Editorial

SEROLOGY: RECENT DEVELOPMENTS, STRENGTHS, LIMITATIONS AND PROSPECTS: A STATE OF THE ART OVERVIEW

Summary Specific antibodies can be used as a surrogate marker for bacterial load in leprosy. Tests to detect antibodies can be used for (i) the classification of patients for treatment purposes [most multibacillary (MB) patients are seropositive, most paucibacillary (PB) patients are not], (ii) the prediction of an increased risk of relapse and (iii) the identification of contacts having an increased risk of developing leprosy. With the advent of fast, robust and easy to perform serological tests such as lateral flow, agglutination and card tests, the application of serology in the field for these purposes becomes a feasible prospect. We hereby present an overview of the current knowledge and new developments in this area and discuss the strengths, limitations and possible applications of antibody detection in leprosy research and control.

Introduction

*Mycobacterium leprae* is the causative agent of leprosy, a debilitating disease that affects over 700,000 new patients each year. Even though there is an effective cure available for leprosy in the form of multidrug therapy (MDT), millions of cured patients still suffer from the consequences of the nerve damage that forms the hallmark of leprosy.

The current strategy for combating leprosy is based on the early detection and treatment of patients to halt transmission. This is based on the assumptions that (i) there is no environmental reservoir for the leprosy bacillus that is important for human transmission; and (ii) only people with clinical symptoms are infectious. This latter point, which has implications for control strategies, is still open for debate.

A number of diagnostic issues hampers the correct and timely diagnosis of leprosy and the correct classification of the patients for treatment purposes: (i) bacterial examinations are often not in use; (ii) there is a tendency to overdiagnose single lesion PB leprosy; and (iii) early (borderline) lepromatous often goes unnoticed due to the absence of sensibility loss and lesions. Approximately 70% of leprosy patients can be diagnosed on the basis of the single sign of skin patches with sensory loss, but 30% of patients, including many MB patients, do not have this clinical sign. The delayed detection of this latter group of patients may be a major cause of continued transmission. In addition, integration of leprosy control programs into general health services, in combination with the fact that leprosy is a rare disease, poses a threat to the continued availability of sufficiently experienced health workers to diagnose
leprosy. Diagnostic tools are thus very much needed to ensure the correct and timely diagnosis and treatment of leprosy patients.

A number of groups have investigated the use of serology for diagnostic, follow-up and control purposes. A small number of articles describe the detection of M. leprae antigens in blood, serum or plasma, but the vast majority of the work has concentrated on the detection of antibodies. This article reviews the current knowledge and new developments, and discusses the strengths, limitations and possible applications of antibody detection in leprosy research and control.

**Antigens under investigation and in use**

In the 1980’s leprosy serology started out with the use of crude M. leprae sonicate. Due to problems with cross-reaction much effort was put into the identification and purification of specific M. leprae antigens. A number of protein antigens were identified of which the 35 kDa protein is still in use, but the most widely used antigen today is the phenolic glycolipid I (PGL-I) of M. leprae. IgM antibodies are raised against its terminal trisaccharide which is considered to be species-specific. No cross reactivity was observed with sera from patients with M. tuberculosis, M. kansasii, M. avium and M. intracellulare infections.²

As M. leprae cannot be cultured in vitro, the production of sufficient quantities of PGL-I for serology was a problem. Moreover, the apolar nature of the lipid part of the molecule made coating of the whole antigen to ELISA plates difficult. To overcome these problems, the groups of Brennan, Fujiwara and Gigg synthesized semi-synthetic derivatives in which the terminal di- or trisaccharide part of PGL-I is coupled with a linker to a protein carrier molecule such as bovine serum albumin (BSA). This has led to a whole range of semi-synthetic antigens for use in leprosy serology. The most widely used ones are currently natural disaccharide linked to BSA via an octyl linker (ND-O-BSA) and natural trisaccharide linked to BSA via a phenyl linker (NT-P-BSA).

