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Long-term Efficacy of 2 Year WHO Multiple Drug Therapy (MDT) in Multibacillary (MB) Leprosy Patients[1]

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ABSTRACT

Relapse rate estimates after 2 year WHO multiple drug therapy (MDT) in multi-bacillary (MB) leprosy vary. Between 1987 and 1994, 500 MB leprosy patients completing 2 year MDT were enrolled in a prospective relapse study. The majority of patients (N = 316) were treated and followed at the physician-staffed Cebu Skin Clinic (CSC), whereas others (N = 184) received therapy from government clinics and were followed by CSC technicians in the field. Relapse definition was an increased bacteriologic index (BI) and new skin lesions, supplemented with mouse footpad inoculations. Through 2002, follow-up was 5368 person-years, with a mean of 10.8 years per patient. The absolute relapse rate was 3% (15/498; 0.28/100 person-years), with a cumulative risk estimate of 3.9% at 15 yrs. For a subset of 217 patients followed for ≥12 yrs or until relapse, relapses occurred in 9% (13/142) attending the CSC, versus 3% (2/75) assessed in the field (p = 0.09). The rate for patients followed at CSC for ≥12 yrs and a pre-treatment BI ≥2.7+ was 13% (13/98). All relapses were BL or LL, with pre-treatment BI's of ≥2.7+. Relapses occurred long after completion of therapy, between 3 and 11 yrs from the midpoint of the examination without relapse to detection, or between 6 to 13 yrs to the actual year of detection, 7 occurring at ≥10 yrs. Lesion material from all relapses contained *M. leprae* that was rifampin and clofazimine sensitive, whereas 3 showed partial or full dapsone resistance. [Follow-up rigor and time], medical expertise, and pre-treatment bacterial load influence relapse rates after 2 yr MDT.

RESUMÉ

Les estimations des taux de rechutes des patients lépreux multibacillaires (MB), après deux années de polychimiothérapie (PCT) selon les recommendations de l'OMS, varient. Entre 1987 et 1994, 500 MB patients hanséniens ayant complété deux années de PCT furent

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enrôlés dans une étude prospective de rechute. La majorité des patients (N=316) furent traités et suivis par l'équipe de médecins de la Cinique des maladies de la peau de Cébu (CSC), tandis que les autres (N = 184) reçurent un traitement des cliniques gouvernementales et furent suivis par des techniciens de la CSC sur le terrain. La définition de la rechute était une augmentation de l'index bactérioscopique (IB) et l'apparition de nouvelles lésions cutanées, complétées par des inoculations à la voute plantaire de souris. Le suivi de 2002 a été de 5368 personnes par année, avec une moyenne de suivi de 10,8 années par patient. Le taux absolu de rechute était de 3% (15/498; 0,28/100 personnes par années) avec une estimation de risque cumulé de 3,9% à 15 ans. Pour une sous-population de 217 patients suivie pendant plus de 12 ans ou bien jusqu'à la rechute, les rechutes représentaient 9% (13/142) des malades suivis pas le CSC, contre 3% (2/75) chez ceux suivis sur le terrain (p = 0,09). Le taux de rechute pour des patients suivis pendant plus de 12 ans et avec un BI avant le traitement de plus de 2,7+ était de 13% (13/98). Toutes les rechutes ont été observées chez des patients BL ou LL, avec un BI avant le traitement de plus de 2,7+. Les rechutes ont eu lieu bien après la fin du traitement, entre 3 et 11 années de la médiane d'examens sans rechute à la détection, soit entre 6 et 13 années après l'année réelle de détection, 7 rechute ayant apparu après 10 années. Toutes les biopsies des personnes ayant rechutées contenaient des *M. leprae* qui étaient toutes sensibles à la rifampicine et la clofasimine, bien que 3 montraient une résistance partielle ou totale à la dapsone. La rigueur et le temps du suivi, l'expertise médicale et la charge bactérienne avant le traitement influencent les taux de rechute après deux années de PCT.

