Multidrug-Resistant Tuberculosis Not Due to Noncompliance but to Between-Patient Pharmacokinetic Variability

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(See the editorial commentary by Dartois, on pages 1827–9.)

Background. It is believed that nonadherence is the proximate cause of multidrug-resistant tuberculosis (MDR-tuberculosis) emergence. The level of nonadherence associated with emergence of MDR-tuberculosis is unknown. Performance of a randomized controlled trial in which some patients are randomized to nonadherence would be unethical; therefore, other study designs should be utilized.

Methods. We performed hollow fiber studies for both bactericidal and sterilizing effect, with inoculum spiked with 0.5% rifampin- and isoniazid-resistant isogenic strains in some experiments. Standard therapy was administered daily for 28–56 days, with extents of nonadherence varying between 0% and 100%. Sizes of drug-resistant populations were compared using analysis of variance. We also explored the effect of pharmacokinetic variability on MDR-tuberculosis emergence using computer-aided clinical trial simulations of 10,000 Cape Town, South Africa, tuberculosis patients.

Results. Therapy failure was only encountered at extents of nonadherence ≥60%. Surprisingly, isoniazid- and rifampin-resistant populations did not achieve ≥1% proportion in any experiment and did not achieve a higher proportion with nonadherence. However, clinical trial simulations demonstrated that approximately 1% of tuberculosis patients with perfect adherence would still develop MDR-tuberculosis due to pharmacokinetic variability alone.

Conclusions. These data, based on a preclinical model, demonstrate that nonadherence alone is not a sufficient condition for MDR-tuberculosis emergence.

Drug-susceptible tuberculosis is currently treated with isoniazid, rifampin, and pyrazinamide as part of a directly observed therapy—short-course strategy (DOTS). However, therapy success is threatened by the emergence of multidrug-resistant tuberculosis (MDR-tuberculosis), defined as simultaneous resistance to isoniazid and rifampin. It is believed that most MDR-tuberculosis arises due to poor adherence, hence the need for a DOTS. The World Health Organization (WHO) has built DOTS into the global approach for tuberculosis control. However, the level of nonadherence associated with the emergence of MDR-tuberculosis is unknown; thus the program requires that all tuberculosis patients be supervised by healthcare workers while swallowing antibiotics. The WHO Director-General has called DOTS the most important health breakthrough of past decades [1].

Most studies that established the utility of directly supervised therapy were retrospective and relied on examining rates of MDR-tuberculosis before and after implementation of DOTS programs [2, 3]. However, DOTS has 5 components, including provision of an adequate and standardized drug supply, which could lead to reduction of MDR-tuberculosis rates independent of adherence. Recently, a meta-analysis questioned the efficacy of direct supervision by a healthcare worker [4].
Moreover, despite >98% adherence, extensive emergence of MDR-tuberculosis still occurs [5]. We were interested in determining whether poor adherence leads to therapy failure and to MDR-tuberculosis emergence. Because DOTS is now accepted worldwide, it would be neither ethical nor desirable to perform a randomized clinical study to answer these questions. Therefore, we examined the questions in our hollow fiber system (HFS) model of tuberculosis.

**METHODS**

**Bacterial Strains**

*Mycobacterium tuberculosis* H37Rv (ATCC 27294) with minimum inhibitory concentrations (MICs) to isoniazid, rifampin, and pyrazinamide of 0.06, 0.125, and 12.5 mg/L, respectively, was used in the experiments. A rifampin-resistant isogenic strain (ATCC 35838) with an MIC of >32 mg/L due to a mutation at codon 531 Ser→Leu in the genes for the β subunit of DNA-dependent RNA polymerase (rpoB) was also used. We generated an isoniazid-resistant *M. tuberculosis* H37Rv strain (MIC = 2 mg/L), which has an AGC→GGC mutation at codon 315 of the catalase-peroxidase gene (*katG*). Storage and propagation of cultures were as described previously [6–9]. Cultures were grown in Middlebrook 7H9 broth supplemented with 10% Oleic acid, albumin, dextrose, catalase (OADC) (herein termed “broth”) at 37°C under 5% CO₂ and shaking conditions. Day 4 cultures in log-phase growth were then used for experiments.

