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Comparative Value of Sputum Smear Examination and Culture Examination in Assessing the Progress of Tuberculous Patients Receiving Chemotherapy*

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Bacteriological response is generally considered the best criterion for assessing the efficacy of chemotherapy in patients with pulmonary tuberculosis. The bacteriological methods most commonly used are examination of sputum smears for tubercle bacilli, culture of bacilli from sputum specimens, and drug-sensitivity tests on positive cultures. Culture examination, though more sensitive than smear examination in detecting tubercle bacilli, is time-consuming and economically impracticable as a routine method in most developing countries. A study was therefore undertaken at the Tuberculosis Chemotherapy Centre, Madras, to determine the relative value of smear examination and culture examination in predicting the outcome of treatment and assessing the efficacy of chemotherapeutic regimens in 515 patients (all with bacteriologically confirmed disease and isoniazid-sensitive organisms on admission) receiving isoniazid, alone or with sodium PAS. The results showed that the value of smear examination of overnight sputum specimens at monthly intervals closely approached that of culture examination in assessing the progress of the patients, the percentages of correct predictions by smear and by culture being of the same order. Smear examination was slightly less effective than culture examination in detecting differences in the efficacies of regimens, but it has been estimated that this disadvantage can usually be compensated for by increasing the study population by about 20 %.

In studies of the chemotherapy of pulmonary tuberculosis undertaken at the Tuberculosis Chemotherapy Centre, Madras, the progress of patients has been assessed mainly on the results of sputum cultures for tubercle bacilli. However, culture facilities are either very limited or unavailable in most tuberculosis clinics in developing countries. The present paper therefore attempts to study the efficacy of the simple method of sputum smear examination for tubercle bacilli, relative to that of culture examination (and isoniazid-sensitivity tests), in assessing the progress of patients receiving chemotherapy and in classifying their response to treatment. The comparisons are based on the ability, during treatment, of these methods to predict the outcome at the end of one year of chemotherapy. The patients for these comparisons have been drawn from the first 3 chemotherapy studies undertaken at the Centre (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1963). In these studies, five regimens, ranging in therapeutic efficacy (as measured by the proportion of patients showing a favourable response, assessed mainly by strict bacteriological criteria) from about 45% to 90%, had been investigated.

PATIENTS

In this report, 532 patients, who were included in the main analysis of three chemotherapy studies (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1963), have been considered. All were aged 12 years or more, had pulmonary tuberculosis

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confirmed by culture examination, had had no previous chemotherapy (except 18 (3%) who had received up to two weeks of chemotherapy), and were excreting *isoniazid-sensitive* tubercle bacilli on admission to treatment. They were prescribed isoniazid plus *p*-aminosalicylic acid (sodium PAS) or one of four regimens of isoniazid alone for 12 months (see footnote to Table 5).

Seventeen patients have been excluded from all the analyses in the present report because their response to the initially prescribed chemotherapy could not be assessed, eight because they died of non-tuberculous conditions and nine because their chemotherapy was changed on account of drug toxicity. Of the remaining 515, who form the subject of this report, 9 died of tuberculosis and 40 had the initially prescribed chemotherapy changed on account of serious radiographic or clinical deterioration. These 49 patients have been classified as having an unfavourable response to treatment and included in all the analyses up to the time of death or change of chemotherapy.

INVESTIGATIONS DURING TREATMENT

All the patients had routine monthly examinations at the Centre which included a chest radiograph and sputum examination. At the end of each month of treatment, they were asked to produce two collection specimens of sputum. In addition, from three months onwards, when it was anticipated that some of the patients would have difficulty in expectorating, a pair of laryngeal swabs was taken from all patients at each monthly examination. The sputum specimens were examined for the presence of tubercle bacilli both by smear and by culture, and the laryngeal swabs by culture only. An isoniazid-sensitivity test was set up on one positive culture, if available, at each monthly examination.

Methods of obtaining specimens

Collection specimens of sputum. The patient was given a sterile screw-capped McCartney bottle (1 oz[1]) to take home and was instructed to expectorate into it until it was nearly full. On the following morning, the bottle was handed in at the laboratory; the specimen was thus material collected overnight and sometimes over 24 hours.

Laryngeal swab specimens. A pair of swabs was obtained at the Centre from each patient as described by Velu, Narayana & Subbaiah (1961).

Bacteriological procedures

Direct smear examination of sputum specimens. A new glass slide was used for the preparation of each smear. Purulent or, failing this, mucoid material or any deposit in the sputum was selected for examination. The whole smear was examined by fluorescence microscopy (Holst, Mitchison & Radhakrishna, 1959) and was reported as positive if it contained a minimum of four acid-fast bacilli of typical morphology.

Culture of sputum specimens and laryngeal swab specimens. From each sputum specimen and from each pair of laryngeal swabs (processed as one specimen), cultures were set up on Löwenstein-Jensen medium without potato starch (Tuberculosis Chemotherapy Centre, Madras, 1959), and examined weekly for 8-9 weeks; they were reported as negative if no growth was present by that time.

Sensitivity tests. Tests of sensitivity to isoniazid were set up on Löwenstein-Jensen medium slopes containing concentrations of isoniazid of 0.2, 1, 5 and 50 μg/ml, as well as on a drug-free control slope, by inoculating approximately 0.01 mg (moist weight) of tubercle bacilli (Tuberculosis Chemotherapy Centre, Madras, 1959). A culture isolated during treatment was classified as isoniazid-resistant if it yielded a growth of 20 colonies or more on the slope containing 0.2 μg/ml.

DEFINITIONS OF FAVOURABLE AND UNFAVOURABLE RESPONSE TO TREATMENT

Patients with a *favourable* response to treatment are defined as those who yielded only negative cultures (usually 7-9) at least at the last three monthly examinations, that is, at 10, 11 and 12 months. In addition, 21 (4%) patients who yielded only one positive culture at 10, 11 or 12 months following at least three consecutive monthly examinations at which all cultures were negative have also been regarded as having had a favourable response for reasons reported earlier (Velu et al., 1960, 1961; Devadatta et al., 1961a; Dawson et al., 1966[2]).

Patients with an *unfavourable* response to treatment are defined as (*a*) those who never had all cultures negative at three consecutive monthly examinations or (*b*) those who had a total of two or more positive cultures at the last three monthly examinations following culture negativity at three or

[1] 1 fluid ounce ≃ 28 ml.

