Clinical evaluation of a complement fixation test using human hydatid cyst fluid antigen for the detection of *Echinococcus granulosus* antibody

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**Introduction**

Complement fixation (CF) is a commonly used serodiagnostic test for hydatid disease, especially recommended for the evaluation of cure after surgery or for checking on treatment with anthelminthic drugs (9). However, the absence of an FDA-approved standardized antigen makes it difficult to assess the performance of different CF tests by using published studies. The objectives of this study were to determine sensitivity, specificity, negative predictive value and positive predictive value of a CF test using human hydatid cyst fluid as antigen and to evaluate the value of this test presently used at the Mayo Clinic in following the progress of treatment.

**Methods**

**Complement-fixation test**

A micro-volume complement-fixation (CF) test was performed according to a technique described elsewhere (26). Briefly, the sera under examination were inactivated at 56°C for 30 min. in a waterbath. Using a dropper pipette 0.025 ml serum, 0.05 ml complement (5CH₅₀) and 0.025 ml antigen were placed in U-shaped microtiter plates. Veronal buffer saline was used as diluent. After the plates had stood overnight at 4°C, 0.025 ml hemolysin with 0.28% sheep erythrocytes was added to each cup. Plates were incubated for additional 30 min. at 37°C and read after centrifugation (3 min. at 300 × g). Fixation greater than 2 + (less than 50% hemolysis) was considered as a positive reaction.

**Antigen:**

The antigen employed was fluid aspirated from a hydatid cyst removed operatively from a patient with echinococcosis of the liver. The aspirated fluid was clear and contained many protoscoleces. After sterile-filtration, the fluid was preserved with 1 : 50,000 merthiolate and stored at 4°C. The same batch of antigen was used throughout the study-period. On the basis of the optimal dilution in a checkerboard titration, the antigen was diluted 1 : 32 to 1 : 12. Cyst fluid remained stable for years when preserved with merthiolate and stored at 4°C (8).
Complement:
Lyophilized guinea pig complement was purchased from Whittaker Bioproducts (Walkersville, Maryland). A checkerboard titration procedure showed the optimal complement activity to be 1:180 to 1:150.

Anti-sheep hemolysin:
Lyophilized hemolysin was purchased from Becton, Dickinson and company (Cockeysville, Maryland). The optimal antibody dilution was found to be 1:2,500 to 1:2,000.

Patients
— Group I:
Patients on whom a CF-test was performed at Mayo between April 11th 1984 and April 20th 1990 were identified from computerized laboratory records. 221 patients tested for Echinococcus antibody were identified: 201 patients were tested once, 20 patients twice. The complete medical record of all patients was reviewed. Information was collected regarding age, citizenship, endemic countries visited, symptoms, signs, species diagnosis, laboratory data, treatment, complications and outcome for all patients with a positive CF result. CF test results obtained during this 6 year-period were used to determine sensitivity, specificity, positive and negative predictive value (2).

— Group II:
Echinococcosis cases seen at Mayo since 1976 were identified by checking the medical records for all patients indexed in the Medical Records Department with the discharge diagnosis hydatid disease. CF-tests were carried out for 25 out of 33 echinococcosis patients seen at Mayo during this 15-year period. 11 patients had sera drawn more than once and were chosen for assessing the value of the CF-test for evaluation of surgery and/or treatment with antihelminthic drugs. Two of these 11 cases are also included in Group I.

The diagnosis cystic hydatid disease was established either by incontrovertible evidence obtained at operation or by combining characteristic radiological/sonographical findings, clinical information, history of previous residence in an endemic area and immunological evaluation (Casoni skin test was performed at Mayo until 1984). The study protocol was approved by the Institutional Review Board at Mayo.