**Definitions**

The standard definition of bactericidal activity is microbial kill of rapidly multiplying bacilli by antibiotics, and the standard definition of sterilizing activity is microbial kill of either semidormant or slowly replicating bacilli in an acidic environment [10, 11]. In the HFS, therapy failure was defined by presence of positive culture at the end of the experiment. The extent of nonadherence was defined as percentage of doses missed based on the Centers for Disease Control and Prevention (CDC) regimen 1, in which drugs are administered daily for 56 days [11]. However, in many parts of the world, therapy is administered for 5 of 7 days each week (total 40 doses) during the initial phase of therapy (5/7 regimen). WHO’s recommendation 2.1B is for 3 weekly doses that can be administered in a low-HIV setting (thrice-weekly regimen) [12].

**Hollow Fiber System**

Technical details of our HFS model have been described previously [6–9]. Each HFS and its contents were incubated at 37°C under 5% CO₂. *M. tuberculosis* was inoculated into the peripheral compartment of each HFS. Drug treatments were administered daily via a computer-controlled syringe pump. We mimicked the pharmacokinetics of isoniazid 300 mg daily, rifampin 600 mg daily, and pyrazinamide 2 grams daily in patients. The HFS achieves decline of antibiotic concentration via dilution of the central compartment with fresh media. We set dilution rates for the drug with the smallest half-life, and achieved the longer half-lives by supplementing drugs with the longer half-life via computer-driven syringe pumps. Drug concentrations achieved in each HFS were measured at 5 time points during the first 24 hours. These concentrations were then modeled using the ADAPT 5 program [13], assuming a 1-compartment model with first-order elimination [6–9]. Bacteria were sampled from the peripheral compartments, then washed twice with normal saline to remove any carryover drug, and cultured on Middlebrook 7H10 agar supplemented with 10% OADC (herein termed “agar”). To enumerate the drug-resistant subpopulations, the samples were cultured on agar supplemented with 0.2 mg/L isoniazid, 2.0 mg/L rifampin, or 300 mg/L pyrazinamide. In addition, we also examined for resistance to 0.125 mg/L isoniazid or 0.0625 mg/L rifampin, based on new susceptibility breakpoints that we have proposed [14]. For pyrazinamide, the agar was acidified to pH 5.8 and supplemented with 10% fetal bovine serum [9]. Cultures were incubated at 37°C under 5% CO₂ and colonies were counted starting 3 weeks after incubation.

**Nonadherence Experiments**

We tested the hypothesis that the extent of nonadherence is directly proportional to the size of the drug-resistant *M. tuberculosis* populations. For bactericidal-effect experiments, *M. tuberculosis* on the fourth day of log-phase growth was inoculated into the peripheral compartment of each HFS in which broth circulated. This was done so that the *M. tuberculosis* would continue in log-phase growth. For sterilizing effect on day 4, log-phase growth cultures were cultured in broth acidified to pH 5.8 for 4 days of further growth (semidormant bacilli) and then inoculated into the peripheral compartment of each HFS with circulating acidified broth.

First, each of 18 HFSs was inoculated with 20 mL of 6.5 log₁₀ colony forming units (CFU) per milliliter of drug-susceptible *M. tuberculosis* in log-phase growth. This mimics the volume of a spherical tuberculosis abscess of approximately 2.2-cm radius for each system. Therapy was administered daily until 0%, 20%, 40%, 60%, 80%, and 100% of total doses in a 28-day treatment period was reached in each of 3 HFSs (Supplementary Table 1). Cultures for total and drug-resistant *M. tuberculosis* were collected on days 3, 7, 10, 14, 21, and 28. For sterilizing-effect studies, semidormant bacilli were inoculated and nonadherence of 0%, 30%, 60%, 70%, 80%, and 100% was examined (Supplementary Table 1), based on bactericidal-effect study results.

