The effect of corticosteroids usage on bacterial killing, clearance and nerve damage in leprosy; Part 3 – Study of two comparable groups of 100 multibacillary (MB) patients each, treated with MDT + steroids vs MDT alone, assessed at 6 months post – release from 12 months MDT

VANAJA PRABHAKAR SHETTY*, FATEMA ABBAS KHAMBATI*, SUNIL DATTATRAYA GHATE*, GOSPI DOLLY CAPADIA*, VIVEK VASUDEV PAI** & RAMASWAMY GANAPATI**
*The Foundation for Medical Research, 84-A, R.G. Thadani Marg, Worli, Mumbai 400 018, India
**Bombay Leprosy Project, Vidyan Bhavan, V.N. Purav Marg, Sion, Chunnabatti, Mumbai 400 022, India

Accepted for publication 25 November 2009

Summary
Objective: To investigate effects of therapeutic usage of corticosteroids on *M. leprae* killing and clearance, on clearance of granuloma and on nerve damage in multibacillary (MB) leprosy patients.

Design: From a cohort of 400 untreated MB patients, a comparable group of 100 each receiving MDT + steroids (group A) vs MDT alone (group B) were assessed at 18 months as compared to month zero with respect to clinical and granuloma regression, *M. leprae* killing and clearance, and nerve functions. Analysis was performed using SPSS version 10.0. The significance of association was tested using Chi square and Fisher’s exact tests.

Results: Regression of lesions assessed clinically and by histopathology was seen in 52% and 53% patients in group A and 46% and 63% in B respectively (*P* not significant). Clearance of bacteria assessed by bacteriological index (BI) in slit skin smears (SSS) and extent and intensity of antigen using anti-BCG staining were also comparable in the two groups. Multiplication of *M. leprae* in the mouse foot pad (MFP) indicating the presence of viable bacilli was seen in 14% and 16% of SSS positive BL-LLs patients in groups A and B respectively (*P* not significant). The occurrence of viable *M. leprae* was higher among patients with repeat reaction (19%)
than single (11%). Using clinical tests (nerve palpation, monofilament and voluntary muscle testing), the proportion of sensory and motor nerves showing improvement or deterioration were similar in the two groups. However using nerve conduction studies, the overall proportion of nerves showing deterioration (22%) was significantly higher than improvement (9%) \((P < 0.001)\).

**Conclusions:** Treatment with MDT + corticosteroids does not adversely affect the clearance of granuloma, *M. leprae* and/or its antigens and *M. leprae* killing. However the continued presence of viable bacteria in > 14% of BL-LLs patients indicate that 12 months of MDT may be insufficient for complete bacterial killing. In both groups nerve conduction studies indicated that deterioration of nerves was high suggesting, MDT + corticosteroids was not very efficacious in the prevention or reversal of nerve damage. A better immuno-modulatory drug or a modified corticosteroid regime is needed.

**Introduction**

Around half of all leprosy patients who either present with or develop reaction or neuritis during the course of the disease receive steroids.\(^1,2\) The effects of corticosteroids in varying doses and duration for the treatment of reaction, neuritis and nerve damage have been studied in India\(^3\) and elsewhere\(^2,4\) and is reviewed in details by Naafs.\(^5\) Their effect in reducing the frequency of reaction has also been assessed in a multi-centric study from different states of India.\(^6\) However there is a dearth of information with regard to the relationship between corticosteroid usage and MDT induced killing and clearance of bacilli or clearance of granuloma. A question that remains unaddressed and is the topic of this study, is whether the usage of corticosteroids hinders the killing of bacilli, or clearance of antigens, or granuloma, thereby increasing the risk of relapse and the occurrence of late reversal reaction and/or nerve damage in the long run. In the current nation-wide strategy of ‘Leprosy Elimination’, there is scant emphasis on follow – up and surveillance which render it difficult to estimate the exact incidence of relapse or recurrence of lesions. A field based study in North India showed that overall around 5% of MB patients presented with recurrence of lesions\(^7\) while the documented relapse rates ranged from 1% to 20%.\(^8,9\) In an earlier study carried out at our centre, 15/25 PB and 21/52 MB relapsed cases registered had history of receiving corticosteroids suggesting a possible associated risk.\(^10,11\)

In tuberculosis, steroids are known to accelerate the activation of dormant organisms.\(^12\) In leprosy failure of MDT to kill *M. leprae* and the persistence of viable *M. leprae* are the main cause of relapse.\(^13\) As peripheral nerves are the major reservoir of *M. leprae*\(^14\) and if prevention and timely treatment of nerve damage is to be a priority, it is important to ensure that a therapeutic intervention such as corticosteroid usage does not promote viability and persistence of *M. leprae*.

