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Use of Nonlinear Mixed-Effects Analysis for Improved Precision of Early Pharmacodynamic Measures in Tuberculosis Treatment

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Nonlinear mixed-effects analysis of serial sputum colony-counting data supports the existence of two bacillary subpopulations in sputum, eliminated at different rates. It distinguishes between combination regimens, removes bias, and greatly improves precision, with significant implications for the analysis of surrogate endpoints of "sterilization" in the development of new antituberculosis regimens.

Improving tuberculosis treatment depends on enhancing the "sterilizing" activity of drug regimens, and surrogate endpoints of this activity are urgently needed (9). Serial sputum colony counting (SSCC) of *Mycobacterium tuberculosis* early in therapy has been advocated for this purpose. "Early-bactericidal-activity" studies measure the effects of single agents over the first 2 days (10, 20), probably reflecting activity against actively dividing bacilli within cavities rather than "sterilizing" activity. Extending the sampling period could provide such a measure (11), but prolonged monotherapy raises ethical concerns. The only SSCC study employing a modern combination regimen and methods without decontamination of sputum was performed in Kenya in 1989 and 1990 (2). Reanalysis of this study (1) using summary measures (SM) (13) supports the concept of extended sampling but does not make optimal use of the data. We describe a nonlinear mixed-effects (NLME) approach offering greater efficiency.

The study design and conduct were described previously (2). A total of 122 new pulmonary-tuberculosis patients received streptomycin and isoniazid and either thiacetazone (the SHT regimen) or rifampin and pyrazinamide (the SHRZ regimen). All harbored drug-sensitive strains and consented to human immunodeficiency virus (HIV) serology (29% seropositive). Sputa were sampled for SSCC on days 0, 2, 7, 14, and 28.

The nlme package (18) in R (19) was used for NLME analysis with monoexponential and biexponential models. Only positive results were included, as in the previous analysis (1). A $\log_{10}$ transformation of the response and a variance function were used to account for heteroscedasticity. With an exponentiated parameterization to enforce positivity of the parameters (5), the biexponential model has the following form:

$$\log_{10} \mathrm{CFU} = \log_{10} (e^{\theta_1} \times e^{-\mathrm{day} \times e^{\theta_2}} + e^{\theta_3} \times e^{-\mathrm{day} \times e^{\theta_4}})$$

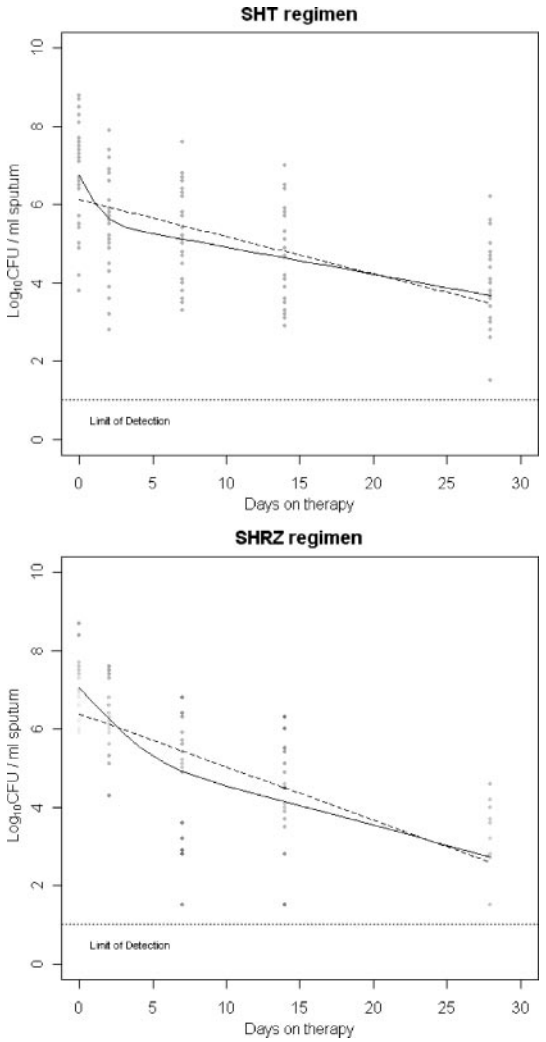

FIG. 1. Locations of the monoexponential (dashed lines) and biexponential (solid lines) regression functions fitted to the subsets of complete profiles for the SHT ($n = 29$) and SHRZ ($n = 19$) regimens.