Next, we examined the effect of the nonadherence pattern on resistance emergence. Emergence of drug-resistant mutants is a stochastic process, which means that a drug-resistant subpopulation may simply fail to arise by chance. To exclude this possibility, inoculum was premixed with 0.5% of isogenic
rifampin-resistant strains for the pattern of nonadherence bactericidal-effect studies. The 0.5% proportion was chosen because it is just below the clinically meaningful proportion of 1%. Three HFSs were then treated with either 80% or 60% nonadherence administered as start-stop, start-stop-start, or random patterns depicting commonly encountered behavior of tuberculosis patients. For the random forgetting pattern, a computer-generated random number sequence was generated, with each integer representing a day of therapy. In order to determine if reduced efficacy during nonadherence simply reflects the effect of a decreased cumulative drug dose, we included 3 additional HFSs in which the entire 28-day cumulative dose was administered during the first 20% of days and then stopped. Finally, expert opinion recommends that if someone misses 2 weeks of cumulative doses during the initial phase of therapy, standard therapy should be started again as if the patient had received no therapy [11]. To examine this, we treated each HFS exposed to different patterns of nonadherence with standard daily therapy at 100% adherence between day 28 and day 42 and compared the kill rates of standard daily therapy after different extents of nonadherence.

To determine the pattern of nonadherence associated with the worst outcomes during sterilizing-effect studies, 4 changes to the bactericidal-effect study were made. First, the nonadherence study period was extended to 56 days (Supplementary Table 1). Second, the entire cumulative 28-day dose was administered during the first 40% period of the first 28 days, as well as the first 40% period of the last 28 days. In order to determine the effect of nonadherence on amplification of a preexistent drug-resistant subpopulation, the inoculum was premixed with both 0.5% of the rifampin-resistant isolate as well as 0.5% of the isoniazid-resistant strain. In order to enhance detection of drug resistance, we emptied the entire bacillary population in each HFS cartridge on day 56 and performed rifampin and isoniazid MICs using Etest strips (AB Biodisk).

**Statistical Analysis**

GraphPad Prism 5 software was used for statistical analysis. Two-way analysis of variance (ANOVA) with Bonferroni post-test correction was used to compare bacterial burden at each time point. Linear regression analysis was performed to compare the kill rates, with 100% adherence in HFS exposed to prior different patterns of nonadherence.

**Monte Carlo Simulations**

We propose that pharmacokinetic variability is an alternative explanation for MDR-tuberculosis emergence. First, when a population of patients is given the same dose of an anti-tuberculosis drug, a wide distribution of drug concentrations are encountered as a consequence of evolution-driven pharmacokinetic variability [15–18]. This results in low drug concentrations in a subset of patients throughout the initial phase of therapy. Given that drug resistance is near certainty when anti-tuberculosis agents are administered as monotherapy [19–21], we hypothesized that the low drug concentrations encountered due to the pharmacokinetic variability lead to effective monotherapy and hence drug resistance in a proportion of patients. Because computer-aided clinical trial simulations that utilize Monte Carlo methods closely predict therapeutic events in tuberculosis patients [7, 9, 22], we applied this mathematical method to determine the proportion of patients who develop MDR-tuberculosis during treatment with standard therapy in Khayelitsha, Cape Town, South Africa. This location was chosen because MDR-tuberculosis is common and the pharmacokinetic variability of anti-tuberculosis drugs in these patients has been well characterized [17, 18].

First, the rifampin exposure below which there is effective isoniazid monotherapy was identified by examining the 0- to 24-hour area under the concentration–time curve (AUC) below which rifampin is ineffective in clinical studies from Cape Town, as well as 1 study from Nairobi, Kenya [23–26]. Regression analysis identified the minimum AUC associated with an early
bactericidal effect as 13.1 mg·h/L and the minimum AUC associated with a sterilizing effect as 7.8 mg·h/L. The rates of M. tuberculosis CFU/mL decline at a variety of AUCs was then determined. Isoniazid was defined as mediating microbial kill only during the first 3 days but continuing to prevent rifampin resistance from day 4 to day 56. Rifampin and isoniazid kills were considered independent of each other and neither synergistic nor antagonistic. Pyrazinamide is inactive at neutral pH [27]. Resistance emergence was only examined for bacilli at neutral pH; therefore, pyrazinamide’s presence was ignored. We assumed that no drug resistance would emerge among semi-dormant bacilli under acidic conditions, given that 3 drugs work on this subpopulation and thus monotherapy situations would be less common.