**A1M**

To investigate effects of the therapeutic usage of corticosteroids on the bacterial killing and clearance and on nerve damage in leprosy. The following parameters were studied as outcome measures and compared at fixed time points (0 and 18 months, i.e. 6 months post-release from MDT). The parameters assessed were: a) clinical regression of lesions,
b) clearance of *M. leprae* and its antigens from the lesions
c) regression of granuloma
d) presence of viable *M. leprae* using the mouse foot pad test and,
e) changes in nerve function assessed clinically and electro-physiologically.

The study findings at 0 and 18 months in two groups each comprising 100 multibacillary (MB) patients, each of whom received MDT + steroids or MDT alone, are presented and discussed.

**PATIENTS AND METHODS**

The inclusion and exclusion criteria of patients have been previously published. 15

**STUDY DESIGN**

A prospective cohort of 400 untreated MB patients were investigated at baseline, and followed up at 6 monthly intervals for a period of 18 months. It was a one centre study carried out at the Foundation for Medical Research. The Bombay Leprosy Project (a field project covering G-North Municipal ward of Mumbai) and Kushtrog Nivaran Samiti (a field project covering Panvel taluka of Raighad district of Maharashtra State) assisted in case finding, case holding and referral of patients to FMR. The target of recruiting 400 patients was worked out on the premise that ~30% of MB cases develop reaction and ~80% of the reaction and/or neuritis occur within the first 6 to 8 months of starting treatment16 necessitating intervention with corticosteroids.

Patients who received steroids during the first 6–8 months of starting MDT were placed under group A while those treated with MDT only were placed under group B (refer Figure 1). On completion of 18 months follow up the patients were subjected to repeat investigations for assessment of parameters listed above.

**GROUP A**

Patients receiving MDT + corticosteroids (MB patients presenting with or developing T1R or T2R with/without neuritis or neuritis alone or silent neuropathy commencing within 6–8 months of registration).

**GROUP B**

MB patients with no symptoms of reaction/neuritis/silent neuropathy of < 6 months duration and treated with MDT only.

Details of study design and baseline findings are published earlier.15

**STUDY POPULATION**

As per the THELEP study report17 regardless of the treatment regimen employed, around 10% of the MB cases carry persister bacilli. To achieve 95% power of demonstrating a statistically significant difference with and without usage of corticosteroids at the 1% level required the recruitment of 100 patients in each of the groups A and B. The patients in the two groups were closely matched with respect to age, bacteriological index (BI) and leprosy class as per the Ridley – Jopling scale.18
All patients were treated with the 12 months MDT regimen for multibacillary leprosy, as per the WHO/NLEP guidelines.19

**TREATMENT FOR REACTION AND/OR NEURITIS OR SN OF < 6 MONTHS**

Group A patients were additionally treated with corticosteroids (Prednisolone – 40 mg daily tapered to 5 mg daily over 12 weeks as per WHO recommendation19 i.e. 40 mg,}

---

**Figure 1. Study Design. Key:** MB-multibacillary, MF–monofilament testing, VMT–voluntary muscle testing, NP–nerve palpation, NCS–nerve conduction studies, MDT–multi drug therapy, NFI–nerve function assessment, T1R – type 1 reaction, T2R – type 2 reaction, SN – silent neuropathy, MFP – mouse foot pad test.
30 mg, 20 mg, 15 mg, 10 mg, 5 mg for 2 weeks each (daily dose) under the supervision of a clinician. The same course was repeated if the patient developed a further reaction episode. We used the rigid steroid regimen to ensure uniformity in the treatment among the study patients.

Definitions of Type 1 (T1R), Type 2 reactions (T2R), Neuritis, Silent neuropathy (SN) < 6 months, nerve function impairment (NFI) are given in a previous publication.\textsuperscript{15}

Methods – The methodology has been detailed in a previous publication.\textsuperscript{15,20}

Briefly the patients were subjected to; examination and investigations at intake and follow up, the latter including a second skin biopsy at 18 ± 2 months.

WHO disability grading

Slit skin smear (SSS)

Nerve function assessment tests included;

(a) Nerve palpation (NP)
(b) Touch sensibility testing using monofilaments (MF)
(c) Voluntary muscle testing (VMT)
(d) Sensory nerve conduction measurements (SNC)
(e) Motor nerve conduction measurements (MNC)

Skin biopsy was subjected to;

(a) Histopathology – Trichrome modified Fite – Faraco (TRIFF) staining,
(b) Assessment of bacterial load/gram weight by tissue homogenisation
(c) Mouse foot pad test for viability studies
(d) Immunohistochemistry – Anti-BCG antibody staining.

Criteria for Grading

In order to assess the difference between the 2 groups, all the semi-quantifiable outcome measures were graded under 3 categories; i.e. very good, good and poor as described below at 18 ± 2 months, as compared to the baseline values.

Few patients \(n = 15\) did not report for the second biopsy exactly at 18 ± 2 months. The biopsy and other assessments were done later for these patients. The above parameters were therefore corrected taking the time factor into consideration where ever applicable.

Regression of skin lesions

Very good – Complete regression and/or disappearance of clinical lesions, of erythema and satellite lesions if any.

