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Serological screening for tuberculosis in the community: an evaluation of the Mycodot procedure in an African population with high HIV-2 prevalence (Republic of Guinea-Bissau)

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Abstract

The immuno-dot-blot assay MycoDot™, which detects lipoarabinomannan (LAM) antibodies, was evaluated for the serological diagnosis of active pulmonary tuberculosis in patients in a rural community in the Republic of Guinea-Bissau. Sera from 269 adults (age >15) and 33 children (age < 5) were assayed for antibodies in a blind manner and the results compared to the clinical status of tuberculosis. The assay had a specificity and a sensitivity of 92.4% and 63.0% respectively, when applied to the adult population. In HIV-2 infected individuals (27/269), the specificity and sensitivity of the assay were similar, 94.7% and 62.5% respectively. The assay did not provide high sensitivity for the diagnosis of tuberculosis in children. Sera from patients with leprosy cross-reacted with the antigen of the assay. It is concluded that this easily performed assay may be useful for the presumptive diagnosis of tuberculosis in adult populations in rural areas of developing countries where routine screening is not readily available.

Keywords: Tuberculosis; HIV-1; HIV-2; Serology; Diagnosis; Lipoarabinomannan

1. Introduction

In developing countries AFB microscopy is the major means of diagnosis of tuberculosis. When accurately performed, AFB microscopy identifies only about half of the patients with pulmonary tuberculosis (TB) [23]. However, in economically disadvantaged countries, such a service may not be readily available. Therefore, a considerably larger number of TB cases may escape detection namely due to the sequential visits required of the patient for AFB microscopy. In addition, AFB microscopy needs both a working microscope and a competent microscopist. Both of which are in short supply in economically deprived countries.

Diagnostic methods readily employed in developing countries can be those that are based on serological procedures. Serological procedures are economical, easy to perform, require little or no equipment, and may require a single patient visit to the health center.

Various serological methods have been evaluated for the diagnosis of TB. However, not all lend themselves well to conditions commonly found in the rural areas of developing countries. As an example, whereas ELISA based techniques that employ purified antigens for the detection of TB seromarkers appear promising [7], they require a number of manipulations, expensive materials (microwell plates), temperature controls (incubators) and instruments that read the results; hence a source of electricity is needed. In contrast, immuno-dot-blot techniques on solid supports (membranes) involve one or two pipetting steps, require no special equipment, and most importantly, yield consistent and reproducible results [4,15].

Diagnosis of TB by serological procedures using a single antigen has been proposed. Despite much controversy with respect to their sensitivity, promising results have been obtained with purified antigens of mycobacterial origin, namely the cell wall glycolipids: phenolic glycolipid

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PGL-Tb1, diacyl trehalose (DAT) (formerly SL-IV) and lipoarabinomannan (LAM) [6,9,13,14,19,20].

Among the cell wall glycolipids, detection of antibodies against LAM has been reported to offer high potential for the screening of active TB, inasmuch as in the most recent report in Denver, CO, USA, the sensitivity by immuno-dot-blot assay was evaluated at 85% [4], in a country that has all that is needed for the diagnosis of TB and where it could be assumed that little would be added to this task by the addition of this type of assay. However, it has precisely those qualities, which make its use very attractive for the diagnosis of presumptive TB in patients who reside in very rural areas such as those commonly present in many areas of Africa.

It was therefore our intention to evaluate the specificity and sensitivity of the currently available MycoDot™ assay, based on the detection of LAM antibodies using an immuno-dot-blot technique, for the identification of active TB in patients from a rural population in the Republic of Guinea-Bissau, a country where TB is highly prevalent and which is also currently the epicenter of HIV-2 infection [5].

2. Materials and methods

2.1. Population studied

The fieldwork was done from February 1995 to March 1996 in the Nhacra sector (Republic of Guinea-Bissau, West Africa), a rural area between the rivers Mansoa and Geba, 26 to 51 km from the capital. A total population of 18 353 was censored (Ministry of Health, 1994). For this, the total number of "tabancas" (traditional villages) within the sector, and of individuals within each "tabanca", were considered. Random sampling of the houses within each "tabanca", and random sampling of the individuals within each house led to the selection of a group of 269 adults (age >15). A second group of 33 children (age < 5), not having received the BCG vaccination, was also examined.

