The ML Flow test as a point of care test for leprosy control programmes: potential effects on classification of leprosy patients

SAMIRA BÜHRER-SÉKULA*, JAN VISSCHEDIJK*, MARIA APARECIDA F. GROSSI**, KRISHNA P. DHAKAL***, ABDULLAHI U. NAMADI +, PAUL R. KLATSER* & LINDA OSKAM*

*KIT (Royal Tropical Institute), KIT Biomedical Research, Amsterdam, The Netherlands

**Secretaria de Estado da Saúde de Minas Gerais, Belo Horizonte, Brazil

***Netherlands Leprosy Relief (NLR), Biratnagar, Nepal

+ Netherlands Leprosy Relief (NLR), Jos, Nigeria

Accepted for publication 31 October 2006

Summary

Objective To evaluate the use of the ML Flow test as an additional, serological, tool for the classification of new leprosy patients.

Design In Brazil, Nepal and Nigeria, 2632 leprosy patients were classified by three methods: (1) as multibacillary (MB) or paucibacillary (PB) according to the number of skin lesions (WHO classification), (2) by slit skin smear examination, and (3) by serology using the ML Flow test detecting IgM antibodies to Mycobacterium leprae-specific phenolic glycolipid-I.

Results The proportion of MB leprosy patients was 39.5, 35.6 and 19.4% in Brazil, Nepal and Nigeria, respectively. The highest seropositivity in patients was observed in Nigeria (62.9%), followed by Brazil (50.8%) and Nepal (35.6%). ML Flow test results and smears were negative in 69.1 and 82.7% of PB patients, while smears were positive in 58.6% of MB patients in Brazil and 28.3% in Nepal. In MB patients, both smears and ML Flow tests were negative in 15.6% in Brazil and 38.3%, in Nepal. Testing all PB patients with the ML Flow test to prevent under-treatment would increase the MB group by 18, 11 and 46.2% for Brazil, Nepal and Nigeria, respectively. Using the ML Flow test as the sole criterion for classification would result in an increase of 11.3 and 43.5% of patients requiring treatment for MB leprosy in Brazil and Nigeria, respectively, and a decrease of 3.7% for Nepal.

Conclusions The ML Flow test could be used to strengthen classification, reduce the risk of under-treatment and minimize the need for slit skin smears.

Correspondence to: S. Bührer-Sékula (Tel: +31 20 5665449; Fax: +31 20 6971841; e-mail: samira@buhrer.net and l.oskam@kit.nl)
Introduction

Due to the long incubation period and the silent transmission of leprosy, efforts to reduce and eventually eliminate the disease require long-term planning and commitment. Leprosy control programmes are being integrated into the general health system and one of the challenges is to maintain high quality sustainable care for leprosy patients. In order to prevent deformities and interrupt transmission, leprosy control programs concentrate on early diagnosis and effective treatment of leprosy patients. Consequently, diagnosis and classification of patients are important, and simple tools that would help to diagnose and classify leprosy patients correctly are desirable.

The diagnosis of leprosy is based on the presence of one or more of the following features: hypopigmented or reddish skin lesion(s) with definite loss of sensation, definite thickening of a peripheral nerve with or without loss of sensation, or skin-smear positivity for acid-fast bacilli (AFB). For treatment purposes patients are classified according to the World Health Organisation (WHO) system by counting the number of skin lesions. Leprosy patients with five or less lesions will receive a paucibacillary (PB) multi drug therapy (MDT) regimen and patients with six or more lesions will receive MDT for multibacillary (MB) leprosy.

A classification system focusing on number of skin lesions alone is potentially open to under diagnosis of leprosy and of MB leprosy in particular. In early MB cases, skin lesions are often few, difficult to see or even invisible. The weakness of using a purely clinical system for classifying leprosy is that smear-positive cases presenting few lesions will be incorrectly classified as PB and will receive insufficient chemotherapy, with the attendant risk of relapse. The determination of the bacterial load in skin smears considerably improves the reliability of the classification, but this service is often not generally available in the areas where leprosy is most common.

Several studies have shown that the presence of IgM antibodies to Mycobacterium leprae-specific phenolic glycolipid-I (PGL-I) correlates with the bacterial load of a leprosy patient: 15–40% of PB patients are seropositive, compared with 80–100% of MB patients. Detection of these antibodies may thus be a useful alternative method for classifying leprosy.

