Leprosy serology using PGL-I: a systematic review

Sorologia da hanseníase utilizando PGL-I: revisão sistemática

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ABSTRACT

Serology using a species-specific antigen for *Mycobacterium leprae*, PGL-I, could be a marker for the bacterial load of patients with leprosy. Various studies have identified the potential use of serology in the classification of patients for treatment purposes, case monitoring, identification of the risk of relapse and selection of household contacts with a higher risk of contracting the disease. A systematic review of the literature was conducted and 26 articles were included in this comparative analysis. The results of the use of PGL-I serology in different situations, its limitations and possible applications were evaluated. Studies show the efficacy of PGL-I serology in the classification of patients, treatment monitoring and as a predictive test for leprosy reactions. To improve early diagnosis and follow-up of the population at greatest risk of developing leprosy, the methodologies used in the past have yet to show a favorable cost-benefit ratio, although studies indicate that the use of the test might positively influence leprosy control programs. With simple and robust techniques, the use of PGL-I serology is viable.

Key-words: Serology. PGL-I. Leprosy. ELISA.

RESUMO

A sorologia utilizando o antígeno espécie-específico do *Mycobacterium leprae*, PGL-I, pode ser um marcador de carga bacteriana em pacientes com hanseníase. Estudos identificaram potencial de uso da sorologia na classificação de pacientes para fins de tratamento, monitoramento de terapia, risco de recidiva e na seleção dos contatos com maior risco de adoecer. Foi realizada uma revisão sistemática e 26 artigos foram incluídos na análise comparativa. Avaliamos os resultados do uso da sorologia PGL-I em diferentes situações, suas limitações e possíveis aplicações. Estudos mostraram eficácia da sorologia PGL-I na classificação de pacientes, monitoramento da terapia, e nas reações hansênicas como teste preditivo. Para diagnóstico precoce e seguimento de população de alto risco, as metodologias utilizadas ainda não demonstraram custo-benefício favorável, porém estudos indicam que a utilização do teste poderá influenciar positivamente nos programas de controle da hanseníase. Com técnicas simples e robustas, o uso da sorologia PGL-I é viável.

Palavras-chaves: Sorologia. PGL-I. Hanseníase. ELISA.

Since 1991, the World Health Organization (WHO) has sought to achieve the goal of the elimination of leprosy as a public health problem, defined as a prevalence rate of one leprosy case per 10,000 population. In 2007, the global prevalence of leprosy registered at the beginning of the year was 224,717 active cases, 13.2% lower than that registered in 2006[83]. The significant decline in prevalence was largely due to the introduction of Multi-Drug Therapy (MDT) as the standard treatment and the marker for disease cure (release from treatment), in contrast with dapsone (DDS) treatment over the rest of the patient's lifetime, as was the case until 1982.

Leprosy is still one of the main causes of physical disability, which contributes to the continuation of stigma and social disadvantage for those who have the disease and their family members[55]. The WHO estimates that 25% of patients have some degree of disability due to leprosy[81], which denotes the existence of late diagnosis related to the operational problems of low coverage and case resolution within the health system, in addition to aspects inherent in the insidious evolution of the disease[79].

Little is known of the real distribution and transmission of leprosy infection and the factors that lead to the onset of disease, mostly due to the fact that it is impossible to cultivate *Mycobacterium leprae* in vitro. Infection with the bacilli is significantly more prevalent than cases of the disease itself. Therefore, further research is necessary, particularly concerning bacillary transmission, the role of subclinical infection, the progression of infection to disease and incidence tendencies[75]. The generally accepted concept is that multibacillary (MB) patients are the principle source of infection. Therefore, a control strategy based on case diagnosis and treatment should reduce the transmission of the organism. Over time, the chain of transmission would be broken and leprosy would disappear naturally[45].

The discovery and elucidation of the chemical structure of the glycolipid specific to *Mycobacterium leprae* in 1981[43], and the discovery that it was antigenic in 1982[58], were great innovations in leprosy research. PGL-I has been used in several studies showing that leprosy patients at the lepromatous end of the spectrum form large quantities of immunoglobulin of the IgM type in response to this antigen (seropositivity of 80-100%), while patients at the tuberculoid end showed specific immunoglobulin at much lower levels (seropositivity of 30-60%)[9 14 22 44 46 57 59].