[2] See article on page 533 of this issue.

more consecutive monthly examinations or (*c*) those who had had their chemotherapy changed owing to serious radiographic or clinical deterioration or (*d*) those who died of tuberculosis.

It will be noted that these definitions do not depend on the results of smear examination of sputum specimens.

RESULTS

Although five regimens with different levels of therapeutic effectiveness were investigated, only the amalgamated results have been presented in Tables 1 to 4; the findings in the individual treatment series are briefly discussed on page 578.

Prognostic value of smear, culture and isoniazid-sensitivity examinations of one sputum specimen during treatment

Table 1 compares the value of smear examination, culture examination and isoniazid-sensitivity tests in predicting favourable response to treatment. The analysis is based on the results obtained with one sputum specimen each month. In order that the comparisons between the three methods are based on the same population, only those patients who had both smear and culture results available on the same specimen, together with an isoniazid-sensitivity test result at that month if the culture was positive, have been included in the analysis.

Considering first the results of smear examination, 65% of 198 patients with a positive smear at one month had a favourable response at one year, as compared with 82% of 282 patients with a negative smear. The corresponding proportions at two months were 54% of 125 and 82% of 365 patients. Thus, in the first 2 months, smear examination was not of much prognostic value. However, this value increased in subsequent months. Thus, 33% of 108 patients with a positive smear at 3 months as compared with 86% of 375 with a negative smear had a favourable response at one year; the corresponding proportions at 6 months were 3% of 70 and 88% of 415, and these remained fairly stationary for the rest of the year, the proportions at 12 months being 0% of 49 and 91% of 399, respectively. An indication of the gradual increase from month to month in the prognostic value of smear examination is provided by the trend observed in the *percentage of correct predictions*,[1] when the results of smear examination at each month are used to predict the outcome at the end of one year of treatment. This proportion was 62% at 1 month, 82% at 3 months, 90% at 6 months, 89% at 9 months and 92% at 12 months.

Culture examination in the first 2 months of treatment was also not of much prognostic value. However, its value, like that of smear examination, increased subsequently. Thus 10% of 98 patients with a positive culture at 6 months had a favourable response at one year, as compared with 93% of 387 patients with a negative culture; the corresponding proportions at 12 months were 6% of 69 and 95% of 379. The proportion of correct predictions [1] was 47% at 1 month, 78% at 3 months, 92% at 6 months, 94% at 9 months and 95% at 12 months.

Considering next the results of isoniazid-sensitivity tests, 18% of 28 patients with a resistant culture at one month had a favourable response at one year, as compared with 78% of 452 patients with an isoniazid-sensitive or a negative culture. The corresponding proportions at two months were 12% of 65 and 84% of 425 patients. Thus, even in the early months of treatment, the isoniazid-sensitivity test was of moderate prognostic value. Thereafter, its value increased further. Thus, 7% of 95 patients with a resistant culture at 6 months had a favourable response at one year, as compared with 93% of 390 with a sensitive or negative culture, and at 12 months the corresponding proportions were 2% of 65 and 95% of 383. The proportion of correct predictions [2] was 78% at 1 month, 84% at 2 months, 87% at 3 months, 93% at 6 months, 94% at 9 months and 95% at 12 months.

On comparing the prognostic value of smear examination with that of culture examination, it is seen that neither examination is very satisfactory in the early months of treatment; thus, at three months, predictions based on smear results would have been correct in 82% of the patients, the corresponding figure for culture results being 78%. At subsequent months, both types of examination yielded very satisfactory results—for example, the

[1] This figure is derived for each month by adding the number of patients with a *negative* result at that month and a *favourable* response at one year to the number with a *positive* result at that month and an *unfavourable* response at one year, and expressing the sum as a proportion of the total patients assessed. For example, considering smear results at three months, the figures are 323 and 72, respectively, resulting in 395 correct predictions out of a total of 483, that is, 82% of correct predictions.

[2] This figure is derived for each month by adding the number of patients with a negative or an isoniazid-sensitive culture at that month and a favourable response at one year to the number with an isoniazid-resistant culture at that month and an unfavourable response at one year, and expressing the sum as a proportion of the total patients assessed.

TABLE 1

FAVOURABLE RESPONSE AT ONE YEAR RELATED TO RESULTS OF SMEAR, CULTURE AND ISONIAZID-SENSITIVITY TESTS ON ONE SPUTUM SPECIMEN, AT MONTHLY INTERVALS DURING TREATMENT

Months after start of treatment		Smear		Culture		Isoniazid sensitivity		Total patients in analysis [a]
		Positive	Negative	Positive	Negative	Resistant	Sensitive or culture-negative	
1	Total patients	198	282	362	118	28	452	480
	Favourable response No.	128	230	248	110	5	353	
	Favourable response %	*65*	*82*	*69*	*93*	*18*	*78*	
	% correctly predicted	*62*		*47*		*78*		
2	Total patients	125	365	241	249	65	425	490
	Favourable response No.	67	298	142	223	8	357	
	Favourable response %	*54*	*82*	*59*	*90*	*12*	*84*	
	% correctly predicted	*73*		*66*		*84*		
3	Total patients	108	375	157	326	87	396	483
	Favourable response No.	36	323	69	290	13	346	
	Favourable response %	*33*	*86*	*44*	*89*	*15*	*87*	
	% correctly predicted	*82*		*78*		*87*		
4	Total patients	79	405	111	373	95	389	484
	Favourable response No.	17	348	23	342	11	354	
	Favourable response %	*22*	*86*	*21*	*92*	*12*	*91*	
	% correctly predicted	*85*		*89*		*90*		
5	Total patients	80	407	102	385	95	392	487
	Favourable response No.	14	360	14	360	9	365	
	Favourable response %	*18*	*88*	*14*	*94*	*9*	*93*	
	% correctly predicted	*87*		*92*		*93*		
6	Total patients	70	415	98	387	95	390	485
	Favourable response No.	2	367	10	359	7	362	
	Favourable response %	*3*	*88*	*10*	*93*	*7*	*93*	
	% correctly predicted	*90*		*92*		*93*		
7	Total patients	61	415	100	376	98	378	476
	Favourable response No.	5	360	10	355	8	357	
	Favourable response %	*8*	*87*	*10*	*94*	*8*	*94*	
	% correctly predicted	*87*		*93*		*94*		
8	Total patients	66	404	92	378	90	380	470
	Favourable response No.	4	361	7	358	5	360	
	Favourable response %	*6*	*89*	*8*	*95*	*6*	*95*	
	% correctly predicted	*90*		*94*		*95*		
9	Total patients	60	405	78	387	75	390	465
	Favourable response No.	5	361	3	363	1	365	
	Favourable response %	*8*	*89*	*4*	*94*	*1*	*94*	
	% correctly predicted	*89*		*94*		*94*		
12	Total patients	49	399	69	379	65	383	448
	Favourable response No.	0	363	4	359	1	362	
	Favourable response %	*0*	*91*	*6*	*95*	*2*	*95*	
	% correctly predicted	*92*		*95*		*95*		

