

The sensitivity and specificity of fluorescent leprosy antibody absorption (FLA-ABS) test for detecting subclinical infection with *Mycobacterium leprae*

JI BAOHONG,* TANG QUANKUI,* LI YENLONG,*
CHEN JIAKUN,* ZHANG JIALING,* DONG
LIWEN,* WANG CHENMIN,† MA JIAJU‡
& YE DALI§

**Shanghai Zeng Yi Hospital; † Hai-an Leprosy Hospital, Jiangsu Province; ‡ Hanzhong Sanitorium, Shanxi Province; § Huzhou Epidemic Prevention Station, Zhejiang Province, People's Republic of China*

Accepted for publication 24 January 1984

Summary From the examination of 854 sera from different sources by the fluorescent leprosy antibody absorption (FLA-ABS) test, the sensitivity and specificity of this test for leprosy has been confirmed. A positive FLA-ABS test in a non-leprosy individual should be considered as an indicator of subclinical leprosy infection. The subclinical infection rates of two endemic areas ranged from 11·4 to 16·3% and were at least 200 times higher than the cumulative prevalence rate of clinical infection. The combination of a positive FLA-ABS test with a negative Mitsuda reaction indicates that the individual has been infected with *Mycobacterium leprae*, but cell-mediated immunity has not been induced. Such individuals are at a greater risk of developing multibacillary leprosy and should be carefully followed up or some prophylactic measures should be considered. Since subclinical infection cannot be differentiated from very early leprosy by the FLA-ABS test alone, it is not a reliable diagnostic test of early leprosy.

Introduction

A technique for the detection of subclinical infection with *Mycobacterium leprae* among the population of leprosy endemic areas is one of the most important requirements for leprosy control programmes. The technique should be highly sensitive and specific for leprosy, the procedures and equipments should be simple and convenient for use in the field. Abe *et al.*¹⁻⁴ have developed the fluorescent

leprosy antibody absorption (FLA-ABS) test, using the indirect fluorescent antibody technique and improving the specificity by absorption of cross-reacting anti-mycobacterial antibodies in the serum. This test has been claimed to be useful for detecting subclinical leprosy infection.¹⁻⁶ The purpose of the present study is to evaluate the sensitivity and specificity of the FLA-ABS test.

Materials and methods

SERA

Approximately 0.5 ml serum was collected from each individual and stored at -20°C . Sodium azide at final concentration 0.1% was added to those specimens which had to be transported for long distances, these specimens being kept in wet-ice thermos flasks. The sera of leprosy patients were collected from Shanghai Zeng Yi Hospital, Hanzhong Sanatorium (Shanxi Province) and the leprosaria of Suzhou Prefecture (Jiangsu Province). The household contacts of leprosy patients were from Hai-an County (Jiangsu Province), Huzhou (Zhejiang Province) and Chenggu County (Shanxi Province). The healthy inhabitants of leprosy endemic areas were collected from Caoyuan People's Commune of Hai-an County and Lianshi People's Commune of Huzhou. The cumulative leprosy prevalence rates of these two areas were 4.8 and 6.2 per thousand respectively. The doubtful leprosy cases were from the Out-patient Department of Shanghai Zeng Yi Hospital and the professional contacts were the staff of the same hospital. Pulmonary tuberculosis patients were provided by the Shanghai Second Tuberculosis Sanatorium. Sera of healthy non-contacts were obtained from donors in Shanghai.

MYCOBACTERIAL SUSPENSION

M. leprae suspensions were prepared from human leproma by the method of Abe *et al.* or from *M. leprae*-infected armadillo livers by Protocol 1/79. The latter *M. leprae* were provided by courtesy of Dr R J W Rees, National Institute for Medical Research, London, under the IMMLEP project of WHO. Two forms of armadillo-derived *M. leprae* were obtained: bacillary suspension at a concentration of 10^{10} AFB/ml and freeze-dried bacilli. The bacillary suspension was diluted to 2×10^8 AFB/ml for preparing the smear of *M. leprae* and the results were the same as in those obtained with human-derived *M. leprae*. The freeze-dried bacilli were used for the additional absorption with *M. leprae*. BCG, *M. vaccae*, *M. tuberculosis* (H37Rv), *M. kansasii*, *M. marinum*, *M. smegmatis*, *M. phlei* and *M. avium* were cultivated on Ogawa's 1% egg medium. The colonies were homogenized, washed and suspended in normal saline.