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The type of leprosy and the level of proximity and relationship between the household contact and the index-case are other factors that weigh in disease risk evaluation. Patients often have no knowledge of any previous contact with the disease and the majority of incident cases do not report having been in contact with other patients[38]. However, evidence exists that the fact that individuals live in the same household with leprosy patients does significantly raise the risk of developing the disease[31 54 76 77].

A study that monitored contacts over a period of 6 years showed that there is a 7.2-fold greater risk of developing leprosy (MB or PB) in seropositive contacts with antibodies to PGL-I when compared to seronegative contacts, increasing to 24-fold greater risk of developing MB leprosy[32]. The percentage of contacts that progress to disease among seropositive contacts suggests that serology with anti-PGL-I could be useful as a prognostic test[18].

Although the detection of antibodies may indicate current or past infection of *Mycobacterium leprae* regardless of the presence of clinical signs[19 35 46], antibody titers appear to be more closely associated to the level of exposure to *Mycobacterium leprae* in the community at large. This is because the distribution of seropositivity in groups of household contacts or leprosy cases has not proved to be higher than non-contacts in highly endemic areas, but significant differences exist between contacts and non-contacts in areas of lower endemicity.

Seropositivity in a general population has a uniform distribution. This may mean that no difference exists between healthy individuals and leprosy cases to distinguish between subclinical infection and disease. In this case, serological tests based on the detection of IgM antibodies against PGL-I should not be used as a diagnostic tool for population screening to detect leprosy cases[37].

The diagnosis of leprosy is clinical and, as per WHO recommendations, operational classification is based on the number of skin lesions, where patients with up to 5 lesions are considered paucibacillary (PB) and those with 6 or more lesions are multibacillary (MB)[53]. Approximately 70% of leprosy patients can be diagnosed via the presence of skin patches with reduced sensitivity. However, 30% of patients, including many MB cases, do not present this sign. Bacilloscopy is an important auxiliary examination, but it is not always available.

Episodes of leprosy reactions are the most significant disease complications and can occur during and/or after treatment, often leaving sequelae. Type I, or reversal reactions are episodes of acute inflammation of the skin and peripheral nerves that are the result of late hypersensitivity to bacilli antigens that occur in as many as 30% of patients[62]. Erythema Nodosum Leprosum, or type II reactions, can occur at the lepromatous end of the spectrum and often begin with the patient in a febrile, weakened state with papular cutaneous nodules accompanied by inflammation in the nerves, eyes and testicles[8].

Research has sought to evaluate PGL-I serology as a tool in the monitoring of treatment efficacy based on the strong correlation between bacilloscopy and the levels of antibodies to PGL-I in clinical samples of patients.

The current study aimed to review serology for the detection of IgM antibodies against PGL-I, its application as an auxiliary test for diagnosis and the classification of HD patients for treatment purposes, case monitoring, relapse risk identification and the selection of household contacts with a greater risk of contracting the disease.

MATERIAL AND METHODS

Criteria for search and selection

A systematic bibliographic review was conducted using PGL-I serology to define the search parameters in bibliographic medical databases such as BIREME/PAHO/WHO, MEDLINE, Cochrane Library, Brazilian Society of Dermatology, reports from international committees, academic thesis and personal experiences from the authors published in indexed and non-indexed sources. The terms used in the search were *PGL**', *leprosy*, *Bacterial index* and *phenolic glycolipid* combined with filters for diagnostic studies such as *diagnosis*, *sensitivity*, *specificity* and *epidemio**.

Criteria for the selection of studies for review

The selected studies were included based on an objective evaluation of the methodology and quality of each work, with criteria adopted for inclusion that sought to group similar studies and exclude those without possibility for comparison (**Table 1**).

Criteria for inclusion:

- Studies that presented a methodology that could be replicated in other contexts;
- Use of synthetic glycolipids (DBSA, ND-O-BSA, NT-P-BSA);
- Samples of patients that had not yet been treated for leprosy (if the objective was to determine sensitivity);
- Research on the presence of IgM antibodies.

Criteria for exclusion:

- Use of native PGL-I;
- ELISA using a cut-off point below 0.150 or over 0.300;
- Studies including patients only from a pool of PB or MB patients;
- Lack of information on the criteria for inclusion/exclusion in the study;
- Lack of information on the criteria used for patient classification.

Data extraction

From a total of 109 works, 57 articles were selected for inclusion, and of these, 26 were selected for comparative analysis (**Figure 1**).