Results
Group I:
Seven out of 221 patients tested for Echinococcus antibody between April 11 1984 and April 20 1990 had current Echinococcosis due to Echinococcus granulosus. A summary of these cases, including the results of the CF-test, organs infected maximum percentage of eosinophils in peripheral blood smears and history of previous surgery for echinococcosis is given in table 1. Four patients with CF-antibody titers ranging from 1:16 to 1:512 underwent surgical and/or medical treatment. Neither surgical nor medical treatment was considered necessary for the remaining 3 patients; one with a titer of 1:4 and two giving negative CF-test results. The 5 patients with true positive CF results accounted for 6 specimens, the patients with false negative CF accounted for 3 sera. All patients with current echinococcosis were American citizens living in Illinois (2 X), Indiana (2 X), Minnesota, Missouri and Wisconsin; all of them were immigrants from endemic areas.
TABLE 1
Summary on 7 echinococcosis cases (current infections), Mayo 1984 to 1990.

<table>
<thead>
<tr>
<th>Pat.</th>
<th>Year</th>
<th>CF</th>
<th>Age/Sex</th>
<th>Disease contracted in:</th>
<th>Location of hydatid cyst(s)</th>
<th>WBC/mm³</th>
<th>Treatment at Mayo</th>
<th>History of previous surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.</td>
<td>1989</td>
<td>1:512*</td>
<td>46 M</td>
<td>Greece</td>
<td>Liver</td>
<td>7,100</td>
<td>Drug</td>
<td>Liver at age 45</td>
</tr>
<tr>
<td>A.</td>
<td>1985</td>
<td>1:128</td>
<td>55 M</td>
<td>Syria</td>
<td>Femur, acetabulum, soft tissue</td>
<td>8,800</td>
<td>Surgery + Drug</td>
<td>Liver at age 6, Lung at age 30, Hip at age 53</td>
</tr>
<tr>
<td>A.</td>
<td>1989</td>
<td>1: 16</td>
<td>50 M</td>
<td>Iraq</td>
<td>Liver (some calcifications)</td>
<td>4,500</td>
<td>Drug</td>
<td>Liver at age 47</td>
</tr>
<tr>
<td>S.</td>
<td>1987</td>
<td>1: 16</td>
<td>49 F</td>
<td>Greece</td>
<td>Liver (calcified)</td>
<td>10,500</td>
<td>Surgery</td>
<td>Liver at age 10</td>
</tr>
<tr>
<td>A.</td>
<td>1985</td>
<td>1:  4</td>
<td>59 F</td>
<td>Greece</td>
<td>Liver (calcified)</td>
<td>5,600</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>K.</td>
<td>1985</td>
<td>neg.**</td>
<td>48 F</td>
<td>Greece</td>
<td>Liver (scattered calcifications)</td>
<td>6,000</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M.</td>
<td>1988</td>
<td>neg.</td>
<td>44 M</td>
<td>India</td>
<td>Liver (calcified)</td>
<td>7,100</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

* = A 2nd specimen (titer: 1:512) was drawn 10 days later.
** = A 2nd specimen (titer: negative) was drawn 24 days later.
n. d. = investigation not done

TABLE 2
Summary on patients with mere history of echinococcosis (past infections), Mayo 1984 to 1990 (accounting for 3 out of 213 sera with true negative CF test result).

<table>
<thead>
<tr>
<th>Pat.</th>
<th>Year</th>
<th>CF</th>
<th>Age/Sex</th>
<th>Present complaints</th>
<th>Echinococcosis contracted in:</th>
<th>Previous surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.</td>
<td>1987</td>
<td>neg.</td>
<td>30 F</td>
<td>None (check up)</td>
<td>Saudiarabia</td>
<td>Liver at age 23 (Mayo, CF 1:23)</td>
</tr>
<tr>
<td>E.</td>
<td>1984</td>
<td>neg.</td>
<td>59 M</td>
<td>None (check up)</td>
<td>Spain</td>
<td>Liver at age 53 + Drug (Mayo, CF 1:32)</td>
</tr>
<tr>
<td>G.</td>
<td>1989</td>
<td>neg.</td>
<td>50 M</td>
<td>Paraparesis secondary to spine deformity</td>
<td>Greece</td>
<td>Lung at age 30 (outside), vertebral at age 40 (laminctomy, outside)</td>
</tr>
</tbody>
</table>

214 patients tested for *Echinococcus* antibody between 1984 and 1990 did not have current echinococcosis. Three of these 214 patients had undergone surgery for hydatid disease in the past (Table 2); none of them gave positive reaction in the CF-test. One serum specimen exerted anticomplementary activity. This sample was drawn for evaluation of a “low density mass” seen on an abdominal CT scan, a mass which turned out to be a “partial volume artifact”. The patient, a 54 year old woman with chronic obstructive pulmonary disease and Still’s disease, died 4 weeks later of cardiac arrhythmia without any signs of hydatid disease.