The ML Flow test is a simple and rapid immunochromatographic flow test for the detection of IgM to PGL-I. It is a point of care test; a test that can be performed when a patient is diagnosed without the need to go to a specialized laboratory or come back later for the results. This test allows implementation of serological testing in leprosy control programs. In a laboratory evaluation of a set of well-characterized patients, the ML Flow test correctly classified MB patients with a sensitivity of 97.4% using a combination of clinical signs, smear positivity and histopathology as the gold standard.

We studied the implementation of the ML Flow test in leprosy control programs in three countries, Brazil, Nepal and Nigeria, and have assessed the acceptability and feasibility of the test as: (1) an additional tool for the classification of new leprosy patients and (2) a tool to identify those contacts of leprosy patients who might have an increased risk of developing leprosy in the future. A classification system in which the ML Flow test is included may affect the distribution of PB and MB patients.
Materials and methods

STUDY AREAS

The study ran from October 2002 to March 2004 in Brazil and Nepal and from November 2002 to April 2004 in Nigeria. The leprosy control programs in which we performed the study are well established and supervised regularly. Forty-nine health facilities participated: 14 (8 health centres, 4 hospitals and 2 reference centres) in Brazil, 7 (4 health centres, 2 hospitals and 1 reference centre) in Nepal and 28 (23 health centres and 5 hospitals) in Nigeria. During the period of 1999–2003, the mean new case detection rates were 6.2, 16.2 and 1.9 per 10,000 population, and the average percentage of patients treated as MB were 71, 44 and 96% for the included health facilities in Brazil, Nepal and Nigeria, respectively.

STUDY POPULATION

During the study period, newly diagnosed patients were invited to participate. All those who accepted gave written informed consent. A total of 2632 leprosy patients were included in the study: 1070 from Brazil, 1066 from Nepal and 186 from Nigeria. In Nepal, only patients from the reference centre were included in the final analysis because only this centre performs slit skin smears. There were no differences in demographic and clinical characteristics between these patients and patients from other health facilities (data not shown).

CASE DEFINITION/CLASSIFICATION CRITERIA

In all three countries the diagnosis was based on the presence of skin lesion(s) consistent with leprosy and with definite sensory loss, with or without thickened nerves. In Brazil and Nepal diagnosis of leprosy is confirmed by a medical doctor.

In Brazil and in the reference centre in Nepal, the presence of AFB in slit skin smears also contributed to the classification. The classification criteria used in the field differed between the three countries. To compare the effect of using serology when classifying patients in field situations, we used the WHO operational classification for our analysis.2 Thus leprosy patients were classified by the number of skin lesions: people with six or more lesions were designated MB and people with five or fewer lesions were designated PB. Finding thickened nerves did not affect the classification.

TRAINING OF HEALTH CARE WORKERS

In each country a training workshop was held in which trainers provided participating health care workers with the relevant background theory and technical skills. Their understanding of leprosy and their capability to collect blood, perform the test, interpret the results, fill in the patient and contact cards for data collection and give the correct information to patients and contacts were all checked.

SEROLOGY

The ML Flow test was performed according to the methods described by Bührer-Sékula et al.6 A finger prick blood sample was placed in the sample port and a dilution buffer was added. The diluted blood runs over an antigen line and a control line. The test was read after five
minutes and the result considered valid if the control line (anti-human IgM) was clearly visible. If *M. leprae*-specific IgM antibodies are present they attach to the antigen (semisynthetic derivate of PGL-I) and a distinct red line appears in the test zone. When no (−) or very faint (+/−) staining is observed, the result is regarded as negative. The quality of all ML Flow test batches sent to the three countries was controlled against the sensitivity and specificity of the reference evaluation batch. Malfunction of the test is rare and was not reported during the study. Nevertheless, health care workers were instructed to perform a second test if the control line was not visible. At least 20% of the tests in each country were re-read by local project supervisors who were blinded to the first test result.

**Microscopic Examination of Slit-Skin Smear**

In all health facilities in Brazil and in the reference centre in Nepal microscopy for the presence of AFB in slit skin smears is routinely performed and a quality control system is in place. Skin smears from four body sites were used, two from the earlobes, one from an elbow and another from a skin lesion. Whenever it was impossible to take a skin smear from a lesion, a smear from the other elbow was used instead. Smears were stained for AFB by Ziehl–Neelsen’s method and 100 oil-immersion fields were examined. Although the laboratories routinely record the bacterial index (BI), for this study we recorded results as either negative or positive.