[a] See text on preceding page.

proportion of correct predictions at six months was 90% with smear results and 92% with culture results; nevertheless, the prognostic value of culture was consistently better than that of smear. For assessing the statistical significance of this difference, it is convenient to present the results as in Table 2. This table shows that, at four months, correct predictions were made by both smear and culture results for 396 of 484 patients and incorrect predictions by both in 40, so that predictions based on smear and those based on culture were equally efficient or inefficient in 436 (90%) of the patients. Of the remaining 48 (10%) patients, 14 were correctly predicted by smear but incorrectly by culture and, conversely, 34 were correctly predicted by culture and incorrectly by smear; this difference is statistically significant ($P<0.01$). The findings at subsequent months also showed a consistent and statistically significant benefit to the culture examination. However, this benefit was usually of the order of only 5% and is, to a certain extent, offset by the fact that cultures are likely to become contaminated; further, it always takes much longer to obtain culture results—according to the procedure used in this laboratory, up to nine weeks—as compared with smear results, which usually can be obtained the same day.

On comparing next the prognostic value of culture examination with that of the isoniazid-sensitivity test, the latter was found to be uniformly better in the early months of treatment; thus, the *difference* in the magnitude of correct predictions was 31% at 1 month, 18% at 2 months, 9% at 3 months, and 1% at most of the subsequent months (Table 1). (However, according to the procedure used in this laboratory, it takes four weeks after a culture has become positive for the sensitivity test result to become available.) An assessment of the statistical significance of this difference must of necessity be confined to patients who produced a positive culture. At one month, there were 362 patients with positive cultures; both types of examination yielded correct predictions in 23 patients and incorrect predictions in 5 (not tabulated here). In the remaining 334 patients, the outcome was correctly predicted by culture results in 91 and by isoniazid-sensitivity results in 243, the difference being highly significant statistically ($P<0.001$). The corresponding differences at two months and three months were also highly significant ($P<0.001$). However, at four months, discrepant predictions were obtained for only 16 patients; the prediction based on culture was correct in 4 cases and that based on isoniazid-sensitivity in 12, the difference just attaining statistical significance ($P\simeq0.05$). At subsequent months, none of the differences was statistically significant.

It may be concluded that the examination by smear of one overnight specimen of sputum at monthly intervals is of great prognostic value, it being possible to predict correctly from four months onwards the outcome of treatment in at least 85% of the patients. Examination by culture increases this proportion to at least 89%, the increase being

TABLE 2

COMPARISON OF THE PROGNOSTIC VALUE OF SMEAR EXAMINATION AND CULTURE EXAMINATION OF ONE SPUTUM SPECIMEN, AT MONTHLY INTERVALS DURING TREATMENT

Prediction based on smear results	Prediction based on culture results	Months after start of treatment													
		4		5		6		7		8		9		12	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Correct	Correct	396	*82*	415	*85*	424	*87*	406	*85*	417	*89*	413	*89*	407	*91*
Incorrect	Incorrect	40	*8*	28	*6*	27	*6*	21	*4*	21	*4*	24	*5*	19	*4*
Correct	Incorrect	14	*3*	11	*2*	11	*2*	10	*2*	6	*1*	3	*1*	5	*1*
Incorrect	Correct	34	*7*	33	*7*	23	*5*	39	*8*	26	*6*	25	*5*	17	*4*
Total		484	*100*	487	*100*	485	*100*	476	*99*	470	*100*	465	*100*	448	*100*
χ^2 [a]		8.33		11.00		4.24		17.16		12.50		17.29		6.55	
P		<0.01		<0.001		<0.05		<0.001		<0.001		<0.001		≃0.01	

[a] Based on the third and fourth rows of figures and having one degree of freedom.

statistically significant. No further benefit is derived after four months (in terms of correct predictions) by the performance of an isoniazid-sensitivity test. However, in the early months of treatment, the proportion of correct predictions can be significantly increased by considering the results of isoniazid-sensitivity tests.

Comparison of the prognostic value of smear examination of one and two sputum specimens during treatment

Since smear examination of one sputum specimen during treatment was found to be of great prognostic value from four months onwards, it was decided to investigate whether the smear examination of an additional specimen would enhance the value, particularly at 1, 2 and 3 months. Analyses were therefore undertaken, along the lines of those in Table 1, on all patients with smear results on two sputum specimens at the monthly examination, irrespective of the availability of culture results.

Analyses, not tabulated here, showed that in the first 3 months of treatment, prognostication based on two smears was unsatisfactory, as was the case with one smear. Further, they also showed that from four months onwards, when the majority of sputum smears were negative, the prognosis of one positive smear plus one negative smear was invariably closer to that of two positive smears than to that of two negative smears; consequently, in Table 3, which presents the findings at 4-9 months and at 12 months, patients have been classified as having " at least one positive smear " or " both smears negative ", when considering the prognostic significance of two smears. These findings show that, at four months, the proportion of patients whose response was correctly predicted by considering the smear result of one specimen was 84%, as compared with 86% by considering the smear results of two specimens; the corresponding figures at five months were 87% and 86%, respectively; neither of these differences attained statistical significance. Both at six months and at seven months, the proportion of correct predictions was significantly (although only slightly) greater when based on smear results of two sputum specimens instead of one; thus, the proportions were 92% and 90% at six months ($P \simeq 0.02$), and 90% and 87% at seven months ($P \simeq 0.001$). At eight months and subsequently, the differences were smaller and were statistically non-significant.

It may be concluded that, from six months onwards, the prognostic value of two smear examinations each month is slightly greater than that of one smear examination.