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<table>
<thead>
<tr>
<th>Pat. Year</th>
<th>CF</th>
<th>Age/ Sex</th>
<th>Place of birth</th>
<th>Reason for ordering CF: sono./ liver eosino- varia</th>
<th>Diagnosis/ history</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. 1987</td>
<td>1:16(1)</td>
<td>38 M</td>
<td>Panama</td>
<td>Cyst — — —</td>
<td>Cerebral cysticercosis</td>
</tr>
<tr>
<td>S. 1986</td>
<td>1:8</td>
<td>41 F</td>
<td>Mexico</td>
<td>Cyst — — —</td>
<td>Cerebral cysticercosis</td>
</tr>
<tr>
<td>S. 1985</td>
<td>1:2</td>
<td>43 F</td>
<td>Mexico</td>
<td>Cyst — — —</td>
<td>Cerebral cysticercosis</td>
</tr>
<tr>
<td>S. 1987</td>
<td>1:2</td>
<td>31 M</td>
<td>Canada</td>
<td>Mass — — —</td>
<td>Bilateral lung nodules representing metastatic soft tissue sarcoma</td>
</tr>
<tr>
<td>M. 1988</td>
<td>1:16</td>
<td>37 F</td>
<td>Cambodia</td>
<td>Mass — — —</td>
<td>Endomterioma in right ovary (2.5 cm), pulmonary tuberculosis</td>
</tr>
<tr>
<td>H. 1988</td>
<td>1:2</td>
<td>39 M</td>
<td>USA</td>
<td>Mass + — —</td>
<td>Hepatic solitary lymphoma</td>
</tr>
<tr>
<td>K. 1985</td>
<td>1:16(2)</td>
<td>52 M</td>
<td>Greece</td>
<td>— + — —</td>
<td>Non-specific granulomatosis of liver and monoclonal gammopathy of undetermined etiology</td>
</tr>
<tr>
<td>E. 1986</td>
<td>1:16</td>
<td>56 M</td>
<td>Italy</td>
<td>— + — —</td>
<td>Biliary cirrhosis secondary to biliary stones</td>
</tr>
<tr>
<td>W. 1990</td>
<td>1:64</td>
<td>34 F</td>
<td>USA</td>
<td>Cyst + — —</td>
<td>Benign livercyst (6 cm diameter)</td>
</tr>
<tr>
<td>B. 1988</td>
<td>1:16(3)</td>
<td>20 F</td>
<td>USA</td>
<td>— — 83.5% of 21,500 WBC/mm³</td>
<td>Episodic angioedema with eosinophilia (died at age 22)</td>
</tr>
<tr>
<td>H. 1986</td>
<td>1:8(4)</td>
<td>58 M</td>
<td>USA</td>
<td>— — 53.3% of 11,400 WBC/mm³</td>
<td>Eosinophilia of unknown etiology (missionary in New Guinea for 13 years)</td>
</tr>
<tr>
<td>T. 1986</td>
<td>1:128</td>
<td>7 M</td>
<td>Indonesia</td>
<td>— — 37.0% of 15,200 WBC/mm³</td>
<td>Eosinophilic meningitis due to Angiostrongulus cantonensis; Trichuris trichiuria</td>
</tr>
<tr>
<td>M. 1989</td>
<td>1:8</td>
<td>20 F</td>
<td>Cambodia</td>
<td>— — — +*</td>
<td>Delivery, because of recent immigration recheck on parasites (E.histolytica IHA 1:4096) 3 months earlier; Hymenolepis nana</td>
</tr>
<tr>
<td>F. 1988</td>
<td>1:2</td>
<td>33 M</td>
<td>USA</td>
<td>— — — +*</td>
<td>Alcoholisms Hepatitis B</td>
</tr>
</tbody>
</table>