TABLE 1

List of articles and abstracts excluded from the study and reasons for exclusion.

Year	Author	Antigen	Serum dilution	Cut-off	Sample	Reason for exclusion
1983	Cho[26]	native PGL-I	NA	NA	87	native PGL-I
1983	Brett[5]	native PGL-I	-	-	70	native PGL-I
1986	Bach[1]	native PGL-I	1/250	-	88	native PGL-I
1986	Levis[49]	native PGL-I	1/300	-	192	native PGL-I
1987	Chanteau[19]	ND-O-BSA	1/250	0.100	724	Cut-off<0.150
1987	Menzel[52]	D-O-BSA	1/100	1.0	207	Cut-off>0.300
1988	Wu[85]	ND-O-BSA	1/200	0.04	213	Cut-off<0.150
1988	Chanteau[21]	NTP	1/250	0.589	19	Cut-off>0,300
1988	Lyons[51]	native PGL-I	1/300	-	77	native PGL-I
1988	Fine[37]	DBSA	1/020	-	6002	native PGL-I
1990	Hussain[44]	DBSA	1/250	0.500	100	Cut-off>0,300
1990	Saad[66]	native PGL-I	-	0.27	357	native PGL-I
1990	Bagshawe[2]	native PGL-I	1/100	0.200	960	native PGL-I
1991	Cho[24]	ND-O-BSA	1/300	0.200	101	treated patients
1991	Soebono[70]	native PGL-I	-	0.180	2430	native PGL-I
1991	Chujor[27]	native PGL-I	1/500	-	147	native PGL-I
1992	Sticht-Groh[72]	DBSA	1/200	0.200	245	Compare buffer
1992	Chin-A-Lien[50]	DBSA	-	0.150	10	treated patients
1993	Prakash[60]	NT-P-BSA	-	1.16	65	Cut-off>0,300
1993	Yamashita[86]	native PGL-I	1/250	0.200	214	native PGL-I
1993	Foss[39]	native PGL-I	-	-	47	native PGL-I
1994	van Beers[78]	NT-P-BSA	-	0.380	1302	Cut-off>0,300
1994	Soares[69]	ELISA	1 /050	0.199	562	patients not classified
1998	Stefani[71]	DBSA	1/300	0.2	255	treated patients
1998	Kumar[48]	native PGL-I	-	0.23	698	native PGL-I
1998	Cunha[28]	PGL-I BSA	-	0.20	272	treated patients
2001	Cho[25]	ND-O-BSA	1/300	-	101	treated patients
2002	Wu[84]	ND-O-BSA	-	-	1061	treated patients
2003	Bührer-Sékula[14]	NT-P-BSA	1/050	-	498	simple size
2005	Cardona-Castro[16]	native PGL-I	1/040	0.394	248	Cut-off>0.300
In Press	Schruring[68]	NT-P-BSA	-	0.200	864	treated patients
In Press	Brito[7]	NT-P-BSA	1/050	-	208	treated patients

NA: not available.

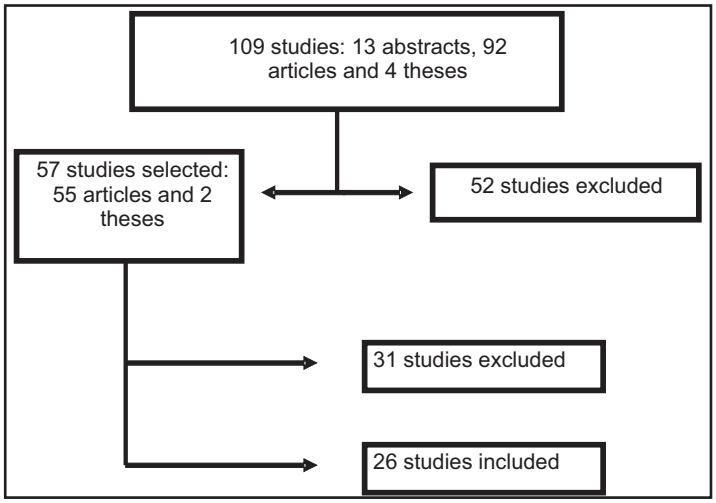

FIGURE 1

Total number of articles and abstracts reviewed.