Comparison of the prognostic value of two smears, one culture and two cultures during treatment

It has been shown that the prognostic value of culture examination of one sputum specimen during treatment is slightly greater than that of smear examination of one specimen from four months onwards. It has also been shown that the prognostic value of smear examination of two specimens is slightly greater than that of one specimen from six months onwards. It was therefore decided to compare the prognostic value of smear examination of *two* specimens with that of culture examination of *one* specimen from six months onwards. This comparison is presented in Table 4, which also includes the results obtained by culture examination of two specimens. Only patients with both smear and culture results available on two specimens of sputum at the monthly examinations have been included in the analysis. As with smears, the prognosis of one positive culture plus one negative culture was invariably closer to that of two positive cultures than to that of two negative cultures, so that patients have been classified in the table as having " at least one positive culture " or " both cultures negative ", when considering the prognostic significance of two cultures.

At six months, the proportion of correct predictions was 92% whether two smears or one culture formed the basis of prediction. However, both at seven and at eight months, predictions based on two smears were correct in 90% of the patients, as compared with 93% in the case of one culture, a significant difference ($P<0.02$). The corresponding proportions were 91% and 94% at nine months ($P \simeq 0.02$), and 92% and 94% at 12 months ($P \simeq 0.05$). It may be concluded that, from six months onwards, the prognostic value of culture examination of one sputum specimen is slightly but consistently greater than that of smear examination of two specimens.

On comparing next the prognostic value of culture examination of one sputum specimen with that of two specimens, little difference was found in the proportions of correct predictions (Table 4).

Findings in the individual treatment series

The patients in the present report received isoniazid plus sodium PAS (PH) or one of four regimens of isoniazid alone, namely, HI-1, HI-2, H and H650 (for details, see footnote to Table 5). It

TABLE 3

FAVOURABLE RESPONSE AT ONE YEAR RELATED TO THE RESULTS OF SMEAR EXAMINATION OF ONE SPUTUM SPECIMEN AND OF TWO SPUTUM SPECIMENS, AT MONTHLY INTERVALS DURING TREATMENT

Months after start of treatment		One smear		Two smears		Total patients in analysis [a]
		Positive	Negative	At least one positive	Both negative	
4	Total patients	76	393	92	377	469
	Favourable response No.	16	336	21	331	
	Favourable response %	*21*	*85*	*23*	*88*	
	% correctly predicted	*84*		*86*		
5	Total patients	85	397	102	380	482
	Favourable response No.	16	348	25	339	
	Favourable response %	*19*	*88*	*25*	*89*	
	% correctly predicted	*87*		*86*		
6	Total patients	71	407	89	389	478
	Favourable response No.	2	360	6	356	
	Favourable response %	*3*	*88*	*7*	*92*	
	% correctly predicted	*90*		*92*		
7	Total patients	64	404	80	388	468
	Favourable response No.	7	351	8	350	
	Favourable response %	*11*	*87*	*10*	*90*	
	% correctly predicted	*87*		*90*		
8	Total patients	69	402	79	392	471
	Favourable response No.	4	360	9	355	
	Favourable response %	*6*	*90*	*11*	*91*	
	% correctly predicted	*90*		*90*		
9	Total patients	58	394	68	384	452
	Favourable response No.	5	352	7	350	
	Favourable response %	*9*	*89*	*10*	*91*	
	% correctly predicted	*90*		*91*		
12	Total patients	49	389	57	381	438
	Favourable response No.	0	354	2	352	
	Favourable response %	*0*	*91*	*4*	*92*	
	% correctly predicted	*92*		*93*		

[a] See text on opposite page.

was found that the HI-1 and H650 regimens were of the same order of therapeutic efficacy (Tuberculosis Chemotherapy Centre, Madras, 1963); this was also true of the HI-2 and H regimens which were, however, less effective (Tuberculosis Chemotherapy Centre, Madras, 1960). The PH regimen was the most efficacious.

Separate analyses were undertaken for patients in the PH, HI-1/H650 and HI-2/H series to determine the extent to which the main conclusions drawn so far may have to be modified by the differences between the regimens in therapeutic efficacy. Table 5 summarizes the findings of analyses based on one sputum specimen collected at monthly intervals during treatment, from four months onwards. First, it is seen that both smear and culture are of least prognostic value in the HI-2/H series, the mean proportion of correct predictions being 83.6% and 89.2%, respectively, as compared with 89.2% and 95.7% in the HI-1/H650 series and 92.1% and 94.3%

TABLE 4. FAVOURABLE RESPONSE AT ONE YEAR RELATED TO RESULTS OF EXAMINATION BY SMEAR OF TWO SPUTUM SPECIMENS AND BY CULTURE OF ONE AND OF TWO SPUTUM SPECIMENS, AT MONTHLY INTERVALS DURING TREATMENT

Months after start of treatment		Two smears		One culture		Two cultures		Total patients in analysis [a]
		At least one positive	Both negative	Positive	Negative	At least one positive	Both negative	
6	Total patients	73	323	81	315	95	301	396
	Favourable response No.	6	296	9	293	14	288	
	Favourable response %	*8*	*92*	*11*	*93*	*15*	*96*	
	% correctly predicted	*92*		*92*		*93*		
7	Total patients	69	324	88	305	98	295	393
	Favourable response No.	6	290	9	287	14	282	
	Favourable response %	*9*	*90*	*10*	*94*	*14*	*96*	
	% correctly predicted	*90*		*93*		*93*		
8	Total patients	66	315	80	301	91	290	381
	Favourable response No.	8	284	8	284	10	282	
	Favourable response %	*12*	*90*	*10*	*94*	*11*	*97*	
	% correctly predicted	*90*		*93*		*95*		
9	Total patients	60	303	65	298	79	284	363
	Favourable response No.	6	275	3	278	10	271	
	Favourable response %	*10*	*91*	*5*	*93*	*13*	*95*	
	% correctly predicted	*91*		*94*		*94*		
12	Total patients	49	310	61	298	67	292	359
	Favourable response No.	2	283	4	281	7	278	
	Favourable response %	*4*	*91*	*7*	*94*	*10*	*95*	
	% correctly predicted	*92*		*94*		*94*		

[a] See text, page 578.

