Smear Results in the Diagnosis of Mycobacterioses Using Blue Light Fluorescence Microscopy

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Examination of 6,880 sputum specimens from untreated patients disclosed that 3.1% were positive for mycobacteria by fluorescence microscopy, and 92.5% of these has positive cultures. There was a positive correlation between the number of organisms seen on smear and growth on culture. All specimens with positive smears and negative cultures contained rare or few acid-fast bacilli on the smear. Eighty-two percent of the specimens with positive cultures and negative smears yielded <25 colonies, whereas there were >25 colonies from 93% of the specimens with positive smears and cultures. In a low prevalence, general hospital population, the primary acid-fast smear continued to be a reliable diagnostic tool.

Although tuberculosis is declining in incidence in this country, and most cases are in older aged groups (20), the need for early diagnosis and treatment is still necessary. Since Mycobacterium tuberculosis takes 3 to 8 weeks to grow (19), the wait for positive cultures prior to the initiation of therapy is undesirable and impractical. Early treatment must still rely on radiological and clinical evidence supported by smear results.

In 1975 Boyd and Marr reported that 55% of the positive smears in their series were false positives (4) and that 81% of the patients represented by these results had negative radiological findings. The false positives were defined as being smear positive and culture negative. As suggested by these workers, if the predictive value of these smear results followed the formula suggested by Vecchio (26), then as the incidence of this disease in the tested population declined, the predictive value of a positive smear result would decline also. Carried to the ultimate extreme, if there were no tuberculosis, 100% of the positive smears would be false positives.

This report of a high percentage of false-positive smears led us to examine the culture and smear results from specimens obtained from the University Hospital (UH), Veteran's Administration Hospital (VAH), and Harborview Medical Center (HMC), all of which were processed at HMC. We were interested in knowing how predictive smears were of culture results and whether there were any differences in the results from specimens submitted by the different hospitals. Each of these hospitals has a different kind of patient population. UH is a referral center, HMC is the City and County Hospital for Seattle and King County and possesses the main tuberculosis ward for this county, and VAH is a 250-bed general medical and surgical VA center.

This report describes the relationship between smear and culture results and discusses the bearing that known factors affecting smear results may have in relation to the observed findings and the value of smears in the primary diagnosis of tuberculosis.

MATERIALS AND METHODS

Specimens. First, morning sputum specimens were delivered from the VAH (from October 1974 to October 1975) and UH (from October 1973 to October 1975) to HMC by bus. They were refrigerated until they were processed. All specimens were processed within 24 h with the exception of those collected on the weekend which were processed within 72 h. Only sputum specimens for which there were smear and culture results were included in the series. All specimens from patients who were on therapy were analyzed separately.

Digestion and concentration of sputum. Specimens were digested and decontaminated by the N-acetyl-L-cysteine-sodium hydroxide method (15).

Microscopy. Smear concentrates were fixed on a hot plate at 60°C for 2 h and stained with 0.1% auramine O. The slides were examined with a Zeiss RA 38 microscope using a blue light source (23). A high intensity tungsten lamp was housed in an illuminator with a collector lens, and critical illumination was obtained by focusing the lamp filament on the specimen plane. A 3-mm BG 12 primary filter and a Zeiss number 53 secondary filter were used. The entire surface of the smear was scanned with a
25× objective, and the presence of acid-fast bacilli was confirmed by using a 63× planachromat lens. All specimens screened by this method were from patients who were not on therapy and who were being evaluated for the diagnosis of tuberculosis. The smears were scored as 3 to 9 per slide, rare; 10 or more per slide, few; and 2 or more per high-powered field, numerous. One or two organisms per smear were reported as rare if the duplicate smear was also positive.

Specimens from patients on therapy were screened by Kinyoun carbol fuchsin stain (24).