RESUMEN

La tasa de recaída después de 2 años de tratamiento con poliquimioterapia (PQT-OMS) en la lepra multibacilar (MB) varía debido a diferentes factores. Entre 1987 y 1994, 500 pacientes MB que completaron 2 años de PQT fueron enrolados en un estudio prospectivo sobre recaídas. La mayoría de los pacientes (N = 316) fueron tratados y supervisados por el personal médico de la clínica de la piel de Cebu (CSC), mientras que otros (N = 184) recibieron la terapia en clínicas periféricas del gobierno y fueron supervisados por técnicos de la CSC en el campo. La definición de recaída fue un aumento en el índice bacteriológico (IB) y nuevas lesiones en la piel. La resistencia o susceptibilidad a drogas se probó por inoculación en la almohadilla plantar del ratón. Durante el año 2002, el tiempo de seguimiento fue de 5368 persona-años, con una media de 10.8 años por paciente. La tasa absoluta de recaída fue del 3% (15/498; 0.28/100 persona-años), con un riesgo acumulativo estimado de 3.9% a 15 años. Para un subconjunto de 217 pacientes supervisados por >12 años o hasta que se presentó la recaída, las recaídas ocurrieron en el 9% (13/142) de los pacientes atendidos en la CSC, contra el 3% (2/75) de los pacientes atendidos en el campo (p = 0.09). La tasa para los pacientes atendidos en la CSC por >12 años y un IB pre-tratamiento de >2.7+ fue del 13% (13/98). Todas las recaídas fueron de casos BL o LL con un IB pre-tratamiento de >2.7+. Las recaídas ocurrieron mucho tiempo después de haberse concluido la terapia (entre 3 y 13 años), ocurriendo 7 casos a >10 años. El material de las lesiones de todas las recaídas contuvieron *M. leprae* que fue sensible a la rifampina y clofazimina, aunque 3 aislados mostraron resistencia parcial o total a la dapsona. El rigor en el seguimiento y el tiempo del mismo, la experiencia médica, y la carga bacteriana previa al tratamiento, influyen en la tasa de recaída después de 2 años de PQT.

Dapsone monotherapy was used to treat patients with all forms of leprosy from the 1940's until the 1990's. However, concerns about dapsone resistance and relapses upon discontinuation of therapy, especially in those with multi-bacillary (MB) disease, led to the development of rifampin-containing bactericidal regimens in the 1970's, modeled after successful short-course rifampin-based regimens for pulmonary tuberculosis ([10]). By 1982, the WHO abandoned dapsone monotherapy and introduced multiple-drug therapy (MDT), a convenient, relatively inexpensive regimen consisting of monthly rifampin and clofazimine, and daily dapsone and clofazimine administered for at least 2 yrs in MB patients. By 1994, MDT was implemented worldwide and the overall prevalence of leprosy, but not incidence, dropped dramatically because patients completing MDT were removed from prevalence lists ([2, 17]). Relapse rates after MDT were purported to be low, but sufficiently discrepant estimates, now coupled with evidence that

patients with high pre-treatment *M. leprae* loads are at higher risk and that mean relapse incubation periods for rifampin-containing regimens are likely beyond 5 yrs, indicate a need for additional, rigorous, long-term studies to more clearly define the protective efficacy of 2 year MDT ([1, 3, 9, 57, 58]).

With the extraordinary time and effort required to treat and then follow patients to accurately measure relapse, coupled with widespread MDT implementation only since the early 1990's, prospective, rigorously conducted studies are scarce ([1, 3, 4, 6, 9, 57]). Indeed, many reports are retrospective analyses expressed in absolute numbers, and lack adequate follow-up time or sufficient participants, or vary in the definition of relapse criteria, making comparisons across studies difficult ([6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18]). For prospective 2 year MDT relapse studies, overall relapse rates range from 0% to 20%, likely reflecting variability in study site, treatment compliance, relapse definition, frequency and operational differences in follow-up, length of follow-up, and pre-treatment bacterial indices (BI) ([1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 57]). Jamet found that post-MDT relapse rates increased from 3% to 20% when mean follow-up was extended from 3.5 to 6 yrs ([9]), and in 2 studies, the proportion of patients relapsing with high pre-treatment bacterial indices (BI; ≥4+) was notably higher ([3, 9]).