Good – Partial regression which means reduction in the size, number of lesions and decrease in erythema.

Poor – No significant change in the existing lesion or increase in the activity of the lesion in size, shape, erythema or appearance of new lesions.
HISTOPATHOLOGY

Five micron thick sections were cut from paraffin embedded tissue biopsy specimens, stained with TRIFF and studied under light microscope. Improvement in granuloma between 0 and 18 months was determined on 2 scores; a) localisation and regressing changes in the infiltrating cells b) decline in cellular infiltrate; these were graded using following criteria:

Very good – Near complete absence of infiltrating cells and/or presence of non specific type of infiltrating cells in localised areas.

Good – Presence of regressing, ill defined infiltrating cells at localised areas.

Poor – Presence of active infiltrating cells, not very different, to that seen at baseline.

Note: Cellular infiltration was assessed with the following grades: 0 = no cellular infiltrate; 1+ = groups of cells; 2+ = moderate cellular infiltration; and 3+ = extensive cellular infiltration. 50 fields/section were observed under light microscope using 40× magnification.

Bacteriology

Bacteriological index (BI) assessed in slit skin smears (graded from 0–6+) and bacterial load/gm wt of tissue biopsy was determined and recorded. Its decline was graded using following criteria at 18±2 months.

Very good: decline in average BI log ≥ 1.5.

Good: decline in average BI log of ≤ 1.5 to 1.0.

Poor: decline in average BI log of < 1.0.

Antigen clearance

Antigen load detected by anti – BCG antibody staining and intensity graded from 0–4+. It should be noted that biopsy sites were different at 0 and 18±2 months.

Very good: Decline in antigen load of ≥ 2 +.

Good: Decline in antigen load of < 2+ to 1 +.

Poor: Decline in antigen < 1 +.

Note: Antigen load and intensity of staining was assessed with the following grades; 1+ = mild in few infiltrating cells, 2+ to 3+ = moderate in 25–75% of cells, 4+ = marked in mostly all cells. 50 fields/section were observed under light microscope using 40× and 100× magnification.

Changes in nerve functions assessed by NP, MF, VMT and NCS tests

NERVE PALPATION (NP)

Improvement – Reduced enlargement of nerve from 3+ or 2+ to 1+/0,

Deterioration – Increased enlargement of nerve to 3+, 2+ or 1+.

MONOFILAMENT (MF) TESTING

Improvement – Restoration of sensory score to normal or improvement in sensory score by ≥ 2 points,
**The effect of corticosteroids usage in multibacillary leprosy**

**Data Analysis**

Analysis was performed using SPSS version 10.0. The significance of association was tested using Chi-square test and Fisher’s exact test.

**Ethical Approval**

No financial incentive was given to the patients. Travel expenses were refunded to the patients on occasion. The study followed the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS/WHO, 1993). The study had the clearance from the Institutional (the Foundation for Medical Research; a non-government organization) ethics committee. Informed written consent was obtained from individual study subjects before inclusion in the study and before the biopsy procedure from each patient.

The Committee for the Purpose of Control and Supervision of experiments on animals (CPCSEA) cleared the use of animals in the study. The Foundation for Medical Research is registered with CPCSEA and has a valid registration number 423/01/1/CPCSEA.

**Results**

*At 18 months (± 2 months) in 100 comparable patients each in groups A and B*

**Age: sex ratio**

Mean age was (31.6) in group A and (29.5) in B. The male to female ratio was 4:1 in both the groups. Using Ridley Jopling scale in group A, 39% patients were classified as borderline tuberculoid (BT), 19% mid borderline (BB), 30% borderline lepromatous (BL), 7% sub-polar lepromatous (LLs) and 5% as pure neural (PN). Similarly in group B, 40% were BT, 18% BB, 28% BL, 7% LLs and 7% PN.
Disability

Proportion of patients showing improvement and deterioration in disability grades 1 and 2 were comparable in the two groups (Table 1).

Clinical and histopathological regression of lesions

Very good + good clinical regression of lesions was seen in 52% patients in group A and 46% in B while histopathological regression was seen in 56% in group A and 63% in B (p not significant) (Table 1).

Bacteriological findings

At month zero, 65% patients in group A and 64% in B were slit skin smear negative. Among the remaining 35% SSS positive patients in group A (6% had BI 1+, 17 were 2–3+ and 12 were > 4+) while in group B (4% had BI 1+, 19 were 2–3+ and 13 were > 4+).

At 18 months very good + good decline in bacterial index (assessed by SSS and load per gram wt. of tissue) were closely comparable in the two groups; (23/35 = 66% and 64% in group A; 19/32 = 60% and 57% in group B respectively) (Table 1).

Further at 18 months in group A, 5/19 (26%) BB, 14/30 (47%) BL and 1/7 LLs patients and in group B, 15/18 (83%) BB and 5/28 (18%) BL patients turned BI negative (by SSS) (results not shown). This indicates that, in group B clearance of bacteria was better among BB ($\chi^2 = 12.09, P < 0.001$) while in group A it was marginally better among BL patients ($\chi^2 = 5.45, P < 0.01$).