**Ethical Clearance and Informed Consent**

Ethical clearance was given by the Research Ethics Committee of Santa Casa, Belo Horizonte, Brazil, the Nepal Health Research Council, Kathmandu, Nepal and the Research Ethics Committee of the Nigerian Institute of Medical Research, Federal Ministry of Health, Lagos, Nigeria. The informed consent forms were written in the local languages using simple phrasing. In the case of minors informed consent was obtained from a parent or guardian.

**Statistical Analysis**

Statistical analysis was performed using Epi Info version 6.04.d. When comparing differences between groups the Chi-squared test was used.

**Results**

**Classification of Patients Based on Number of Skin Lesions and ML Flow Test Results**

The classification of patients according to the WHO operational classification in relation to ML Flow test results for the patient study populations in the three countries is presented in Table 1. The proportion of MB leprosy patients was lowest in Nigeria (19.4%) (*P* < 0.01). There was no significant difference between the proportion of MB patients in Brazil (39.5%) and Nepal (35.6%) (*P* = 0.06). The highest seropositivity was observed in Nigeria (62.9%), followed by Brazil (50.8%) and Nepal (35.6%). Of patients classified as PB based on the number of skin lesions 29.6% (192/648), 17.2% (118/687) and 57.3% (86/150) were positive.
Table 1. ML Flow positivity for PB and MB patient groups classified according to WHO by the number of skin lesions

<table>
<thead>
<tr>
<th></th>
<th>Brazil</th>
<th>Nepal</th>
<th>Nigeria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
<td>NEG</td>
<td>Total</td>
</tr>
<tr>
<td>PB WHO (%)</td>
<td>192 (17.9)</td>
<td>456 (42.6)</td>
<td>648 (60.5)</td>
</tr>
<tr>
<td>MB WHO</td>
<td>352 (32.9)</td>
<td>71 (6.6)</td>
<td>423 (39.5)</td>
</tr>
<tr>
<td>Total</td>
<td>544 (50.8)</td>
<td>527 (49.2)</td>
<td>1071</td>
</tr>
</tbody>
</table>
for the ML Flow test in Brazil, Nepal and Nigeria, respectively. From the MB patients 16.8% (71/423), 41.4% (157/379) and 13.9% (5/36) were negative for the ML Flow test.

**DISTRIBUTION OF THE PATIENTS ACCORDING TO NUMBER OF LESIONS, ML FLOW AND BI RESULTS**

Figure 1 shows that only 22.7% (243/1071) and 8.9% (95/1066) of all patients from Brazil and Nepal, respectively, had six or more lesions and were positive for both the ML Flow test and smear examination for the presence of AFB. The overall smear positivity was higher in Brazil (27.1%) than in Nepal (11.6%).

**PROBABILITY THAT NUMBER OF SKIN LESIONS CORRECTLY CLASSIFIES PATIENTS WHEN COMPARING WITH ML FLOW AND SKIN SMEAR RESULTS**

Table 2 shows the proportions of patients for which WHO classification is confirmed by the smear examination and/or ML Flow test results. The ML Flow test and slit skin smear examination for the presence of AFB were negative in 69.1 and 82.7% of the PB patients and positive for 57.4 and 25.1% of the MB patients from Brazil and Nepal, respectively.

AFB were present for 6.4 and 2% of the PB patients from Brazil and Nepal, respectively. Both the smear examination and ML Flow test were negative for 15.6 and 38.3% of the MB patients from Brazil and Nepal, respectively.

**Discussion**

Even though serological tests cannot be used on their own for the diagnosis of leprosy, they could be used for classification of patients for the appropriate choice of treatment. For this application, we evaluated the ML Flow test as a point of care test in three routine leprosy control programmes and we discuss its influence on the classification of leprosy patients.

The proportions of PB and MB patients in a population of leprosy patients will affect the apparent effectiveness of any method of classification, including the ML Flow test. Classification based on microscopy is highly specific but has low sensitivity. Thus, if the presence of AFB is considered as a ‘gold standard’, any other test has increased sensitivity and reduced specificity. As seen in Fig. 1, a large number of smear negative patients (109 in Brazil and 127 in Nepal) were classified as MB leprosy by both the ML Flow test and the WHO system. The observation that the presence of AFB correlates well with ML Flow positivity, while at the same time many ML Flow positive patients are smear negative, suggests that the ML Flow test is a more sensitive indicator of bacterial load than smear examination. This hypothesis is supported by the observation that BI positive patients without detectable antibodies to PGL-I and given short course treatment did not relapse during 5 years of follow-up. Slit skin smear results depend on the site and quality of the smear, and the size of the sample collected, while the ML Flow test result represents more accurately the systemic bacterial load. We consider serology is more sensitive than slit skin smear examination for detecting MB patients.