FLA-ABS TEST

The steps of the test, including cross-reactions with other mycobacteria and additional absorption with *M. leprae* or other mycobacteria, were those described by Abe *et al.*^{3, 4} A fluorescent microscope, Model BHF, Olympus Ltd, Japan, was used throughout this study. The filter system included BV and interference system, Model F-FITC, Olympus Ltd, Japan. The specificity of the green fluorescence as shown by the bacilli under the BV system were confirmed by using the interference system. The criteria for fluorescent microscopic examination were the same as Abe *et al.*³ and two plus or more fluorescence caused by the 1:40 or higher dilution of sera were considered to be positive.

Results

FLA-ABS TEST AMONG LEPROSY PATIENTS

Out of 161 active leprosy patients, 146 (90.7%) were proved positive by the FLA-ABS test. The results of the tests on patients throughout the spectrum of leprosy are shown in Table 1. The positive rate was highest in lepromatous patients and gradually decreased towards the tuberculoid end. However, 73.9% of TT patients were still positive. The differences in positive rates between LL vs BT or TT, BL vs TT were statistically significant ($P < 0.05$ or $P < 0.01$). The distribution of the antibody titres down the spectrum of leprosy showed the same tendency as the positive rate, i.e. the mean antibody titre was highest at the lepromatous end and gradually decreased towards the tuberculoid end. The differences in mean antibody titre between each type of patient, except BL vs BB or BT vs TT, were statistically significant although there were striking variations within each type.

Table 1. FLA-ABS test among active leprosy patients

| Classification of leprosy | No. of cases | Distribution of antibody titre (10×4^x) | | | | | | | | |
|------------------------------|--------------------|--|------|-------|----|----|----|----|----------------|------|
| | | Positive No. | % | $x =$ | | | | | Mean of x | SD |
| | | | | 0 | 1 | 2 | 3 | 4 | | |
| LL | 45 | 45 | 100 | 0 | 3 | 4 | 14 | 24 | 3.31 | 0.90 |
| BL | 50 | 47 | 94.0 | 3 | 9 | 22 | 11 | 5 | 2.12 | 1.02 |
| BB | 16 | 15 | 93.8 | 1 | 5 | 0 | 7 | 3 | 2.38 | 1.31 |
| BT | 27 | 22 | 81.5 | 5 | 7 | 12 | 2 | 1 | 1.52 | 1.01 |
| TT | 23 | 17 | 73.9 | 6 | 7 | 5 | 4 | 1 | 1.44 | 1.20 |
| Total | 161 | 146 | 90.7 | 15 | 31 | 43 | 38 | 34 | 2.28 | 1.26 |

Table 2. FLA-ABS test in leprosy patients; grouping by bacterial load or activity of infection

| Classification of leprosy | Group | No. of cases | No. | Positive % | <i>P</i> | Distribution of antibody titre (10×4^x) | | | | | | | |
|------------------------------|-----------|--------------------|-----|---------------|----------|--|----|----|----|----|---------------------|------|------|
| | | | | | | <i>x</i> = | | | | | Mean of <i>x</i> | | |
| LL | Smear(+) | 45 | 45 | 100 | — | 0 | 3 | 4 | 14 | 24 | 3.31 | 0.90 | — |
| | Smear(−) | 33 | 30 | 90.9 | <0.05 | 3 | 3 | 11 | 8 | 8 | 2.46 | 1.23 | <0.0 |
| TT | Active | 23 | 17 | 73.9 | — | 6 | 7 | 5 | 4 | 1 | 1.44 | 1.20 | — |
| | Quiescent | 36 | 12 | 33.3 | <0.01 | 24 | 10 | 2 | 0 | 0 | 0.39 | 0.60 | <0.0 |

Table 2 suggested that the results of the FLA-ABS test were well correlated with the bacterial load of the host or the activity of the leprosy infection. The positive rate and mean antibody titre were significantly lower in the smear negative LL group than in those in the smear positive LL group, and the same tendency was observed in the quiescent TT group (where the skin lesions had subsided for at least 3 years) as compared with the active TT group.

FLA-ABS TEST AMONG NON-LEPROSY INDIVIDUALS

Five hundred and forty-nine non-leprosy cases were examined and the results are summarized in Table 3. All sera from sputum-positive pulmonary tuberculosis patients and healthy non-contacts gave negative results. Among the healthy