* = ordered inadvertently while screening for intestinal parasites

(1) = 2nd specimen (titer: 1:16) drawn 43 days later
(2) = 2nd specimen (titer: 1:2) drawn 97 days later
(3) = 2nd specimen (titer: negative) drawn 4 days later
(4) = 2nd specimen (titer: negative) drawn 3 years later
14 patients showed false positive CF titers. All but two of the false positive titers were less or equal to 1:16; a 7 year old boy from Indonesia with eosinophilic meningitis due to *Angiostrongulus cantonensis* and intestinal *Trichuris trichiura* infestation presented a titer of 1:128. A 34 year old factory worker who had lived in Arkansas until she moved to Oklahoma at age 24 presented a titer of 1:64. A summary of those cases including the reasons for ordering the CF-test is presented in table 3.

Using data from patients group I, the sensitivity was found to be 66.7% calculated by dividing the number of the true positive sera (n = 6) by the sum of the number of true positives and false negatives (n = 9). Specificity, as the number of true negatives (n = 212) divided by the sum of numbers of true negatives and false positives (n = 228), was 93%. Negative predictive value, as the number of true negatives divided by the sum of the number of true negatives and false negatives (n = 215), was found to be 98.6%. The positive predictive value was found to be 30% by dividing the number of true positives by the sum of the number of true positives and false positives.

**Group II:**

CF testing was done for 25 out of 33 patients with *Echinococcus granulosus* infection seen at Mayo from 1976 to March 1990. In 11 of those 25 patients serum was also drawn after initiation of therapy (9 were seen at Mayo before 1984, 2 after 1984); CF results of these cases were compared with the final outcome of therapy. Data from 5 cases in which the patients failed to undergo treatment of echinococcosis are summarized in table 4. A check-up 2 years after surgical removal of a liver cyst (surgery performed at an outside hospital) revealed a Greek female patient to be still reactive in the CF-test, despite the absence of any other sign of hydatid disease (peripheral blood smear: 0% eosinophiles); 14 years later she returned, at this time with multiple cysts involving spleen, omentum, lung and chestwall (19.5% eosinophiles). A Greek male patient was found to be seropositive one year after surgical removal of 2 liver cysts; the patient had experienced 2 syncopal episodes 3 years before (retrospectively considered to be allergic reactions caused by cyst leakage); clinical investigation revealed recurrence of multiple small liver cysts. A patient from Saudiarabia fell into anaphylactic shock during surgery (removal of 5 of 7 liver cysts). This case of intra-operative spillage was reflected by an increase of CF antibody (5 titer steps) 6 weeks after operation despite mebendazole therapy. Two years later he presented with multiple recurrent liver cysts. Failure of mebendazole therapy was also correctly reflected by persisting CF antibodies in 2 other patients who did not receive surgical treatment.

Data from 6 patients with a successful outcome of therapy (accounting for 16 CF results, including one test done outside) are summarized in table 5. All 6 patients undergoing successful surgery and/or medical treatment showed low CF antibody titers. In one case a specimen was drawn on day 23 after surgery: a decrease of one titer-step was found at this early point of time. A decrease of 3 titer-steps was observed as early as 36 days after surgery, conversion to “negative” as early as 3 and 4 months after removal of the cysts or after initiation of medical treatment. Mebendazole therapy was successfully applied only in a patient with “early stage of echinococcosis”; CF was negative 4 months after initiation of medical treatment.

**Discussion**

The first human infection with hydatid cyst in the United States was diagnosed in 1808 (4). Although the majority of patients still acquire this infection abroad, there are endemic foci of this disease in the USA (7, 20). In the present study all 7 patients with hydatid infection seen at Mayo between 1984 and 1990 were immigrants from endemic areas.
TABLE 4
CF-test results of 5 patients unsuccessfully treated for echinococcosis, Mayo 1976 to 1990.