Even among the studies selected, the characteristics of study design, criteria for definition of the groups selected, level of patient exposure to *Mycobacterium leprae* and immunological response of those infected (in endemic areas or not) differ. In the selection of patients, the gold standard used to define PB or MB varied among the studies and influenced the level of sensitivity observed. The studies that used enzyme-linked immunosorbent assay (ELISA) differed in technique in relation to the type and concentration of antigens, dilution of samples and definition of cut-off points for positivity. These differences interfered in the sensitivity and specificity of the tests.

Analysis

No significant differences occurred between the results using ELISA or faster methods of antibody detection[41 52 64 74]. A close correlation was observed between studies using samples in filter paper (finger prick)[25 42 67 69] and those collected by venopunction, despite the fact that the serological titers detected on samples that passed through filter paper were generally lower[74].

PGL-I in patient classification

Several studies observed a correlation between the levels of antibodies detected with ELISA and bacteriological indexes (BI)[9 11 12 13 14 15 18 22 25 27 42 57 61 67 73], justifying studies which use serology as an auxiliary tool in patient classification.

To facilitate the visualization and comparison of the results, the studies were grouped according to their study populations. **Table 2** demonstrates the positivity in studies involving MB and PB patients using diverse techniques, including ELISA, dipstick, ML Flow and Passive Hemoagglutination (PHA).

Average seropositivity among the studies for MB and PB patients was 78% and 23%, respectively, varying between 51.2% and 97.4% in the MB group and from 6.9% to 57.3% in PB. Variations in the classification criteria (PB and MB) used are not clear in many studies, given that the WHO criteria were changed in the middle of the period in question and were not always completely followed. For example, the classification of patients as MB or PB in the 1980s required a smear bacilloscopy. At the end of the 1990s, this classification was changed based on the number of skin lesions present without regard to the number of nerve trunks affected[82]. In general, seropositivity in PB cases presented lower percentages in the studies that used bacilloscopy as the gold standard. In contrast, studies that showed elevated seropositivity also used classification based on the number of lesions or combined this approach with bacilloscopy. This variation in seropositivity percentages is related to the differences in immunological response in different populations. For example, the implementation of research for ML Flow[15] in Nepal showed almost half the seropositivity (31.9%; 340/1066) level of that observed in Brazil (50.8%; 544/1071) and Nigeria (62.9%; 117/186). The low bacterial production in Nepalese patients was confirmed by both bacilloscopy and ML Flow negative results for 38.3% and 15.6% of MB patients classified by the number of skin lesions in Nepal and Brazil, respectively.

Analysis of the results showed that the use of serology as a tool for patient classification would lead to a reduction in the number of patients treated as MB. This is because the counting of skin lesions is a functional operational tool, but has not been well-received by health professionals. When laboratory tests like bacilloscopy and histopathology are not available, there is a strong tendency to classify patients as MB, as seen in the Nigerian study, where a large proportion of patients received the MB treatment regimen unnecessarily[15]. Part of this fear may be explained by the fact that

TABLE 2

PGL-I serology results in multibacillary and paucibacillary patients.

							Positives/sample (nº)		Seropositivity (%)	
Year	Author	Country	Antigen	Technique	Serum dilution	Cut-off	MB	PB	MB	PB
1988	Petchclai	Thailand	ND-BSA	PHA	1/064	-	37/38	6/24	92.0	25.0
1989	Chanteau	Hait	NT-P-BSA	ELISA	1/250	0.200	26/27	12/35	96.0	34.0
1990	Groenem	Zaire	DBSA	ELISA	1/080	0.200	8/14	4/58	57.0	6.9
1993	Cellona	Philippines	ND-O-BSA	ELISA	1/200	0.160	163/193	22/147	84.5	15.0
1998	Bührer-Sékula	Manaus - Brazil	DBSA	ELISA	1/300	0.200	80/108	14/103	74.1	13.6
				Dipstick	1/050	-	86/108	14/103	79.6	13.6
1998	Bührer-Sékula	Manaus - Brazil	DBSA	ELISA	1/300	0.250	63/123	8/55	51.2	12.0
2000	Bührer-Sékula	RJ - Brazil	ND-O-BSA	Dipstick	1/050	-	100/130	10/134	76.9	7.4
2001	Bührer-Sékula	Manaus - Brazil	DBSA	Dipstick	1/050	-	57/67	23/103	85.1	22.3
2003	Bührer-Sékula	Brazil, Indonesia, Philippines	NT-P-BSA	ML Flow	1/050	-	111/114	34/85	97.4	40.0
2006	Schruring	Bangladesh	NT-P-BSA	ELISA	1/167	0.199	204/294	138/731	69.4	18.9
		Nigeria					31/36	84/150	86.1	57.3
2007	Bührer-Sékula	Brazil	NTP-BSA	ML Flow	1/050	-	352/423	192/648	83.2	29.6
		Nepal					222/379	118/687	58.6	17.2
2008	Parkash	India	NT-P-BSA	ML Flow	1/050	-	23/25	39/122	92.0	32.0

