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SMEAR-POSITIVE AND CULTURE-NEGATIVE RESULTS OF ROUTINE SPUTUM INVESTIGATIONS FOR THE DETECTION AND THERAPY CONTROL OF PULMONARY TUBERCULOSIS

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Summary

The frequency of smear-positive and culture-negative results (M+C−) is an important factor in the supervision by the Central Laboratory of its own activities and those of the laboratories in the periphery. Under the conditions of the present study, which included evaluation of 6415 specimens positive on smear during 5 years and studied simultaneously by microscopy and culture, about one half of the M+C− results (3 per cent of the total) were thought to be probably laboratory errors and the other half were from patients being treated by chemotherapy. It is necessary to know, at least approximately, the size of this latter group of persons before the contribution of laboratory errors can be estimated and the necessary action taken to reduce their frequency.

Résumé

La fréquence de résultats positifs à l'examen du frottis et négatifs à la culture (M+C−) est un facteur important dans la supervision exercée par le Laboratoire Central sur ces propres activités et sur celles des laboratoires plus périphériques. Dans les conditions de la présente étude, qui comportait l'évaluation de 6415 échantillons positifs à l'examen du frottis, réalisés au cours de 5 années et étudiés simultanément par microscopie et culture, environ la moitié des résultats M+C− (3 % du total) ont été considérés comme étant probablement des erreurs de laboratoire et l'autre moitié correspondait à des malades sous traitement chimiothérapique. Il est nécessaire de connaître au moins approximativement la dimension de ce second groupe de sujets avant de pouvoir estimer la part des erreurs de laboratoire et d'apprécier l'action à prendre pour réduire leur fréquence.

Resumen

La frecuencia de baciloscopías al directo y negativas al cultivo (M+C−) constituye un factor importante para la supervisión por parte del Laboratorio Central de las actividades propias así como de las de los laboratorios periféricos. Bajo las condiciones del presente estudio, que incluyó la evaluación de 6.415 muestras positivas al exámen directo a lo largo de 5 años estudiadas simultáneamente con microscopía y cultivo, alrededor de la mitad de los resultados M+C− (3 % del total) se consideró como probablemente debida a errores de laboratorio y la otra mitad correspondió a pacientes en tratamiento con quimioterapia. Es necesario conocer, al menos aproximadamente, la cuantía de este último grupo de personas antes de poder estimar la contibución de los errores de laboratorio y de poder tomar las medidas necesarias para reducir su frecuencia.

Introduction

It is assumed that a 'case' of pulmonary tuberculosis refers to a person with bacteriologically confirmed disease (WHO Expert Committee on Tuberculosis, 1964). For the developing countries, however, bacteriologically confirmed disease actually means patients with acid-fast bacilli in the sputum on smear only, because of lack of culture facilities both for case detection and the control treatment. The former was discussed extensively by Raj Narain and others (1968; 1971), the latter by Devadatta and others (1966). For both purposes, therefore, especially because of the limited facilities for the treatment available in developing countries and of the high defaulter rates, it is imperative to know the real value of a positive smear, that is to say the extent of the error inherent in the whole procedure, from the collection of specimens to the reading of the smears under the particular conditions for an institution in a country. Such studies also form an important part of proficiency testing in laboratories for the bacteriology of tuberculosis where the extent of over- ('false positive') or under-reading ('false negative') must be continuously monitored.

The present study covers our experiences in the period 1969 to 1973 both at the level of the Central Laboratory and in the periphery.

Materials and methods

During the 5 years mentioned, a total of 69 902 sputum specimens were submitted to microscopic examination and culture, resulting in 7402 positive smears. The specimens were received from the hospital and from dispensaries and 'health centres' in the periphery. They were collected by either the 'spot' or 'overnight' technique (Padmanabha Rao and others, 1966). In some cases it was not known whether the specimen was from a newly diagnosed patient or one being treated. The essential data were available in 6415 (87 %) of the cases.

For microscopy, the fluorescence method described by Holst, Mitchison and Radhakrishna (1959) was used: purulent particles were transferred by means of a wooden applicator to the slide and a fine, uniform smear was prepared. Then it was heat-fixed, stained for 10 minutes with auramin O-phenol without heating, washed, decolourized with acid–alcohol for 4 minutes, washed again and counter-stained with potassium permanganate for 30 seconds. The smears were examined with a ×400 dry objective and the findings in 100 visual fields were registered according to the following scale (Manual de Bacteriologia de la Tuberculosis, 1973):

− = no acid-fast bacilli seen

+ = less than 1 bacillus per field in 100 fields

++ = 1–10 bacilli per field in at least 50 fields

+++ = more than 10 bacilli per field in at least 20 fields.

Smears with less than 3 acid-fast bacilli in all 100 fields were regarded as negative (Raj Narain and others, 1968).