A higher proportion of patients scored positive in the anti-BCG antigen staining (137/200 = 69%) as compared to AFB detected by the conventional methods such as SSS (74/200 = 37%), HL (70/200 = 35%) indicating greater sensitivity of the former technique.

Clearance of antigen assessed using anti-BCG staining was also comparable in groups A = 21/60 (35%) and B = 28/62 (45%) (P not significant) (Table 1).

Detection of viable bacteria as assessed by growth in mouse foot pad (MFP) test

At month zero among the slit skin smear (SSS) negative patients; 25/55 (45%) in group A and 19/57 (33%) in B scored positive in MFP while among SSS positive patients; 20/34 (59%) and 28/34 (82%) in groups A and B respectively scored positive (results not shown).

At 18 months, none of the SSS negative patients (BT – BB) in either groups scored positive (results not shown) whereas among SSS positive (BL-LLs) patients, 5/35 (14%) in group A and 5/32 (16%) in B scored positive in MFP (Table 1). These 10 patients testing positive at 18 months had a BI range of 1+ to 4+ at month zero. At 18 months 4/10 among them turned BI negative while 6/10 had a BI range of 1 to 3+.

Notably none of the 25 BL-LLs patients with high BI i.e. >4+ at month zero (results not shown) tested positive in mouse foot pad at 18 months. Moreover in group A, among the 5 patients testing positive at 18 months, 4 presented with Type 1 reaction (T1R) and/or neuritis (N) and one had silent neuropathy (SN) of < 6 months duration. However none
<table>
<thead>
<tr>
<th>Variables Description</th>
<th>Group A</th>
<th>Group B</th>
<th>( P ) value, Sig. between groups A &amp; B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(At 18 months)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(At 0 month)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) No. (%) of patients showing improvement/deterioration of WHO disability grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DG0</strong></td>
<td><strong>DG1</strong></td>
<td><strong>DG2</strong></td>
<td><strong>DG0</strong></td>
</tr>
<tr>
<td>Improvement</td>
<td>-</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Deterioration</td>
<td>7</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>No change</td>
<td>55</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>b) No. (%) of patients showing clinical regression of skin lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>41</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>48</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>c) No. (%) of patients showing histopathological regression of lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>19</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>37</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>44</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>d) No. (%) of patients showing decline in bacteriological index (BI) assessed by SSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>0</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Good</td>
<td>-</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Poor</td>
<td>-</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>No change</td>
<td>65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>e) No. (%) of patients showing clearance of antigen assessed by anti-BCG staining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>0</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Good</td>
<td>-</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Poor</td>
<td>-</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>No change</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>f) No. (%) of slit skin smear +ve patients showing growth in mouse foot pad</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 0 month</td>
<td>20/34 (59)</td>
<td>28/34 (82)</td>
<td></td>
</tr>
<tr>
<td>At 18 months</td>
<td>5/35 (14)</td>
<td>5/32 (16)</td>
<td></td>
</tr>
<tr>
<td>% decline</td>
<td>75%</td>
<td>82%</td>
<td></td>
</tr>
</tbody>
</table>
of the 7 patients presenting with Type 2 reaction (T2R) tested positive at 18 months. Both these findings are probably attributable to one of the known limitations of the MFP test.23

Nerve function assessment findings

The sensory and motor nerve functions assessed using clinical tests i.e. nerve palpation (NP), monofilament (MF) testing and voluntary muscle testing (VMT) respectively are presented in Tables 2 and 3. Overall the proportions of sensory and motor nerves showing improvement and deterioration were comparable. The improvement and deterioration of sensory and motor nerves in groups A and B with respect to clinical tests were also comparable.

By NCS (Table 4) the proportion of sensory and motor nerves showing deterioration was significantly higher (22%) than improvement (9%) ($\chi^2 = 57.47, P < 0.001$). Secondly proportion of sensory nerves showing deterioration was significantly higher in group A ($P < 0.001$) while in case of motor nerves the difference was not significant.

Distribution of single vs repeat reactions in relation to type of reaction and leprosy class

In group A, 73% patients had single and 27% had repeated episodes of reaction, the latter receiving therefore more than one course of corticosteroids. Majority of BT 34/39 (87%) and BB 14/19 (74%) followed by 18/30 (60%) BL, 4/7 (57%) LLs and 3/5 (60%) PN patients presented with single episode of reaction. Repeat reactions were more common among BL 13/30 (40%), LLs 3/7 (43%) and PN 2/5 (40%) patients as compared to BT 5/39 (13%) and
The effect of corticosteroids usage in multibacillary leprosy

BB 5/19 (26%). Majority of patients with single; 59/73 (81%) and repeat; 25/27 (93%) reactions showed T1R and/or neuritis.