PHA: passive hemoaglutination, MB: multibacillary, PB: paucibacillary.

classification using the number of lesions ignores the size of the patches and health care workers observe the important relation between the size of lesions and clinical form of the disease. Recently, lesion size was identified as an important aspect in the treatment decision[67].

PGL-I in case holding

The serological methods based on PGL-I can be used in leprosy case holding. In the majority of patients, antibody levels drop once treatment is initiated, so they are obviously much higher at diagnosis[25] and fall 25 to 50% annually afterwards[23 24 33 47 63]. This decline varies widely among patients, in that this decline can be linear and quickly become negative or take several years after the end of treatment to become so[40].

PGL-I as a predictor of reactions and relapse

Few studies assessed the utility of serology for diagnosis or to predict which patients may have reactions or relapses; however, they do tend to indicate the same risk factors for reactions and relapse after release from treatment.

As an auxiliary tool in the diagnosis of type I or II reactions during treatment, serological tests did not prove efficient, because similar levels were obtained in patients without reactions and even among the healthy population[71]. However, patients with high concentrations of anti-PGL-I IgM at the onset of treatment presented a higher risk of developing type 1 reactions, thus identifying patients for monitoring and early treatment may reduce nerve damage and disability[65]. In posttreatment reactions, patients with a positive PGL-I serology when released from treatment showed a 10.4-fold greater chance of developing reactions compared to those with negative serology[7].

Research using serology is necessary to identify the patients at higher risk of developing reactions in order to define the best approach to case holding and patient monitoring.

Seropositivity may be the first indicator of leprosy relapse[50 84]; however, in immunosuppressed patients, seropositivity may not be present[50]. In a clinical trial to reduce the treatment period for leprosy, seropositivity proved to be essential for predicting relapse and only one out of nine patients diagnosed as a relapse case showed negative serology at the onset of treatment and even the one exception was a drug-resistant case[12]. A study conducted in Brazil observed a significant association between a higher risk of relapse in patients with a certain set of characteristics, such as being closer to the lepromatous end of the spectrum and being positive for BI and anti PGL-I[56]. This shows that the bacilli can remain relatively protected from the immunological effects of treatment, subsequently proliferating under more propitious conditions.

Household contacts

The high prevalence of seropositives among household contacts of leprosy patients demonstrates that subclinical infection with *Mycobacterium leprae* is common[30 52 66] and is related to the leprosy type of the patient under study[20 30 70].

Studies in contacts have shown seropositivity in as many as 18.4% (**Table 3**), with lower levels obtained in contacts of PB patients and higher levels in MB contacts. Monitoring of contacts provided evidence that those who tested seropositive had a higher risk of developing MB leprosy than those who were seronegative[32]. The BCG vaccine seems to have a protective effect, given that the majority of seropositive contacts that were vaccinated developed only the PB form of leprosy[36].

Research in Rio de Janeiro verified the positive influence of decentralized healthcare in the increase of new case detection, leading to earlier diagnosis, thereby reducing the number of patients that developed disabilities[29]. Therefore, the detection of antibodies against PGL-I can help to identify infected household contacts without clinical signs or symptoms and may be a useful tool in control programs.

TABLE 3

PGL-I serology results in household contacts.