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TABLE 1. Point estimates and 95% confidence intervals of the fixed effects and goodness of fit for the two regimens from pooled and mixed-effects fits using the whole data set (SHT $n = 54$, SHRZ $n = 46$)

Regimen	A ($\log_{10}$ CFU)	α ($\log_{10}$ CFU/ml/day)	B ($\log_{10}$ CFU)	β ($\log_{10}$ CFU/ml/day)	AIC[a]	LR[b] test
SHT						
Monoexponential	6.081 (5.851–6.311)[c]	0.102 (0.086–0.122)			769.4	
Biexponential	6.633 (6.282–6.984)	0.621 (0.241–1.132)	5.380 (4.916–5.844)	0.066 (0.045–0.099)	751.9	<0.0001
Biexponential mixed effects	6.650 (6.264–7.037)	0.881 (0.651–1.191)	5.463 (5.114–5.812)	0.076 (0.060–0.095)	688.6	<0.001
SHRZ						
Monoexponential	6.397 (6.148–6.647)	0.146 (0.128–0.167)			622.2	
Biexponential	6.845 (6.509–7.180)	0.456 (0.243–0.858)	5.472 (4.868–6.075)	0.100 (0.072–0.140)	606.4	<0.0001
Biexponential mixed effects	6.834 (6.509–7.159)	0.519 (0.409–0.658)	5.307 (4.824–5.790)	0.094 (0.079–0.113)	526.7	<0.001

[a] AIC, Akaike information criterion.
[b] LR, likelihood ratio.
[c] Values in parentheses are 95% confidence intervals.

Fitted models were compared using the Akaike information criterion, the likelihood ratio test, and residual plots. For comparability, parameter estimates were reexpressed as intercepts A and B (θ_1 and θ_3 divided by 2.303) and rate constants α and β [e.g., $\beta = (e^{\theta_4})/2.303$] on the $\log_{10}$ scale (1). An analytical approximation, θ_3/e^{θ_4}, was used to compute the elimination times of subpopulations. A linear model was used for covariate effects on the intercepts. Inferences relied on Wald tests, but other methods were used to explore and confirm the findings (quantile-quantile plots and the Shapiro-Wilks W test for normality, unpaired Student or Welch *t* tests, and Wilcoxon and Mann-Whitney rank sum U tests for tests of significance, as appropriate).

One hundred subjects (54 SHT; 46 SHRZ) had more than one data point available, and 48 subjects had five positive data points. For both data sets, model fitting unequivocally selected a biexponential model (Table 1 and Fig. 1), suggesting that at least two subpopulations of bacilli, eliminated at different rates, are present in sputum (referred to henceforth as "fast" and "slow"). Parameter estimates were consistent with previous SM estimates (1) and the effects of these regimens in clinical trials (7), with the "slow" rate constant, β, larger for SHRZ than for SHT (0.094 versus 0.063 log CFU/ml/day for regimen contrast within the whole data set [$P = 0.013$]; 0.094 versus 0.076 log CFU/ml/day for fitted data by regimen [$P < 0.001$]). The addition of random effects significantly improved the fit ($P < 0.001$) (Table 1), accounting for high variability in parameters between subjects, greater in the intercepts A and B than the rate constants α and β.

Differences in the A intercept by HIV status were confounded by the extent of cavitation ($P = 0.432$ for HIV and 0.018 for the cavitation score), but controlling for this, the B intercept remained reduced in the HIV-positive group ($P = 0.008$). β did not differ by HIV status for either regimen (SHT, $P = 0.928$; SHRZ, $P = 0.367$). For the SHT and SHRZ regimens, the predicted elimination times were 7.5 (5.6 to 9.5) and 13.2 (10.3 to 16.0) days for the "fast" subpopulation and 74.3 (68.8 to 79.8) and 56.2 (52.9 to 59.5) days for the "slow" subpopulation.