In Brazil and Nepal, most PB patients (69.1 and 82.7%) are classified correctly by the WHO classification system, as shown by the absence of AFB and negative ML Flow test
Figure 1. Distribution of results (ML Flow, BI and classification based on skin lesions) for patients (A) from Brazil and (B) from the reference center in Nepal. In each figure, the number of ML Flow positive patients (ML Flow POS) inside the darker circle is distributed according to the other results, in the circle on the left side, the number of patients presenting six or more skin lesions (WHO MB) and in the circle below the number of BI positive patients (BI POS) are given. Numbers in italics represent number of patients classified as MB according to number of lesions but ML Flow negative. Numbers underlined represent number of patients who are ML Flow positive but classified as PB according to number of lesions.
results (Table 2). Smear positivity confirmed misclassification occurred in only 6.4 (34 + 8/648) and 2% (13 + 1/687) of PB patients in Brazil and Nepal, respectively.

Major differences were found in Brazil and Nepal as regards MB patient classification. In the MB group, a big difference is observed between Nepal and Brazil: the MB classification was confirmed by the presence of AFB in 59% (243 + 5/423) of the Brazilian MB patients, but only in 28% (95 + 12/379) of the patients in Nepal (Table 2). The method used for slit skin smear examination in both countries was the same, and a QC system is in place. However, the quality of the slit skin smear examination may differ between the two countries. The proportion of smear positive MB patients is significantly lower in Nepal than in Brazil, (P < 0.001). The unexpectedly large group of MB patients with a positive ML Flow result who were smear negative (25.8 and 33.5% of the MB patients in Brazil and Nepal, respectively) is another indication that slit skin smear examination may not be sensitive enough. These smear negative seropositive patients may represent additional confirmed MB cases. The proportion of MB patients who are both ML Flow and smear negative is surprisingly high in Brazil (66/423 = 15.6%) and even higher in Nepal (145/379 = 38.3%) (Table 2). We assume that these patients are being misclassified by the WHO system. This difference in proportions may be influenced by differences in the immune response between the two populations and/or differences in the severity of infection. It may be possible that the ideal cut off point for the number of lesions classifying patients as MB could differ among countries.

In Nigeria, the ML Flow test was only compared with the WHO classification, as slit skin smear examination is not routinely performed in the facilities involved in the study. The data from Nigeria presented here use the WHO criteria to define MB leprosy, but in the field there was a greater tendency to classify patients as MB, since a much lower threshold of five lesions plus affected nerves was used. According to assessment by the health care workers, 55.9% (104/186) of patients had five skin lesions and 73.1% (136/186) had multiple-nerve involvement. This led to 95.7% of patients actually being treated as MB, as opposed to 19% if the WHO criteria had been strictly applied (data not shown). It supports the study observation (data not shown) that health care workers may over-diagnose MB leprosy in an attempt to avoid under-treatment. In both Brazil and Nepal, slit skin smear results were available and

<table>
<thead>
<tr>
<th>Classification based on WHO lesion counting</th>
<th>Confirmatory tests</th>
<th>Brazil</th>
<th>Nepal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ML Flow</td>
<td>BI</td>
<td>n</td>
</tr>
<tr>
<td>PB patients POS POS</td>
<td>34</td>
<td>5.2</td>
<td>13</td>
</tr>
<tr>
<td>NEG POS</td>
<td>8</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>POS NEG</td>
<td>158</td>
<td>24.4</td>
<td>105</td>
</tr>
<tr>
<td>NEG NEG</td>
<td>448</td>
<td>69.1</td>
<td>568</td>
</tr>
<tr>
<td>Total</td>
<td>648</td>
<td>100</td>
<td>687</td>
</tr>
<tr>
<td>MB patients POS POS</td>
<td>243</td>
<td>57.4</td>
<td>95</td>
</tr>
<tr>
<td>NEG POS</td>
<td>5</td>
<td>1.2</td>
<td>12</td>
</tr>
<tr>
<td>POS NEG</td>
<td>109</td>
<td>25.8</td>
<td>127</td>
</tr>
<tr>
<td>NEG NEG</td>
<td>66</td>
<td>15.6</td>
<td>145</td>
</tr>
<tr>
<td>Total</td>
<td>423</td>
<td>100</td>
<td>379</td>
</tr>
</tbody>
</table>

* Based on BI result.
there was no significant difference in the level of MB over-diagnosis. Whereas in Nigeria slit skin smear results were not available possibly making health care workers even less confident resulting in a tendency to over-diagnose MB leprosy.