The laboratory diagnosis of TB and other associated pathologies was carried out at the Centro de Medicina Tropical in Bissau.

Tuberculosis was diagnosed based on the signals and symptoms of the disease followed by clinical examination and laboratory data (in particular AFB microscopy and erythrosedimentation; chest X-rays were not available). Individuals with negative AFB microscopy and no clinical signs of active disease were considered non-tubercular. Conversely, all individuals with positive clinical evaluation and AFB microscopy, obtained from one sputum sample, were considered as actively infected with pulmonary TB. In children, non-BCG vaccinated and less than 5 years of age, Mantoux positive reactions ($\geqslant 10$ mm) were considered recent primary infections.

Patients with leprosy were referred to by the Hospital of "Mal de Hansen" of Cumura based on clinical observation.

The HIV status of the individuals was carried out using serological techniques (HIV-SPOT, E.Y. Laboratories For Genelabs Diagnostics, Pte Ltd, Singapore). If the tests were positive they were confirmed by enzyme-linked immunosorbent assay (ELISA) (Enzygnost anti-HIV-1, and Enzygnost anti-HIV-2, Behringewerke, Germany), performed in the Laboratório Nacional de Saúde Publica (LNSP), Bissau. When discordant they were reconfirmed by Western blotting (New Lav Blot I, New Lav Blot II, Sanofi Pasteur, France) at the Microbiology Laboratory of the IHMT, Lisbon.

2.2. Serology

The MycoDot™ serological assay was purchased from Mossman Associates (Blackstone, MA, USA). It is based on the detection of specific immunoglobulin G antibodies against the LAM antigen, fixed onto a solid support consisting of a plastic comb designed to fit into the wells of a microtiter plate. The principle of the test is that each comb is first incubated in wells containing diluted serum, where specific antibodies from the sample, if present, bind to the antigen. Then it is incubated in wells containing a suspension of colored particles that will bind to the bound anti-LAM antibodies generating a red spot. Likewise, 48 tests may be performed per 96 well microtiter plate. No special equipment is necessary. The readings are carried out with the naked eye, and positive results are evaluated according to the color intensity of the red spot as compared to that of a reference comb. Incubation with the sera was prolonged to 60 min, as suggested for increasing the yield of the test [4]. The interpretation of the test results was done as recommended by the manufacturer. Spots with low color intensity (borderline reactions), of subjective interpretation, were considered negative. All specimens were analyzed in a blind manner and the results of each test correlated with the TB status of the patient.

3. Results

Sera from the selected population of 269 adults were assayed for anti-LAM IgG antibodies in a blind manner using the MycoDot™, and the results compared to the clinical status of tuberculosis (Table 1). These serum samples were from 46 individuals with definite pulmonary TB, and from

Table 1
Results of the MycoDot™ test in the rural population between the rivers Mansoa and Geba in Nhacra, Republic of Guinea-Bissau

MycoDot™ results	Clinical condition		Total
	Smear positive tuberculosis cases	Non-tuberculosis cases	
Positive	29	17	46
Negative	17	206	223
Total	46	223	269

Sensitivity = 63.0%. Specificity = 92.4%. Positive predictive value = 63.0%. Negative predictive value = 92.4%.

a control group of 223 individuals, with no laboratory or clinical evidence of pulmonary TB. Thirty-seven of the 269 individuals (13.8%) were also found to be seropositive for HIV, with 27/37 positive for HIV-2, 4/37 positive for HIV-1, 2/37 having serology positive for both HIV-1 and 2, and 4/37 with a HIV species undetermined. In addition, the presence of other infections was determined, including 21 cases of leprosy (7.8%), of which 3/21 were concomitant with active TB.

The sensitivity and and specificity of the MycoDot™ assay was calculated for this population with results of 63.0% and 92.4% respectively (Table 1). The positive predictive value was 63.0%, and the negative predictive value was 92.4%.

