratories 'A' and 'B', according to Ridley and Jopling's classification, for leprosy patients (CREDESH - HC/UFU, 2008).

Clinical	Laboratory 'A'									Laboratory 'B'								
Diagnosis	I	TT	TT/BT	BT	BB	BL	LL	Inc	Agreement nº(%)	I	TT	TT/BT	BT	BB	BL	LL	Inc	Agreement[a] nº(%)
I	4	—	—	—	—	1	—	—	4/5 (80.0)	2	2	—	—	—	1	—	—	2/5 (40.0)
T	5	10	11	1	—	—	—	—	10/27 (37.0)	4	20	1	1	1	—	—	—	20/27 (74.0)
BT	11	7	14	10	—	3	—	—	10/45 (22.2)	4	17	2	14	7	1	—	—	14/45 (31.1)
BB	7	—	1	2	3	9	2	1	3/25 (12.0)	—	1	2	4	9	4	4	1	9/25 (36.0)
DL	1	—	—	1	1	5	6	1	5/15 (33.3)	—	—	—	1	1	7	6	—	7/15 (46.6)
LL	—	—	—	—	—	3	30	—	30/33 (90.9)	—	—	—	1	2	1	29	—	29/33 (87.9)
Total	28	17	26	14	4	21	38	2	62/150 (41.3)	10	40	5	21	20	14	39	1	81/150 (54)

Inc: inconclusive, I: indeterminate, TT: tuberculoid, BT: borderline-tuberculoid, BB: borderline-borderline, BL: borderline-lepromatous, LL: lepromatous.

DISCUSSION

The limitations of using a purely clinical system in the classification of leprosy patients without considering laboratory exams may lead to inadequate measures in the patient's treatment, resulting in some patients taking toxic drugs unnecessarily and others being administered an inefficient treatment, exposing the community to a source of infection and maintaining the transmission of the disease[10 11].

The proper classification of leprosy cases is an indispensable tool for understanding the disease and its evolution in patients. The constant difficulties and controversies surrounding its diagnosis

highlight the importance of seeking more precise criteria for this procedure. However, there is a lack of studies describing the resources that should be used correctly in health services[30].

Correlation between clinical and histopathological classification has been the focus of permanent studies over the last few years[8]. A previous report demonstrated that it is necessary to perform biopsies in all leprosy cases and to correlate biopsy results with those of the clinical diagnoses in order to improve patient classification and prognosis[13].

There is a visible standardization in studies that evaluate clinical and laboratorial agreement in various countries, presenting higher indices of agreement for the polar spectral forms (TT and LL) and lower indices for the interpolar forms (borderline). However, the results of these studies have not been uniform and some of them present correlations based on of very small patient samples[2].

The clinical and laboratorial agreement of 67.6% obtained in the present study indicates the importance of a more elaborate clinical diagnosis, since this result was obtained at a National Reference Center for leprosy and this strategy should be the main tool for the detection and classification of the disease[38]. Due to the wide spectrum of clinical manifestations of leprosy[14 17], the use of histopathological and immunological criteria could increase both the sensitivity and the specificity of the procedures involved in the diagnosis and in the correct allocation of the patient in differentiated treatment schemes[9 27], and is a requirement that could be established by Reference Centers for research, attendance and teaching concerning this disease[35].

Regarding the operational classification according to the number of lesions, skin-smear bacilloscopy aided the diagnosis in about 5% of cases, indicating the importance of this exam, which the WHO considers the *golden standard* for a more correct classification[10]. The serum ML Flow test reclassified as PB approximately 10% of the patients considered MB based on the number of lesions. Patients classified as MB, according to the WHO criteria, should present a positive ML Flow due to the presence of a large quantity of anti-PGL1 antibodies, a specific antigen of *Mycobacterium leprae* in the peripheral blood[19]. In the present work, the level of agreement between the WHO and the final operational classification was substantial agreement, with a Kappa score of 0.75 ($P<0.001$). The degree of disagreement of approximately 10% indicated the importance of laboratory exams for the diagnosis and classification of leprosy, and in this case, principally due to the bacterial index and ML Flow. Several studies have demonstrated that the presence of specific antibodies against *Mycobacterium leprae*, detected by ML Flow, is directly proportional to the patient's bacterial load. PB patients are serum-negative, while MB patients are serum-positive[5 12 34].

Agreement between the final clinical form defined for the patients according to Ridley and Jopling[32] and the operational classification according to the WHO criteria showed a high value (above 80%) when compared with the result observed in a previous study[37]. The BT patients showed a lower agreement than that observed for the other forms, probably because this clinical form can be treated as PB or MB, and this classification was defined principally by the positivity of the BI and the ML Flow. A lower agreement was also obtained for the PB forms, as described by other researchers[10], which might result in many of these individuals receiving treatment as MB.

This study revealed important differences in the results of the bacterial index exams of skin biopsies carried out by the two laboratories. The positivity index of Laboratory 'A' (38.4%) was lower than the results reported in the literature[18], which showed a value of 52.1% and which, in turn, was lower than the index obtained by Laboratory 'B' (62.9%).