In 1987, we started a prospective longitudinal study to assess for relapses in MB patients who had satisfactorily completed 2 year MDT. Mean follow-up time was about 10 years per patient, longer than other similarly designed studies ([1, 3, 9]). Relapse was defined by 2 field-expedient criteria, including new skin lesions consistent with leprosy and an increasing BI. Supplemental assays included histology and mouse footpad assays, the latter assessing *M. leprae* viability and sensitivity to the individual components of MDT.

METHODS AND MATERIALS

Protocol. Enrollment was conducted from 1987 to 1994 at the Cebu Skin Clinic (CSC), an established leprosy referral center for Cebu province. Any patient with MB leprosy who had satisfactorily completed 2 years of WHO-MDT and was willing to comply with long term follow-up was eligible. The cohort included patients treated and followed at the leprologist-staffed CSC, as well as those treated at local government clinics with a documented diagnosis of MB leprosy and satisfactory completion of 2 year WHO-MDT. MDT consisted of 24 observed doses of rifampin (600 mg) and clofazimine (300 mg) given at monthly intervals within a 24 to 30 month period, and unobserved daily dapsone (100 mg) and clofazimine (50 mg), with no additional efforts to enhance self-administration ([24]).

Patients were excluded if they resided in a leprosaria or more than 100 kilometers away from the CSC, or had tuberculosis, malignancy, or other chronic systemic illness. Volunteers were advised on follow-up requirements and potential benefits, and a field team monitored some patients without transportation. All volunteers received vitamins and travel reimbursement.

To the greatest extent possible, clinical examinations, lepra reaction monitoring, and slit skin smears for acid-fast bacilli (AFB), following a standard 6 site sampling procedure ([25]), were done annually. Unless otherwise specified, all BI values refer to the mean value obtained from the 6 sites. Generally, patients treated at the CSC received follow-up by physician-leprologists every 1 to 2 years, and those recruited from the local government clinics received follow-up by a CSC field team every 2 to 3 years. When more than 1 year had elapsed between smears for a relapsed patient, time to relapse was estimated by selecting the "midpoint" year of the period between most recent smear and the year of smear positivity ([9]).

Relapse was defined as the appearance of new skin lesions consistent with leprosy and a BI increase of ≥2+ at any site ([6, 9]). Lesion tissue was then harvested for histology and mouse footpad inoculation, and the patient treated with ROM, consisting of 12 monthly, supervised doses of rifampin (600 mg), ofloxacin (400 mg), and minocycline (100 mg) ([26]). Lepra reactions, including reversal reaction (RR), characterized by swelling and erythema of existing leprosy lesions and erythema nodosum leprosum (ENL), characterized by crops of tender papulo-nodules, fever, and malaise, were graded as mild, moderate or severe ([27]). Reactions were treated with oral corticosteroids until resolution.

Histology and mouse footpad studies. For each relapse, mouse footpad tests were

TABLE 1. *Demographic characteristics of volunteers.*

Demographics			
Enrolled	500		
1987	79		
1988	58		
1989	85		
1990	50		
1991	71		
1992	68		
1993	86		
1994	4		
Male : Female	377:123		
Mean age (yr), (range)	32.5 (10–69)		
Clinical diagnosis pre-MDT		Mean BI Pre-MDT (range)	Mean BI at MDT completion (range)
BT	24	1.3 (0.5–1.3)	0.2 (0–1.5)
BB	16	2.1 (0.8–2.3)	0.1 (0–1)
BL	253	2.9 (0.2–5)	0.2 (2.4)
LL	207	4.1 (1.2–5.5)	1 (0–4.3)
		All	All
		3.34 (0.2–5.5)	0.5 (0–4.3)

BT = borderline tuberculoid, BB = borderline, BL = borderline lepromatous, LL = lepromatous

used to assess viability of *M. leprae* organisms and characterize drug sensitivities as previously described ([28, 29, 30]). A skin punch biopsy from a clinically active lesion was obtained and processed for inoculation into inbred CBA/J mice. Approximately 5×10^3 acid-fast bacilli (AFB) were inoculated into each hind footpad. One group of untreated mice was used to confirm *M. leprae* viability, whereas other groups of mice received clofazimine or dapsone in the feed (0.0001%, 0.001% and 0.01%), or rifampin twice weekly by gastric gavage (5 mg/kg, 10 mg/kg and 20 mg/kg). For all experiments, both hind footpads of each mouse were pooled, processed, stained (Fite-Faraco), and counted ([28, 31]). *M. leprae* viability was determined by periodically sacrificing 1 or 2 untreated mice, starting at 6 months. Upon viability confirmation, defined as $\geq 1.5 \times 10^5$ *M. leprae*/footpad, treated mice were assessed for *M. leprae* growth. Drug resistance was defined as a harvest of $\geq 10^5$ *M. leprae*/footpad (≥20-fold increase in the inoculum).