TABLE 5. COMPARISON OF THE PROGNOSTIC VALUE IN INDIVIDUAL TREATMENT SERIES OF SMEAR EXAMINATION AND CULTURE EXAMINATION OF ONE SPUTUM SPECIMEN AT MONTHLY INTERVALS DURING TREATMENT

Treatment series [a]	Assessment	Percentage of correct predictions									
		Months after start of treatment									Mean
		4	5	6	7	8	9	10	11	12	
PH	Smear	*88*	*88*	*92*	*92*	*92*	*93*	*94*	*94*	*96*	*92.1*
	Culture	*89*	*93*	*94*	*95*	*96*	*95*	*95*	*97*	*95*	*94.3*
HI-1/H650	Smear	*89*	*88*	*90*	*85*	*92*	*88*	*90*	*91*	*90*	*89.2*
	Culture	*94*	*96*	*95*	*95*	*94*	*96*	*98*	*97*	*96*	*95.7*
HI-2/H	Smear	*76*	*87*	*85*	*81*	*85*	*85*	*85*	*82*	*86*	*83.6*
	Culture	*84*	*87*	*87*	*88*	*91*	*90*	*91*	*93*	*92*	*89.2*

[a] PH: 3.9-5.5 mg/kg body-weight (200 mg for 100-lb (45.4-kg) patient) of isoniazid plus 0.2-0.3 g/kg body-weight of sodium PAS (10 g for 100-lb patient), daily in two divided doses, for 12 months.
HI-1: 7.8-9.6 mg/kg body-weight (400 mg for 100-lb patient) of isoniazid alone, daily in one dose, for 12 months.
HI-2: 7.8-9.6 mg/kg body-weight (400 mg for 100-lb patient) of isoniazid alone, daily in two divided doses, for 12 months.
H: 3.9-5.5 mg/kg body-weight (200 mg for 100-lb patient) of isoniazid alone, daily in two divided doses, for 12 months.
H650: 12.5-15.2 mg/kg body-weight (650 mg for 100-lb patient) of isoniazid alone, daily in one dose, for 12 months.

TABLE 6

THERAPEUTIC EFFICACIES OF ANTITUBERCULOSIS REGIMENS IN THREE CONTROLLED STUDIES, AS ASSESSED BY SMEAR RESULTS AND CULTURE RESULTS

Study	Treatment series [a]	Total patients	Patients with a favourable response			
			Classification based on culture results [b]		Classification based on smear results [c]	
			No.	%	No.	%
First	Sanatorium (PH)	81	73	*90*	72	*89*
	Home (PH)	81	67	*83*	69	*85*
Second	PH	86	78	*91*	77	*90*
	HI-1	64	47	*73*	48	*75*
	HI-2	66	38	*58*	43	*65*
	H	86	38	*44*	41	*48*
Third	H650	51	37	*73*	38	*75*

[a] For details, see footnote to Table 5.
[b] For details, see page 574.
[c] For details, see text below.

in the PH series, respectively. Secondly, the contrast in prognostic value between smear and culture examinations is smallest in the PH series, the mean difference in the proportion of correct predictions being 2.2% as compared with 6.5% in the HI-1/H650 series and 5.6% in the HI-2/H series; the differences in these figures between the PH series and each of the other two series attain statistical significance ($P<0.02$). These findings suggest that the value of both types of examination, particularly the smear examination, is adversely affected if the regimen is of low efficacy and that both are prognostically more efficient and yield closely similar results if the regimen is of high efficacy.

Comparison of smear and culture examinations in assessing the therapeutic efficacies of antituberculosis regimens

At the Centre, the therapeutic efficacy of antituberculosis regimens is assessed mainly on the basis of culture results of sputum specimens collected at 10, 11 and 12 months. It is now proposed to study the value of similar assessments based solely on smear results. Such a study would be of special interest to centres in developing countries with limited or no culture facilities.

In the following analyses, the definition of favourable response, based on smear results only, is " all smears (usually 7-9) negative at 10, 11 *and* 12 months ", and that of unfavourable response is " at least one positive smear at 10, 11 *or* 12 months, or change of chemotherapy owing to serious radiographic or clinical deterioration, or death from tuberculosis ". The corresponding definitions based on culture results have been stated on page 574.

Both sets of definitions were applied to patients in three studies undertaken at the Centre and the findings are presented in Table 6. The first study was a comparison of the efficacy of treatment with isoniazid plus PAS at home and the same treatment in sanatorium, for 12 months (Tuberculosis Chemotherapy Centre, Madras, 1959). The second was a comparison of the efficacies of four antituberculosis regimens, three of isoniazid alone and one of isoniazid plus PAS, in the domiciliary treatment of pulmonary tuberculosis for 12 months (Tuberculosis Chemotherapy Centre, Madras, 1960). In the third, which was a controlled study of isoniazid toxicity, only one antituberculosis regimen, namely a high dosage of isoniazid, was used (Tuberculosis Chemotherapy Centre, Madras, 1963).

In the first study, 90% of 81 patients treated in sanatorium were classified, on the basis of culture results, as having had a favourable response, as compared with 89% on the basis of smear results. The corresponding proportions for 81 patients treated at home were 83% and 85%, respectively. In the second study, the proportions classified as having had a favourable response on the basis of culture results were 91% of 86 PH, 73% of 64 HI-1,

58% of 66 HI-2 and 44% of 86 H patients; the corresponding proportions based on smear results were 90%, 75%, 65% and 48%, respectively. In the third study, 73% of the 51 patients had a favourable response as assessed by culture results, as compared with 75% as assessed by smear results.

Thus, there is not much difference between the smear assessment and the culture assessment of the therapeutic efficacy of the regimens. Further analyses (not tabulated here) showed that, on the average, the two assessments yielded an identical classification in 95% of the patients (range, 92%-99%). However, an inspection of the proportions of patients with a favourable response to the various regimens shows that the variation from regimen to regimen is greater for the classification based on culture results than for that based on smear results. This is reflected by the range of these proportions, which is 44%-91% for the former and 48%-90% for the latter. This suggests that the ability to detect differences in efficacy between regimens is greater with cultures than with smears. In the Annex (page 586), this conclusion is confirmed on a larger number of regimens obtained by including three more studies undertaken at the Centre. It is also shown there that this shortcoming of a chemotherapy study based on smear examination alone can usually be offset by a 20% increase in the number of patients admitted to study.

