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Evaluation of the Bordier Em18 antibody ELISA for the detection of human active *Echinococcus multilocularis* infection

Using stored and fresh serum samples obtained from patients with different forms of echinococcosis, we evaluated the Bordier Em18 antibody ELISA in our laboratory. We could compare the results with those obtained from the Institute of Parasitology, Berne, Switzerland, an ELISA test that has been used as the standard in the management of our patients so far. We also have obtained sera from our colleagues in Ulm (Drs. Beate Grüner and Julian Schmidberger) and have classified those and our sera into i) before/after radical surgery, ii) before/after nonradical surgery, iii) progressive diseases, iv) stable disease, v) inactive disease. Some of the antibody measurement results could also be compared with the so-called ADAMU-Em18 test.

Patients w/o evidence for alveolar echinococcosis

Table 1 shows the details for patients with varying diagnoses (CE=cystic echinococcosis, UE=undifferentiated echinococcosis, No E=alternative diagnoses).

Table 1. Details of patients with negative Bordier ELISA results and comparison with other antibody tests for alveolar echinococcosis.

		Bordier		IfP, Berne		ADAMU
Sample/patient ID number	Final diagnosis	Em18 Lot 2	Em18 Lot 3	Em18	Em2	Em18 1:6
#10	CE	0,15	0,31	nd	nd	neg
#9	CE	0,0	0,08	0	0	nd
#7	UE	1,00	0,48	1	2	neg
#19	UE	0,25	0,41	nd	nd	neg
#26	UE	0,58	0,79	nd	nd	nd
#11	No E	0,0	0,04	0	11	neg
#16	No E	0,76	0,27	nd	nd	neg
#18	No E	0,22	0,53	nd	nd	neg

As can be seen, the Bordier Em18 tests were consistently negative, as were the Em18 antibody tests from alternative producers/laboratories.

Patients with alveolar echinococcosis

Samples, sometimes multiple stored samples from the same patient, all with different stages of alveolar echinococcosis (AE) were tested with the Bordier ELISA.

Table 2. Results for sera tested with Em18 ELISA obtained from patients with alveolar echinococcosis.

Sample/patient ID number	Sampling date	AE diagnosis	Bordier		IfP, Berne Em18
			Em18 Lot 2	Em18 Lot 3	
#2	23.09.16	2011	0,23	0,12	0
#15	15.09.16	2012	0,23	0,44	0
#21	08.11.16	2015	**4,31**	**3,70**	**18**
#27	09.01.17	2008	**17,41**	**3,60**	**19**
#14	22.08.16	2016	**7,84**	**5,42**	**31**
#12	04.08.16	2012	**2,40**	**1,41**	**7**
#20	14.11.16	2011	**4,17**	**6,21**	**42**
#22	21.11.16	2016	**5,06**	**3,61**	nd
#23	01.12.16	2016	**2,32**	**2,60**	nd
#24	05.12.16	2016	1,02	0,23	nd
#28	16.01.17	2016	0,56	0,60	nd
#3	17.03.16	2011	**2,81**	**9,32**	**14**
#5	21.03.16	2014	0,42	0,52	0
	19.09.16		0,37	0,82	0
#6	11.04.16	2007	**5,34**	**3,34**	**18**
	16.01.17		**5,07**	**6,21**	**18**
#1	7.03.16	2014	**28,02**	**17,76**	**110**
	12.12.16		**20,84**	**80,66**	**110**
#17	20.10.16	2016	**9,85**	**8,75**	nd
	28.11.16		**8,66**	**8,75**	nd
	16.01.17		**6,79**	**8,17**	nd
#4	21.03.16	1985	**19,58**	**11,63**	**60**
	24.05.16		**22,24**	**11,41**	**60**
	22.08.16		**22,63**	**11,71**	nd
	22.08.16		**11,15**	**16,61**	nd
	22.08.16		**11,54**	**16,87**	nd

Of these 17 patients with AE, 12 were positive in the Bordier Em18 ELISA test, and 5 were negative. For 12 patients, in part with multiple samples, we had results from the comparative test available. As shown in table 2, the Bordier Em18 test results were in complete agreement with the IfP-Berne Em18 results.

We next performed dilutional analyses on several positive samples. The results are shown in Table 3. With the exception of the samples from patient #4 (high antibody concentration) the extinction values decreased consistently with increasing dilution.

We also assayed the same samples on different days and found a satisfactory agreement for negative samples and limited agreement on the samples with high antibody concentration (patient #4) (table 4).