Data collection and analysis. Data were recorded on standardized case report forms, entered into a computerized database (Excel 2000), and cross-checked for agreement. Graphical and statistical analyses were done by Sigmaplot 8 (SPSS, UK), SigmaStat (version 2.03, SPSS, UK), SPSS (UK), and Minitab (version 13). The primary outcome was relapse, and secondary outcomes were BI-relapse relationships, mouse footpad studies, and incidence of lepra reactions. Relapse rates were expressed as the percentage of patients relapsed, with incidence densities calculated as the number of relapsed patients divided by the total person-years of follow-up. The Kaplan-Meier method was used to estimate the cumulative risk of relapse during follow-up ([32, 33]). Lepra reactions were reported as proportions.

RESULTS

Volunteers. From 1987 to 1994, 500 patients were enrolled, including 184 government clinic referrals with documented satisfactory MDT completion. Recruitment and demographics are shown in Tables 1 and 2. Clinical classification was according to Ridley and Jopling ([34]). For bacterial loads, 181 volunteers had a "high" BI (≥4.0+) (36%) and 319 had a "low" BI (<4.0+) (64%). At MDT completion, 219 volunteers (44%) remained smear positive, with a downward trend through year 6, when all smears were negative. In general, BI values fell about 1 log per year. Through 2002, 83 patients were no longer in follow-up due to

TABLE 2. *Range of BI pre-MDT, and proportion of positive smears during and after MDT.**

BI range	Number pre-MDT	Number at MDT completion	Number of smear positives yearly post-MDT				
			Yr 1	Yr 2	Yr 3	Yr 4	Yr 5
5.0–6.0+	**63**	0	0	0	0	0	0
4.0–4.9+	**118**	2	1	0	0	0	0
3.0–3.9+	142	11	1	0	0	0	0
2.0–2.9+	119	30	4	2	2	0	0
1.0–1.9+	48	72	7	11	0	0	0
0.1–0.9+	10	104	29	16	15	5	1
Total	500	219/500 (43.8%)	42/202 (20.8%)	29/211 (13.7%)	17/207 (8.1%)	5/250 (2.0%)	1/208 (0.48%)

* All smears negative at 6th year.

relapse (N = 15), death unrelated to leprosy (N = 32), ingestion of anti-leprosy drugs (N = 3), and relocation (N = 33). Overall, 465 volunteers remained in follow-up for ≥5 years or relapsed (Figure).

Relapses. Two volunteers were excluded from analysis because they failed to return for any follow-up. Through 2002, follow-up was 5368 patient-years, with a mean of 10.8 years per patient. Overall, the relapse rate was 3.0% (15/498), with an incidence density of 0.28/100 patient-years. A Kaplan-Meier survival curve (Figure) estimated cumulative risks of relapse at 5, 7, and 15 yrs of 0.64% (95% confidence interval [CI], 0.03%–1.25%), 1.7% (95% CI, 1.0%–2.4%), and 3.9% (95% CI, 2.2%–5.6%), respectively.