Table 3. FLA-ABS test among non-leprosy individuals

| Group | No. of cases | Positive | | Distribution of antibody titre (10×4^x) | | | | | | |
|---|--------------------|----------|------|--|----|----|---|---|----------------|------|
| | | No. | % | $x =$ | | | | | Mean of x | SD |
| | | | | 0 | 1 | 2 | 3 | 4 | | |
| Healthy non-contacts | 30 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tuberculosis patients | 30 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| Endemic area | | | | | | | | | | |
| Healthy adults (Lianshi) | 147 | 24 | 16.3 | 123 | 21 | 3 | 0 | 0 | 0.18 | 0.43 |
| Healthy schoolchildren (Lianshi) | 44 | 5 | 11.4 | 39 | 5 | 0 | 0 | 0 | 0.11 | 0.32 |
| Healthy schoolchildren (Caoyuan) | 117 | 16 | 13.7 | 101 | 13 | 3 | 0 | 0 | 0.16 | 0.43 |
| Household contacts of multibacillary leprosy | 88 | 58 | 65.9 | 30 | 36 | 17 | 5 | 0 | 0.97 | 0.88 |
| Household contacts of paucibacillary leprosy | 45 | 8 | 17.8 | 37 | 6 | 2 | 0 | 0 | 0.22 | 0.52 |
| Professional contacts | 48 | 20 | 41.7 | 28 | 8 | 9 | 3 | 0 | 0.73 | 0.98 |

Table 4. Comparison of FLA-ABS test in professional contacts: grouping by seniority

| Group | No. of cases | | Positive % | P* | Distribution of antibody titre (10×4^x) | | | | | | Mean of x | SD | P* |
|---------------------------|--------------|-----|------------|----------|--|---|---|---|---|--|-------------|------|----------|
| | No. | No. | | | 0 | 1 | 2 | 3 | 4 | | | | |
| Seniority ≥ 15 years | 26 | 19 | 73.1 | — | 7 | 7 | 9 | 3 | 0 | | 1.31 | 1.01 | — |
| Seniority < 5 years | 22 | 1 | 4.6 | < 0.01 | 21 | 1 | 0 | 0 | 0 | | 0.05 | 0.21 | < 0.01 |
| Total | 48 | 20 | 41.7 | — | 28 | 8 | 9 | 3 | 0 | | 0.73 | 0.98 | — |

* Compare with the group of seniority ≥ 15 years.

inhabitants, excluding the household contacts from the two endemic areas, the positive rates ranged from 11.4 to 16.3% and the mean antibody titres ranged from 0.11 to 0.18%. No significant difference has been observed between the two areas or two age groups from the same area. Both the positive rate and mean antibody titre were very significantly higher in household contacts of multibacillary patients than in those of paucibacillary patients. The so-called professional contacts were the staff working in a leprosy hospital mainly for smear-positive patients. The total positive rate of that group was 41.7%. If subdivided according to seniority in the hospital, the positive rate and mean antibody titre were much higher among the staff with seniority ≥ 15 years than in those with seniority < 5 years (Table 4).

Mitsuda lepromin (1.6×10^8 AFB/ml) testing was undertaken immediately after blood collection in 340 healthy inhabitants or household contacts in leprosy endemic areas. The Mitsuda reaction was read at 21–28 days and was considered positive if the average diameter of the nodule was greater than 3 mm. As shown in Table 5, no correlation has been found between the Mitsuda reaction and the FLA-ABS test ($\chi^2 = 0.264$, $P > 0.05$). According to the results of both tests, there were four combinations, i.e. both positive, both negative, Mitsuda positive/

Table 5. Correlation between FLA-ABS test and Mitsuda reaction

| Mitsuda reaction | FLA-ABS test | | Total |
|------------------|--------------|-------------|--------------|
| | Positive | Negative | |
| Positive | 34 (10.0%) | 152 (44.7%) | 186 (54.7%) |
| Negative | 19 (5.6%) | 135 (39.7%) | 154 (45.3%) |
| Total | 53 (15.6%) | 287 (84.4%) | 340 (100.0%) |

FLA-ABS negative or Mitsuda negative/FLA-ABS positive. Nineteen (5.6%) out of these 340 individuals were Mitsuda negative but FLA-ABS positive. Seventeen had an antibody titre of 1:40 and 2 of 1:160.

FLA-ABS TEST AMONG DOUBTFUL LEPROSY CASES

The so-called doubtful cases were those who had skin lesion(s) or neural symptoms resembling leprosy when they first appeared. FLA-ABS tests had been carried out in 75 patients of this group and the results are shown in Table 6. The