On examining single vs repeat reactions in relation to bacteriology assessed by slit skin smears it was observed that single episode of Type 1 reaction were most common 56/65 (86%) and repeat reactions were less 9/27 (14%) among SSS negative MB patients. On the other hand, repeat reactions were more common among SSS positive patients (18/27 = 67%).

Single vs repeat reaction groups were also compared to determine whether there existed difference in the outcome measures i.e. clinical regression, decline in bacterial index,

Table 3. No. of sensory and motor nerves showing improvement and deterioration assessed using monofilament testing (MF) and voluntary muscle testing (VMT) in groups A and B at 18 ± 2 months (n = 200 nerves each)

<table>
<thead>
<tr>
<th>Sensory nerves</th>
<th>Ulnar (At 0 month)</th>
<th>Median</th>
<th>R.cut</th>
<th>Sural</th>
</tr>
</thead>
<tbody>
<tr>
<td>(At 18 months)</td>
<td>N</td>
<td>Ab</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>19</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>Deterioration</td>
<td>11</td>
<td>14</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>No change</td>
<td>132</td>
<td>11</td>
<td>9</td>
<td>149</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Deterioration</td>
<td>8</td>
<td>2</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>No change</td>
<td>143</td>
<td>12</td>
<td>4</td>
<td>161</td>
</tr>
<tr>
<td><strong>Motor nerves</strong></td>
<td>Ulnar (At 0 month)</td>
<td>Median</td>
<td>C.peroneal</td>
<td>P.tibial</td>
</tr>
<tr>
<td>(At 18 months)</td>
<td>N</td>
<td>Ab</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Deterioration</td>
<td>4</td>
<td>8</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>No change</td>
<td>148</td>
<td>17</td>
<td>2</td>
<td>189</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>13</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Deterioration</td>
<td>9</td>
<td>0</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>No change</td>
<td>165</td>
<td>4</td>
<td>6</td>
<td>195</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between groups</th>
<th>A</th>
<th>B</th>
<th>Chi-square, P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical significance of nerves by MF testing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>89 (11%)</td>
<td>83 (10%)</td>
<td>6·10, P &lt; 0·01</td>
</tr>
<tr>
<td>Deterioration</td>
<td>74 (9%)</td>
<td>42 (5%)</td>
<td>0·071, P &gt; 0·25</td>
</tr>
<tr>
<td>No change (N)</td>
<td>535 (67%)</td>
<td>577 (72%)</td>
<td>4·33, P &lt; 0·05</td>
</tr>
<tr>
<td>No change (Ab)</td>
<td>70 (9%)</td>
<td>67 (8%)</td>
<td>0·071, P &lt; 0·25</td>
</tr>
<tr>
<td>No change (0)</td>
<td>33 (4%)</td>
<td>30 (4%)</td>
<td>0·071, P &lt; 0·25</td>
</tr>
<tr>
<td>Statistical significance of nerves by VMT assessment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>26 (3%)</td>
<td>17 (2%)</td>
<td>1·93, P &lt; 0·25</td>
</tr>
<tr>
<td>Deterioration</td>
<td>17 (2%)</td>
<td>10 (1%)</td>
<td>1·84, P &lt; 0·25</td>
</tr>
<tr>
<td>No change (N)</td>
<td>726 (91%)</td>
<td>758 (95%)</td>
<td>8·14, P &lt; 0·005</td>
</tr>
<tr>
<td>No change (Ab)</td>
<td>24 (3%)</td>
<td>5 (1%)</td>
<td>12·77, P &lt; 0·001</td>
</tr>
<tr>
<td>No change (0)</td>
<td>4 (1%)</td>
<td>7 (1%)</td>
<td>0·148, P &lt; 0·25</td>
</tr>
</tbody>
</table>

Key: N – normal, Ab – abnormal, 0 – total sensory loss/muscle weakness.
antigen clearance, occurrence of viable *M. leprae* (Table 5) and changes in nerve function at 18 months as compared to month 0.

**Side effects of corticosteroids**

Except for weight gain and moon face, none of the patients were recorded with major corticosteroid related complications during the course of this study. However it must be mentioned that supporting tests (i.e. X-ray and blood tests) were done only when there was clinical suspicion (results not shown).

---

### Table 4. No. of sensory and motor nerves showing improvement and deterioration assessed by sensory and motor nerve conduction studies (SNC & MNC) in groups A and B at 18 ± 2 months (*n* = 200 nerves each)

