

The use of serology as an additional tool to support diagnosis of difficult multibacillary leprosy cases: lessons from clinical care

O uso da sorologia como ferramenta adicional no apoio ao diagnóstico de casos difíceis de hanseníase multibacilar: lições de uma unidade de referência

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ABSTRACT

Seven multibacillary leprosy and two suspected cases assisted in different situations during clinical care activities at the University in Rio de Janeiro City are described. All cases presented some difficulties for diagnosis, since they evolved with few or no cardinal signs or symptoms of leprosy. A serological test used as an auxiliary tool was helpful in the diagnosis or exclusion procedure of each case, facilitating academic discussions at the time of case examination. Considering serology and bacilloscopy (skin smear) as the only rapid and relatively cheap available tests for confirmation of atypical MB leprosy, the advantages and disadvantages of their use were discussed. Both tests support the diagnostic procedure and the classification of cases for treatment purposes. The advantage of bacilloscopy is its capacity for diagnosis confirmation. The advantages of serology are: (a) its applicability for direct use by health workers, providing immediate results; (b) the potential for patient participation in the process; and (c) it provides a learning opportunity, allowing for improved teaching of leprosy pathogenesis.

Key-words: PGL-I serology. Leprosy. Multibacillary leprosy. Transmission. Early diagnosis.

RESUMO

Sete casos de hanseníase multibacilar (MB) e dois casos com suspeição de hanseníase atendidos em situações distintas do atendimento clínico-dermatológico na Universidade Federal do Rio de Janeiro são descritos. Todos apresentaram dificuldades no diagnóstico visto que não tinham sinais e sintomas cardinais da hanseníase. Um teste sorológico utilizado como ferramenta auxiliar foi útil no processo de diagnóstico ou exclusão de cada caso e facilitou as discussões acadêmicas na hora do exame clínico. A sorologia e baciloscopia de linfa são consideradas como os únicos instrumentos rápidos e de baixo custo para a confirmação de casos MB atípicos, e as vantagens e desvantagens de cada exame são discutidas. Ambos os testes complementam o processo diagnóstico e classificação dos casos para fins terapêuticos. A vantagem da baciloscopia está na sua capacidade de confirmação do diagnóstico. As vantagens da sorologia são: (a) sua aplicabilidade para uso direto por profissionais de saúde no momento da consulta, visto que os resultados são imediatos, (b) a possibilidade da participação dos pacientes no processo, e (c) oferece uma oportunidade para melhor ensino da patogênese da hanseníase.

Palavras-chaves: PGL-I sorologia. Hanseníase. Hanseníase multibacilar. Transmissão. Diagnóstico precoce.

The case detection rate of leprosy in Brazil has remained high and generally stable in recent years and has been the source of scientific debates and research studies¹. Among all the factors that contribute to this, the long incubation period of *Mycobacterium leprae* and the existence of a large pool of unidentified infected individuals not on multidrug therapy (MDT) are two important ones². Another possibility is the silent transmission by individuals with asymptomatic disease or infection³ who present a high bacterial load.

Several studies have shown that the presence of IgM antibodies to the *Mycobacterium leprae*-specific phenolic glycolipid-I

(PGL-I) correlates with the bacterial load of a leprosy patient: 15-40% of paucibacillary (PB) patients are seropositive, compared to 80-100% of multibacillary (MB) patients. While detection of these antibodies cannot be used for diagnosis, they are a useful tool for confirming the diagnosis of MB disease.

Serology is highly sensitive for detecting the presence of antibodies to *Mycobacterium leprae* in the bloodstream, rather than in the lesions from a specific part of the body, as determined by acid fast bacilli (AFB) in slit skin smears. The advantage of bacilloscopy is its capacity to confirm diagnosis. However, the operational drawbacks are that it is a time-consuming, invasive exam composed of several steps that increase the possibility of error. Skin biopsy is performed in many cases and its reliability and the resulting histopathological information often help classify the form of the disease. This probably contributes to the excessive demand of this exam observed in Brazil⁴, but it is rarely available in remote areas.

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In our University Hospital (UH), the leprosy team assists out-patients referred by basic health units (BHU), as well as in-patients in dermatology, rheumatology, infectious diseases and general medicine. Additionally, university extension activities are promoted, including active case finding campaigns. They involve local health workers (HW) and the interaction of dermatology residents in communities where leprosy is prevalent. All of the cases presented were diagnosed at the UH and complementary exams were performed at its laboratory.

