Em2-ELISA for the follow-up of alveolar echinococcosis after complete surgical resection of liver lesions

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Abstract

Alveolar echinococcosis, a serious and often fatal human disease, can be efficiently cured only by complete surgical resection of the *Echinococcus multilocularis* lesion. The present study showed that the determination in patients who had undergone surgery of antibody activity directed against the antigen Em2 reliably reflected complete or incomplete surgical resection. From 9 patients with pre-operative positive results in the Em2 enzyme-linked immunosorbent assay (Em2-ELISA) and successful surgical resection, 6 converted to negative within one year and the remaining 3 patients within 4 years after surgery. Six of 7 additional patients who showed recurrences in an average of 6 years after surgery despite assumed complete surgical resection, were positive by Em2-ELISA at the time of recurrence. Discrimination was not possible between these 2 groups of patients when using an ELISA employing crude antigen obtained from *E. granulosus* hydatid cyst fluid.

Introduction

Alveolar echinococcosis, caused by the larval stage of *Echinococcus multilocularis*, is one of the most lethal helminth infections of humans. Only surgical complete removal of the entire parasite lesion offers a prospect for curative treatment. It has been reported that only 26% of cases were resectable in Alaska (SCHANTZ *et al.*, 1983). The development of new immunodiagnostic techniques for early detection of the infection (GOTTSTEIN *et al.*, 1987) and improved methods for clinical diagnosis and surgical treatment (SCHRÖDER & ROBOTTI, 1986; GILLET *et al.*, 1988) resulted in an increased rate of radical resectability, which is presently estimated to be about 40% regarding central European patients. The 'radicality' of the resection is very difficult to determine by the surgeons, as microlesions and root-like parasite protrusions (ECKERT *et al.*, 1983; MEHLHORN *et al.*, 1983) may remain unseen in apparently healthy liver tissue. In this way, some of these patients may subsequently develop recurrences. Serological tests have been used, among others, for post-operative follow-up studies. In most studies, crude antigens derived from *E. granulosus* served to detect parasite-specific immunoglobulins (reviewed by SCHANTZ *et al.*, 1983) or immunoglobulin classes (GOTTSTEIN *et al.*, 1984; VUITTON-DROUHARD, 1985). Generally, a decrease of antibody concentration was observed after surgery. This decrease was more marked in parasite-specific immunoglobulin (Ig) E and IgA levels, compared to the parasite-specific IgG. Most of the patients investigated were under permanent mebendazole or albendazole treatment, which may have influenced the course of serology. Nevertheless, antibodies were still detectable for long periods after surgical intervention. Based on the interpretation of the test results, clear statements regarding the actual status of the disease in individual patients were not possible in many cases. A recent study performed on 3 patients with alveolar echinococcosis indicated that antibodies detected by the use of a purified *E. multilocularis* antigen (Em2 antigen) (GOTTSTEIN, 1985) in an enzyme-linked imunosorbent assay (Em2-ELISA) declined dramatically within months after radical operation (LANIER *et al.*, 1987). In order to evaluate this method further as a follow-up technique, a study was carried out with 18 selected Swiss patients suffering from alveolar echinococcosis.

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Patients and Methods

Patients

All 18 patients investigated were under long-term medical supervision by the 'Swiss Echinococcosis Study Group'. The diagnosis of alveolar echinococcosis had been confirmed clinically, histologically and serologically (WOODTLI *et al.*, 1985; AMMANN *et al.*, 1989). All patients had received surgical treatment with subsequent long-term mebendazole chemotherapy (for details see AMMANN *et al.*, 1989). The following groups of patients with confirmed alveolar echinococcosis of the liver were included in the study.

Group (a). Eleven persons with complete surgical resection of the parasite lesion. No recurrences had been observed up to the end of October 1988 (cut-off point). The average follow-up period after surgery was 7 years (range 2–11 years). More detailed information on the patients is given in Table 1.