Applicability of the findings in situations where smear examination alone is available for diagnostic purposes

All the analyses in this report are based on 515 patients who had at least one positive culture on admission to treatment and who were excreting isoniazid-sensitive bacilli (see page 574). However, not infrequently, unsatisfactory situations are encountered where facilities for culture examination (and sensitivity tests) are not available, and where the initial diagnosis of tuberculosis is based mainly on the results of smear examination. It is therefore of interest to know how the conclusions in the foregoing pages would be affected in such situations, since patients without a positive smear would not be included while those with isoniazid-resistant organisms (and at least one positive smear) would not be excluded. In the present instance, there were 43 patients in the former category and 28 in the latter; on excluding the 43 and including the 28, the modified population for analysis became 500.

Analyses were undertaken on the modified population to compare the prognostic value of smear examination and culture examination of one sputum specimen at monthly intervals during treatment. These showed that, apart from decreasing very slightly (1% to 3%) the proportion of correct predictions, the changes in the population did not have any important effect either on the prognostic value of smear or on that of culture. It therefore seems reasonable to conclude that the findings in the present paper are also applicable to situations where smear examination alone is available for diagnostic purposes; however, it must be emphasized that this conclusion is based on data from a clinic where excretors of acid-fast bacilli of non-tuberculous origin are uncommon.

DISCUSSION

The progress during treatment of patients with pulmonary tuberculosis is usually followed by periodic bacteriological, radiographic and clinical examinations. In economically favoured countries, it is possible *to prescribe* regimens of chemotherapy which produce a favourable response in virtually 100% of the patients (Crofton, 1960; Canetti, 1962). In such situations, despite current practice, it is obviously not important to follow closely the progress of patients receiving chemotherapy provided it is ensured that they are in fact receiving the prescribed drugs. On the other hand, in developing countries, regimens which *do not* produce a favourable response in 100% of the patients often have to be prescribed for economic reasons (Lauckner, 1959; Canetti, 1962; *Tubercle (Lond.)*, 1963; Fox, 1964). Under these circumstances, it is important to assess periodically the progress of patients so that those whose disease is not responding to chemotherapy may be detected as early as possible.

Of the various methods used to follow the progress of patients, bacteriological assessments are the most valuable (American Trudeau Society, 1959). It has been shown at the Centre that the erythrocyte sedimentation rate is an unsatisfactory measure of progress, as an elevated rate is compatible with bacteriologically quiescent disease at the end of a year of chemotherapy (Tuberculosis Chemotherapy Centre, Madras, 1959) or at the end of a 2-year (Devadatta et al., 1961a) or a 4-year (Dawson et al., 1966[1]) period of follow-up. Also, serial radiography is of limited value in assessing the progress of patients since radiographic deterioration—for instance, an increase in the size of cavities—can occur

[1] See article on page 533 of this issue.

in the presence of persistent bacteriologically quiescent disease (Devadatta et al., 1961a), and radiographic improvement is not necessarily associated with a favourable bacteriological response (Devadatta et al., 1961b).

The bacteriological methods most commonly used are examination of sputum smears for organisms morphologically resembling tubercle bacilli, culture examination for tubercle bacilli and tests of sensitivity to the drugs prescribed. Culture examination and drug-sensitivity tests take many weeks to yield results, require more skilled staff, and are economically not possible as routine methods in most tuberculosis clinics in developing countries at the present time. Moreover, cultures may become contaminated with other organisms; this occurred in 9.1% of the 11 445 cultures set up during treatment from sputum specimens obtained from the 515 patients who form the subject of this report. Smear examination has the disadvantage of being less sensitive than culture examination in detecting tubercle bacilli (Corper & Cohn, 1933; Andrews & Radhakrishna, 1959). It is also more likely to produce false positive results since it is not usually possible to distinguish tubercle bacilli from other acid-fast organisms. However, this occurred very rarely in the present study; thus, of 2098 sputum specimens examined during treatment which yielded a positive smear, only 0.1% were subsequently found to be due to acid-fast saprophytes. Another disadvantage is that a smear-positive culture-negative result would be regarded (often wrongly) as indicating the presence of viable tubercle bacilli if culture examination is not undertaken (Tuberculosis Chemotherapy Centre, Madras, 1959; Great Britain, Medical Research Council, 1962); in this study, of the 2098 sputum specimens with a positive smear, 7.1% yielded a negative culture.

Smear examination, however, does not need complicated laboratory facilities, is easy to perform, and results can be obtained on the same day that specimens are submitted to the laboratory; further, under Indian conditions, it is approximately 10 times less expensive than culture examination and 20 times less expensive than a sensitivity test to a single drug (E. M. Mackay-Scollay, quoted by Fox, 1964). It was decided, therefore, to compare the value of smear examination with that of culture examination and isoniazid-sensitivity tests in assessing the progress of patients receiving chemotherapy. The smears were examined by fluorescence microscopy; this method yields at least as many positive results as the Ziehl-Neelsen method, no more false positives, and has the advantage of a very great saving of time in the preparation and examination of smears (Holst, Mitchison & Radhakrishna, 1959).

The findings in the present study are based on the results of smear and culture examination of sputum specimens collected at monthly intervals from 515 patients receiving isoniazid, either alone or in combination with PAS; *all* 515 patients had bacteriologically confirmed pulmonary tuberculosis with isoniazid-sensitive organisms on admission to treatment. The results revealed that, from four months onwards, the examination by smear of one sputum specimen per month is a very valuable method of assessing the progress of patients receiving chemotherapy. For example, 97% of 70 patients with a positive smear result at six months had an unfavourable response at one year and 88% of 415 with a negative smear result had a favourable response. (The response to treatment was classified as favourable or unfavourable, mainly on the basis of culture results of a total of 7-9 sputum specimens, obtained at 10, 11 and 12 months.) If the smear result at six months had been used to predict the response to treatment, correct predictions would have resulted for 90% of the patients, and this proportion remained fairly constant for the rest of the year. Examination by culture of one sputum specimen was of slightly more prognostic value. Thus, if the results of one culture examination at six months had been used to predict the outcome of treatment, correct predictions would have resulted for 92% of the patients. However, in situations where sputum conversion occurs unusually late—there is evidence that this happens in Hong Kong (British Tuberculosis Association/ Hong Kong Tuberculosis Treatment Services, 1964) —the value of both methods of examination at six months in predicting the outcome at the end of one year of chemotherapy might be less.