During these activities a serological test was used, the ML Flow test, which detects IgM antibodies to PGL-I. The test is stable at 28°C for 3 years and can be performed by health workers at any level. The test was performed using 5µl of whole blood obtained by finger pricking and results were read after 5 minutes. The color intensity of the test line can be read as negative, or ranging from 1+ to 4+⁵. The fact that each step of the test can be followed by the patient and trainees benefits the teaching regarding the pathogenesis of the disease and provides an opportunity for learning.

Through the use of case report lessons⁶, our group aimed to (a) highlight the potential benefit of the ML Flow test as an auxiliary tool in diagnosing difficult MB leprosy cases (lepromatous (LL) and borderline-lepromatous (BL)), and (b) demonstrate that a problem still exists concerning MB leprosy diagnosis when clinical signs and symptoms are absent or are not easily recognized in general health services.

CASES REPORTS AND DISCUSSION

The detection of AFB in skin smears confirms active disease, while PGL-I serological tests detect antibodies against *Mycobacterium leprae*; this indicates infection that might never evolve to disease. Nevertheless, serology is useful for classifying patients as PB or MB in field conditions⁷ and as a tool to assist in diagnosis, as illustrated in the following cases. The test was used for (1) helping identify differential diagnoses in cases 1, 2 and 3; (2) reinforcing clinical suspicion of MB asymptomatic cases in cases 6, 8, and 9, and (3) confirming the suspicion of active leprosy in cases 4, 5 and 7. The objective was to discuss the cases and report how the test was used. **Table 1** summarizes the patients' histories and only relevant aspects of clinical features are described.

Case 1. The patient was referred to the UH to investigate recurrent erythema nodosum on the trunk and limbs lasting for 12 years. At the time of examination, he presented only erythematous and painful nodules spread over the entire body (**Figure 1**). The ML Flow test was performed and its negative result triggered further investigation for other infectious diseases as a possible explanation for the clinical symptoms. The patient was positive for the hepatitis C virus (HCV), which is also a cause of erythema nodosum. The histopathological exam showed no features of leprosy (**Figure 2**); therefore, leprosy was excluded.

Case 2. Admitted to the infectious disease department of the UH, this patient presented with fever and enlargement of the lymph nodes (cervical/inguinal), liver and spleen. AIDS was suspected and tuberculosis (TB) was diagnosed due to an AFB-positive sputum result. Soon thereafter, anti-TB treatment was initiated (rifampicin, isoniazid and pyrazinamide) but his symptoms



FIGURE 1

Generalized Erythema Nodosum Leprosom.

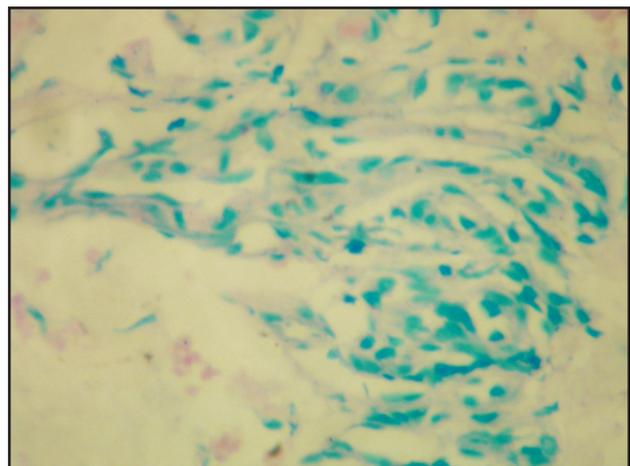


FIGURE 2

Wade 1000x: No acid fast bacilli in the perianexial infiltrate.

worsened with a reactional episode, despite the fact that all three sputum cultures were negative for *Mycobacterium Tuberculosis*. Therefore, confusion between the two diagnoses occurred due to the possibility of *Mycobacterium leprae* present in the upper respiratory tract. The positive ML Flow test performed during the dermatological consultation induced further examination of material from lymph node biopsy and slit skin smears. The skin infiltration observed in the face was doubtful and fibular nerve was painful upon palpation. No other signs or symptoms were observed. The biopsy was stained according to Wade and LL was confirmed (**Figure 3**). Multidrug therapy (MB) was initiated, combined with 200mg thalidomide for two months. After the regular 12 doses of MDT the patient was released from treatment. Leprosy and tuberculosis are both endemic in Brazil and, in most cases, show distinct clinical courses; however, differential diagnosis between these diseases can be challenging, especially in early lepomatous leprosy cases.