Year	Author	Country	Antigen	Technique	Serum dilution	Cut-off	Positives/sample (nº)	Soropositivity (%)
1989	Desforges	New Caledonia	NT-O-BSA	ELISA	1/250	0.257	44/309	14.2
			ND-O-BSA			0.174	30/309	9.70
1990	Sulçebe	Albania	DBSA	ELISA	1/300	0.200	7/53	13.3
1991	Krishnamurthy	India	ND-O-BSA	ELISA	1/040	0.200	58/402	14.5
1993	Chanteau	Polynesia	NT-P-BSA	ELISA	1/250	0.200	204/1201	17
1993	Cellona	Philippines	ND-O-BSA	ELISA	1/200	0.160	39/601CMB 5/71CPB	6.5CMB 7.0CPB
1998	Bührer-Sékula	Manaus - Brazil	DBSA	ELISA	1/300	0.250	2/42	4.0
1998	Bührer-Sékula	Manaus - Brazil	DBSA	ELISA	1/300	0.200	4/108	3.7
				Dipstick	1/050	-	2/108	1.90
1999	Roche	Nepal	DBSA	ELISA	1/300	0.200	8/47	17
2004	Sinha	India	ND-O-BSA	ELISA	1/300	0.200	81/2994	2.70
2005	Calado	RJ - Brazil	NT-P-BSA	ML Flow	1/050	-	158/860CMB 76/679CPB	18.4%CMB 11.3%CPB
2008	Duppre	RJ - Brazil	NT-P-BSA	ML Flow	1/050	-	265/1567CMB 76/560CPB	16.9%CMB 13.6%CPB

CMB: household contact of multibacillary index case, CPB: household contact of paucibacillary index case.

TABLE 4

PGL-I serology results comparing endemic and non-endemic populations.

Year	Author	Country	Antigen	Technical	Serum dilution	Cut-off	Positives/sample (nº)		Soropositivity (%)	
							endemic population	non endemic population	endemic population	non endemic population
1990	Cartel	French Polynesia	NT-P-BSA	ELISA	1/250	0.200	157/3567		4.3	
1990	Groenem	Zaire	DBSA	ELISA	1/080	0.200	29/1524		2.90	
1992	Douglas	Philippines (end) USA (n end)	ND-O-BSA	ELISA	1/500	0.150	5/398	4/426	1.30	0.70
			NT-O-BSA				10/398	6/426	2.50	1.40
			ND-P-BSA				6/398	6/426	1.50	1.40
			NT-P-BSA				6/398	7/426	1.50	1.60
1993	Cellona	Philippines	ND-O-BSA	ELISA	1/200	0.160	7/401		1.70	
1996	Gonzalez-Abreu	Cuba	ND-A-BSA	ELISA	1/200	0.199	938/24.293		3.80	
1999	van Beers	Indonesia	PGL-I MLPA	MLPA	-	-	506/1876	67/959	27.00	7.00
2002	Bakker	Indonesia	NT-P-BSA	ELISA	1/500	0.200	96/4.140		2.30	
2004	Bakker	Indonesia	NT-P-BSA	ELISA	1/500	0.200	98/3.271		3.00	

end: endemic population, n end: non endemic population.

Study population

Table 4 demonstrates that the seropositivity rate varies and appears to be dependent on the leprosy incidence rate in the community[3 4 17 18 34 41 42 80].

While different rates of seropositivity in endemic areas, as opposed to non-endemic areas, may reflect subclinical infection[46], as yet, no evidence exists of a correlation between seroprevalence of PGL-I and the incidence of leprosy. Additionally, studies have not yet shown how to use serology in the evaluation of leprosy control activities.

In school children aged 10 to 12, different patterns of seropositivity distribution in endemic countries, such as Indonesia and Brazil, did not permit confirmation of PGL-I seropositivity as an indicator of the magnitude of the disease in a given area[10 80]. In Indonesia, such a correlation was shown, but could not be confirmed in Brazil. Despite the fact that the two studies included similar populations, the methods differed; in Indonesia, children almost exclusively in that age group were included, while clusters of similar age were used in Brazil. The study of clusters may not be appropriate to represent the infection rate in the population, but the cost-benefit ratio of including all children in a particular age group would not justify the use of serology as a simple tool to evaluate leprosy endemicity in a determined region[13 78].

Conclusion

Serologic tests to detect IgM immunoglobulin to PGL-I are useful to assist in diagnosis when the results are considered together with clinical information[2 6 11 67]. They may be used to classify patients as MB or PB and in the monitoring of treatment efficacy, which must be accompanied by reduced circulating antibody titers[1 22 26 25 85].

For leprosy reactions, PGL-I also proved to be useful as a predictive tool[7 65].

For early diagnosis and monitoring of those at higher risk, the methodologies used to date have still not shown a favorable cost-benefit ratio, although studies indicate that the use of this type of test could positively influence leprosy control programs.

Almost thirty years after the identification of PGL-I, we affirm that the evolution of related research has generated simple and robust methodologies that are useful for epidemiological studies and as auxiliary tools in the classification of and treatment definition for leprosy.

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