Group (b). Seven persons with complete surgical resection of the visible parasite liver lesions reported by the surgeons. The 7 patients developed recurrences at time intervals of 3 years and 5 months to 11 years and 2 months (average 6 years) after operation in spite of continuous chemotherapy. The average follow-up period was 13 years (range 10–21 years). More detailed information on these patients is given in Table 2.

Serological assays

Parasite-specific serum IgG was detected by ELISA. All serum samples had been kept frozen (−80°C) and were examined at the same time and under uniform conditions in order to avoid intertrial variations. Between 3 and 14 serum samples, during the

Table 1. Information on the patients of group (a), showing no recurrence after complete surgical resection of the parasite lesions

Patient's identification	Age at diagnosis (years)	Sex	Organs involved		Duration of chemotherapy (months)
			Primary disease	Recurrence	
Aff (1920)	57	f	liver	no	29
Bar (1956)	24	f	liver	no	24
Hub (1917)	67	m	liver	no	19
Mat (1947)	38	f	liver	no	23
Mos (1932)	49	f	liver	no	25
Reu (1926)	51	m	liver	no	73
Sb (1912)	67	f	liver	no	12
Sa (1949)	29	f	liver	no	73
Sw (1936)	47	f	liver	no	56
Sz (1925)	53	f	liver	no	?
Cd (1942)	44	m	liver	no	9

Table 2. Information on the patients of group (b), showing recurrence after surgical resection of the parasite lesions

Patient's identification	Age at diagnosis (years)	Sex	Organis involved		Duration of chemotherapy (months)
			Primary disease	Recurrence	
Eb (1918)	57	m	liver	liver, kidney, retroperitoneal, lungs	77
Rm (1921)	55	f	liver	liver	67
Vw (1958)	18	m	liver	liver	100
Ak (1929)	48	f	liver	liver coeliac plexus	117
Alt (1930)	45	f	liver	liver	62
Pfr (1939)	31	f	liver	liver	79
Rei (1945)	22	f	liver	liver	112

Table 3a. Follow-up by Em2-ELISA of patients of group (a), showing no recurrence after complete surgical resection. The last result for each patient corresponds to the serum sample at the cut-off point

Patient's identification	AU at surgery[a]	Years after surgery									
		1	2	3	4	5	6	7	8	9	10
Aff	12	0	0	0	0	0	0	0	0	0	
Bar	16	4	0	0	0	0	0	0			
Hub	17	25	0								
Mat	37	0	0	0							
Mos	0	0	0	0	0	0	0	0			
Reu	60	6	9	10	0	0	nd[b]	0	0	nd[b]	0
Sb	10	0	0	0							
Sa	70	0	nd[b]	0	0	0	0	0	0	0	
Sw	30	0	0	0	0	0					
Sz	0	0	0	0	0						
Cd	22	0	0								

[a]AU=antibody units, as percentage of a positive reference serum (see Patients and Methods); 0=no antibodies detectable (negative).
[b]nd=Not done (no serum available).

periods indicated in Tables 3 a, b and 4 a, b, were examined per patient. The ELISA was performed as described previously (GOTTSTEIN *et al.*, 1984; GOTTSTEIN, 1985), using the criteria for interpretation of seropositivity reported by GOTTSTEIN *et al.* (1984). The results are expressed in arbitrarily defined antibody units (AU), as a percentage of the antibody activity of a positive reference serum. (The reference serum was arbitrarily set at 100 AU, corresponding to 100% reference antibody reactivity and simultaneously to the absorbance at 404 nm measured for this serum.) Patients were regarded as sero-negative when antibodies could not be detected (AU=0). Two antigens were included in this study: a purified species-specific antigen (Em2) derived from *E. multilocularis* and a crude *E. granulosus* antigen (EgHF) from hydatid cyst fluid of bovine origin (GOTTSTEIN *et al.*, 1983, 1984).

Results

The results of serological follow-up of the patients from groups (a) and (b) are shown in Tables 3 and 4.