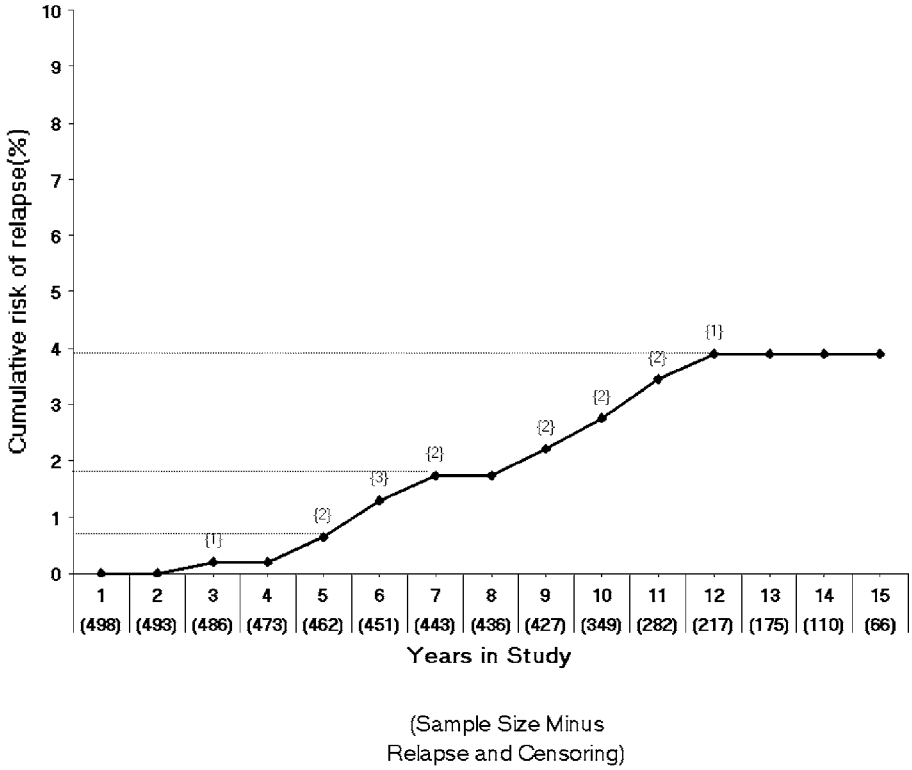
All relapses were borderline lepromatous (BL) or polar lepromatous (LL) before MDT and had pre-treatment BIs ≥2.7+. For the subset of patients followed at the CSC by physician-leprologists for ≥12 years, 13/142 (9%) relapsed vs. 2/75 (3%) followed in the field (p = 0.09, Fischer's exact test). For a smaller group of patients within the CSC subgroup followed for ≥12 yrs and who had an initial BI ≥2.7+, the relapse rate was 13% (13/98) vs. 0% (0/44) in those with an initial BI <2.7 (p <0.001, Fisher's exact test). There was no correlation between relapse incubation time and BI magnitude at relapse ($r^2 = 0.26$; p = 0.35).

Using midpoint estimates, relapses were recorded between 3 and 12 yrs, with a mean relapse incubation time of 7.9 yrs (95% CI, 6.4 to 9.3 yrs) (Table 3). Based on actual year of detection, relapses occurred between 6 and 13 yrs (7 relapses between 10 and 12 yrs), with a mean relapse incubation time 9.0 yrs (95% CI, 7.7 to 10.4 yrs).

All patients fulfilling the criteria for relapse presented with new papules, nodules, or plaques and a rising BI therein (5+ [N = 12], 4+ [N = 2], and 3+ [N = 1]). In all but a single relapse (R-341), the BI had become 0 at all 6 sites sampled on the examination prior to relapse detection. In 10 relapses, AFB were present in new skin lesions as well as earlobes, 8 bilaterally, with BIs ranging from 1+ to 5+ (mean 3.3+), whereas all other standard smear sites were negative for AFB. Overall, 5 relapsed volunteers had a BI of 0 on MDT completion, with all but one attaining a BI of 0 prior to relapse detection. For those with a high pre-MDT BI, 2 of 6 attained a BI of 0 at MDT completion.

Post-MDT reversal reaction (RR) and erythema nodosum leprosum (ENL), recorded only for volunteers recruited at CSC, were reported in 71 of 316 volunteers (23%), with 62 being graded as mild (Table 4) ([27]). The majority of RR (61%) and all ENL occurred within 5 years of MDT completion. Some volunteers developed multiple episodes of the same reaction type, but RR and ENL was never reported in the same patient. All reactions resolved with oral corticosteroids, without permanent sequelae.