<table>
<thead>
<tr>
<th>Pat. Year</th>
<th>CF</th>
<th>Age/Sex</th>
<th>Echinococcosis contracted in:</th>
<th>Location and size of cyst(s)</th>
<th>Treatment at Mayo</th>
<th>Follow up till</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. 1977</td>
<td>1:16</td>
<td>50 F</td>
<td>Greece</td>
<td>Spleen (12 cm)</td>
<td>Surgery</td>
<td>1990</td>
</tr>
<tr>
<td>P. 1989</td>
<td>1:512</td>
<td>46 M</td>
<td>Greece</td>
<td>Liver (2 cysts, 1.5 and 10 cm)</td>
<td>Surgery</td>
<td>1990</td>
</tr>
<tr>
<td></td>
<td>1:512</td>
<td></td>
<td></td>
<td>recurrence of multiple small liver cysts</td>
<td>Surgery of 5 cysts with anaphylactic reaction + mebendazole for 3 months</td>
<td>1982</td>
</tr>
<tr>
<td>T. 1980</td>
<td>1:32</td>
<td>34 M</td>
<td>Saudiarabia</td>
<td>Liver (7 cysts, 2 to 10 cm)</td>
<td>Surgery</td>
<td>1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>six weeks after surgery: CF 1:1024 recurrence of 9 liver cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O. 1982</td>
<td>1:16</td>
<td>59 M</td>
<td>Spain</td>
<td>Cervical spine (multiple cysts, 2 to 3 cm)</td>
<td>Surgery + continuous mebendazole</td>
<td>1986</td>
</tr>
<tr>
<td>A. 1989</td>
<td>1:16</td>
<td>50 M</td>
<td>Iraq</td>
<td>Liver (1.5 cm, calcifications)</td>
<td>mebendazole</td>
<td>1990</td>
</tr>
</tbody>
</table>

1 = History of pat. G.: Liver cyst (surg. at age 34, outside). At age 36 at Mayo: “all tests o. k., except CF: +, no clinical evidence for having another cyst”.
2 = History of pat. P.: 1987 two “indeterminate syncopial episodes”.
3 = History of pat. O.: Since 1979 on mebendazole because of recurrent intradural cysts in lumbar spine (outside).
4 = History of pat. A.: Liver cyst (surg. at age 47, and surg. + mebendazole at age 49), since then (Nov. 1988) on mebendazole (outside).

The complement fixation test applied to the diagnosis of cases of suspected hydatid infection has been used in many variations since first introduced by GHEDINI in 1906, who used hydatid fluid from human cysts as antigen (10). The choice of antigen and the technique still remain the main problems in the use of CF (13). Hydatid fluid antigens of humans origin were considered superior or equal to animal fluids in various techniques by some workers, inferior by others (11). The CF-test also reacts differently according to the type of antigen employed, i.e. hydatid fluid, protoscoleces or laminated membrane of the cyst (24), which may account for the discrepancies in the literature on the sensitivity of this reaction.

The sensitivity of the CF-test (as reported in the literature) ranges from a mere 36% (13) to the remarkable figure of 97% (21). Based on data from 25 publications KAGAN found the approximate average sensitivity to be 69% (13). CHEMTAI et al. evaluated a CF-test used in the diagnosis of 141 surgically-proven echinococcosis patients and reported the average sensitivity to be 63.3% (5). The sensitivity found for our CF-test was 66.7%. CF is not the only method in echinococcosis serology hampered by low sensitivity: at the Centers for Disease Control (CDC) the sensitivity of a routinely used double diffusion test applying the arc 5-antigen was found to be 66% (19). The CDC presently confirms positive IHA results with an immunoblot assay using an Echinococcus antigen with an apparent molecular weight of 8 kDa; this test shows a sensitivity of 91% for surgically confirmed Echinococcus granulosus hydatid disease of the liver (19).
TABLE 5
CF test results of 6 patients treated successfully for echinococcosis, Mayo 1976 to 1990.