Group (a) (radical resection, no recurrence)

Em2-ELISA. Initially (before surgery), 9 of 11 patients had detectable anti-Em2-antibodies. One year after surgery, 6 of these 9 patients had already converted to negative (anti-Em2). Of the remaining patients, 2 were negative (anti-Em2) 2 years after surgery and one 4 years after surgery. After conversion to negative, none of the patients showed any further anti-Em2-antibody activity up to the cut-off point (Table 3a).

Table 3b. Follow-up by EgHF-ELISA of patients of group (a), showing no recurrence after complete surgical resection. The last result for each patient corresponds to the serum sample at the cut-off point

Patient's identification	AU at surgery[a]	Years after surgery 1	2	3	4	5	6	7	8	9	10
Aff	95	28	23	14	24	19	17	18	20	20	
Bar	91	25	13	7	0	3	3	5			
Hub	87	73	70								
Mat	38	0	0	0							
Mos	39	12	8	0	0	0	0	0			
Reu	78	51	42	56	31	41	nd[b]	22	10	nd[b]	6
Sb	46	3	11	0							
Sa	79	12	nd[b]	19	20	16	9	4	4	0	
Sw	87	63	54	30	17	17					
Sz	72	15	17	8	14						
Cd	75	21	4								

[a]AU=antibody units, as percentage of a positive reference serum (see Patients and Methods); 0=no antibodies detectable (negative).
[b]nd=Not done (no serum available).

Table 4a. Follow-up Em2-ELISA of patients of group (b), showing recurrence after surgical resection. The last result for each patient correponds to the serum sample at the cut-off point of the present study. The year 0 corresponds to the date of diagnosis of recurrence

Patient's identification	AU at the following times (in years before and after recurrence)[a] –7	–6	–5	–4	–3	–2	–1	0	1	2	3	4	5	6	7	8	9	10
Eb	18[b]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	14	22	17	16	16	12	10	27				
Rm			9[b]	nd[c]	nd[c]	6	nd[c]	37	3	0	0	0	0	0				
Vw					5[b]	0	0	4	0	0	0	0	0	0	0	0		
Ak						8[b]	2	0	0	0	0	0	0					
Alt								48[d]	19	11	1	11	0					
Pfr								33[d]	0	18	12	0	0	0	0	0	0	0
Rei								107[d]	16	70	58	47	30	0	0	5	0	

[a]AU=antibody units, as a percentage of a positive reference serum (see Patients and Methods); 0=no antibodies detectable (negative).
[b]Initial values at the date of surgery.
[c]nd=Not done (no serum available).
[d]No serum available of the date at surgery.

Table 4b. Follow-up by EgHF-ELISA of patients of group (b), showing recurrence after surgical resection. The last result for each patient corresponds to the serum sample at the cut-off point of the present study. The year 0 corresponds to the date of diagnosis of recurrence

Patient's identification	AU at the following time (in years before and after recurrence)[a] –7	–6	–5	–4	–3	–2	–1	0	1	2	3	4	5	6	7	8	9	10
Eb	96[a]	nd[b]	nd[b]	nd[b]	nd[b]	nd[d]	79	85	90	85	59	69	75	74				
Rm			123[a]	nd[b]	nd[b]	63	nd[d]	92	100	40	52	62	66	51				
Vw					67[a]	36	25	21	19	21	4	0	3	0	0	0		
Ak						14[a]	9	4	7	0	0	2	47					
Alt								48[c]	38	28	17	20	13					
Pfr								47[c]	37	55	46	44	25	24	28	31	31	20
Rei								45[c]	63	70	61	55	28	36	24	18	10	

[a]AU=antibody units, as percentage of a positive reference serum (see Patients and Methods); 0=no antibodies detectable (negative).
[a]Initial values at the date of surgery.
[b]nd=Not done (no serum available).
[c]No serum available of the date at surgery.

EgHF-ELISA. Anti-EgHF-antibodies were initially detected in all of 11 patients. One year after surgery, only one patient had converted to negative. At the cut-off point, 7 patients still had anti-EgHF-antibodies detectable, while the 3 other patients had converted to negative (Table 3b).