**Culture.** Several drops of the specimen concentrate were inoculated onto each of two Lowenstein-Jensen slants and one Middlebrook 7H11 plate (6). The plates were incubated in 10% CO₂ for 3 weeks, and the Lowenstein-Jensen slants were incubated in CO₂ for 1 week in a horizontal position with loosened caps (11). The caps were then tightened, and the tubes were removed to an aerobic incubator for the remaining 7 weeks. The number of colonies were recorded as the total number per culture.

**Identification.** Acid-fast organisms were identified by the methods described by Runyon et al. (24), and identifications were confirmed by the Seattle-King County Health Department.

**RESULTS**

From 6,880 specimens there were 213 smears (3.1%) positive for acid-fast bacilli. Among those specimens positive by smear, 92.5% were also positive by culture (Table 1). Ninety-seven percent of the specimens that gave negative smears also had negative cultures. All of the positive smears from specimens that did not have positive cultures had either rare or few acid-fast bacteria. When these results were compared with the culture failures from specimens of patients on therapy, there was a significant increase in culture failures in the latter group as revealed by Chi-square analysis ($P < 0.001$) (Table 2).

Fifty percent of the specimens with positive cultures had negative primary smears. A review of these specimens revealed that of 82% with 25 colonies or less on culture, 90% yielded less than 10 colonies (Table 1). Of the specimens yielding positive cultures and having positive smears, 93% had >25 colonies. This was significantly more than the 17.5% of specimens with negative smears ($P < 0.005$) having that number of colonies.

A review of the species of mycobacteria isolated from specimens in this series revealed some striking differences between hospitals. Of the mycobacteria isolated from UH specimens, 39% of the isolates were *M. tuberculosis*; whereas, this species comprised 84% and 93% of VAH and HMC isolates, respectively. In addition, all of the specimens with atypical mycobacteria had a small number of colonies and negative smear results, with the exception of three isolates of *M. chelonei* var. *abscessus* from a UH patient in whom this infection was clinically significant. The latter specimens were consistently positive by fluorescence microscopy.

**DISCUSSION**

The evaluation of test results has recently come under scrutiny with respect to the relationship between the prevalence of disease in a given population and the probability that an individual patient with a positive test has the disease (4). Several workers have reported on the mathematical considerations that give these probabilities (25, 26). It is apparent from these reports that in the range representing 1 to 10% incidence, the probability of a reliable result drops off precipitously with decreased incidence of disease. Since the incidence of tuberculosis is declining in many parts of the world, it is necessary to reevaluate the reliability of smear results in populations with low incidences.

Some important considerations regarding acid-fast smear and culture results have emerged through the years. Many factors, such as staining method (1, 3, 7, 28), contamination of smears by organisms in water (2, 3, 8, 10), severity of the disease in the population which affects the number of organisms in the specimens (1, 19, 21, 22), chance distribution of organisms (5, 9), limits of sensitivity of the methods (5, 12-18, 27), and therapy (1), may all affect results.

Parrot et al. found that the numbers of organisms seen on smear had a bearing on the
culture results, and there was a 50% chance of a negative culture when only 1 organism per 100 fields was viewed (21). Ten out of 16 of the negative cultures in our series had 3 to 9 organisms per slide (700 to 800 fields), and the rest had greater than 10 per slide but fewer than 2 per high-powered field. Lower numbers of organisms have been correlated with less severe disease in which the opportunity for obtaining a negative culture would be greater (21). In our series, 90.5% of the cultures with fewer than 25 colonies had negative smears. The sensitivity of smear results is such that it takes a minimum of 10³ organisms per ml to give a positive smear (12). Parrot et al. found that the smear was positive only 50% of the time when there were 3 to 9 colonies per culture tube (21). It would appear that numbers of organisms and severity of disease are important factors in obtaining positive smears and cultures.

In three nontuberculosis hospitals located in a metropolitan area with a new case rate of less than 14 out of 100,000 population, acid-fast smears remained a reliable specific screen for tuberculosis with a greater than 92% overall correlation between smears and cultures. The low sensitivity of the smears with respect to positive cultures appeared to be related to low numbers of organisms in the samples.

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LITERATURE CITED