**Tests in use**

Even though a variety of tests have been developed over the years for the detection of anti-M. leprae antibodies, such as the radioimmunoassay (RIA) and the monoclonal antibody inhibition test, the most widely used test at the moment is the direct enzyme linked immunosorbent assay (ELISA). In this ELISA M. leprae antigen is coated to the wells of the ELISA plate after which the blood product to be measured is added to the well. If antibodies are present, these bind to the antigen and can then be detected by a labeled conjugate.

Antibodies can be detected in serum, plasma, whole blood, capillary blood and filter paper blood. One comparative study showed that there was a strong correlation between the results obtained with serum and filter paper blood but that the titers obtained with filter paper blood were lower at the same dilution.³

The ELISA is a widely used and versatile technique, but its main drawbacks in the endemic settings where leprosy is most prevalent are its requirements for trained personnel, expensive equipment and consumables that need to be stored in a refrigerator. Also, it takes typically about 1 day before results are available.
In order to make the application of serological tests more convenient in field situations, a number of rapid, robust and easy-to-use tests have been developed. The first test that became available was the *M. leprae* particle agglutination test (MLPA test). In 1999 Roche et al. described the use of a card test for the detection of antibodies directed against the 35 kDa protein of *M. leprae*. Unfortunately these tests are no longer available due to discontinuation of the production. In 1998 Bührer et al. described the development and evaluation of a simple dipstick test that was capable of detecting anti-*M. leprae* IgM antibodies within 3 h, with results showing a 97.2% agreement with ELISA. Further development led to a lateral flow test which can detect antibodies in 10 min (Bührer et al., submitted for publication). Figure 1 shows this ML flow test.

As leprosy is a poverty-related disease, the development and production of leprosy diagnostics has never been a priority for industry, as witnessed by the discontinuation of the production of both the MLPA test and the 35 kDa card test.

Over the years a vast number of studies have been devoted to the evaluation and application of serological tests, particularly ELISA assays, for leprosy. One should be careful when comparing the results obtained in the various studies: not only may the antigen used vary, so may the protocol for performing the ELISA, especially with regard to: (i) the dilution

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**Figure 1.** The lateral flow test for the detection of anti-*M. leprae* IgM antibodies in blood or serum.
of the blood product (varying between different studies from 1:20 to 1:500), (ii) the substrate, and (iii) the criteria used to determine seropositivity. Although a standardized procedure has been published, this is not widely in use. An additional complicating factor for comparing results obtained with the different studies is the fact that the WHO endorsed classification definitions of patients for treatment purposes have changed over the years. In the 1980s an MB patient had to have a positive skin smear result of at least 2+; this later changed to any positive skin smear result and since the end of the 1990’s an MB patient is any patient with more than 5 lesions. All these variables make the comparison of serology evaluation studies difficult, but it is possible to draw a number of general conclusions as shown in the following chapters.

**Serology for the diagnosis of leprosy and reactions**

Antibody production probably does not play a protective role in leprosy but it varies along the leprosy spectrum as a consequence of the graded bacillary load from the tuberculoid to the lepromatous pole, as shown in Figure 2. Numerous studies have shown that antibody levels can be used as a surrogate marker for the bacterial load, with a widely varying but positive correlation between antibody levels and bacterial index (BI). Antibody levels are particularly elevated in MB patients. Studies show that typically between 75 and 100% (and mostly well over 90%) of the MB patients are seropositive. In PB patients the situation is very different: only 15–40% of the PB patients elicit an antibody response detectable by ELISA with the seropositivity rate being higher in those PB patients that have a higher disability grade. In patients with pure neural (PN) leprosy the seropositivity is around 50%. This means that serology cannot be used as a single diagnostic test for the identification of leprosy patients as at least 50% of the PB and PN patients would be missed. Serological

![Figure 2. Relationship between antibody levels and other leprosy parameters.](image-url)
results always have to be considered together with other clinical and diagnostic information. However, especially for PN leprosy seropositivity is important for confirmation, as this form of leprosy is notoriously difficult to diagnose.