Since HIV-2 is prevalent in this area, with other types of HIV representing only a small number of cases, we examined the serological results in the predominant HIV-2-positive group (27/37). In this subgroup the sensitivity and specificity of the test was 62.5% and 94.7% respectively (Table 2). The positive and negative predictive values were 83.3% and 85.7% respectively. In the HIV positive group, when the HIV type was not discriminated (37/37), the sensitivity of the assay dropped to 50.0% (Table 3), and specificity was 96.0%. The positive and negative predictive values were 85.7% and 80.0% respectively.

The presence of leprosy contributed to a rise in the false-positives. After eliminating the cases of leprosy from the population of the study, the assay had a specificity of 94.1%, with sensitivity, positive predictive and negative predictive values of 60.5%, 68.4% and 91.9% respectively (data not shown). Cross-reactions with other diseases were not apparent.

Table 2
Results of the MycoDot™ test in the HIV-2-positive populations

MycoDot™ results	Clinical condition		Total
	Smear-positive tuberculosis cases	Non-tuberculosis cases	
Positive	5	1	6
Negative	3	18	21
Total	8	19	27

Sensitivity = 62.5%. Specificity = 94.7%. Positive predictive value = 83.3%. Negative predictive value = 85.7%.

Table 3
Results of the MycoDot™ test in HIV-positive and HIV-negative populations

MycoDot™ results	Clinical condition				Total	
	Smear-positive tuberculosis cases		Non-tuberculosis cases			
	HIV+	HIV−	HIV+	HIV−	HIV+	HIV−
Positive	6	23	1	16	7	39
Negative	6	11	24	182	30	193
Total	12	34	25	198	37	232

HIV-positive
Sensitivity = 50.0%.
Specificity = 96.0%.
Positive predictive value = 85.7%.
Negative predictive value = 80.0%.

HIV-negative
Sensitivity = 67.6%.
Specificity = 91.9%.
Positive predictive value = 59.0%.
Negative predictive value = 94.3%.

Table 4
MycoDot™ results in children less than 5 years of age with respect to the Mantoux reaction

Mantoux	MycoDot™		Total
	+	−	
⩾ 10 mm*	0	4	4
5–9 mm	0	1	1
⩽ 4 mm	2	26	28
Total	2	31	33

* Mantoux-positive reactions (⩾ 10 mm) in non-BCG-vaccinated children less than 5 years of age were considered recent primary infections.

In the group of 33 children, 4 cases were considered recent primary TB infections from their Mantoux reaction (⩾ 10 mm). The MycoDot™ assay was negative in all four cases, as well as in one dubious case (Mantoux reaction 5–9 mm) (Table 4).

4. Discussion

The aim of this study was to evaluate a serological assay MycoDot™, which has been proposed for the presumptive diagnosis of active TB in an African community in the Republic of Guinea-Bissau, West Africa. This community was also chosen so as to compare the performance of the assay in HIV-positive patients, as serological tests for the diagnosis of TB are believed to be less accurate in HIV-infected persons [2,10,11,18]. However, in previous studies there was no discrimination between HIV-1 and HIV-2 infections. To our knowledge this is the first field study to evaluate a TB serological assay in the presence of a high prevalence of HIV-2 infection.

The MycoDot™ assay is based on an immuno-dot-blot technique for the detection of immunoglobulin G antibodies against the LAM antigen. Our interest in this assay was based on the simplicity of the technique, its inexpensiveness, the absence of requirements for specialized equipment and its applicability in rural areas of economically deprived countries.

A number of antigenic glycolipids identified in the mycobacterial cell wall have been envisaged for the serodiagnosis of TB: phenolic glycolipid PGL-Tb1, diacyl trehalose (DAT) (formerly SL-IV) and lipoarabinomannan (LAM) [6,9,13,14,19,20]. Among these, LAM, an abundant lipopolysaccharide constituent of the cell wall, has been reported to offer high potential for the screening of active TB [2,4,8,10,11,17–19,21]. A number of these studies were performed using the commercially available MycoDot™ assay [2,8,10,11,17,21].

Results from these different studies have shown that the specificity of the serological detection of LAM antibodies in smear-positive pulmonary TB is high (91–100%), whereas much more variable results have been obtained for sensitivity. These have generally been low, 72% [19], 18.5% [10],

56% [11], 63.2% [17], and 34.8% [8], except in two independent studies showing sensitivities of 85% and above [4, 18]. This variability in sensitivity continues to fuel the controversy concerning the diagnostic value of LAM antibody detection for the presumptive diagnosis of active TB.