The close agreement between predictions based on smear and culture examinations can be understood by considering the changes in the bacterial population in any patient receiving chemotherapy. Thus, if the disease is responding, the sputum first becomes negative on smear examination because of a decrease in the bacterial population and then negative on culture examination, the disease eventually attaining bacteriological quiescence. On the other hand, if the disease stops responding to the chemotherapy, the sputum remains positive, or eventually becomes positive, on smear examination because of an increase in the bacterial population.

Furthermore, the frequency of smear-positive culture-negative results and of acid-fast saprophytes—the two main causes of false positive results on smear examination—was not high among the specimens in this study.

Culture examination of two sputum specimens at monthly intervals did not prove to be of greater prognostic value than that of one specimen. However, with a contamination rate of 9%, the examination of two specimens instead of one would substantially reduce the number of patients with *no information* available on account of contaminated cultures. Isoniazid-sensitivity tests increased the prognostic value of positive cultures during the first 4 months of treatment. However, this benefit is not of much practical value because it might take one month after isolation of a positive culture to obtain the isoniazid-sensitivity result. After four months of treatment, no benefit was derived by considering the results of isoniazid-sensitivity tests, the explanation for this finding being that nearly all positive cultures obtained in later months from patients receiving chemotherapy containing isoniazid are resistant to that drug (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1963; East African/British Medical Research Council Isoniazid Investigation, 1960; Selkon et al., 1964).

This study has shown that it is possible to draw accurate conclusions regarding the efficacies of antituberculosis regimens from controlled trials, even if sputum specimens are examined by smear only instead of by culture. However, in such trials, the likelihood of detecting differences in efficacy between regimens would be slightly decreased, a disadvantage which can usually be offset by a 20% increase in the number of patients admitted to study. These findings suggest that it would be inappropriate not to *consider* controlled chemotherapy studies in developing countries merely because facilities for culturing tubercle bacilli are very limited. Such countries usually have large numbers of tuberculous patients and, provided an *efficient* smear service can be set up, accurate conclusions can be drawn from controlled studies in which smear examination only is undertaken during treatment, although a slightly larger number of patients would have to be admitted. Even in these circumstances, it would be *very desirable* to undertake culture and drug-sensitivity examinations on sputum specimens obtained from patients *before* admission to the study. If facilities for quick and efficient transportation of the sputum specimens are available, such tests could be arranged either at a central laboratory in the same country or in a more developed country. For instance, a central laboratory in Madras is successfully undertaking cultures and drug-sensitivity tests on sputum specimens collected from centres in various parts of India (P. R. J. Gangadharam, personal communication, 1965). Such tests have also been carried out successfully in London on sputum specimens collected from patients in Hong Kong (Hong Kong Government/British Medical Research Council Investigation, 1964), and from patients in Rhodesia (Jean F. Heffernan, personal communication, 1966).

It is concluded that the value of smear examination of overnight specimens of sputum at monthly intervals approaches closely that of culture examination and drug-sensitivity tests in assessing the progress of tuberculous patients receiving chemotherapy, and in assessing the therapeutic efficacies of regimens.

SUMMARY

1. This paper compares the value of smear examination for tubercle bacilli of overnight specimens of sputum, month by month, with that of culture examination and isoniazid-sensitivity tests in assessing the progress of patients treated with isoniazid, either alone or with sodium PAS, in three chemotherapy studies. The comparisons are based on the ability of these bacteriological methods to predict, during treatment, the response at the end of 12 months, which was classified as favourable or unfavourable, mainly on the basis of culture results at 10, 11 and 12 months.

2. Smear examination, from four months onwards, of one sputum specimen was a very valuable method of assessing the progress of patients receiving chemotherapy. For example, 97% of 70 patients with a positive smear at six months had an unfavourable response at one year and 88% of 415 with a negative smear had a favourable response. If the results of one smear examination at six months had been used to predict the outcome of treatment, correct predictions would have been made in 90% of the patients.

3. Culture examination of one sputum specimen was of slightly greater prognostic value than smear examination from four months onwards. Thus, if the result of a culture examination at six months had been used to predict the outcome of treatment, correct predictions would have been made in 92% of the patients. However, this slight advantage,

which is statistically significant, is partially offset by the delay in obtaining the results and loss of information due to contamination.

4. There was evidence suggesting that the value of smear and culture examinations, particularly the former, was adversely affected if the regimen was of low efficacy and that both were prognostically more efficient and yielded closely similar results if the regimen was of high efficacy.

5. The prognostic value of smear examination of two sputum specimens was slightly greater than that of one specimen, but less than that of culture examination of one specimen, from six months onwards.

6. Culture examination of two sputum specimens offered no further benefit over that of one specimen except in substantially reducing losses due to contamination.

7. Isoniazid-sensitivity tests were found to increase the prognostic value of positive cultures during the first 4 months of chemotherapy only; moreover, the test results might not become available until as long as one month after the culture had become positive.

8. Reliable conclusions could be drawn regarding the therapeutic efficacies of regimens by considering the results of smear examination, since the two types of assessment (smear and culture) yielded, on the average, identical classifications in 95% of the patients. However, smear examination was slightly less sensitive than culture examination in detecting differences in the therapeutic efficacies of various antituberculosis regimens; this disadvantage can usually be offset by admitting about 20% more patients.

RÉSUMÉ

Dans ce travail, les chercheurs du Centre de Chimiothérapie de la Tuberculose, à Madras, établissent une comparaison entre trois méthodes (recherche, à intervalle d'un mois, du bacille tuberculeux par examen direct; culture; épreuves de sensibilité) permettant de suivre l'évolution de malades traités par l'isoniazide, seul ou associé au PAS. Ils ont tenté de définir leurs avantages respectifs pour la prévision, en cours de traitement, de la réponse favorable ou défavorable (déterminée principalement par cultures aux 10e, 11e et 12e mois) à 12 mois de chimiothérapie.

Dès le 4e mois, l'examen microscopique direct offre d'excellentes possibilités d'évaluer les progrès de la guérison. Chez 97% des 70 malades positifs à l'examen direct au 6e mois, on a noté une réponse défavorable après un an de traitement; lorsque cet examen était négatif (415 malades), la proportion des réponses favorables a atteint 88%. Sur la base des résultats des examens de crachats effectués au 6e mois, on aurait pu prévoir avec exactitude la nature de la réponse au traitement dans 90% des cas.

