A simple dipstick assay for leprosy: development, evaluation and application

Bührer-Sékula, S.

Citation for published version (APA):
Summary and Conclusions

*Mycobacterium leprae* is an acid-fast, slow growing, obligate intracellular bacillus that preferably invades the nerve Schwann cells and macrophages. The rate at which leprosy spreads in a community depends on the proportion of susceptible individuals in the population, the opportunity for contact with an infected person and the force of infection in the community. The outcome of infection is based on the capacity of the host to mount an effective immune response. Leprosy has a long incubation period and it generally takes two to five years for the disease to occur. In the great majority of infected persons the cellular immune response is efficient and clinical symptoms never appear (subclinical infection). When clinical symptoms appear the disease manifestation varies according to the immune response of the patient, the so-called leprosy spectrum.

Physically and psychologically, leprosy is a striking disease. Initially leprosy symptoms may be hardly noticed. During the course of the disease skin lesions become more visible and nerves are damaged irreversibly resulting in deformities, and handicaps. The consequent stigma generates an extensive fear in both patients and the general public.

Lesions are frequently noted on exposed surfaces of the skin but can be present all over the body. They may be single or numerous, varying widely in form, appearance, and color.

Early diagnosis is a key to the interruption of leprosy transmission. Silent transmission is facilitated by the slow growth characteristic of the bacilli, the long incubation period of leprosy, the slow progress of the irreversible nerve damage and the stigma of leprosy. The diagnosis of leprosy is based on clinical signs and symptoms that can be recognized by a health worker after a period of training. In endemic countries the diagnosis is usually made clinically on the presence of skin lesions, anesthesia of the skin lesions, thickened nerves, and, if suitable laboratory facilities are available, the presence of acid-fast bacilli in slit skin smears.

Serological techniques for leprosy are aimed at the detection of specific anti-*M.leprae* antibodies, which are reflective of current infection and useful for follow-up during therapy and for the assessment of the prevalence of the disease and the spread of infection in the community. Although the backbone for serology is at the moment the ELISA for the detection of IgM antibodies to PGL-1, this assay is too complicated to be applied in most areas were leprosy remains a public health problem. In order to simplify the use of serology in leprosy control a simple assay is urgently required. This thesis describes the development, evaluation and application of a simple dipstick assay (ML Dipstick) for the detection of IgM antibodies to PGL-1 of *M.leprae*. 
Chapter 1 gives a general introduction on leprosy. Epidemiology, clinical diagnosis, classification, treatment and control are all discussed after which an extensive overview of the knowledge in the field of leprosy serology is given.

Chapter 2 describes the development of the ML Dipstick for the detection of IgM antibodies to PGL-I of *M. leprae*. A high degree of agreement was observed between the ELISA and the dipstick assay when tested on 435 sera. No significant difference was found between the dipstick assay and ELISA when seropositivity rates obtained in groups of leprosy patients, household contacts, and controls were compared. The interpretation of the dipstick results as positive or negative was unequivocal. The test does not require any specialized equipment and the highly stable reagents make the test robust and suitable for use in tropical countries.

Chapter 3 describes a further simplification of the ML Dipstick assay by using whole blood and an evaluation of the assay performance in the leprosy endemic area of Amazonas in Brazil. The agreement with the "gold" standard ELISA was 94.9%. This simple assay may be useful to identify those at risk of developing leprosy, for example among contacts of leprosy patients at lower levels in the health services.

Classification of leprosy patients into PB and MB determines the duration of their treatment. MB patients are treated for a period of 24 months with a monthly supervised combination therapy consisting of rifampicin, clofazamine and dapsone, whereas PB patients are treated for 6 months with rifampicin and dapsone. MB patients are thought to be the main source of transmission, therefore it is especially important to diagnose MB patients timely and correctly. Misclassification increases the risk of relapse due to insufficient treatment if a multibacillary (MB) patient is classified as paucibacillary (PB), thereby also prolonging the time that the patient is infective.

Chapter 4 shows how ML Dipstick can contribute to improved classification of leprosy patients for treatment purposes. In this chapter the results of ML Dipstick were combined with clinical classification by counting the number of lesions. Results were compared with the classification based on the bacteriological index. In this study 264 leprosy patients were investigated. The classification based on the number of lesions only was found to be 85% sensitive and 81% specific (using the BI as the gold standard) at detecting MB cases among the study population. Sensitivity would have increased if patients would have been classified according a combination of the number of lesions and the dipstick result. This combination method was found to be 94% sensitive and 77% specific, which is an improvement of 9% in the sensitivity compared to lesion counting only. The results of this study indicate that testing with the dipstick all patients initially classified by lesion counting as PB can significantly contribute to improved classification of leprosy patients for treatment purposes.
Summary and Conclusions

With the integration of leprosy control into the general health system, diagnosis and classification will be primarily in the hands of less experienced professionals. Misclassification leads to a higher risk of relapse. Identifying those patients who have high antibody levels would in all probability recognize patients that have a high bacterial load and consequently should receive longer treatment.

Chapter 5 investigates whether ML Dipstick is capable of identifying patients with a higher risk of relapse after treatment. The sensitivity of the dipstick test for detection of MB patients was 85.1%, the specificity 77.7%. We found that of the 71 dipstick negative PB patients 35.2% were clinically cured at the end of treatment, compared to only 9.5% of the 21 dipstick positive PB patients. Nine of 170 (5.3%) patients in the study population relapsed within the 5-year follow-up period. Seven were MB patients, all of which were dipstick positive. Two PB patients relapsed, one was dipstick negative and one was dipstick positive. From this it can be concluded that dipstick positivity is a risk factor for the future development of relapses, especially in those groups of patients who receive a shorter-than-usual course of treatment and that the dipstick can be used as an additional, simple tool for classification of patients and for identification of those patients who have an increased risk of relapse.

Chapter 6 shows the results of an epidemiological study performed in Brazil using ML Dipstick for the detection of seropositivity among 7073 school children in three different leprosy endemic states. It was examined whether seropositivity rates could be related to leprosy detection rates and whether seropositivity could be used as a proximal indicator to predict the leprosy incidence in other areas. As such it would be useful to detect the effect of control measures. This study shows a widely varying distribution of seropositivity in the communities independent from the number of leprosy cases detected. No differences in the patterns of seropositivity between ELISA and dipstick were observed. Based on these results it is not possible to make definite conclusions on the hypothesis that seropositivity and leprosy prevalence in a community correlate.

In summary, this study shows that ML Dipstick test is simple and robust and suitable for use in tropical countries. The results obtained correlate well with the ELISA results and the ML Dipstick can be used on both serum and whole blood. It was also shown that ML Dipstick can be used as an additional tool for the classification of leprosy patients into PB and MB for treatment and to identify patients who have an increased risk of developing a relapse. It may be used for early identification of infection in contacts. As such the test has a wide applicability in the field of leprosy control and should be a valuable addition to the leprosy field worker’s diagnostic tools.