



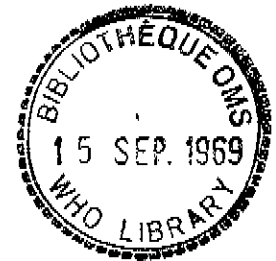
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COMPARATIVE EVALUATION OF DIAGNOSTIC TOOLS

by

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1. INTRODUCTION

There are two major problems in the diagnosis of amoebiasis. The first is the demonstration of the presence of Entamoeba histolytica and this, although simple in theory, presents many difficulties in practice. The second is how to decide whether the infection with the parasite has any casual relationship to the patient's symptoms.

2. DEMONSTRATION OF INFECTION

2.1 Intestinal infection

2.1.1 Faecal specimens

The difficulties surrounding demonstration of intestinal infection are concerned in finding the amoebae and in their certain identification. One of the few aspects of amoebiasis on which there seems to be general agreement is that when surveying asymptomatic subjects only about 30 per cent. of the total positives will be found by examination of a single specimen (Dobell, 1917; Kershaw, 1946; Stamm, 1957; Svensson, 1935; Wenyon & O'Connor, 1917).

The amoebae are not evenly distributed throughout a stool specimen so that selection of the part to be examined is important; the parasitologist should receive a whole stool specimen from which to select a likely part, rather than be presented with a small portion randomly chosen by inexperienced staff. Furthermore, even when only cysts are present, it is important that the specimen should be as fresh as possible since the finer morphological characteristics are quickly lost. When immediate examination is not practicable the morphology of cysts can be preserved by emulsification of a suitable portion in formol-saline; trophozoites can be preserved by fixing a smear in FVA fixative (Brooke & Goldman, 1949).

The excretion of amoebae tends to be intermittent (Marsden & Smith, 1946; Schensnovich & Smirnova, 1946) and some workers have thought that there was a definite rhythmical periodicity (Kershaw, 1946; Lincicome, 1942). There is evidence that examining three preparations from the same faecal specimen is as productive as examining one preparation from three consecutive specimens (Stamm, 1957). In view of the intermittence of excretion, Marsden and Smith (1946) recommended making stool examinations at weekly intervals rather than on consecutive days.

The results of the questionnaire distributed by WHO and analysed by Elsdon-Dew (1964) demonstrated clearly the enormous variation in methods and criteria used for diagnosis at the various centres.

Since there are no objective tests, such as sugar reactions in culture, which can be used for the identification of Entamoeba histolytica, identification is subjective and depends on the experience of the pathologist and technician. Under these circumstances, complete standardization cannot be achieved; an experienced faecal microscopist may be able to give a valid opinion on a simple saline preparation where a less experienced worker would need to see a haematoxylin stained preparation.

Ridley & Hawgood (1956) considered that one concentrated specimen would reveal as many positives as three direct examinations; many would disagree with this. Robinson (1968) has developed a routine cultural technique which he claims will reveal more positives than either direct microscopy or concentration; even if this is so, it has the disadvantage that identification in culture requires considerable experience.

Under present circumstances it would seem that initial identification must be left to the discretion and taste of the individual worker, but whenever a survey or drug trial is being conducted, preserved specimens for cysts and stained smears for trophozoites should be kept for independent assessment.

There is a great need for an objective method of identification of E. histolytica simple enough to be used in a routine laboratory. Immunofluorescent staining seems to offer the possibility of achieving such a test. Goldman (1953) described a method of specific immunofluorescent staining of E. histolytica in culture and this could greatly assist in making Robinson's cultural technique suitable for use by the inexperienced. Immunofluorescent staining of E. histolytica in faeces presents greater difficulties largely because of the autofluorescence of faecal preparations. However, the specific staining of Shigella sonnei in faeces has been successfully achieved (Taylor et al. 1964) and there seems to be no good reason why a similar technique should not be applied to E. histolytica. Such a technique would save a great amount of time spent in microscopy, would provide a method which could be used by the inexperienced, and would go far to standardization of the accuracy and comparability of work at different centres.

Whatever method of search is used a decision must always be made on how many stool specimens should be examined.

In the dysenteric stool there is usually no difficulty in finding haematophagous trophozoites, provided the specimen is examined really fresh (i.e. within minutes of being passed) on a warm slide; certainly three such examinations, completed where necessary by examination of a scraping taken from an ulcer during sigmoidoscopy will reveal trophozoites in all but the most exceptional case.

The difficulty in finding trophozoites or cysts becomes increasingly greater on passing from amoebic dysentery down the scale of severity through non-dysenteric intestinal amoebiasis, to asymptomatic amoebiasis. It may be necessary to examine as many as 21 stools from an asymptomatic subject before finding it positive. It is clear therefore that any advice on the number of stools which should be examined must be a compromise between the scientifically desirable and the economically possible. The most which is likely to be ever economically possible is six consecutive specimens, and the minimum which should be regarded as clinically acceptable for diagnostic or follow-up purposes is three. In view of the variability of excretion of cysts, three specimens taken at weekly intervals can be expected to reveal more positives than three taken on consecutive days.