For culture examination, sputum was treated with about twice its volume of 3.75 % sodium hydroxide by mechanical agitation. After centrifuging for 15 minutes, the supernatant was discarded and the sediment neutralized with use of a *p*H indicator. Each specimen was seeded (0.1 ml) on to 3 slopes of Löwenstein–Jensen medium with glycerin and without potato starch. All batches of media were checked by seeding them with known suspensions of the H37Rv strain of *M. tuberculosis*. The media were incubated at 37 °C and read weekly for 12 weeks.

Results

Among the 7402 positive microscopy results there were 483 (6.5 %) with negative cultures, the proportion in each of the 5 years ranging from 3.8 % in 1970 to 13.8 % in 1972 and 11.2 % in 1973. The majority of the smears (52 to 81 %) were graded +; but 10 % were +++.

In 410 M+C− specimens from a total of 6415 it was possible from the available data to assess the probable cause of the result. In 7 cases the patients had leprosy and the acid-fast organisms may have been *M. leprae*. In 198 (3.1 % of the total 6415 specimens) the patient was receiving chemotherapy. In 205 (3.2 %) specimens (25 of which came from patients in whom there was no abnormality in the chest x-ray and a negative tuberculin reaction) the positive report was judged likely to have been due to laboratory errors, such as inefficient staining and the misinterpretation of appearances on the slide.

The proportion of 410 M+C− results attributable to the effects of chemotherapy was 48 %, the proportions in each year ranging from 39 % in 1970 and 1973 to 51 % in each of the other 3 years.

Discussion

The possible factors producing an M+C− result have been analysed by a number of investigators. Padmanabha Rao and others (1966) investigated the interval between collection of the specimen and inoculation of the culture. In agreement with others (see Pollak, Urbanczik and Quiñones, 1970 for review) they concluded that probably up to a week there is hardly any adverse effect of transport and/or storage on the positivity of cultures. As for the type of sputum collection, 'overnight' specimens yielded more positive results on both smear and culture than 'spot' sputa, but the difference was not statistically significant. The possible superiority of fluorescence microscopy over the Ziehl–Neelsen method was also suggested as a factor. According to Venezuelan experiences (Pollak, 1970), however, among 556 culture-positive specimens only 2.3 % were positive on smear by fluorescence and negative by the Ziehl–Neelsen technique, which is not a statistically significant difference. Thus, in agreement with Holst, Mitchison and Radhakrishna (1959), we would rather stress the operational advantages, particularly the speed, of fluorescence microscopy. A major factor may be the reliability of the Ziehl–Neelsen technique when performed at a peripheral 'health centre'. In India this question was studied by Nagpaul and others (1968): had a single centre among 8 institutions investigated performed with the same efficiency as the others, over-reading in the periphery would have been reduced to 1.9 %. A very limited study reported by Boulahbal and Larbaoui (1973) showed that, for a trained microscopist, a single reading of smears is sufficient to enable him to classify the slides precisely; the practice of dual reading of the same slide by either the same or another person will add very few to the total number of positive results. From a separate study not reported here our experience is in agreement with the findings described. One point, however, should be stressed: such studies represent a very important part of the supervision by the Central Laboratory of peripheral institutions and they must be carried out continuously. As reported recently by Messias Dias Filho (1975) in similar trials the proportion of 'false positive' (7.6 %) or 'false negative' (8.7 %) results in 1971 dropped to 2.2 and 0.7 % respectively, in 1973.

One factor, however, may be of importance. Parrot and others (1971) reached the following conclusion based on examination of 35 613 specimens from 4480 patients by fluorescence microscopy and culture: extensive cavitary disease and M+C− results for more than 3 months after the last positive culture were found in 15 % of persons treated with izoniazid and in 29 % of persons receiving rifampicin therapy. The sharp increase in the proportion of M+C− results in Table I in the last 2 years of observation is coincident with the introduction of rifampicin for therapy on a limited scale in the Hospital General del Sur. In the experience of Parrot and others (1971), about 68 % of persons with cavernous pulmonary tuberculosis

Table I. The proportions of M+C− results and of the 3 grades of positivity in the years 1969 to 1973

Year	Total M+C−	Percentage			
		Of all M+ results	Graded +	++	+++
1969	93	4.8	53	34	13
1970	73	3.8	52	40	8
1971	94	5.0	66	24	10
1972	172	13.8	81	12	7
1973	51	11.2	61	21	18

treated with rifampicin displayed M+C− results for more than 1 month after the last positive culture; a comparable group in the Hospital General del Sur showed M+C− results in over 50 % of patients. It is obvious that the total of M+C− results may be considerably influenced by even a small group of patients treated with such highly effective, possibly bactericidal, drugs as rifampicin and isoniazid.

The total proportion of evaluable M+C− results in this study was 6.4 %, 3.1 % were probably due to intensive chemotherapy and in about 3.2 % some sort of laboratory error could be either traced back or at least strongly suspected. In other words, about 3 % 'cannot be helped', whereas another 3 %, approximately, constitute failures of technique which should be dealt with. These 3 % are an important indicator of bacteriological efficiency in our particular situation, which should be closely followed year by year as part of the general supervision by the Central Laboratory of its own activities and of the health centres in the periphery.

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