TABLE 1
Summary of the clinical symptoms and tests results of the cases.

Case	Sex Age	Contact status	Relevant aspects of patient's history	Clinical features	Sensitivity testing	ML Flow	BI skin smears	Histopathology	Other tests	Diagnosis
1	Male 40	no	recurrent erythema nodosum lasting for 12 years	only nodules disseminated to entire body	normal	Neg	Neg	erythema nodosum without AFB, not compatible with leprosy	Pos HCV; neg for all other lab screening	Hepatitis C
2	Male 23	no	6 kg weight loss in 4 months	fever; lymph node (cervical/inguinal); liver and spleen enlargement; suspected AIDS; initially diagnosed as TB	normal but the fibular nerve was painful on palpation	Pos 3 +	AFB Pos in sputum	granulomatous infiltration in inguinal lymph node; presence of AFB; LL	3 Neg TB cultures Neg HIV	LL
3	Male 26	no	initially diagnosed as drug allergy was treated with corticosteroids; weight loss (8 kg), 3 "flu episodes" (nasal obstruction) plus malaise in the last 6 months	few red nodules in the legs; suspicion of nodular vasculitis; in return visit after 20 days no longer showed skin lesions or symptoms	normal	Pos 3 +	Neg	presence of AFB in lymph nodes biopsy; LL	Neg HIV	ENL LL
4	Male 42	no	unspecific and diffuse lesions	thickened skin in the shoulders and right arm presenting paraesthetic sensation	normal	Pos 3 +	Pos	note done	NR	BL
5	Male 38	PB daughter 2 years before	treated and cured of leprosy when he was 12 years old (RMP 90 days plus DDS daily for 2 years)	plaque enlarging on his abdomen; light infiltration	loss sensation in the centre of lesion	Pos 4 +	Pos	presence of AFB; borderline lepromatous	NR	MB relapse
6	Male 55	2 PB nephews <15	acrocyanosis (smoking 3 packs cigarettes daily), detected during active contact examination, potential silent leprosy	neither skin lesions nor nerve enlargement; acrocyanosis and reticularis livedo	normal	Pos 3 +	Pos	presence of AFB; borderline lepromatous	NR	BL
7	Female 38	daughter LL	LL cases detected during active contact examination, typical natural leprosy evolution during 16 years	reported recurrent ulcers and being under treatment of varicose ulcers for almost a year; had painful nodules suggestive of erythema nodosum in both legs; especially during her 4 pregnancies.	discrete loss sensation in both plantar regions (tibial posterior nerve region)	Pos 4 +	Pos	erythema nodosum leprosum; presence of AFB	NR	LL
8	Male 11	case 7 plus 2 LL cases	family cluster (3 cases), detected during active contact examination	no typical leprosy lesions; nasal obstruction; moderate acrocyanosis; livedo in both legs and a doubtful skin infiltration of earlobes and face	normal	Pos 4 +	Pos	discrete lymphocyte and histiocytes infiltration; presence of AFB	NR	LL
9	Female 70	3 LL (father and brothers)	In 2003 she was examined and was negative in both dermatological/neurological inspection and serological test	acrocyanosis, reticularis livedo and one erythematous plaque in her left thigh	doubtful loss of sensation	Pos 4 +	Neg	granuloma annulare elastolytic features; absence of AFB	NR	granuloma annulare + leprosy?

NR: noting remarkable; LL: lepromatous; BL: borderline lepromatous; ENL LL: erythema nodosum leprosum lepromatous; AFB: acid fast bacilli; BI: bacillary index. HCV: hepatitis C virus; HIV: human immunodeficiency virus. TB: tuberculosis. Neg: negative, Pos: positive.