Table 6. FLA-ABS test among doubtful leprosy cases grouping by clinical followed up results

| Group | No. of cases | Positive No. | Positive % | Distribution of antibody titre (10×4^x) | | | | | | |
|---------------------------------------|--------------------|-----------------|---------------|--|----|------------|---|---|----------------|------|
| | | | | 0 | 1 | $x =$ 2 | 3 | 4 | Mean of x | SD |
| Confirmed leprosy during follow-up | 4 | 3 | 75.0 | 1 | 2 | 1 | 0 | 0 | 1.00 | 0.82 |
| Requiring further follow-up | 13 | 7 | 53.9 | 6 | 4 | 2 | 1 | 0 | 0.85 | 0.99 |
| Ruled out leprosy during follow-up | 58 | 9 | 15.5 | 49 | 7 | 2 | 0 | 0 | 0.19 | 0.48 |
| Total | 75 | 19 | 25.3 | 56 | 13 | 5 | 1 | 0 | 0.35 | 0.67 |

total positive rate was 25.3%. They had been followed up clinically in the Out-patient Department for at least 2 years before the test. About 1 year after the test, these patients were divided into three groups, as indicated below. Histopathological examinations were carried out on a sample of two-thirds of all patients studied. Group A with 4 cases whose lesion(s) gradually progressed with unequivocal leprosy, 3 (75%) were FLA-ABS positive. Group B with 13 cases whose lesion(s) or symptoms had not significantly changed and still need further follow-up, 7 (53.9%) were FLA-ABS positive. Group C with 58 cases, in whom the diagnosis of leprosy can be ruled out, 9 (15.5%) were FLA-ABS positive. The differences in positive rate and mean antibody titre between group A vs B has no statistical significance but both groups were significantly higher than group C. To analyse the outcome in groups A and C, the χ^2 test was used to check the correlation between the FLA-ABS test and the follow-up results (Table 7). The χ^2 test was 7.384 and $P < 0.01$. Thus, the FLA-ABS positive doubtful cases were more prone to develop leprosy than the FLA-ABS negative cases. Among the 9 patients who were positive to FLA-ABS in group C, 7 cases came from leprosy endemic areas but the other 2 did not. The reasons for the positive tests in the latter 2 cases are unknown.

Table 7. Correlation between FLA-ABS test and clinical follow up results of doubtful cases

| FLA-ABS test | Clinical follow up results | | Total |
|--------------|----------------------------|-------------------|-------|
| | Confirmed leprosy | Ruled out leprosy | |
| Positive | 3 | 9 | 12 |
| Negative | 1 | 49 | 50 |
| Total | 4 | 58 | 62 |

CROSS-REACTION AND ADDITIONAL ABSORPTION OF POSITIVE FLA-ABS SERA

In order to show that a positive FLA-ABS test was not due to the retention of cross-reactive antibody to other mycobacteria in the sera after absorption with cardiolipin, lecithin, BCG and *M. vaccae*, 53 positive sera from non-leprosy individuals in Table 3 were tested for cross-reactivity with 6 other species of mycobacteria, i.e. *M. tuberculosis*, *M. kansasii*, *M. marinum*, *M. smegmatis*, *M. phlei* and *M. avium*. Only 5 demonstrated cross-reactivity with 1 or 2 species of mycobacteria, namely 3 to *M. smegmatis*, 2 to *M. marinum* and 1 to *M. tuberculosis*. These 5 sera were further absorbed with their corresponding mycobacteria. The cross-reaction disappeared in 4 sera but 1 was still positive to *M. smegmatis* though the antibody titre was reduced from 1:160 to 1:40. The antibody titre to *M. leprae* was not influenced by additional absorption with other mycobacteria. Ten positive FLA-ABS sera, 5 multibacillary patients, 4 household contacts and 1 professional contact, were tested by additional absorption with *M. leprae*, i.e. 0.1 ml serum absorbed by the routine procedures (resulting in a 10-fold dilution) was mixed with 0.5 mg freeze-dried *M. leprae* and incubated at 37°C for 30 min. Before absorption with *M. leprae*, the antibody titre was 1:160 in 1, 1:640 in 5 and 1:2560 in 4. After absorption with *M. leprae*, the anti-*M. leprae* antibodies were completely removed.

Discussion

It is impossible to measure directly the sensitivity of FLA-ABS test for detecting subclinical leprosy infection, because at present no other specific reference systems are available. However, the results of the FLA-ABS test in leprosy patients and also in contacts can provide useful information. The present study revealed the following observations: (1) By the FLA-ABS test, anti-*M. leprae* antibodies have been detected in more than 90% of active leprosy patients. Even though the positive rate was least in TT patients, it still gave 73.9% (Table 1). (2)

The positive rate and mean antibody titre correlated well with the bacterial load of the host or the activity of leprosy infection (Table 2). (3) Theoretically, subclinical infection should be greater in contacts and chances of subclinical infection should correlate with the infectivity of the index case, the intensity and the duration of exposure. The positive rate and mean antibody titre of the FLA-ABS test were much higher in household contacts of multibacillary patients than in those of paucibacillary patients.

contacts with longer seniority than in those with shorter seniority (Table 4). From these findings, the FLA-ABS test seems to be quite sensitive for detecting subclinical leprosy infection. The following observations suggested that the FLA-ABS test is specific for leprosy infection: (1) All specimens from sputum-positive pulmonary tuberculosis patients and healthy non-contacts gave negative results (2) Only a small proportion of positive FLA-ABS sera from non-leprosy individuals showed cross-reaction to other mycobacteria and this can be differentiated by additional absorption with corresponding mycobacteria. (3) The reaction against *M. leprae* is completely removed when positive sera are further absorbed by *M. leprae*.