<table>
<thead>
<tr>
<th>Sensory nerves</th>
<th>Ulnar</th>
<th>Median</th>
<th>R.cut</th>
<th>Sural</th>
<th>Total No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Ab 0</td>
<td>N</td>
<td>Ab 0</td>
<td></td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>9</td>
<td>7</td>
<td>17</td>
<td>93 (8)</td>
</tr>
<tr>
<td>Deterioration</td>
<td>46</td>
<td>9</td>
<td>16</td>
<td>17</td>
<td>125 (26)</td>
</tr>
<tr>
<td>No change</td>
<td>76</td>
<td>7</td>
<td>46</td>
<td>101</td>
<td>123 (12)</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>15</td>
<td>4</td>
<td>24</td>
<td>82 (12)</td>
</tr>
<tr>
<td>Deterioration</td>
<td>21</td>
<td>8</td>
<td>26</td>
<td>1</td>
<td>70 (18)</td>
</tr>
<tr>
<td>No change</td>
<td>97</td>
<td>19</td>
<td>36</td>
<td>107</td>
<td>110 (18)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Motor nerves</th>
<th>Ulnar</th>
<th>Median</th>
<th>C.Peroneal</th>
<th>P.Tibial</th>
<th>Total No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Ab 0</td>
<td>N</td>
<td>Ab 0</td>
<td></td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>14</td>
<td>6</td>
<td>15</td>
<td>71 (9)</td>
</tr>
<tr>
<td>Deterioration</td>
<td>53</td>
<td>17</td>
<td>26</td>
<td>25</td>
<td>110 (18)</td>
</tr>
<tr>
<td>No change</td>
<td>57</td>
<td>7</td>
<td>100</td>
<td>28</td>
<td>70 (12)</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>–</td>
<td>17</td>
<td>2</td>
<td>16</td>
<td>66 (7)</td>
</tr>
<tr>
<td>Deterioration</td>
<td>59</td>
<td>10</td>
<td>15</td>
<td>37</td>
<td>100 (12)</td>
</tr>
<tr>
<td>No change</td>
<td>41</td>
<td>65</td>
<td>4</td>
<td>107</td>
<td>70 (9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Between groups</th>
<th>A</th>
<th>B</th>
<th>Chi square, <em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Improvement</td>
<td></td>
<td>7.96, <em>P</em> &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Deterioration</td>
<td></td>
<td>12.75, <em>P</em> &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>No change (Ab)</td>
<td></td>
<td>4.7, <em>P</em> &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>No change (0)</td>
<td></td>
<td>8.86, <em>P</em> &lt; 0.005</td>
</tr>
</tbody>
</table>

Statistical significance of nerves by MNC study

|                | Improvement |         | *P* = 0.199          |
|                | Deterioration |         | 5.22, *P* < 0.01     |
|                | No change (N) |        | 0.72, *P* < 0.25     |
|                | No change (Ab) |   | 2.23, *P* < 0.1      |
|                | No change (0) |       | 0.39, *P* < 0.5      |

Key: N – normal, Ab – abnormal, 0 – no recordable sensory/motor potential.
Discussion

In this study of 200 MB patients, clinical regression of lesions, regression of granuloma through histopathology, decline in bacteriological index, clearance of bacterial antigen, bacterial viability and changes in nerve function were assessed at 0 and 6 months post release from treatment in two patient groups A and B. The former received MDT + steroids for reaction, the latter without reaction received MDT alone. The objective was to determine whether usage of corticosteroids had an adverse effect on the above parameters.

The study shows that the steroid regime of 40 mg daily tapered to 5 mg daily over 12 weeks does not negatively influence the clearance or killing of *M. leprae*. In accordance with the earlier documented findings\textsuperscript{1,2,24} no major side effect of steroids was observed in any of the patients during the course of the study.

In both the groups, a significant proportion of smear positive (BL-LLs) patients showed presence of viable *M. leprae* (14\% in A & 16\% in B) as demonstrated through an unequivocal growth in the footpads of non-immunosuppressed Swiss White mice. The percentile decline in the occurrence of viable bacteria was also comparable (Tables 1 and 5). This on one hand shows that use of corticosteroids does not significantly alter the killing of *M. leprae* but on the other hand shows that occurrence of viable bacteria is indeed high (~15\%) among smear positive BL-LLs patients receiving 12 months of MDT.

Table 5. Comparison of findings in patients with single (S) vs repeat (R = 2–3) reactions