Mouse footpad studies. Lesion samples from all relapses grew in untreated mice, confirming viability. All relapse isolates demonstrated sensitivity to all 3 tested rifampin and clofazimine dosage schedules. *M. leprae* from 12 relapsed patients were found to be fully dapsone sensitive. Relapse



THE FIGURE. Cumulative risk estimates (Kaplan-Meier) of relapse after 2 year MDT. Numbers in parentheses in the graph represent relapses, and numbers along the x-axis represent patients in follow-up.

isolates R-499 and R-398 (Table 3) grew in mice treated with only the lowest concentration of dapsone (mild to negligible resistance), whereas isolate R-285 grew in mice treated with all 3 concentrations, compatible with "high" grade resistance.

DISCUSSION

Relapse was studied in a cohort of 500 MB leprosy patients treated reliably with 2 year MDT and followed prospectively by an experienced study site for a mean of 10.8 yrs, longer than most other studies ([57]). For a subset of 217 patients followed ≥12 yrs or until relapse, relapse rates were 9% (13/ 142) among those attending the leprologist-staffed CSC and 3% (2/75) assessed by a CSC field team, a notable difference suggesting that relapse detection may have been affected by medical expertise. Within a smaller subset of patients followed at CSC for ≥12 yrs and a pre-treatment BI ≥2.7, the relapse rate was 13% (13/98). In contrast, the relapse rate was 0% (0/44) in those followed for ≥12 yrs with pre-MDT BIs <2.7+ supporting contentions that higher pre-treatment BIs influence relapse risk [(6)]. Overall, a total of 15 relapses yielded an absolute relapse rate of 3% (0.28/100 person-years) and a Kaplan-Meier survival analysis (adjusts for differences in follow-up) estimated the cumulative risk of relapse to be 3.9% at 15 yrs.

Other notable findings included 8 of 15 relapses occurring within 7 years of MDT completion, and in agreement with other studies, most were in patients with high pre-MDT BI (≥4+) ([57]). Five of 15 relapses attained a BI of 0 by MDT completion, with all but one attaining a BI of 0 prior to relapse detection, emphasizing smear negativity is not necessarily protective against relapse ([3]). All relapses, fulfilling our 2 field-expedient relapse criteria of new skin lesions with an increased BI, were subsequently found to contain *M. leprae* that multiplied in mice, underscoring the reliability of our relapse definition. Especially encouraging was that none of the relapses were associated with peripheral neuropathy or disability. Post-

TABLE 3. *Clinical characteristics of relapses.*

Patient code		New skin lesions at relapse	Age at relapse	Pre-MDT diagnosis	Pre-MDT BI	Post-MDT BI	Year enrolled	Prior negative skin smear	Relapse smear after MDT***	Midpoint year to relapse skin smear after MDT**
R-341	M	Macules, papules, localized infiltration	43	BL	3.7	1.3	1987	1987	1993 (6)	1990 (3)
R-158	F	Plaques	27	LL	**4.5***	2.3	1989	1992	1996 (7)	1994 (5)
R-285	F	Papules, localized infiltration	32	BL	3.0	0	1990	1994	1996 (6)	1995 (5)
R-366	M	Macules, papules, localized infiltration	16	LL	**5.0***	0.5	1992	1997	1999 (7)	1998 (6)
R-408	M	Nodules, localized infiltration	44	LL	**4.5***	0.3	1993	1997	2001 (8)	1999 (6)
R-499	M	Nodules, papules, localized infiltration	48	LL	**4.0***	2.3	1988	1994	1996 (8)	1995 (7)
R-082	M	Localized infiltration	20	BL	**4.0***	0	1991	1997	1998 (7)	1998 (7)
R-207	F	Papules, localized infiltration	21	BL	3.6	0	1992	1998	1999 (7)	1999 (7)
R-053	M	Macules, nodules	25	BL	2.7	0	1990	1997	2001 (11)	1999 (9)
R-162	M	Macules, papules	39	LL	3.8	2.3	1987	1995	1997 (10)	1996 (9)
R-110	M	Localized infiltration	67	BL	3.7	1.8	1987	1994	1999 (12)	1997 (10)
R-161	M	Macules, localized infiltration	32	BL	2.7	0.7	1987	1997	1997 (10)	1997 (10)
R-422	M	Macules, papules, localized infiltration	40	BL	3.7	1.3	1988	1997	2000 (12)	1999 (11)
R-081	M	Macules, papules, localized infiltration	26	LL	3.3	1.3	1988	1997	2000 (12)	1999 (11)
R-398	M	Macules, localized infiltration	32	BL	**4.0***	0	1989	2000	2001 (13)	2001 (12)

* pre-MDT "high" BI (≥4.0+)
** date (number of elapsed years)

TABLE 4. *Lepra reactions after 2 year WHO-MDT.*

Reaction	Total*	Severity	Within 5 years of MDT completion	Range (years)
RR	62/316 (20%)	57 mild 5 moderate	38 (61%)	0–11
ENL	9/316 (3%)	4 mild 5 moderate	9 (100%)	0–5

* There were no enrollees with both reversal reaction (RR) and erythema nodosum leprosum (ENL).