Group (b) (radical resection, but with subsequent recurrence)

Em2-ELISA. Of 7 patients investigated, all demonstrated anti-Em2-antibody activity initially (before surgery). Two of them converted to negative within 2 years after surgery. One of the latter remained negative despite a recurrence, the other had a rise in anti-Em2-antibody concentration during recurrence. Overall, at the time of recurrence, 6 of 7 patients were Em2-positive and most of them maintained anti-Em2-antibody activity for a period of years following recurrence. At the end of the follow-up period, 2 of 7 patients were still Em2-positive (Table 4a).

EgHF-ELISA. All 7 patients were initially positive by EgHF-ELISA, as well as at the time of recurrence. At the end of the follow-up period, 6 of 7 patients showed anti-EgHF-antibody activity (Table 4b).

Discussion

The present study extends previous preliminary observations (LANIER *et al.*, 1987) indicating that Em2-ELISA might prove useful for monitoring patients with alveolar echinococcosis following surgical treatment. Our results indicate that 9 patients with anti-Em2 antibodies at the time of surgery converted to negative within 1 to 4 years after operation. In most of the patients (7 of 9) antibody levels had decreased to zero within the first year after operation, independent of the initial antibody concentration at the time of surgery. On the other hand, 6 of 7 patients with an assumed radical resection, but with recurrence demonstrable on the average 6 years after surgery, showed anti-Em2-antibody activity at the time of recurrence. Although the number of patients selected for this study was relatively small, the present results, combined with those of LANIER *et al.* (1987), tend to indicate that after successful radical operation a significant decrease of anti-Em2 antibody concentration may be expected, provided that no recurrence occurs. On the other hand, a persistence of anti-Em2 antibody concentration may indicate the persistence of parasite material and in this way indirectly also indicate the possibility of recurrence. The use of common serological tests employing crude antigens such as *E. granulosus* hydatid fluid (EgHF) does not permit such a discrimination, as most sera from both groups of patients remained positive for a long period after surgery. Nevertheless, a tendency of the anti-EgHF-antibody concentration to decrease was observed in the group of patients with successful resection, confirming similar observations published earlier (GOTTSTEIN *et al.*, 1984). Several points must be considered concerning the results presented above. One concerns the observation that the presence of anti-Em2-antibodies correlates only with the presence of parasite material and not with its viability. This was shown by RAUSCH *et al.* (1987) who, conducting a sero-epidemiological survey by Em2-ELISA, were able to detect 5 Em2-positive patients, with lesions in which the larval *E. multilocularis* had died spontaneously at an early stage of infection. In all of these cases, the surgical resection of the dead lesions was followed by a rapid decrease of anti-Em2-antibody concentration to zero (R. L. Rausch, personal communication). In the present study, because of these observations, we deliberately did not include patients with inoperable or partially operable lesions or who had received palliative surgery followed by mebendazole or albendazole therapy. Measurement of humoral immunity has been shown, up to now, to be of limited applicability for assessing the course of the disease during chemotherapy in such groups (MÜLLER *et al.*, 1982; SCHANTZ *et al.*, 1983; GOTTSTEIN *et al.*, 1984; LANIER *et al.*, 1987). We definitely need new immunological tools which may reliably reflect the actual biological status of a treated, but not completely removed, parasite lesion. Some indications in this direction may have been given by measuring circulating or immune-complexed parasite antigens (GOTTSTEIN, 1984; CRAIG & NELSON, 1984), but such tests will have to be adapted specifically to problems of infections with *E. multilocularis*. Another point of discussion is the observation that all 15 patients whose serum was tested at the time of surgery were positive by EgHF-ELISA, whereas only 13 of them were positive by Em2-ELISA, indicating lower diagnostic sensitivity of the latter test. This problem may be overcome by purifying new highly specific antigens which can be employed in addition to the Em2-antigen for diagnostic purposes. To this end, we have recently synthesized a recombinant *E. multilocularis* antigen in *Escherichia coli* (VOGEL *et al.*, 1988), which proved to be useful for the purpose in question, as in both of the cases mentioned above antibodies were detected against the recombinant antigen.

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