A partir du 4e mois, les résultats des cultures ont une valeur pronostique légèrement supérieure à celle des données fournies par l'examen direct. Les cultures effectuées au 6e mois auraient permis d'établir une prévision correcte dans 92% des cas. Les avantages de cette méthode, quoique statistiquement significatifs, sont cependant partiellement éclipsés par suite de la lenteur du procédé et du risque de contamination des cultures.

La valeur pronostique des cultures et surtout celle de l'examen direct s'affaiblissent lorsque le traitement appliqué est peu actif; en revanche, l'utilité de ces deux méthodes est manifeste, et du même ordre, en cas d'emploi d'un schéma chimiothérapique particulièrement efficace.

La qualité des prévisions s'améliore légèrement si l'examen microscopique direct est effectué sur deux échantillons de crachats au lieu d'un seul, mais leur précision reste cependant inférieure à celle du pronostic basé sur les résultats de la culture d'un échantillon effectuée au 6e mois. La culture de deux échantillons au lieu d'un seul a pour unique avantage de réduire fortement la proportion des échecs dus à la contamination.

Pratiquées au cours des quatre premiers mois du traitement, les épreuves de sensibilité à l'isoniazide accroissent la valeur pronostique des cultures positives. Ce n'est cependant qu'après un certain délai, qui peut atteindre un mois après l'obtention des cultures positives, que l'on peut recueillir les résultats de ces épreuves.

Les auteurs estiment que l'on peut faire confiance aux résultats fournis par l'examen direct de l'expectoration lorsqu'il s'agit d'apprécier l'efficacité de différents schémas thérapeutiques. Les deux méthodes d'évaluation, examen direct et culture, permettent en effet d'opérer un classement des malades qui, en moyenne, est identique dans 95% des cas, bien que la première se soit montrée légèrement moins sensible. Il semble que l'on pourrait pallier ce désavantage en augmentant de 20% le nombre des malades concernés par l'enquête.

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Annex

RELATIVE VALUE OF SMEAR AND CULTURE EXAMINATION IN ASSESSING THE THERAPEUTIC EFFICACIES OF ANTITUBERCULOSIS REGIMENS

The relative value of smear examination and culture examination in detecting differences in efficacy between antituberculosis regimens may be assessed from Table 7, in which the results obtained with eight regimens used at the Centre since 1956 have been summarized. The details regarding the first five regimens—namely, PH, HI-1, HI-2, H and H650—have already been presented (see footnote to Table 5); those for the last three—namely, SHTW, TH and P_6H/H—are given below:

SHTW. 12.5-16.1 mg/kg body-weight (650 mg for 100-lb (45.4-kg) patient) of isoniazid in one dose and a uniform dosage of 1 g of streptomycin, administered together twice weekly, for 12 months.

TH. 4.6-8.0 mg/kg body-weight (300 mg for 100-lb patient) of isoniazid daily plus 2.3-4.0 mg/kg body-weight (150 mg for 100-lb patient) of thioacetazone daily, in one dose, for 12 months.

P_6H/H. A uniform dosage of 200 mg of isoniazid daily plus 6 g of sodium PAS daily in one dose for the first 6 months, followed by 6.2-7.7 mg/kg body-weight (300 mg for 100-lb patient) of isoniazid alone in one daily dose for the second 6 months.

The dispersion of the percentages of patients with a favourable response to these eight regimens, as measured by their variances, is larger for assessments based on cultures than for those based on smears (see columns headed P_c and P_s in Table 7). For assessing the statistical significance of this difference, the usual variance ratio test of Fisher is not applicable since the variances, being based on the same set of patients, are not independent.

TABLE 7

THERAPEUTIC EFFICACIES OF EIGHT ANTITUBERCULOSIS REGIMENS, AS ASSESSED BY SMEAR RESULTS AND CULTURE RESULTS OF SPUTUM SPECIMENS

Regimen [a]	Number of patients	Percentage of patients with a favourable response	
		Classification based on culture results [b] (P_c)	Classification based on smear results [c] (P_s)
PH	380	*86*	*88*
HI-1	64	*73*	*75*
HI-2	66	*58*	*65*
H	86	*44*	*48*
H650	143	*68*	*68*
SHTW	72	*94*	*90*
TH	65	*82*	*83*
P_cH/H	63	*67*	*68*
Range		*44-94*	*48-90*
Variance		257.14	193.84
Standard deviation		*16.0*	*13.9*

[a] PH was studied on four occasions, H650 on two and all the remainder on only one occasion.

[b] For details, see page 574.

[c] For details, see page 581.

However, it is evident that, whatever the correlation between the variances of P_c and P_s may be, the sum (P_c+P_s) and the difference (P_c-P_s) of the two assessments of efficacy will be uncorrelated if the variances of P_c and P_s are equal. From this, it follows that the statistical significance of the difference in variances may be assessed by computing the correlation coefficient, r, between (P_c+P_s) and (P_c-P_s) and testing for its deviation from zero. In the present instance, $r = 0.67$, $P \simeq 0.07$.

To visualize what this difference means in practice, it will now be expressed in terms of the number of patients required, if smear examination alone is undertaken, to attain the same amount of discrimination between regimens as is obtained with 100 patients assessed by culture examination. For this purpose, an unfavourable response was scored as 0, a favourable response as 1, and analyses of variance undertaken (Table 8).

The variance ratio measures the differences in mean efficacy between regimens, relative to variations in response between patients receiving the same regimen. Obviously, the larger the variance ratio, the greater is the discrimination between the regimens. It is seen from Table 8 that the variance ratio obtained from smear results (14.05) is smaller than that from culture results (16.62). Using component analysis (Snedecor, 1956), it is found that a 20% increase in the number of patients would result in an increase in the variance ratio for smear results from 14.05 to 16.62.

It may be concluded that the ability to detect differences in efficacy between various antituberculosis regimens is less with smear examination than with culture examination and that this difference borders on statistical significance ($P \simeq 0.07$), but can usually be compensated for by an increase of about 20% in the number of patients admitted to study.

TABLE 8

ANALYSES OF VARIANCE FOR DATA IN TABLE 7

Source	Degrees of freedom	Classification based on culture results			Classification based on smear results		
		Sum of squares	Mean square	Variance ratio	Sum of squares	Mean square	Variance ratio
Between regimens	7	19.1747	2.7392	16.62	15.8354	2.2622	14.05
Between patients receiving the same regimen	931	153.4643	0.1648		149.9366	0.1610	
Total	938	172.6390			165.7720		