<table>
<thead>
<tr>
<th>Pat. Year</th>
<th>CF</th>
<th>Age/ Sex</th>
<th>Echinococcosis contracted in:</th>
<th>Location of cyst(s)</th>
<th>Treatment at Mayo</th>
<th>Follow up till</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>1:256</td>
<td>70 F</td>
<td>Greece</td>
<td>Liver (2 cm) (partially calcified)</td>
<td>Surgery + mebendazole for 1 month</td>
<td>1990</td>
</tr>
<tr>
<td>1979</td>
<td>1:32</td>
<td>53 M</td>
<td>Spain</td>
<td>Liver (8 cm)</td>
<td>Surgery + mebendazole for 3 months</td>
<td>1989</td>
</tr>
<tr>
<td>1981</td>
<td>neg.</td>
<td>37 M</td>
<td>Iraq</td>
<td>* Two pyogenic liver abscesses due to enterococci following resection of an Echinococcosis cyst (outside)</td>
<td></td>
<td>1981</td>
</tr>
<tr>
<td>1983</td>
<td>1:8</td>
<td>51 F</td>
<td>Mexico</td>
<td>Liver “inflammatory infiltrate with eosinophilia; no organisms seen”</td>
<td>mebendazole for 6 months</td>
<td>1984</td>
</tr>
<tr>
<td>1983</td>
<td>1:16</td>
<td>60 F</td>
<td>Greece</td>
<td>Liver (10 cm) (some calcifications)</td>
<td>Surgery</td>
<td>1984</td>
</tr>
<tr>
<td>1980</td>
<td>1:32</td>
<td>23 F</td>
<td>Saudi-arabia</td>
<td>Liver (10 cm)</td>
<td>Surgery</td>
<td>1987</td>
</tr>
</tbody>
</table>


* Three months earlier patient S. underwent resection of a liver-cyst (8 cm) at an outside hospital (CF 1:32; test also performed outside).

The specificity of our CF-test was found to be 93%. CHEMTAI et al. in 1981 stated the specificity of their CF-test to be 90% (5) and KERTESZ et al. — looking at 116 hospital patients, in 21 of whom hydatid disease was confirmed surgically — found the specificity of the CF-test to be 90% (16). The above-mentioned review of publications made by KAGAN shows the average specificity to range from 95% to 99% (13).

Laboratory workers have an interest in determining performance characteristics like sensitivity and specificity in order to assess the relative diagnostic value of different tests; clinicians, on the other hand, are more likely to want to compare positive and negative predictive values of tests, in order to choose the best therapy in response to a positive or negative result.

The negative predictive value, the probability that a patient with a negative test result is in fact free of the condition in question, was 98.6%. Cysts of the two patients with false
negative CF results showed calcification. Calcified cysts are supposed to be dead and are removed only if causing symptoms or if secondarily infected (18); partially calcified cysts often have viable protoscoleces in some areas because the cysts die slowly and by regions (12).

The positive value, the probability that a patient with a positive test result does in fact have echinococcosis, was only 30%. The high number of false positive CF results limits the value of CF in the primary diagnosis of echinococcosis. Indirect haemagglutination, latex agglutination, enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent-antibody tests are more accurate than the complement-fixation test, but have the disadvantage of remaining positive for many years after successful removal of the hydatids (13).

While only 1 out of 7 patients with current echinococcosis had mild eosinophilia (781 eosinophils per microliter), marked eosinophilia was present in 3 of our patients with false positive results. The literature states that generalized eosinophilia is present in only 20 to 25% of the echinococcosis cases (14). Reviewing the literature on Echinococcus serology we only found one paper mentioning idiopathic eosinophilia as a disease which can cause false positive results in the CF-test: KERTESZ et al. reported 9 patients with false positive results of CF-tests; malignant tumor in 6 patients, idiopathic eosinophilia in 2, and cholecystitis once (16).

The nonspecific reactivity in the CF-test of serum from patients with malignancies is well known (27) and was found in 2 (sarcoma, lymphoma) of our 14 patients giving false positive CF results.