Table 3. Results for sera in different dilutions tested with Em18 ELISA.				
Sample/patient ID number	Sampling date	Dilution	Bordier	
			Em18 Lot 2	Em18 Lot 2
#3	17.03.2016	und	2,81	9,32
		1:2	1,39	5,88
		1:4	0,7	3,28
#4	22.08.2016	und	11,54	16,87
		1:2	17,82	8,77
		1:4	11,87	6,68
#12	04.08.2016	und	2,4	1,41
		1:2	1,15	0,74
		1:4	0,84	0,54
#17	20.10.2016	und	9,85	8,75
		1:2	4,79	4,1
		1:4	2,97	2,81
#20	14.11.2016	und	4,17	6,21
		1:2	3,36	3,34
		1:4	2,63	2,21

In order to evaluate whether negative results for Em18 antibody in patients with AE were indicating inactive disease, we tested a set of sera obtained from patients in different stages/phases of their disease (table 5).

Table 4. Test to test variance of the Em18 ELISA.			
Sample/patient ID number	Test date	Bordier	
		Em18 Lot 2	Em18 Lot 2
#15	16.09.2016	0,23	0,44
#15	23.09.2016	0,41	0,49
#4	16.09.2016	22,63	11,71
#4	23.09.2016	11,15	16,61

In order to evaluate whether negative results for Em18 antibody in patients with AE were indicating inactive disease, we tested a set of sera obtained from patients in different stages/phases of their disease. Patients were classified as having had radical (n=14), non-radical (n=12) or no surgery (39), being actually treated or not with albendazole or mebendazole, and considered "cured" (no relapse after therapy discontinuation [n=15] or without any treatment [n=2]) or having progressive (n=7) or stable disease. Em18 titers in the post-surgery samples fell in all 12 patients and were significantly lower than in pre-surgery specimens. Among all 9 (out of the 12) patients with paired pre- and post-surgery serum samples and elevated pre-surgery Em18 antibodies (median index 9.4, IQR 7.5-22, mean of the measurements for each), titers fell to an index <2 (<1 considered negative) – independent on whether the surgery was radical or not (details in table 5), and all 17 patients considered "cured" also had an Em18 antibody index <1 (data not shown).

Of the 25 patients without surgery but with stable disease on antiparasitic chemotherapy 7/25 (28%) were negative in the Em18 ELISA while among patients without prior surgery and progression while on chemotherapy only 1/7 was (6%) negative (not significant) (data not shown).

The titer index for patients without surgery was significantly lower in stable (median 6.3, IQR 3.8-8.2)) versus progressive disease (median 13.8, IQR 8.6-18.9).

Table 5. Test results for patients with AE before and after surgery (most patients were also treated with albendazole).

					Bordier	
Sample/patient ID number	Surgery date	Sampling date		Type of surgery	Em18 Lot 2	Em18 Lot 2
MH			before	radical	27,0#	
			after		0,5	
FS			before	radical	22,0#	
			after		<0.1	
HE			before	radical	>100#	
			after		1,9	
U14HH	22.09.2014	24.07.2014	before	non-radical	16,57	11,86
		10/2016	after		1,42	1,56
U12GS	29.02.2016	25.01.2016	before	non-radical	12,47	9,36
		10/2016	after		1,35	1,75
U1FV	20.10.2014	25.07.2014	before	radical	10,20	7,66
		10/2016	after		1,54	2,55
U34BH	27.12.2012	09.11.2012	before	non-radical	9,52	7,48
		11/2016	after		0,04	0,09
U4SP	19.11.2014	24.10.2014	before	radical	3,92	3,10
		10/2016	after		0,41	0,41
U8LE	11.09.2014	23.07.2014	before	radical	2,60	2,28
		10/2016	after		0,18	0,10
U25WJP	02.04.2013	07.12.2012	before	radical	0,42	0,28
		11/2016	after		0,28	0,12
U42	03.12.2013	09.10.2013	before	radical	0,93	0,85
		11/2016	after		0,38	0,21
U17UA	07.10.2014	17.09.2014	before	radical	0,93	0,87
		10/2016	after		0,01	0,28

Only results of IfP-Berne ELISA avaiable

<u>Conclusions</u>

The Bordier Em18 ELISA test in our hands appeared to give consistent results in terms of indicating active alveolar echinococcosis and to differentiate between CE and (active) AE. Lot to lot and test to test variation appeared to be acceptable. Although at high antibody concentrations the current assay appears to be out of the linear measurement ranges, the ELISA values declined after surgery, and there were very limited fluctuation around the cut-off.

This test will be very useful for AE disease activity monitoring.

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