MDT reactions responded well to oral corticosteroids and resolved without permanent sequelae.

A notable observation was the proportional increase in relapse rates found in patients followed by physicians at CSC vs. those in the field. As care of leprosy patients is shifted to the general health service, wherein even rudimentary follow-up may not be feasible, much less long term follow-up, diagnosis of relapse MB leprosy may be delayed. This is problematic because early relapses, like here, may present with only new skin lesions. However, if relapses are detected only late, the risk for neurologic morbidity and deformity increases. Furthermore, since failure of MDT is generally confined to those with BL or LL and high BIs, the loss worldwide of the availability of skin smears and histopathology lessens the ability to identify those patients requiring the most rigorous follow-up. This argues for the maintenance and training of leprologists, emphasis on the importance of the diagnosis of LL and its relapse, and the re-establishment of skin smears and histopathology.

Recent reports suggest the relapse incubation periods after rifampin-containing regimens like MDT likely extend beyond a previously advocated range of 3 to 7 yrs ([57]). Indeed, Pattyn found that relapses after an intensive 6 week rifampin-containing regimen began at 6 yrs, but with a doubling in years 8 to 9 ([58]). Here, 7 of 15 relapses occurred at 9 or more years (midpoint estimate) after therapy, for an overall mean incubation period of 7.9 yrs. After characterizing 15 relapses through 2002, 3 others occurred in 2003, at years 13, 14, and 15 of follow-up, further underscoring the importance of extended follow-up for defining relapse rates. Notwithstanding, relapse incubation periods will invariably depend on relapse definitions, as would rates, in that a more rigorous definition might require longer follow-up to qualify as a relapse ([3, 9, 57]).

Irrespective of relapse definitions, it is impossible to know whether relapses reflect reactivation of the "persister" organisms that have survived MDT or re-infection ([35, 36, 37, 38, 39, 40, 41, 42]). Indeed, although all

TABLE 5. *Mouse footpad tests for viability and drug sensitivity.*

Patient code	Control group	Rifampicin by gavage			Clofazimine in diet			Dapsone in diet		
		20 mg	10 mg	5 mg	0.01	0.001	0.0001	0.01	0.001	0.0001
R-341	16/16	0/2	0/2	0/2	0/10	0/10	0/10	0/9	0/7	0/10
R-158	7/7	0/6	0/6	NH	NH	0/8	0/10	0/9	0/9	0/10
R-499	14/14	0/6	0/8	0/6	NH	0/8	0/7	0/7	0/8	8/8*
R-285	10/10	0/6	NH	0/6	NH	0/7	0/6	2/8**	8/8	8/8
R-162	14/14	0/3	NH	0/6	NH	0/8	0/10	0/10	0/10	0/10
R-161	2/2	0/2	0/2	0/1	0/2	0/2	0/2	0/2	0/2	0/2
R-082	8/8	0/1	0/5	NH	NH	0/7	0/6	0/7	NH	NH
R-110	10/10	0/2	0/5	0/3	NH	0/6	0/6	0/6	0/6	0/6
R-207	6/6	0/2	0/3	0/2	0/2	0/3	0/7	0/7	0/7	0/7
R-081	2/2	0/2	NH	NH	NH	0/6	0/6	0/5	0/8	0/2
R-366	6/6	0/1	0/2	0/2	NH	0/6	0/6	0/6	0/6	0/6
R-422	13/13	0/2	0/3	0/2	NH	0/9	0/7	0/8	0/7	0/9
R-053	7/7	0/4	0/7	0/5	0/6	0/4	0/6	0/4	0/6	0/3
R-408	5/5	0/5	0/5	0/5	0/5	0/6	0/6	0/6	0/6	0/6
R-398	2/2	0/6	0/5	0/5	0/6	0/6	0/6	0/5	0/5	4/6*

