Transactions of the Royal Society of Tropical Medicine and Hygiene 104 (2010) 546–550

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Correlation between ELISA and ML Flow assays applied to 60 Brazilian patients affected by leprosy

Rozana C. Da Silva[a,*], Sandra Lyon[a], Ana C. Lyon[a], Maria A.F. Grossi[b], Silvia H. Lyon[a], Samira Bührer-Sékula[c,1], Carlos M.F. Antunes[d]

[a] Serviço de Dermatologia Sanitária, Hospital Eduardo de Menezes, Fundação Hospitalar do Estado de Minas Gerais, 30622-020, Belo Horizonte, MG, Brazil
[b] Coordenação Estadual de Dermatologia Sanitária, Secretaria Estadual de Saúde de Minas Gerais, 30130-006, Belo Horizonte, MG, Brazil
[c] KIT Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands
[d] Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal de Minas Gerais, 30130-100, Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 27 July 2009
Received in revised form 4 May 2010
Accepted 4 May 2010
Available online 17 June 2010

Keywords:
Leprosy
Mycobacterium leprae
PGL-1 antigen
Serological assays
ML Flow
ELISA

ABSTRACT

Serological tests can be helpful in classifying leprosy patients as having either the paucibacillary or the multibacillary form. The aim of this study was to evaluate the concordance between two serological assays, i.e. ML Flow and ELISA, in a population of leprosy patients in Brazil. The investigation involved 60 patients with newly diagnosed leprosy. Together with the application of the serological assays, selected demographic, clinical and epidemiological data relating to the study population were recorded. ML Flow detected anti-PGL1 antibodies in 70% of the leprosy patients, while ELISA was positive in 53.3%. The degree of concordance between the tests was substantial (83.3%). A positive correlation was demonstrated between the results obtained in the semi-quantitative ML Flow test and ELISA absorbance values. We concluded that both serological assays were found to be efficient in detecting anti-PGL1 antibodies. The ML Flow test may be a cheaper and easier to perform alternative to ELISA in leprosy patients.

1. Introduction

Leprosy is a disease of significant epidemiological importance within many countries in Latin America. In Brazil, for example, some 44 436 new cases of the disease (2.4/10 000 inhabitants) were registered in 2006, and of these 7.9% occurred in individuals aged less than 15 years.[1] During 2007, the prevalence of leprosy in the country had risen to 3.2/10 000 inhabitants with 60 567 recorded cases of the disease.[2] In the State of Minas Gerais, located in the southeast of Brazil, there were 2547 new cases of leprosy (1.2/10 000 inhabitants) in 2006, 5.3% of which were in individuals younger than 15 years old.[3] The frequency of leprosy patients presenting at diagnosis with a degree of incapacity sufficient to imply that the disease had reached a late stage was much higher in Minas Gerais (10.2%)[3] than in Brazil (4.7%)[2] or worldwide.[4]

From an epidemiological point of view it is important to detect potential sources of *Mycobacterium leprae*, the causative agent of leprosy, in order to prevent the transmission of infection amongst the population. Within this context, new laboratory tools are essential for the identification of the disease at a very early (subclinical) stage of infection.[5] Phenolic glycolipid-1 (PGL-1), a specific immunogenic antigen of the bacterium, was first described in 1980.[6] Since that time various semi-synthetic analogues,

* Corresponding author. Present address: Avenida do Contorno, 4852, Sala 601, Bairro Funcionários, 30110-032, Belo Horizonte, MG, Brazil. Tel.: +55 31 32270092; fax: +55 31 32270092.
E-mail addresses: rozanacastorina@globo.com, cemepe2006@hotmail.com, cemepe@cemepemg.com.br (R.C. Da Silva).
[1] Present address: Tropical Pathology and Public Health Institute, Federal University of Goiás, 74605-901, Goiânia, Goiás, Brazil.

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doi:10.1016/j.trstmh.2010.05.001

representing the unique sugar moiety of *M. leprae* PGL-1 linked (typically) to bovine serum albumin (BSA), have been produced including monosaccharide-octyl-BSA (M-O-BSA) and natural trisaccharide-phenyl-BSA (NT-P-BSA). These synthetic antigens offer various advantages over their native counterparts in that they can be obtained in large quantities, are water soluble,[7] and can be employed in epidemiological studies in which serological assays are carried out on a large number of samples.[8] In this context, enzyme-linked immunosorbent assays (ELISA) have been applied extensively in the detection of anti-PGL1 antibodies, and particularly IgM antibodies[9] More recently, Bührer-Sékula and co-workers[10] developed a single-step immunochromatographic lateral flow test for *M. leprae* known as the ML Flow test. However, the efficient and accurate diagnosis of leprosy, particularly during the early stages of the disease, depends on the specificity and sensitivity of the test employed. The objective of the present study was, therefore, to evaluate the concordance between the results obtained using the ML Flow and ELISA tests in a population of leprosy patients in Brazil.