<table>
<thead>
<tr>
<th>Variables</th>
<th>S (n = 73)</th>
<th>R (n = 27)</th>
<th>$\chi^2$ value (Sig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Male: female ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>52</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>21</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>b) No. (%) of patients showing improvement/deterioration of disability grades 1 and 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>7 (09)</td>
<td>2 (7)</td>
<td></td>
</tr>
<tr>
<td>Deterioration</td>
<td>3 (04)</td>
<td>4 (15)</td>
<td></td>
</tr>
<tr>
<td>c) No. (%) of patients showing clinical regression of skin lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>6 (08)</td>
<td>5 (19)</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>32 (44)</td>
<td>9 (33)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>35 (48)</td>
<td>13 (48)</td>
<td></td>
</tr>
<tr>
<td>f) No. (%) of patients showing histopathological regression of lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>6 (8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>38 (52)</td>
<td>12 (44)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>29 (40)</td>
<td>15 (66)</td>
<td></td>
</tr>
<tr>
<td>g) No. (%) of patients showing decline in bacteriological index assessed by SSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>4/19 (21)</td>
<td>7/16 (43)</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>8/19 (42)</td>
<td>4/16 (25)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>7/19 (37)</td>
<td>5/16 (31)</td>
<td></td>
</tr>
<tr>
<td>h) No. (%) of patients showing clearance of antigen assessed by anti-BCG staining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>4/38 (11)</td>
<td>3/22 (14)</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>9/38 (24)</td>
<td>5/22 (23)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>25/38 (66)</td>
<td>14/22 (63)</td>
<td></td>
</tr>
<tr>
<td>i) No. (%) of slit skin smear +ve patients showing growth in mouse foot pad</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 0 month</td>
<td>11/19 (58)</td>
<td>10/16 (63)</td>
<td></td>
</tr>
<tr>
<td>At 18 months</td>
<td>2/19 (11)</td>
<td>3/16 (19)</td>
<td></td>
</tr>
<tr>
<td>% decline</td>
<td>82%</td>
<td>70%</td>
<td></td>
</tr>
</tbody>
</table>
Notably none of the slit skin smear negative (BT-BB) patients test positive in mouse foot pad (MFP) at 18 months although 44/112 (39%) among them scored positive at month 0. The finding was despite the well known poor sensitivity of the MFP test. However when individual patients scoring positive were examined, no linear relationship was observed between the quantum of bacterial load and of viable M. leprae. All the 10 patients testing MFP positive belonged to a medium BI group, ranging between 1+ to 4+ at baseline while none of the 25 patients with high BI (>4+) tested positive. This may have two explanations; a) due to inherent limitation of the MFP technique i.e. a small number of viable bacteria in a pool of large number of dead bacilli fails to multiply in the foot pads of normal mice. If this is true, the number of patients with viable bacteria may be much higher than recorded in this study. b) Second possibility is that, bacterial persistence is unrelated to the quantum of bacteria. However one of the most intriguing incidental finding is the positive correlation seen between the occurrence of viable M. leprae and Type 1 reaction and/or neuritis suggesting that patients carrying viable bacteria are more likely to show repeated T1R.

Steroids have physiochemical effects on the cytokine levels, and help in reducing the edema and inflammation during both Type 1 and 2 reactions. In this study 46% patients in group B showed very good + good regression of lesions while regression of granuloma in the biopsy tissue was seen in 63% indicating that clinical and histopathological regression of lesions may not go hand in hand. Decline in bacteriological index assessed by the conventional methods such as slit skin smears (SSS) and bacterial load/gm wt. of tissue (HL) were comparable in groups A and B. It has been documented that during a reaction episode mainly T1R, there is an influx of immunologically competent cells which help in faster break – down and/or clearance of bacteria and bacterial antigens. However there was also no significant difference between the two groups in the clearance of antigen either assessed using peroxidase anti-peroxidase (PAP) staining technique (Table 1).

EFFECT OF CORTICOSTEROIDS ON NERVE FUNCTION IMPAIRMENT

By monofilament testing and voluntary muscle testing, percentage of nerves showing improvement and deterioration were comparable in the two groups. However by nerve conduction studies (NCS), improvement was seen in a very small proportion of sensory and motor nerves with higher proportion showing deterioration. This finding is in agreement with higher sensitivity of NCS in comparison to clinical nerve function impairment (NFI) tests. At 18 months, sensory and motor nerves in both the groups (results not shown) were equally affected that was Unlike at month 0 where sensory predominated over motor. Among the functionally important nerves, the median nerve (11%) showed better improvement at 18 months while deterioration was high among the radial cutaneous (31%) and ulnar – motor (36%) nerves which were more affected even at onset (Table 4). This reiterates that the quality of improvement is dependent on the pre-existing extent and severity of damage and further shows that early detection and treatment is a factor in reducing nerve damage.

Further questions that arise are; a) would the deterioration in group A been higher than recorded if corticosteroids had not been given?

Comparing groups A and B, by nerve conduction studies percentile deterioration of only sensory nerves was high in group A (P < 0.001), suggesting that corticosteroids is not very efficacious in preventing sensory nerve damage.
On the other hand, motor nerve deterioration was comparable in the two groups. In the absence of a control arm, 2 plausible explanations are; 1) use of steroids might have helped in keeping the motor nerve damage under control or, 2) motor nerves were less affected at month 0 in both the groups, therefore at 18 months the difference probably was not discernable. Important to note is that SAP amplitude is measured in microvolts while motor response is in millivolts. Hence visible changes are better seen in the latter. The former being already small can remain absent or unrecordable even when improvement has occurred. Thus motor action potential is a more reliable parameter than sensory potential for measuring changes in nerve pathology and evaluating appropriate therapeutic regimens.

b) If corticosteroids had been used in group B, would the deterioration been minimised or prevented? A study carried out in south Asian countries using low dose steroids showed reduction in the incidence of new reaction and nerve function impairment in the short term (4 months) but the effect was not sustained at one year.30