Serology can play an important role in aiding the correct classification of leprosy patients that are newly diagnosed on the basis of clinical symptoms. The combination of lesion counting and serology is especially useful in those situations where skin smear examination services are not in use and leprosy control workers have to rely on lesion counting alone. Bührer et al.\textsuperscript{22} showed that a combination of lesion counting and serology led to a significant decrease (9\%) in misclassification of MB patients compared to lesion counting only. This study demonstrates that serological testing of patients with fewer than six lesions and putting any seropositive patient on MB treatment may prevent under-treatment.

Despite the fact that reactions are the major cause of nerve damage in leprosy, only few studies have addressed the question whether serology could be used to diagnose or even predict reactions and results are conflicting. One study showed that in patients with type 2 reactions (erythema nodosum leprosum, ENL) the occurrence of ENL had no significant effect on antibody levels.\textsuperscript{23} Two other studies found that ENL was related to a decrease in antibody titre.\textsuperscript{24,25} For type 1 reactions (reversal reactions, RR), there is also conflicting evidence suggesting that titres either decrease\textsuperscript{26} or increase\textsuperscript{21,27} before episodes of RR. Further research is needed to clarify these issues.

Serology for monitoring of treatment and prediction of relapse

Antibody levels start to decline in most patients once treatment has been started. The decline is typically 25–50\% per year,\textsuperscript{13,21,29–31} but this may vary widely between individual patients: in some patients antibody level declines are linear and quick, in others the decline is slow and the patient remains positive for years after official cure.\textsuperscript{32} Some authors suggest that this may be due to dead or dormant bacteria that are still present.\textsuperscript{33,34}

It is interesting to notice that an increase in antibody levels may be a first indicator of a relapse.\textsuperscript{35,36} However, this can be suppressed in patients treated with immunosuppressive drugs for reversal reactions.\textsuperscript{35} A study on serology in combination with a treatment trial showed that seropositive patients had an increased risk for the future development of relapse, especially in those groups of patients that had received a shorter-than-usual course of treatment.\textsuperscript{37}

Serology cannot distinguish between past and current infection,\textsuperscript{28} nor can it be used as a sole tool to distinguish between clinical and sub-clinical infection.

Serology for screening contacts

Contacts of leprosy patients run an increased risk of developing leprosy compared to the general population in an area. It is common practice in most leprosy control programs to examine household contacts of leprosy patients for the presence of signs and symptoms of leprosy. However, the majority of the new patients may be missed in this way. A study in Indonesia\textsuperscript{38} showed that, whereas 28\% of the incident leprosy patients were household contacts of other leprosy patients, at least 80\% of all new leprosy patients could be related to other patients by broadening the definition of ‘contact’ to include neighbors and social
contacts. This is analogous to the stone-in-the-pond principle for contact examination in tuberculosis, in which contacts are defined in concentric circles around the index case (see Figure 3).

A number of studies have addressed the differences in seropositivity between patients, household contacts and the general population or controls. Not all studies find higher percentages of contacts positive compared to the general population or controls. It seems that this difference may be due to varying prevalences of leprosy: whereas significant differences in seropositivity may\textsuperscript{39,40} or may not\textsuperscript{41,42} be found in low and medium endemic areas, no differences were observed in high endemic areas.\textsuperscript{20,28,43–45} This latter observation may be due to the fact that in high endemic areas (prevalence >1%) a large part of the population and not only contacts is exposed to \textit{M. leprae} on a regular basis.

Research in the Philippines among 559 household contacts showed that seropositive household contacts run a highly increased risk of developing leprosy (relative hazard 8) and especially of developing MB leprosy (relative hazard 25) compared to seronegative household contacts (Douglas \textit{et al.}, submitted for publication). This is a strong indication that seropositivity can be used for the identification of contacts at risk of developing leprosy in future. This may have direct implications for leprosy control: one could either give seropositive contacts chemoprophylactic treatment or monitor them closely to prevent delayed diagnosis of newly developing leprosy. Further research is needed to determine whether seropositive asymptomatic persons are also sources of transmission.