Further evaluations concluded that LAM is satisfactory in the serodiagnosis of TB as long as HIV is not highly prevalent in the population. The sensitivity values observed were definitely lower in cases of TB associated with HIV: 57% [18], 10.6% [2], 14.6% [10], 25% [11] and 40.1% [17], which refuted the usefulness of the test in regions where HIV is highly endemic. HIV-1 and HIV-2 types were not discriminated in these studies.

The usefulness of the serological test is also questionable in cases of diagnosis of new TB cases as opposed to relapse TB. In one study, 47.6% of the patients with relapse TB cases were MycoDot™-positive, whereas only 9.8% of the patients with newly acquired TB were positive [10].

The population that was selected for our study was from the Nhacra sector, a rural area between the rivers Mansoa and Geba in the Republic of Guinea-Bissau, West Africa. Among the 269 adults selected for the study, 46 definite cases of definite TB were diagnosed (17.1%). The prevalence of the HIV infection was also high, 37 cases out of 269 (13.8%), mainly corresponding to HIV-2 infections (29 cases, 10.8%). A total of 21 cases of leprosy were also diagnosed (7.8%).

In this population we obtained a sensitivity of 63.0% and a specificity of 92.4%, a positive predictive value of 63.0%, and a negative predictive value of 92.4%. These results were similar in the HIV-2-infected subgroup (27/269), with a specificity and sensitivity of the assay of 94.7% and 62.5% respectively. In this case the positive and negative predictive values were 83.3% and 85.7% respectively. However, in the HIV-positive group, when the HIV type was not discriminated for (37/37), the sensitivity of the assay dropped to 50.0% (Table 3). In this case specificity was 96.0%, with the positive and negative predictive values were 85.7% and 80.0% respectively.

The apparently higher sensitivity of the test when performed on the HIV-2 infected subgroup as compared to that of HIV-1, may reflect recent reports showing differences in the pathogenicity of the two species. These reports have shown that HIV-2 is much less pathogenic, producing a longer asymptomatic period, than HIV-1 [1]. However, as the interplay between HIV-2 and the host immune system has only recently been addressed, further studies including a larger group of patients would be necessary to clarify our results, suggesting that, contrary to HIV-1, the serological screening of the TB infection may be achievable in HIV-2-prevalent populations.

If we consider the diagnosis of TB in the group of 33 children, the sensitivity of the MycoDot™ assay was not sufficient to confirm the diagnosis with respect to the Mantoux reaction (Mantoux reactions $\geqslant$ 10 mm considered as recent primary infections), as none of the 5 children who were either Mantoux-positive or Mantoux-dubious (5–9 mm) were MycoDot™-positive. In this respect our results confirm those of Julian and collaborators who considered MycoDot™ inappropriate for the diagnosis of newly acquired TB [10].

In our study, the presence of leprosy contributed to a rise in false-positives, indicating the non-specificity of this antigen in mycobacteria. These results are in agreement with those of previous authors showing that the LAM antigen also gave promising results in the serodiagnosis of leprosy, as anti-LAM antibodies are also found in the leprosy patients [12]. While, in a purely statistical perspective, this cross-reaction with leprosy is a drawback, it might be an asset in the field , since the clinical diagnosis of leprosy is highly characteristic.

We concluded that this easily performed serological assay may be useful for the presumptive diagnosis of active pulmonary TB in adults living in rural areas of developing countries where routine screening is not readily available. These results were confirmed in an area where HIV is endemic (37/269), and HIV-2 predominant (29/37). Likewise, the importance of TB in the HIV-2 infection was also confirmed by these observations, as in a previous report by one of us [5].

The serological diagnosis of TB has also been suggested in cases where patients are unable to produce adequate sputum, are smear-negative, or are suspected to have extrapulmonary TB [22], the latter possibly being more common in HIV-positive patients [3,16].

It is also recommended that LAM be tested in a multiantigen immunoassay which may contribute to improving the sensitivity of the test [9].

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