We performed Monte Carlo simulations of 10,000 patients treated for drug-susceptible tuberculosis. Rifampin and isoniazid population pharmacokinetic parameters derived in Cape Town tuberculosis patients [17, 18] were embedded in the PRIOR subroutine of the ADAPT 5 program. A distribution of AUC and peak concentrations was generated for patients treated with

![Figure 2](image-url)

**Figure 2.** Effect of the nonadherence dosing schedule during bactericidal activity. The color-coded solid boxes represent the days when corresponding treatment was administered for each pattern of nonadherence. A, 80% nonadherence. B, 60% nonadherence. C, Delivery of the cumulative 28-day dose as an 80% start-stop nonadherence (20% adherence) demonstrated decreased killing after therapy was stopped. D, Rifampin-resistant subpopulations as measured by growth on the standard 0.2-mg/L concentration as well as the recently proposed 0.625-mg/L concentration [14]. E, Isonia-zid-resistant subpopulation utilizing 2 susceptibility breakpoints. Abbreviations: CFU, colony-forming unit; M. tuberculosis, Mycobacterium tuberculosis.

Table 1. Effect of Treatment With Standard Regimen in Systems That Were Initially Exposed to Different Extents and Patterns of Nonadherence

<table>
<thead>
<tr>
<th>Prior degree of nonadherence</th>
<th>Slope (log_{10} CFU/mL/day)</th>
<th>95% CI</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>−0.18</td>
<td>−0.24 to −0.12</td>
<td>0.99</td>
</tr>
<tr>
<td>80% start-stop</td>
<td>−0.13</td>
<td>−0.42 to −0.16</td>
<td>0.97</td>
</tr>
<tr>
<td>80% random</td>
<td>−0.11</td>
<td>−0.14 to −0.07</td>
<td>0.95</td>
</tr>
<tr>
<td>60% start-stop</td>
<td>−0.15</td>
<td>−0.24 to −0.07</td>
<td>0.87</td>
</tr>
<tr>
<td>60% start-stop-start-stop</td>
<td>−0.15</td>
<td>−0.56 to −0.27</td>
<td>0.95</td>
</tr>
<tr>
<td>60% random</td>
<td>−0.23</td>
<td>−0.41 to −0.04</td>
<td>0.75</td>
</tr>
<tr>
<td>28-day cumulative dose given as 80% nonadherence</td>
<td>−0.12</td>
<td>−0.17 to −0.08</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Abbreviations: CFU, colony-forming unit; CI, confidence interval.
standard doses of isoniazid and rifampin daily during the first 56 days. Rates of microbial kill were then calculated for each of the 10,000 patients. The lower limit of detection of *M. tuberculosis* in sputum was set at 0.699 log_{10} CFU/mL [28]. To be conservative, we assumed that not all patients on monotherapy would develop drug resistance. Therefore, we constrained drug-resistance emergence rates in subjects on “monotherapy” to be proportional to rates observed in clinical studies of patients treated with monotherapy during the first 2 months of therapy [19, 21, 29].

In a second set of Monte Carlo simulations, we determined the time to emergence of resistance in those patients on effective monotherapy. We assumed that drug-resistant mutants have a prevalence of 3.5 × 10^{-6} and 3.1 × 10^{-8} for isoniazid and rifampin, respectively [30], and that the number of resistant cells after drug exposure follows a Poisson distribution. Drug-resistant bacilli were assumed to have a doubling time of 1–6 days [31]. We assumed that the common *katG* S315T and *rpoB* S531L mutations have negligible fitness costs [32].

**RESULTS**

**Effect of Nonadherence on Bactericidal Activity**

Pharmacokinetic analysis of drug concentrations observed within each HFS confirmed that different pharmacokinetic profiles for each drug were achieved within each HFS (Figure 1A). Regarding efficacy, ANOVA demonstrated that 70.5% of the total variance of the *M. tuberculosis* population was attributable to nonadherence (*P < .0001*). HFS with 0%–40% nonadherence had similar *M. tuberculosis* kill rates throughout the entire 28-day period (Figure 1B). HFS with 60% nonadherence had slower rates of kill until day 14 (*P = .001*). However, with 80% nonadherence, therapy failed. Therefore, the breakpoint of nonadherence was 60%–80% of doses of the daily regimen. Regarding drug resistance, the pyrazinamide-resistant subpopulation was 3.55% of the inoculum, but the proportion decreased with time in all systems and fell below the clinically meaningful 1% proportion by day 14. Thus, nonadherence did not amplify the pyrazinamide-resistant subpopulation. There was no emergence of any isoniazid- or rifampin-resistant *M. tuberculosis* subpopulations, even in HFS in which therapy failed. Thus, the therapy failure associated with nonadherence was due to isoniazid- and rifampin-susceptible *M. tuberculosis*.