Since the FLA-ABS test is quite sensitive and specific for leprosy infection, the positive reaction in non-leprosy cases may be considered as an indication of subclinical infection. Because the positive rate in quiescent TT cases was much lower than in active cases, it would seem unlikely that the FLA-ABS test could detect past aborted infections and, therefore, the subclinical infection rate detected by this test might be an underestimate. Even though the figures in Table 3 demonstrated that the subclinical infection rate was at least 200 times higher than the cumulative prevalence rate of clinical infection at the same area, they are similar to those reported by Abe *et al.*³ A far greater proportion of the population are infected with *M. leprae* than develop clinical disease, indicating that the infections are aborted at the subclinical stage.

There was a correlation between a positive FLA-ABS test and the outcome in individuals with doubtful leprosy, in that they were more prone to develop leprosy later than those with a negative test. However, many doubtful cases came from endemic areas. It is difficult to differentiate the subclinical infection from very early leprosy by the FLA-ABS test. Hence, a positive FLA-ABS test is not a reliable indicator for the early diagnosis of leprosy.

The main purpose for detecting subclinical leprosy is to identify the individuals who have been infected and prone to develop into multibacillary leprosy. The FLA-ABS test alone can hardly fulfil this purpose because the great majority of leprosy patients are positive and the distribution of levels of antibody titres overlap between multibacillary and paucibacillary patients. It is possible to overcome this difficulty by including a lepromin test, because most multibacillary patients are in an anergic state, and therefore lepromin negative. A combination of a positive FLA-ABS test and a negative Mitsuda reaction indicates that the individual has been infected with *M. leprae* but cell-mediated immunity has not

been induced. These individuals are more likely to develop multibacillary-type leprosy and most urgently need to be protected. Abe *et al.* used the frequency of individuals in this category as an index of susceptibility to leprosy.⁴ This index in the present study was 5.6%. Since the antibody titres of the FLA-ABS tests were rather low in all these individuals in our present study, we have followed them up carefully, including physical examination, lepromin and FLA-ABS tests. If this specific immunological status persists or the antibody titre tends to be increasing while the Mitsuda reaction is still negative, then prophylactic measures have been adopted.

Acknowledgment

This investigation received financial support from the Immunology of Leprosy (IMMLEP) and Chemotherapy of Leprosy (THELEP) components of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. The authors express sincere thanks to Dr M Abe, National Institute for Leprosy Research, Tokyo, Japan for helping in standardization of FLA-ABS test and supplying part of reagents used in the test, and to Dr R J W Rees, National Institute for Medical Research, London, UK for his kind supply of *M. leprae*, and for his valuable suggestions and advice in preparing this manuscript.

References

- ¹ Abe M, Izumi S, Saito T, Mathur, SK. Early serodiagnosis of leprosy by indirect immunofluorescence. *Lepr India*, 1976; **48**: 272–6.
- ² Abe M, Yoshino Y. Antigenic specificity of *M. leprae* by indirect immunofluorescence. *Int J Lepr*, 1978; **46**: 118.
- ³ Abe M, Minagawa F, Yoshino Y, Ozawa T. Fluorescent leprosy antibody absorption (FLA-ABS) test for detecting subclinical infection with *Mycobacterium leprae*. *Internat J Lepr*, 1980; **48**: 109–19.
- ⁴ Abe M, Ozawa T, Minagawa F, Yoshino Y. Subclinical infection in leprosy. Its detection and control by fluorescent leprosy antibody absorption (FLA-ABS) test. *Lepr Rev*, 1981; **52** (Suppl. 1): 263–73.
- ⁵ Bharadwaj VP, Ramu G, Desikan KV. Fluorescent leprosy antibody absorption (FLA-ABS) test for early serodiagnosis of leprosy. *Lepr India*, 1981; **53**: 518–24.
- ⁶ Bharadwaj VP, Ramu G, Desikan KV. A preliminary report of subclinical infection in leprosy. *Lepr India*, 1982; **54**: 220–7.