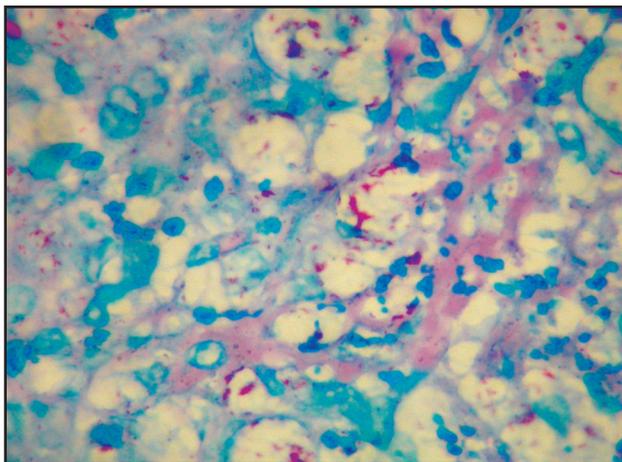


FIGURE 3

Wade 1000x: Granulomatous Infiltration lymphnode, presence of acid fast bacilli.

Case 3. The patient was initially diagnosed with a drug allergy and treated with corticosteroids. After three episodes, the patient was sent to the skin disease out-patient clinic of the UH. He reported substantial weight loss (8kg), 3 flu-like episodes (nasal obstruction) and malaise in the preceding 6 months. Only a few red nodules on the legs were observed. Biopsy of a nodule was performed under suspicion of nodular vasculitis, tuberculosis, and/or drug allergy. In addition, a HIV test was performed, which was negative. After 20 days the patient returned and no longer showed any lesions or symptoms. Unexpectedly, the result of histopathology was erythema nodosum leprosum (ENL). The *ML Flow* was positive and MDT/MB treatment initiated.

Erythema nodosum is a syndromic event with many causal possibilities, among these is MB leprosy. This case is an example of the immunopathology of silent LL, showing that the type-2 reaction was triggered by viral infection. The biopsy was performed due to suspicion of adverse drug effects and leprosy was not considered. In a leprosy-endemic area, a rapid test used for screening could be used to test patients presenting recurrent erythema nodosum. In positive cases, bacilloscopy could be performed, thus avoiding biopsy. The patient was treated with MDT MB in the original health facility.

As an example, in **Case 4**, a positive *ML Flow* test reinforced the clinical suspicion of leprosy and the biopsy was avoided. The patient was referred to the UH due to thickened skin in the shoulder and right arm. The only symptom was paresthesia on an unspecific skin lesion (**Figure 4**), which was not easily recognized by inexperienced field workers. The clinical suspicion was BL. A skin smear examination was performed, the case was confirmed and treated with MDT MB in the health facility.

The following two cases are probably part of the silent MB cases mentioned in the literature as the main source of leprosy transmission⁶. Since no other family members presented MB leprosy, cases 5 and 6 probably acted as transmission agents for PB secondary cases.

Case 5. The patient was treated and cured of leprosy when he was 12 years old with the MB scheme used at that time (90 days



FIGURE 4

Paresthetic sensation on unspecific skin lesion.

rifampicin plus dapsone daily for two years). Two years before the present admission, his daughter was diagnosed with tuberculoid leprosy. Only a few months later, he observed an enlarged plaque on his abdomen (**Figure 5**). Skin smears performed in a basic health facility were negative. The biopsy at that time was inconclusive, but compatible with indeterminate leprosy. Referred to the out-patient clinic of the UH, evolution and minor infiltration of the initial plaque was observed and a new one appeared. Cotton wool, pinprick and monofilaments tests showed loss of sensation in the center of the lesion. A positive *ML Flow* test, clinical aspects and medical history reinforced the diagnosis of a MB relapse and MDT/MB was initiated immediately. The slit skin smears were reexamined and AFB were detected. The histopathological results confirmed an active BL leprosy case. The positive *ML Flow* test was helpful in diagnosing a possible reinfection/ relapse case. The



FIGURE 5

Hypoesthetic, enlarged plaque on the abdomen. *ML Flow* +4.

12 doses MDT/MB were completed and the patient is currently undergoing corticoid therapy for leprosy reactions.

