The Role of Recombinant Antigens in the Serodiagnosis of Human Cystic Echinococcosis

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Introduction and Purpose

The diagnosis of human cystic echinococcosis (CE; Figure 1) is based on imaging (Figure 2). Serological tests, still based on hydatid fluid (HF), are marred by poor sensitivity and specificity in certain contexts. Serology is also of little use for follow-up of treated patients, due to the persistence of antibodies after cure. As recombinant antigens may represent an alternative to HF, several recombinant molecules have been tested, but without taking into account important details such as cyst stage, number, location and others. Here, we present a study on two recombinant antigens that has included such clinical details.

	Number
Cyst location	
Liver	942
Other	162
Cyst number	
Single	662
Multiple	464
Serum collection	
Before treatment	661
After treatment	458
Classification	
CE1	119
CE2	81
CE3a	105
CE3b	325
CE4	266
CE5	134

Table 1. Sera from cystic echinococcosis patients used in the study. Clinical data

Figure 1. Life cycle of *Echinococcus granulosus*

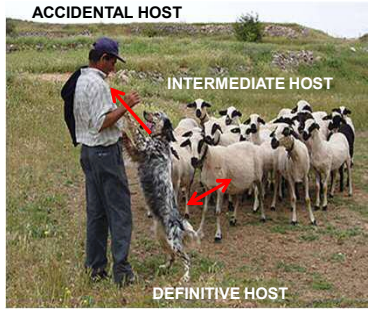
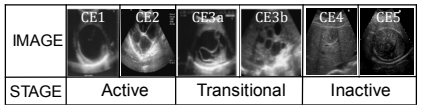


Figure 2. Cyst classification according to WHO-IWGE



Methods

Recombinant antigens B2t and 2B2t (Hernández-González *et al.*, 2012) have been tested on sera from CE patients (1,128 sera; Table 1), patients with cross-reactive diseases (cysticercosis –NCC-, n=104; alveolar echinococcosis –AE-, n=50) and donors (n=86), and compared with the HF in ELISA and chromatography for the detection of IgG. Clinical variables potentially influencing serological results were statistically assessed.

Results

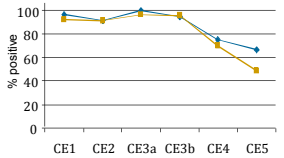
Preliminary results using the AgB-derived recombinant proteins show that these antigens detect active cysts with high sensitivity and specificity (>90%), influenced by cyst classification (Table 2).

		ELISA-HF		ELISA-rB2t		ELISA-r2B2t	
		B-OR	M-OR	B-OR	M-OR	B-OR	M-OR
Cyst number	Single	1	1	1	1	1	1
	Multiple	3,5 (2,2-5,5)	3,1 (1,9-5,1)	2,6 (2,1-3,4)	2,5 (1,9-3,3)	2,4 (1,9-3,1)	2,2 (1,6-2,9)
Cyst location	Liver	-	-	1	-	1	-
	Other	-	-	2 (1,4-2,9)	-	2,6 (1,7-3,7)	-
Cyst classification	CE4-5	1	1	1	1	1	1
	CE1-2-3	5,7 (3,7-8,7)	3,9 (2,5-6,1)	5,3 (4-6,9)	4,5 (3,4-6,01)	7,4 (5,6-9,8)	6,4 (4,7-8,5)
Serum collection	Before tr.	1	1	1	1	1	1
	After tr.	13,3 (6,5-27,6)	10,2 (4,6-22,4)	2,7 (2,1-3,5)	2,2 (1,6-2,9)	2,8 (2,2-3,7)	2,3 (1,7-3,1)

Table 2. Logistic regression analysis of the influence of clinical variables on the outcome of serological tests. Statistically significant differences are marked in red.

Sensitivity of the 2B2t recombinant antigen in strips is also >90% for active and transitional cysts (Figure 3). Specificity was hindered mainly by cross-reactions with NCC patients (Table 4).

Figure 3. Comparative sensitivity of immunostrips containing HF (blue) or 2B2t antigen (brown) with sera from CE patients. Results are shown for each cyst classification group.



	STRIPS HF n+ (%)	STRIPS 2B2t n+ (%)
Donors, n=86	2 (94.4%)	0 (100%)
AE, n=50	33 (34%)	16 (68%)
NCC, n=104	17 (83.6%)	50 (51.9%)

Table 4. Specificity of immunostrips containing HF or 2B2t antigen with sera from donors, AE patients and NCC patients.

Notably, the 2B2t antigen can be used to assess outcome in patients on post-treatment follow-up, showing a decline in specific antibodies in cured patients, but not in those with cysts unresponsive to treatment.

Conclusions

Further development of antigen candidates is needed before production of serological tests that could be implemented in clinical settings. This will be tackled in the frame of the EU-funded FP7 project ""Human cystic echinococcosis research in central and eastern societies (HERACLES)", including: building of biobanks with human samples, clinical database and protocols development, production/testing of recombinant antigens, and development of "lab-on-a-chip" devices for the diagnosis and follow-up of CE.

For further information, visit www.heracles-fp7.eu

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