In our data analysis we used only the number of skin lesions in order to classify patients, but this is not what happens in practice. In all three countries, in addition to the number of lesions as recommended by the WHO classification system, enlarged nerves were also taken into account. Our results show that the WHO classification system is in fact an adequate operational tool, although poorly adhered to in practice and probably very difficult to implement appropriately. In everyday practice, the ML Flow test will be at least as reliable, because it is a less emotive and more objective measure and will give the health worker increased confidence in the classification of especially PB leprosy. The proposed WHO solution to the operational problems is to avoid classification entirely by instituting uniform MDT (UMDT) whereby all patients would receive the same drugs for the same length of time. However, UMDT may not be sufficient for certain groups of patients with high bacterial loads while over treating those patients with PB disease and needs to be evaluated further before it is implemented. The distinction between multibacillary disease and paucibacillary disease is also important for reasons other than allocation of treatment, notably for estimating the risk of reactions and of nerve function impairment and the need for close supervision during and after MDT. We suggest the ML Flow test would provide a means of classification that would be acceptable and applicable in field settings for all these purposes.

If under-treatment is to be prevented, then only patients classified as PB would need to be tested by the ML flow test, and patients who test positive would be treated as MB. Used in this way, the ML Flow test would also detect the vast majority of patients who are smear positive but who have been misclassified as PB because their skin lesions were scanty or difficult to see: 81% (34/42) in Brazil and 93% (13/14) in Nepal (Fig. 1).

Another possibility would be to use the ML Flow test result as the sole criterion for classification of patients for treatment purposes. In this scenario all ML Flow positive patients would be regarded as MB and all ML Flow negative patients as PB. As a result, in Brazil, Nepal, and Nigeria respectively 25% (192 + 71/1071), 26% (118 + 157/1066) and 48.9% (86 + 5/186) of the patients would receive a different treatment regimen from that determined by the WHO classification (Table 1). It would represent an increase of 11.3% and 43.5% of patients treated as MB in Brazil and Nigeria, respectively, and it would represent a decrease of 3.7% in Nepal. From the group of smear positive patients, 4.5% (5 + 8/290) and 10.7% (12 + 1/121) would be incorrectly classified by the ML Flow test in Brazil and Nepal respectively and treated as PB patients, as compared with 2.8% (8/290) and 0.8% (1/121) who would have been missed by the WHO classification (Fig. 1). Further studies will be necessary to investigate whether this group of patients under-classified as PB has indeed a higher risk of relapses and/or reactions.

Currently, studies are ongoing to evaluate the efficacy of 6 months UMDT for all patients. UMDT has the advantage of convenience of administration but the disadvantage that the regimen may be too short for heavily infected MB patients.8 A simple test that would be able to identify heavily infected MB patients who need longer treatment would be useful, and would give confidence to health care workers to accept the shorter regimen for the others. At the moment the ML Flow test is too sensitive for this task because many PB and smear negative patients were seropositive. The test sensitivity could, however, be adjusted to suit this particular task if required.
In conclusion, in leprosy control programmes in which slit skin smear examination is not available, the ML Flow could be used to improve treatment decisions. It may reduce the need for slit skin smears which are invasive and potentially hazardous. Even if developments in chemotherapy were to lead to a uniform treatment regimen for all patients, it should be remembered that PB and MB patients have different risks for reactions, nerve function impairment, relapse and disability. Distinguishing between patients with low and high bacterial loads will therefore remain important for appropriate clinical management.9

Acknowledgements

The Netherlands Leprosy Relief (NLR) financially and logistically supported this project. The NT-P-BSA used for the production of the ML Flow tests was kindly provided by Dr Fujiwara, Institute for Natural Science, Nara University, Nara, Japan. We would like to thank all participants of the study for their input. We are thankful to the two anonymous referees and especially to Dr Anthony Bryceson, the sub-editor, for their constructive criticism.

Authors’ contributions

S. B. S. participated in the design of the study, trained the participants, supervised the study, performed operational data analysis and drafted the manuscript. J. V. participated in the design and supervision of the study. M. A. G., K. P. D. and A. U. N. supervised the study and raw data collection in their respective countries. P. K. and L. O. participated in the design of the study. All authors participated in the interpretation of data, read and approved the final manuscript.

References

7 Buhrer-Sekula S, Visschedijk J, Grossi MAF et al. Combining operational and anthropological studies; experience with a point of care test for leprosy in Brazil, Nepal and Nigeria. Unpublished data.