Case 6. The patient was examined because he was a household contact of 2 paucibacillary (PB) leprosy children diagnosed during a leprosy elimination campaign in a peripheral area of RJ City. Neither leprosy skin lesions nor nerve enlargement were detected. He presented acrocyanosis and discrete erythema on the upper legs that could have been related to his cigarette addiction; however, since he was a candidate as the index case of the two PB cases, MB leprosy was also a feasible diagnosis. Therefore, the ML flow test was performed and the positive result justified additional investigation. Bacilloscopy and biopsy showed periaxial granulomatous infiltration (**Figure 6**) with AFB in the Wade stain confirming BL. The test results changed the patient's attitude, because he initially refused to go to the health facility for

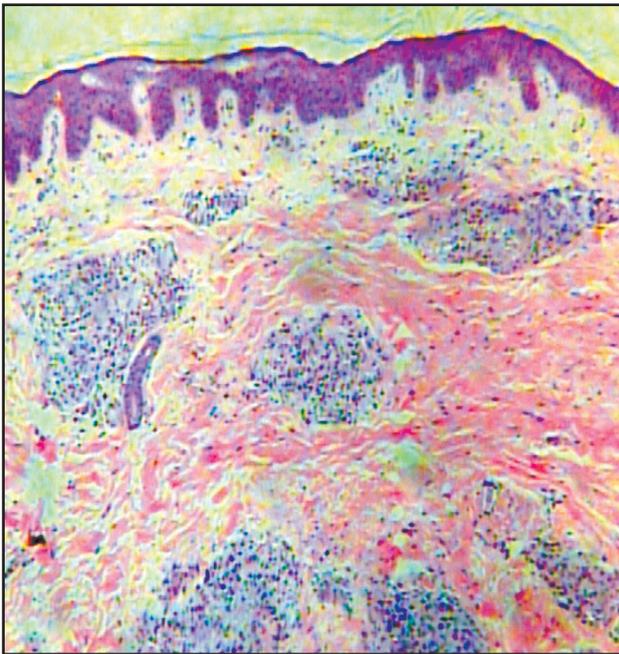


FIGURE 6

Wade40x - Perianaxial granulomatous infiltrate.

examination and the team had to go to his residence. Following the test result, he went willingly for further testing. This exemplifies that the use of the ML Flow test under field conditions reinforced clinical suspicion of silent MB leprosy and encouraged both: (i) the field health worker to refer the case for further investigation and (ii) the patient to go to the reference unit.

Case 7. A woman brought her daughter to the UH, because leprosy was suspected at the local level, and a LL case was confirmed. Therefore, the woman was examined as a household contact and leg ulcers were observed (**Figure 7A**). She reported current treatment for varicose ulcers for almost a year. She also reported recurrent ulcers and painful nodules suggestive of erythema nodosum in both legs during the preceding 16 years, especially during her four pregnancies (**Figure 7B**). The question of active or inactive disease was raised and the strong positive serology result demanded for further investigation. Bacilloscopy and biopsy confirmed the LL case, possibly spreading AFB for 16 years. The patient had discrete



FIGURE 7

7A) Leg ulcers. 7B) Atrophic lesions, probable erythema nodosum leprosum sequelae.

loss of sensation in both plantar regions (*tibialis posterior* nerve region). Despite the residual aspects of her skin lesions and the presence of madarosis and a nasal ulcer without correspondent facial skin infiltration, she reported she had never been diagnosed or treated for leprosy. This was confirmed by an investigation of the leprosy case notification system of RJ. The ML Flow test was strongly positive and she was diagnosed with active LL and treated with MDT MB. The diagnosis was confirmed by histopathology (**Figure 8A and B**). Her 18 year-old son had previously been examined and diagnosed as an LL case. Another son was examined as her contact and the case is presented below. Thus, she was the index case of three children with MB leprosy.

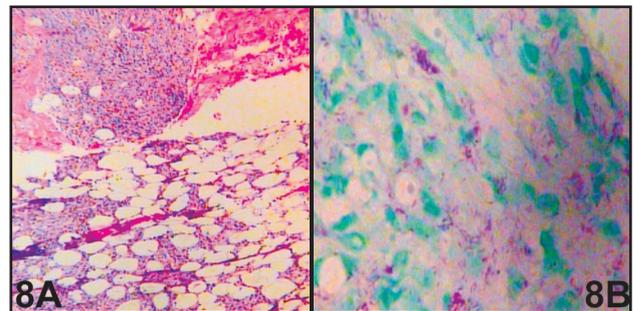


FIGURE 8

8A) 100x - Lymphohistiocytic infiltrate in the hypodermis. 8B) Wade 1000x - acid fast bacilli in the biopsy of leg lesions.