We compared the NLME estimates of β with different SM estimates, with respect to bias (Table 2) and precision (Table 3). Any SM estimator involving day 2 overestimated β by 16 to 139%, reflecting the persistence of the "fast" component within the first week, but over later periods agreed to within 5 to 29%. Overall, comparing the SM β (2 to 28) coefficient with NLME estimates of β, the percent coefficient of variation was reduced sixfold, from 62% to under 10%.

We conclude that a biexponential model fits the data best, a finding consistent with the presence of multiple bacillary subpopulations. Alternative explanations, such as selection of drug-tolerant bacilli from an initially homogeneous population

TABLE 2. Bias: magnitude of SM and NLME estimators of β and their ratio[a]

Parameter[b]	SHRZ		SHT	
	Value ($\log_{10}$ CFU/ml/day)	Ratio	Value ($\log_{10}$ CFU/ml/day)	Ratio
KI(2–7)	0.225	2.39	0.121	1.61
KI(2–28)	0.115	1.22	0.060	0.80
β(2–28)	0.109	1.16	0.057	0.76
KI(7–14)	0.099	1.05	0.091	1.21
KI(14–28)	0.099	1.05	0.053	0.71
NLME β	0.094	1.00	0.075	1.00

[a] Methods for the SM estimates are given in reference 1.
[b] β(*a*–*b*) refers to the coefficient of a linear regression fitted to all the data points between and including times *a* and *b*. KI(*a*–*b*) refers to the slope of a straight line connecting data points at time *a* and *b*.

TABLE 3. Precision: standard errors and percent coefficients of variation of SM and NLME estimators of β

Parameter	Point estimate ($\log_{10}$ CFU/ml/day)	Standard error[a]	CV%[b]
Pooled SM β(2–28)	0.077	0.048	62.3
SHT			
Complete profiles β	0.069	0.014–0.017	20.2–25.2
All data β	0.066	0.012–0.015	18.4–22.5
Mixed effects β	0.076	0.008–0.010	10.9–12.6
SHRZ			
Complete profiles β	0.100	0.022–0.027	21.4–27.3
All data β	0.100	0.016–0.019	15.7–18.6
Mixed effects β	0.094	0.008–0.009	8.8–9.7

[a] A range of standard errors is given, since the confidence intervals on the $\log_{10}$ scale derived from NLME analysis were obtained by an exponential transformation of the confidence interval for the original parameter θ_4 and hence are asymmetrical.
[b] CV%, percent coefficient of variation.

during therapy, seem less plausible given the spatial compartmentalization of bacilli in pulmonary tuberculosis (4, 6), the pharmacodynamic properties of combination chemotherapy (14, 15), and the conditions for induction of drug tolerance in vitro (16). Some studies have reported only a single population in their data (8, 12, 21) but invariably employed sputum decontamination, presumably selectively eliminating any "fast" subpopulation to various extents (3). Since the only observed population then declines at a constant rate, these data support the hypothesis of multiple baseline subpopulations.

Since the biexponential model better represents the data, it can eliminate bias in SM estimates computed over periods prior to elimination of the "fast" subpopulation. It can in principle discriminate between effects on subpopulations, a critical methodological issue for accurate estimation of "sterilizing" activity. The activities of antituberculosis drugs against different subpopulations vary in vitro (17). Agents active against multiple subpopulations or interacting pharmacodynamically with other components of a regimen in vivo could create unpredictable bias in SM estimates.

The estimated elimination times of the "slow" component raise the question of whether this subpopulation is the one from which relapses subsequently arise and therefore an appropriate model for ultimate sterilization and cure. A third subpopulation at an abundance below 10 to 10^3 CFU/ml of sputum may not be detected by SSCC (3), and a lack of degrees of freedom restricts the complexity of the models fitted here. Our findings need to be supported and extended by further experimental work and clinical trials. Though SSCC parameters have greater prima facie plausibility than early-bactericidal-activity measures, whether they accurately reflect clinical endpoints of "sterilization," like relapse, requires ongoing evaluation.

NLME analysis can explicitly model complicated error structures, account for interindividual variability, and make efficient use of incomplete data. It may be a useful method of reducing bias and improving precision in assessment of the "sterilizing" activities of antituberculosis regimens.

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