* low dapsone resistance, ** high dapsone resistance, NH (no harvest)

relapses were essentially sensitive to the MDT components, arguing against the development of drug resistance, murine monitoring of several rifampin-based regimens for MB leprosy indicate that "persisters" surviving rifampin therapy regularly occur in 9% of MB patients ([43]), coinciding with the 9% relapse rate among those with ≥12 yrs, CSC physician-based follow-up. In tuberculosis (TB), RNA restriction fragment length polymorphisms show that in AIDS populations, almost half the infections are new, whereas in populations with low endemicity, the majority are reactivation ([44, 45]). Genotypic assays that detect differences among *M. leprae* strains may help to distinguish reactivation from reinfection in leprosy, as well as whether certain *M. leprae* strains predispose to relapse and which biochemical anomalies confer treatment failure ([46, 47, 48]).

An important goal of MDT is the prevention of multiple drug-resistant *M. leprae*. Here, relapse *M. leprae* was uniformly sensitive to rifampin and clofazimine, and only 3 (20%) isolates showed dapsone resistance (2 low level, 1 fully resistant), surprising in that up to 52% of our untreated patients in the early 1990's harbored primary dapsone resistant *M. leprae* (low, moderate and high) ([28]). Clearly, our current data argue that 2 year MDT prevented the emergence of drug-resistant *M. leprae*, paralleling observations in effective multi-drug TB regimens ([10]).

In this study, even within subsets of patients suggesting that the relapse rate may be as high as 9% or even 13%, MDT provided a reasonably high cure rate. In contrast, other prospective relapse studies report rates of up to 20%, with even higher rates among patients with a high pre-MDT BI, usually characterized by a still positive BI after MDT completion ([9, 14, 19, 49, 57]). In particular, the Marchoux Study Group (Mali) reported relapses in 20% (7/35) of MB patients with a mean follow-up of 6 years, but a rate of 39% for those with a pre-treatment BI ≥4+ ([5]). Girdhar (India) reported relapses in 7% (20/260) of MB patients with a mean follow-up of 4 years, with a 17% (18/170) rate among those with a pre-treatment BI ≥4+ versus 1% (2/153) with a BI <4+ ([6]). Late relapses here and in Pattyn's study, however, suggest that relapse rates after 2 year MDT in Mali and Agra may have increased with additional follow-up ([50]).

In 1998, the WHO reduced the duration of MDT to 1 year for MB patients, with an eventual goal of 6 months for all forms of leprosy and a cumulative relapse rate of ≤5% at 5 yrs, paralleling accepted rates for pulmonary TB ([41]). Previously, relapse rates greater than 5% after therapy of pulmonary TB were rejected by the British Medical Research Council as inadequate ([10]), and recent findings of a 9% relapse rate after 6 months therapy for cavitary TB ([51]), like MB leprosy with a high bacterial burden, prompted calls to extend therapy to 9 months ([52]). Analagously, our MDT relapse data, along with others, might argue that even 2 year MDT is inadequate in MB leprosy, especially for patients with high BI before treatment, and moreover, that 5 years of follow-up is insufficient to accurately define relapse risks.

Alternatives to 2 year MDT include: (i) the proven modality of lifelong dapsone therapy after 5 years of daily dapsone and rifampin ([55]), (ii) extending MDT, or (iii) highly bactericidal combinations that include rifampin or ofloxacin (or congeners), clarithromycin, or minocycline ([54]). Indeed, in the Agra study, the 17% relapse rate found in MB patients treated for 2 years with an initial BI ≥4 was reduced to 4% when MDT was extended until smear negativity, an average of 5 years on treatment ([3]). According to observations in mice whereby minocycline alone or in combination with moxifloxacin added to the bactericidal activity of rifamycin ([54, 55]), and in the highly bacilliferous and immunosuppressed neonatally thymectomized Lewis rat whereby minocycline + rifampin, but not rifampin alone, consistently eliminated all viable *M. leprae* ([56]), minocycline and perhaps moxifloxacin may be especially beneficial components to any future rifamycin-containing regimen.

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