**Effect of Pattern of Nonadherence During Bactericidal Activity**

Different patterns of nonadherence were associated with different therapeutic responses in log-phase growth bacteria, as shown in Figure 2. The start-stop pattern fared worse because microbial kill stopped when the regimen was stopped and the bacteria started to regrow. The start-stop-start-stop pattern, which occurs when patients forget to take therapy but restart each time prior to an encounter with healthcare workers, led to intermediate results. The random pattern of nonadherence, which occurs when patients randomly forget to take antibiotics, fared best (Figure 2A and B). The pattern of microbial kill in HFS in which the entire 28-day cumulative dose was administered during the first 20% of days is shown in Figure 2C. This demonstrates rapid microbial kill until the therapy was stopped such that by day 28 this regimen had killed fewer than had the same cumulative dose administered as daily therapy for 28 days (*P < .01*). This means that both the cumulative drug dose and the duration of therapy when there is 100% adherence independently contribute to therapy failure during nonadherence.

The pyrazinamide-resistant *M. tuberculosis* response pattern was similar to that encountered in the first bactericidal effect study described above. Rifampin-resistant isolates were encountered in all systems with a total bacillary population above detection limits. Comparison of the day 28 drug-resistant bacterial burden is shown in Figure 2D, which demonstrates that the rifampin-resistant subpopulation never increased above the 0.5% introduced in the original inoculum. An isoniazid-resistant subpopulation was encountered in some HFS on day 28 (Figure 2E). However, the isoniazid-resistant subpopulation was below the 1% threshold, even with nonadherence. Finally, when all HFSs were treated with standard therapy starting on day 28, slopes of *M. tuberculosis* kill rates were as shown in Table 1, which demonstrates that there was good microbial kill in all HFSs regardless of prior degrees of nonadherence. The kill rates were similar to the rate of $-0.16$ (95% confidence interval, $-.21$ to $-.10$) log_{10} CFU/mL/day ($r^2 = .93$), which was encountered during the first 14 days of therapy with 100% adherence. This means that no MDR *M. tuberculosis* or tolerance was encountered at sufficient levels to limit effectiveness of standard therapy despite prior poor adherence.

**Effect of Poor Adherence During Sterilizing Activity**

The extent of nonadherence associated with therapy failure during sterilizing effect is shown in Figure 3. HFS with 70% nonadherence killed much slower; 80% nonadherence failed. Thus,
the breakpoint nonadherence associated with therapy failure during sterilizing effect was between 70% and 80% of the daily therapy regimen. There was no emergence of isoniazid- or rifampin-resistant subpopulations in any of the systems. Moreover, no HFS that received therapy had any pyrazinamide-resistant bacilli by day 28.

**Effect of Pattern of Nonadherence During Sterilizing Activity**

The effect of different patterns of nonadherence during the entire 56-day initial phase of therapy, given inoculums with 0.5% isoniazid-resistant and 0.5% rifampin-resistant subpopulations, is shown in Figure 4. There was virtually no difference in total bacterial burden between the start-stop and random nonadherence patterns. For the 60% nonadherence with the start-stop strategy, although there was faster kill on day 14 than with the random pattern (M. tuberculosis burden of 3.39 ± 0.09 vs 4.56 ± 0.08 log_{10} CFU/mL; P < .01), there was no difference by day 56. However, neither isoniazid-resistant nor rifampin-resistant subpopulations of >1% proportion were detected throughout the 56 days of therapy. Even when the entire 20-mL contents of each HFS was emptied on day 56, the rifampin and isoniazid MICs were similar to those in the starting inoculums, further demonstrating no amplification of drug-resistant population.