Echinococcus antigen is also known to cross-react readily with cysticercosis antibodies (6). In our study, sera from 3 out of 14 patients with false positive CF-test results had cysticercosis. Cross-reactivity has also been associated with other helminthic diseases due to a common lipoprotein antigen (25). Angiostrongylus cantonensis, a nematode that usually lives in the pulmonary artery of rats, was the cause of eosinophilic meningitis in a 7 year old boy. In the human host, larvae of this nematode migrate to the brain, spinal cord or eye, where they become immature adults. They usually die in the meninges or the parenchyma of the brain, giving rise to meningeal symptoms. As in our 3 cysticercosis cases the positive CF-reaction did not cause differential problems, indeed drew attention to the possible involvement of a helminthic infection as the cause of the child’s illness. Hymenolepis nana infestation, diagnosed in another patient 3 months before CF testing, could be the reason for a further false-positive result. Thus, 5 out of 14 false positive reactions (= 35.7%) were associated with helminthic disease other than echinococcosis. Although we did not see patients with alveolar hydatid disease during the study period, antibodies to Echinococcus multilocularis are also known to crossreact in our CF-test. Recently we investigated sera from 5 Austrian patients surgically diagnosed as infected with E. multilocularis and found antibody titers of 1 : 2, 1 : 8, 1 : 8, 1 : 64 and 1 : 128 (unpublished data).

Surgery is still the mainstay of treatment (23). The post-operative evaluation of the hydatid patient is of great importance because the excision of the visible cyst may not necessarily imply complete cure (17). Frequently a metacestode of E. granulosus grows for 5 to 20 years before it is diagnosed (4). In the absence of clinical or radiological signs, the behaviour of circulating antibodies may be the earliest or the only indica-
tion of recurrent disease, as was seen in a 50-year old Greek woman, who consulted Mayo 2 years after surgical removal of a liver cyst. The CF-test correctly indicated recurrent disease in the absence of any other clinical evidence. Spillage during operation in another patient was reflected by a 5-fold titer rise 6 weeks after surgery.

According to PAULUZI et al. the CF-test becomes negative approximately 6 months after surgery (24). In our study cure after surgery and/or treatment with antihelminthic drug was accompanied by a negative CF as early as 3 months after successful surgery and 4 months after initiation of medical treatment.

Our findings are in line with the observation that CF antibodies are mainly associated with IgM immunoglobulins (21). Complement-fixing antibodies were usually detected during periods of antigenic activity and disappeared soon after the removal or death of the cyst. This study confirms that the CF reaction is a valuable and helpful indicator for reinfection or continuation of infection, despite its limitations as a diagnostic tool in cystic hydatid diseases. Studies done over recent years have significantly improved the sensitivity and specificity (3, 19). The great advantage brought by the new testing methods (immunoblot assay, enzyme-linked immnosorbent assay) is clearly the elimination of the erroneously positive results. The considerable potential of today’s serological methods for primary diagnosis of echinococcosis was elucidated recently (1).

Summary

A complement fixation (CF) test using human hydatid cyst fluid antigen for the detection of *Echinococcus granulosus* antibody was carried out for 221 patients, including 7 with current hydatid disease, seen at the Mayo Clinic (Rochester) between 1984 and 1990. The CF test was evaluated for sensitivity (66.7%), specificity (93%), negative predictive value (98.6%) and positive predictive value (30%). Cross-reactivity occurred in sera from 14 patients presenting with other helminthic diseases, cancer, liver disorders and marked eosinophilia. The high number of false positive results impedes the use of CF as a diagnostic tool in cystic hydatid disease. The correlation of antibody titer changes (over time) with the final clinical outcome found for 11 echinococcosis-patients seen at Mayo from 1976 to 1990 confirms the value of the CF method in evaluating the success of surgery and the follow-up of treatment with antihelminthic drugs.

Key words

*Echinococcus granulosus*, echinococcosis, serology, complement fixation.

Zusammenfassung

Die klinische Bewertung eines Komplementbindungstests mit einem Antigen aus humanen Hydatidentenzysten zum Nachweis von *Echinococcus granulosus*-Antikörpern


Schlüsselwörter

Echinococcus granulosus, Echinokokkose, Serologie, Komplementbindungsreaktion.

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