Patients with single vs repeat reactions

While the regression of lesions, decline in bacterial index and antigen clearance were comparable in the two groups, decline of granuloma index (GI) in the tissue was better ($P < 0.001$) in patients with single reaction episode. Following T1R, upgrading of the patients towards tuberculoid end of the spectrum has been documented.31 Several studies have also recorded increase in bacterial break down during a reaction episode.32 However its effect on viable $M. leprae$ has not been studied so far. Though the number is small viable $M. leprae$ was higher (19%) among BL-LLs patients with repeat reactions as compared to single (11%). This may indicate that Type 1 reaction does not accelerate killing of bacteria in the host. Secondly there may be a sub-population of $M. leprae$ that is refractory to the effect of MDT and is unrelated to the usage of steroids but strongly associated with the occurrence of T1R, is persuasive.

In keeping with the earlier findings15,33 that the reaction episodes predispose the nerves to further damage, higher percentage of sensory nerve deterioration; 72/216 (33%) was seen among patients with repeat reactions as compared to single 132/584 (22%); $\chi^2 = 9.55\ P < 0.005$ (results not shown). More than one episode of particularly T1R was recorded in 25/27 patients, the majority of whom were smear positive (16/37 = 43%).

The corticosteroid regime needs to be questioned. Would the proportion of nerves showing improvement be more or repeat reactions reduced, if the duration of corticosteroids had been extended or the dose increased? A study by Garbino et al. demonstrated a dose-related (1mg/kg/day to 2mg/kg/day) effect of steroids in the treatment of severe leprosy neuropathy during reactions, especially for an initial short period when inflammation with edema formation is a major component.34 It has been emphatically stated by Naafs35 that 12 weeks course of corticosteroids may not be adequate in the control of T1R and nerve damage. A study by Rao et al.6 using recurrence of reaction as an outcome measure demonstrates that the duration of corticosteroids is more important than the dosage. Prevention of repeat reactions is indeed important and a longer duration of corticosteroids may be justifiable among smear positive BL-LLs patients. Fact that the proportion of both sensory and motor nerves showing deterioration were higher than improvement in both the groups indeed
plead for a better anti-inflammatory or immuno-modulatory drug or regime in tackling this problem.

Conclusions and Implications

(1) Use of 12 weeks regimen of corticosteroids (40 mg Prednisolone tapered to 5 mg) as an adjunct to multi-drug therapy (MDT) does not significantly affect the clearance or killing of *M. leprae* thus dispelling a major apprehension.

(2) No major side effects were recorded in any patients during the course of the study implying that the corticosteroid regime used in this study is safe.

(3) Repeated reaction seen in 27% patients were mainly Type 1 (T1R) and higher among BL-LLs patients implying that, it might be justifiable to administer a longer duration of corticosteroids in these patients.

(4) There was a positive correlation between the presence of viable *M. leprae* and T1R implying that patients carrying viable bacteria are more prone to repeated T1R.

(5) A significant proportion of BL-LLs patients in both groups showed presence of viable *M. leprae* in the mouse foot-pad test indicating that 12 months MB-MDT regime might not be adequate for these patients.

(6) A higher occurrence of viable *M. leprae* among BL-LLs patients with repeat reactions indicate that; a) prolonged usage of steroids without the coverage of MDT may not be advisable b) MDT may need to be prolonged in patients with reaction and/or neuritis and treated with steroids. Also, patients presenting with late reversal reaction should not be treated with steroids alone at any point in time.

(7) Another important issue is the extent to which corticosteroids help in the protection of nerves. Our study findings show that MDT and adjunct corticosteroid therapy is not very efficacious in the prevention and/or reversal of nerve damage.

(8) Early detection and treatment is most important in minimising the nerve damage. However laudable, the effect of steroids in reaction, the search must continue for an alternative drug or regimen for prevention of repeated reactions/nerve damage.

Acknowledgements

The authors are grateful to all the patients who participated in this study. We are grateful to late Dr NH Antia (Founder Director of FMR) for his all round support for this study. Our thanks are due to Mr. Uday Thakkar and management of Kusthrog Nivaran Samiti, Shantivan Panvel for referral of some of the patients and providing premises for an out patient clinic facility at Panvel. Thanks to Dr. NF Mistry (Director, FMR) for her valuable suggestions. Thanks to Dr. Shubhada Pandya for her guidance and inputs in the nerve conduction studies. Our thanks to Mr. K. Gandewar, Biostatistician, Department of Preventive Social Medicine, Sion Municipal Hospital, Mumbai for statistical help. We are indebted to the staff at FMR; Mrs. Anju Wakade for mouse foot pad study, Ramchandra Chile and Harish Poojari for technical help.

**Funding:** This study was funded by the American Leprosy Mission, ILEP Grant code number 7.01.03.46.
References


17. WHO. Study group on chemotherapy of leprosy for control programmes, 1982; TRS, 675: 1–33.


23. WHO. Laboratory techniques for leprosy, 1987; WHO/CDS/LEP/86.4.