2. Materials and methods

2.1. Study population

The investigation was carried out in 2006 and involved 60 patients who had been recently diagnosed with leprosy at the Dermatology Centre of Hospital Eduardo de Menezes. The criteria for exclusion were subjects who had received leprosy treatment for more than three months and those affected by leprosy-associated diseases.

2.2. ML flow test

The ML Flow test was performed according to the method previously described[10] and employed kits kindly donated by the Koninklijk Instituut voor de Trapen (KIT) and Netherlands Leprosy Relief (Amsterdam, The Netherlands). The testing device comprised a nitrocellulose detection strip to which the semi-synthetic antigen NT-P-BSA had been immobilised at a central test zone and flanked at one end by colloidal gold-labelled anti-human IgM antibody and at the other by an absorption pad. The test was performed with 5 μl of whole blood and 130 μl of running buffer (phosphate-buffered saline (PBS) containing 0.66 mg of BSA/ml and 3% Tween 20). The assays were assessed after 5 min and were considered to be positive when a distinct red staining of the test zone was observed or negative when no staining or only faint staining was detected. The results were expressed on a qualitative (−, +) or semi-quantitative (0, +1, +2, +3 and +4) basis. Two readings of the ML Flow test were taken for each sample by two independent researchers.

2.3. ELISA

ELISA detection of IgM antibodies against *M. leprae* PGL-I was carried out as described previously.[11] NT-P-BSA antigen (0.0023 μg of sugar/ml) was diluted in ammonium acetate-carbonate buffer (pH 8.2), coated onto the wells (50 μl/well) of microtiter plates (Nunc-Immunoplates-II, Life Technologies, Taastrup, Denmark), and left to dry at room temperature. BSA (0.1 μg/ml) was used to coat the control wells. The plates were blocked for 60 min with 100 μl of 1% (w/v) BSA in PBS, and subsequently washed six times with PBS containing 0.1% (v/v) Tween-20. Serum samples were diluted 1:300 in 0.1 M Tris buffer (pH. 6.8 ± 0.1) containing 0.1% Kathon CG, 0.02% gentamicin sulphate and 0.01% carminic acid, and 50 μl aliquots were then added to each well. The plates were incubated at 37 °C for 60 min and submitted to a further washing step. An aliquot (50 μl) of peroxidase conjugated anti-human IgM conjugate (Capple/Organon Teknika, Turnhout, Belgium) was added to each well at a 1:2000 dilution in 10 mM Tris buffer (pH 6.8 ± 0.1) containing 0.1% Kathon GC, 0.02% gentamicin sulphate and 0.01% carminic acid, and the plates were incubated at 37 °C for 60 min. Following incubation, the washing procedure was repeated and 50 μl of the substrate 3,3′,5,5′- tetramethylbenzidine (Sigma, St. Louis, MO, USA) was added to each well. In order to control the plate-to-plate and day-to-day variation, a positive reference serum sample was included in quadruplicate in each plate. The reactions were stopped with 50 μl of 1.25 M sulphuric acid when the absorbance (at 450 nm) of the reference control serum sample attained a value of 0.6. The final absorbance values of serum samples were calculated from the differences between the absorbance values of wells coated with BSA alone and those of the test wells coated with NT-P-BSA. All serum samples were assayed in duplicate and the ELISA results were expressed as mean absorbance at 450/630 nm. In order to derive positive or negative indications from the ELISA results, a cut-off absorbance value for a positive reaction was taken to be 0.157, a value that was determined from the Receiver Operating Characteristic (ROC) curve as the one that maximized the sum of sensitivity and specificity values in the studied population.

2.4. Statistical analysis

Statistical analyses were carried out with the aid of Epi-Info version 6.0 (CDC, Atlanta, GA, USA), SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) and Answer Tree version 3.0 software (SPSS Inc., Chicago, IL, USA). All calculations were performed using Microsoft Excel® 2003 (Microsoft Corp., Redmond, WA, USA). Descriptive statistics involved the determination of mean values, amplitude and variance. Statistical analyses of the results were performed using the Kruskal-Wallis test, followed by the multiple-comparison Dunn test. The degree of concordance between the mean levels of anti-PGL1 antibodies determined in a set of samples using different assay procedures was estimated from the calculated kappa (κ) values. Interpretation of the κ values was based on the criteria of Landis and Koch[12] as follows: 1.0 (perfect agreement), 0.81–0.99 (almost perfect), 0.61–0.80 (substantial), 0.41–0.60 (moderate), 0.21–0.40 (slight), 0–0.20 (very slight), <0 (poor).

3. Results

Table 1 presents the demographic, clinical and epidemiological characteristics of the population of leprosy