Case 8. The 11 years old son of case 7 also exemplified silent MB leprosy and the importance of investigating nasal symptoms and acrocyanosis as indicative of LL leprosy. He presented nasal obstruction, moderate acrocyanosis, livedo reticularis in both legs (**Figure 9**) and possible skin infiltration of the earlobes and face, but no typical leprosy lesions. The ML Flow test was strongly positive and active LL was confirmed by skin smears and histopathology. The patient presented recurrent reactions that were difficult to control.

Case 9. A leprosy case? This case was a household contact of three LL cases diagnosed in 2003/04. In 2003 she was examined and was negative in both dermatological/neurological aspects and serological testing. In 2004 she presented with acrocyanosis, livedo reticularis and one erythematous plaque on her left thigh with loss of sensation. The ML Flow test was performed and showed a reversal to a positive result (4+). LL was suspected



FIGURE 9

Case 8 - Livedo reticularis , ML Flow 4+.

and she underwent skin smears and biopsy. Following routine program recommendations, MDT/MB was promptly initiated. The presence of AFB was not confirmed. The histopathology study showed granuloma annulare features, (**Figure 10A and B**). As this result was determined three months later, the decision to complete 6 months of MDT was taken. This case illustrates a difficult differential diagnosis presenting clinical signs, a highly endemic living situation and a positive serology that inaccurately influenced the treatment decision. It is possible that the evolution

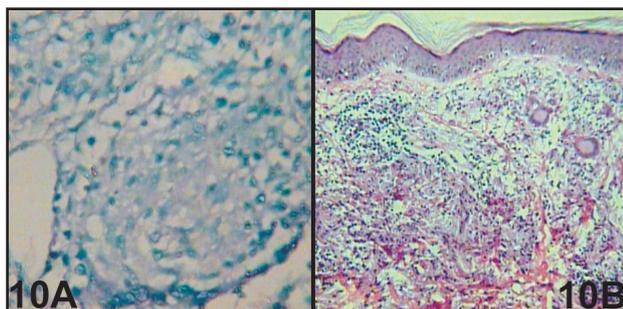


FIGURE 10

10A) 400x. No acid fast bacilli visualized. **10B)** 100x Tuberculoid granuloma with giant cells, characteristic of Granuloma Annulare.

of subclinical infection could result in leprosy disease. The decision to treat for at least 6 months was based on the fact that disease could manifest itself and it probably would be a MB case³, thus the early treatment would benefit the patient. Perhaps, if no treatment had been provided directly after seroconversion, during follow-up of this case, the appearance of signs and symptoms of leprosy would have been observed. Nevertheless, this case illustrates how careful health professionals should be when interpreting the ML Flow test results.

CONCLUSIONS

One criticism to learning practices in medical training is the excess of complementary exams to confirm clinical suspicions of leprosy, even when patients present clear cardinal signs and symptoms of leprosy⁴. This could negatively influence the practices of new doctors at basic health care levels, where they must diagnose leprosy using minimal technology. In fact, 70% of

leprosy cases, especially those with paucibacillary leprosy (PB), could be diagnosed only by anamnesis, epidemiological history, skin and nerve examinations⁸.

Even so, it is difficult to diagnose and correctly treat around 30% of multibacillary leprosy (MB) cases that do not show cardinal signs and symptoms⁸. The availability of a highly sensitive, specific, simple and cheap test would be ideal. Since a large number of PB cases do not have detectable levels of acid-fast bacilli (AFB), this compromises the sensitivity of any serological test. On the other hand, when the detection of antibodies to PGL-I is applied only to cases of suspected MB, this problem disappears and both AFB smears and serological testing are highly sensitive and specific.

As shown in these illustrative situations, in a general hospital in a leprosy-endemic country, a rapid and specific test could be useful when a diagnostic procedure must be performed during in- and out-patient consultation.

In conclusion, it is likely that the greater part of leprosy cases can be diagnosed and treated without any laboratory exams; however, it should be noted that almost all of these are PB cases. At a regional level, many technicians would benefit if important tools, such as serology, could help them with difficult MB cases. It is possible to infer that late diagnoses of these MB cases, which are not easily diagnosed, are responsible for the maintenance of leprosy transmission.

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