**Pharmacokinetic Variability and the Emergence of MDR-tuberculosis**

We performed Monte Carlo simulations to identify the effect of pharmacokinetic variability on efficacy and on emergence of drug resistance. An examination of resultant pharmacokinetic parameters revealed that they closely recapitulated the original pharmacokinetic data [17, 18], internally validating the simulations. The microbial kill rates in sputum of 10000 simulated Cape Town patients are shown in Figure 5A. There was a bi- phasic pattern in the sputum bacillary counts (Figure 5A), which is characteristic of response encountered in actual tuberculosis patients on therapy [26, 33], thereby externally validating our assumptions. The calculated 2-month sputum conversion rate in the simulated subjects was 58.32%. This is similar to the 51%–60% observed in clinical studies of patients with tuberculosis who are on DOTS in the Western Cape of South Africa [34, 35], which further validates that our simulations predict the true clinical effect.

Rifampin concentrations below those that kill M. tuberculosis (AUC <13.1 mg*h/L during first 3 days, and <7.8 mg*h/L subsequently) were encountered in 969 patients. Therefore, M. tuberculosis in these patients was exposed to isoniazid monotherapy, known to lead to drug resistance [29]. These patients had stopping and repeating this starting day 28 took longer to sterilize compared with daily dosing with 100% adherence. Abbreviations: CFU, colony-forming unit; M. tuberculosis, Mycobacterium tuberculosis.

![Figure 4](image)
MDR-tuberculosis Not Due to Nonadherence

DISCUSSION

Global health policies that affect millions of patients should be subject to hypothesis testing in the laboratory. We tested the nonadherence hypothesis in the HFS. The HFS has no immune system and can achieve large bacillary loads, which are 2 important risk factors for emergence of drug resistance [36]. Drug resistance is encountered within 1–3 weeks when each of the drugs is administered as monotherapy in the HFS [7–9]. Thus, it was surprising that there was no emergence or amplification of either rifampin- or isoniazid-resistant subpopulations with nonadherence in multiple experiments. The “pyrazinamide-resistant” subpopulation that was encountered early during therapy merely reflects the fact that critical concentrations of pyrazinamide do not kill >90% of bacilli in liquid culture [37–40]. In any case, this subpopulation was transient. Thus, nonadherence was not a sufficient condition for MDR-tuberculosis emergence, or even monoresistance, during the initial phase of therapy.

We propose pharmacokinetic variability as a working hypothesis for the emergence of MDR-tuberculosis. We used Monte Carlo simulations, which have been shown to correctly predict efficacy of anti-tuberculosis drugs [7, 39], and achieved microbial kill indices and patterns similar to those encountered in the clinic [33–35]. The simulations demonstrated that between-patient pharmacokinetic variability will lead to a considerable proportion of patients being on monotherapy throughout the entire initial phase of therapy, leading to drug resistance. However, this hypothesis needs further testing in the clinic. Nevertheless, if proven to be correct, this problem lends itself to a scientific solution of either optimizing doses for local populations by taking into account pharmacokinetic variability or, better still, individualization of each patient’s doses if resources are available.

Our findings also have immediate clinical and public health implications. Although poor adherence did not lead to MDR-tuberculosis, it nevertheless led to therapy failure in the HFS. Failure was encountered at >60% nonadherence with the daily therapy regimen. On the other hand, the 5/7 regimen is equivalent to our start-stop-regimen, with 29% nonadherence. Thus, if patients took all 40 doses in that regimen, there would be no failure of therapy. Ours is the first evidence to demonstrate that the regimens have equivalent efficacy. However, the 5/7 regimen is less forgiving if nonadherence occurs.

For the thrice-weekly regimen, practically any nonadherence leads to therapeutic failure. Thus, it should be questioned whether it is wise to recommend this regimen.

Our experiments also answered another practical question. Based on expert opinion, if a patient misses 2 weeks of cumulative doses during the initial phase of therapy, then standard their pills. The time to emergence of >1% proportion drug resistance is shown in Figure 5C.
therapy should be started again as if the patient had not taken any therapy [11]. Here, we provide the first experimental evidence that standard therapy will work even after nonadherence (Table 1). However, the therapy should be restarted from the beginning only if the extents of nonadherence associated with failure were reached.

In summary, therapy failure occurred only at high rates of nonadherence. No MDR-tuberculosis emerged with nonadherence in repeated experiments in the HFS. Instead, we propose pharmacokinetic variability as a more likely cause of MDR-tuberculosis emergence.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/).

Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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