Although complete agreement between the two laboratories would be practically impossible, since certain forms present too few bacilli to be detected under a microscope [31], the notably low detection by Laboratory 'A' may indicate possible deficiencies in the procedures for obtaining cuts by Ziehl Neelsen staining[14] and/or in their analysis. Other problems observed by pathologists involved in the diagnosis of the disease include the subjectivity inherent to the method, the training they undergo, their experience, and the particular context of each investigation[15].

Therefore, quality control of all the aspects of laboratory methods is essential for the effective implementation of programs to control this disease[1].

In the analysis of interobserver agreement, analysis of the results revealed a greater number of TT/BT cases diagnosed by Laboratory 'A', the majority of which were classified as TT by Laboratory 'B', significantly reducing agreement for this form. The large number of dubious cases could indicate a lack of standardization concerning the number of histological sections that should be made and/or in slide staining and analysis, as reported in an earlier work[33].

The large number of patients diagnosed as *I* by Laboratory 'A' might be explained by the fact that difficulties occurred in observing poorly developed granulomas, with few specific cells of an epitheloid and/or spumous aspect in the histological sections of patients in transition from group I to the clinical forms established in the spectrum of the disease[16 21 33].

On the other hand, Laboratory 'B' diagnosed a relevant number of patients as TT who were actually BT, underestimating the higher potential for neural damage and risk for developing permanent disabilities, as well as the progression of this form in the spectrum of the disease[7 21 29 33].

The association between the clinical diagnosis and the results of the histopathological exams of Laboratories 'A' and 'B' showed a higher agreement at the latter. Laboratory 'A' showed a significantly lower agreement, which was close to 45% and below that observed in previous reports[13].

At Laboratory 'A', low agreement was obtained for the forms of the TT pole and the borderline group, with the lowest agreement occurring for the BT and BB forms. Due to the difficulty that pathologists face in differentiating the TT from the BT forms, since they present similar granulomatous reactions[16], this Laboratory classified a large part of the pole TT patients as TT/BT (dubious cases), which considerably decreased the agreement for these clinical forms of the disease. Other researchers also obtained low

agreement for the BT form[23]. It is worth noting the large number of I patients diagnosed by Laboratory 'A', which considerably lowered the agreement for the other forms. The validation of histological diagnoses is controversial, since the criteria adopted for their classification are not standardized among pathologists[26].

At Laboratory 'B', the indices of agreement were higher than those of Laboratory 'A', showing values in agreement with other researchers[13] and disagreeing only with respect to the TT (agreement at Laboratory 'B' was higher) and BT forms (agreement was lower). Once again, it is worth emphasizing the difficulty of histopathologically differentiating the TT pole forms and the fact that most of the BT forms were classified as TT by this Laboratory.

In both laboratories, the greatest contribution to the overall agreement was conditioned by the LL form, due to the singular aspect of this form in both the clinical and the histopathological diagnoses[16 29]. Nevertheless, low indices of agreement were obtained for the borderline forms, in line with the findings of other researchers[2 13 37]. Since most of manifestations of leprosy evolve slowly and progressively, patients classified as *BB* develop an intermediary aspect between the BT and BL forms.

In this work, the exams and their classifications presented differences in relation to their efficacy, when analyzed comparatively, which implies the need for critical analysis, using as reference the objectives of the control programs and the reality of the different endemic areas and the standardization of classification adopted in the international literature.

The Immunopathology Committee of the 10th International Leprosy Congress, held in Bergen (1973), recommended the use of the Ridley and Jopling[32] classification, to establish a general nomenclature that would result in uniform diagnostic criteria and to standardize scientific research in several countries[8]. It also reported that the generalized use of this classification requires human and infrastructural resources that do not always exist in developing countries, but that the creation of reference laboratories that can meet the needs of different regions should be an important goal in the study and control of this disease.

Even though classifications are important to improve understanding of the disease, they are often not standardized in health services[38], where the majority have adopted the simplified criteria proposed by the WHO, dividing patients into only 2 groups, PB and MB. However, such over-simplification of this complex host-pathogen relationship is unfortunate and unacceptable for reference centers for patient attendance, teaching and research on leprosy[35]. As an educator and producer of knowledge, the University should take on the role of a reference for the diagnosis and control of leprosy.

Keep in mind that the basis for understanding leprosy is the recognition that the LL form differs from the BL form, and the BT from the TT form clinically, histologically and immunologically. This classification system recognizes the natural diversity of the immune response in leprosy that has challenged immunology for almost half a century. A more thorough understanding concerning the basis of this diversity and the mechanisms involved will be required before the disease can be eradicated[35].

The present study demonstrates that clinical and laboratorial discrepancies in the diagnosis and classification of leprosy should be investigated carefully. The spectrum classification is important for clearer understanding of the disease and its proper treatment, but it is not used in health services, which use the simplified WHO criteria. The appropriate clinical classification may be complemented by ML Flow testing, which reclassified 10.7% of the patients and discriminated the risk of incapacities. Such simplification is unacceptable for Leprosy Reference Centers in patient attendance, teaching and research, for which the standardization of the Ridley-Jopling classification is recommended.

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