The finding of E. histolytica in a stool specimen should always be followed by sigmoidoscopy. When haematophagous trophozoites are present in the faeces, ulcers can usually be seen and surveillance after treatment should continue until these have healed. When no haematophagous trophozoites are present, sigmoidoscopy can help to assess whether there is tissue invasion and whether there is a casual relationship between the presence of the parasite and any symptoms the patient may have; this is of particular importance in areas where there is a high prevalence of infection and other causes of abdominal symptoms are common. Whenever ulcers are seen on sigmoidoscopy, either wet preparations should be examined immediately or smears should be treated with PVA fixative.

2.1.2 Biopsy or autopsy specimens

The distribution of amoebae in infected tissue may be extraordinarily irregular. I have had a grossly ulcerated length of colon removed at operation from which one histological block provided sections with an abundance of amoebae in every section; five other blocks from the same tissue were cut right through and not a single amoeba could be found. Robinson (1968) records a similar experience with autopsy material.

Amoebae can be difficult to see in sections stained by most routine histological techniques. When formalin-fixed tissue is stained by the PAS technique, the amoebae stand out pink or red owing to their content of glycogen; this method is very useful as a screening procedure, but the detailed morphology is usually not well shown and confirmation is necessary by a method designed to demonstrate morphology, e.g. Heidenhain's iron haematoxylin.

2.2 Hepatic amoebiasis

Amoebae causing a liver abscess are mainly in the tissues surrounding the abscess and not in the pus and all are in the trophozoite phase. When a liver abscess is aspirated, it is therefore essential that the pus be examined immediately or smears be made and fixed at once. The last part of the aspirate tends to have the greatest concentration of amoebae and if the pus is very thick it is helpful to incubate portions of it with streptokinase or streptodoinase.

3. RELATIONSHIP OF INFECTION TO SYMPTOMS

The final analysis of the relationship of a demonstrable infection with E. histolytica to the patient's symptoms must be one of clinical judgement, but there are certain technical aids which can help towards the decision.

3.1 Sero-immunological methods

The value of these methods has greatly increased over the last few years largely due to improvements in the antigens available. Further comparative evaluations in different centres are required before anything dogmatic can be said about the relative value of the various techniques available.

3.1.1 Comparative value

Some of the facts which seem to be established are as follows: the haemagglutination test and the complement-fixation test give a high percentage of positives in unequivocal invasive amoebiasis, but the haemagglutination test seems to give a higher proportion of positives in asymptomatic infection; the fall in titre after treatment is quicker with the CFT and the test is usually negative at the end of a year (Kessel et al. 1965).

Gel diffusion, haemagglutination and phagocytosis appear to give very similar results (Halpern et al. 1967). There is evidence that the gel diffusion and haemagglutination tests depend on different antibodies, since absorption of the haemagglutinins fails to remove the precipitins (Maddison et al. 1965). A similar difference may account for the apparent difference in sensitivity between the haemagglutination test and the CFT.

Not such large series have been reported with the fluorescent antibody test but the results seem to be of the same order as with the other tests with a rapid drop in titre after treatment. This test has certain technical advantages whereby it should be comparatively easy to make it available in routine laboratories by the central supply of the necessary test reagents (Boonpucknavig & Nairn, 1967; Coudert et al. 1967; Jeanes, 1966).

There is increasing evidence that positive serological tests indicate present or past tissue invasion by E. histolytica, and there is strong correlation between the serological titres and the virulence of the infecting amoebae to rats (Neal et al. 1968).

3.1.2 Practical value

The specificity and sensitivity of all these tests are excellent in the diagnosis of liver abscess and it is here that they have their most obvious value in the clinical field, since in this condition it is often impossible to demonstrate the presence of the parasite.

The diagnosis of symptomatic gut infections will probably always depend primarily on demonstration of E. histolytica in the stools, but the serological methods may provide a good basis for deciding whether the amoebae are in fact the cause of the patient's complaints, and how persistent the search of faecal specimens should be. When more is known about the persistence of antibodies, serology may be useful in follow-up studies after treatment.

Even now serological population surveys would provide a more accurate measure of the comparative geographical prevalence of amoebiasis than examination of faeces, since the techniques can be standardized and are objective. Powell (1968) has reported on the use of a very simple capillary-tube precipitin test; it appears to be somewhat less sensitive than the more sophisticated techniques but may well be of great value for the small laboratory, the field worker and for survey work.

3.2 Other laboratory tests

Apart from the identification of E. histolytica and the sero-immunological tests there are no laboratory tests which show any consistent pattern in amoebiasis. In amoebic liver abscess the erythrocyte sedimentation rate is almost always raised, often to very high levels (over 100 mm in 1 h) but the total white count is raised in only about 50 per cent. of cases. Jaundice is rare and there is no pattern of liver function tests which is characteristic of the condition.

3.3 Radiology

Patients with amoebic liver abscess often have linear striations at the base of the lung on the affected side, and a raised or deformed diaphragm with limitation in its movement; the examination should always include visual screening as well as a straight radiograph.

The induction of a pneumo-peritoneum and radioactive scanning are two recent techniques which can help in locating the site of a liver abscess but are likely to be reserved for the more difficult cases and are unlikely to gain extensive use.

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