**Serology for screening the general population**

General IgM antibody levels vary with age and reach peak levels in young adulthood; they are also consistently higher in females than males at all ages. Anti-\textit{M. leprae} antibody levels show a unimodal rather than bimodal distribution in endemic areas, probably due to widespread exposure of the population to the leprosy bacillus. There is no clear-cut distinction between seropositives and seronegatives,\textsuperscript{20,44} so any criterion for positivity is arbitrary and can be the subject of discussion.

Total population surveys suggest that subclinical infection is far more common than overt disease as antibodies against \textit{M. leprae} can be detected in 1.7–31% of the endemic population.\textsuperscript{28,39,41,43,46} This percentage varies widely with the prevalence of leprosy in the area and thus with the potential exposure of the population to the bacterium.
Sometimes seropositivity is assumed to be at least partly due to cross-reactivity with certain environmental mycobacteria, but this has never been proved.

In two studies, the potential of using school children surveys to obtain an impression of the leprosy situation in an area was investigated. School surveys target an easily accessible, stable and standardized population and if there would be a correlation between the seroprevalence in this particular group and the leprosy prevalence this type of studies could be used for a quick evaluation of the leprosy situation and, when used repeatedly, to evaluate the effect of control measures. Van Beers et al. found that in Sulawesi, Indonesia the seroprevalence among school children in two different areas correlated well with the leprosy prevalence. These results could not be repeated in a more extensive study by Bührer et al. (manuscript in preparation) in Brazil where antibody levels in school children from nine different municipalities were investigated: there was no correlation between seroprevalence in school children and leprosy incidence in the municipalities. The difference may be due to the different methodologies used and/or to the fact that the variety in the socio-economic background of the study population was much higher in Brazil.

Future developments and applications

A number of opportunities can be identified for further development and application of serological tests. First of all, the availability of rapid, robust and easy-to-use field tests will allow a much wider use of serology than what was until recently possible. Also, the sequencing of the whole *M. leprae* genome and the subsequent data analysis may identify novel antigens that could be of use in serology, especially for the early prediction of reactions. Also, the accurate diagnosis of PB leprosy may become feasible, if not by serology then by novel, antigen-based skin tests.

A further evaluation of (PGL-I) serology as a marker for high risk groups suitable for chemophylaxis or other interventions is necessary. Two further major applications for serology apart from the applications mentioned above are: (i) in field trials where new treatment regimens are evaluated (most notably the uniform 6 months treatment regimen). Serology may be of use to identify patients with high bacterial loads that require longer treatment in order to prevent relapse; and (ii) for research in combination with the measurement of cellular immune responses to get a better insight into the transmission of leprosy, a subject of which much remains unknown but which is crucial to the ultimate elimination of leprosy.

Conclusions

The most widely used serological test at the moment is the ELISA, using PGL-I derived antigens for the detection of anti- *M. leprae* IgM antibodies. This test is mainly suitable for research purposes or use in reference laboratories, but the newly developed rapid tests, such as the ML Flow test, make the incorporation of serological testing into peripheral levels of health care and routine leprosy control programs feasible.

Serology cannot be used as a single diagnostic test for leprosy as the majority of the PB patients are seronegative. Neither can it be used for population screening or for distinguishing past and present infection. However, serology is useful for (i) the classification of patients
into MB and PB for treatment purposes, (ii) the early prediction of relapses, (iii) for the identification of persons that run an increased risk of developing leprosy among high risk groups such as contacts and (iv) (to a limited extent) for follow-up of treatment.

Further research into the use of serology for studying transmission and into the identification of new antigens, especially for the early prediction of reactions, is needed.

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