New biomarkers for post-surgery follow-up of CE patients

Coralie BARRERA & Louis BOHARD
Karima Achour / Laurence Millon / Maria Mar SILES LUCA

Introduction & objectives

Elevated relapse after surgery 4.6% – 22 % (Kapan 2006; Prousalidis 2012; Sielaff 2001)
Even if combined with antihelmintic treatments (Velasco-Tirado, 2017)

Gold standard = ultrasound scan

Current/commercially immunoassays : Antibodies elevated for a longtime.
No early serological assays availables to detect relapse.

Follow-up CE : Many approaches tested : antibodies, cytokines, parasitic antigens.

Recombinant Antigens **B2T & 2B2T** : promising biomarkers for follow-up but large number of pre-operatively non-responsive CE cases. (Hernandez-Gonzalez 2018)

A peak in immune and serological responses in many cases shows up at 1 month after surgical treatment of CE (Ben Nouir 2009; 2008)

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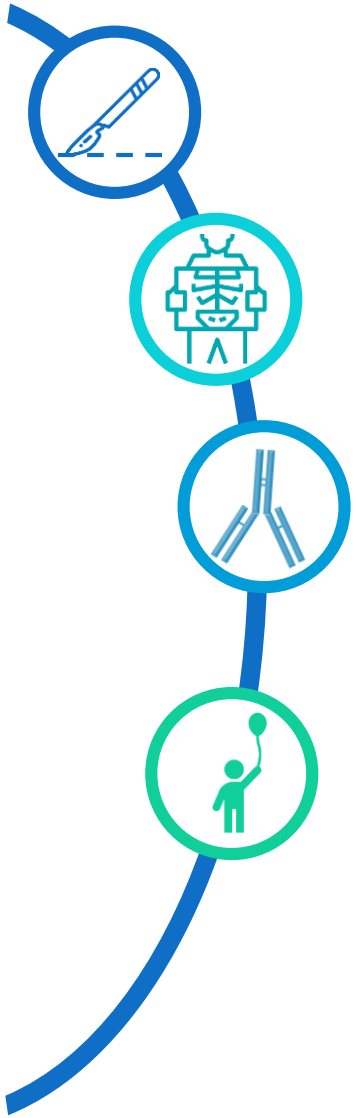
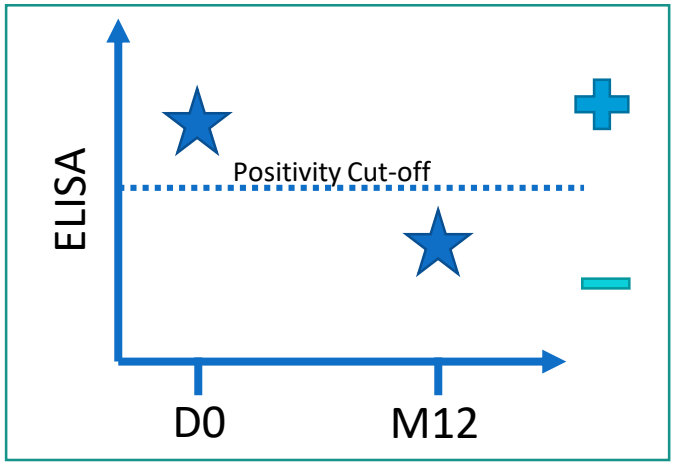
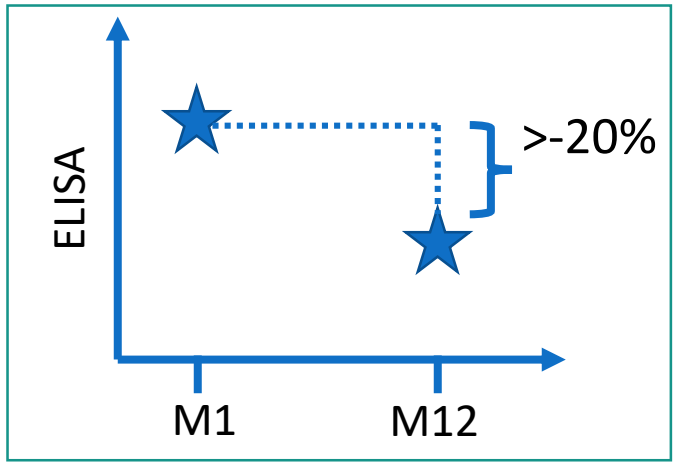




% of patients with negativization



Significative decrease for iterative comparison

Iterative comparison between Ab levels at M1 and Ab levels at M12

More informative for the follow-up than a positivity cut-off

Salah *et al. Parasites Vectors* (2021) 14:180
https://doi.org/10.1186/s13071-021-04679-5

Parasites & Vectors

RESEARCH Open Access

Promising proteins detected by Western blot from *Echinococcus granulosus* protoscoleces for predicting early post-surgical outcomes in CE-affected Tunisian children

Eya Ben Salah[1], Coralie Barrera[2], Sana Mosbahi[3], Bruno Gottstein[4], Mar Siles-Lucas[5], Samia Belhassen[3], Abdellatif Nouri[3], Hamouda BABBA[1], Laurence Millon[2] and Wahiba Sakly[1*]

Table 2 Number of CE patients according to the evolution of IgG levels observed between D030 and D365 for anti-B2t, anti-2B2t, and both antibodies (2B2t or B2t), and determination of the probability of being relapse-free

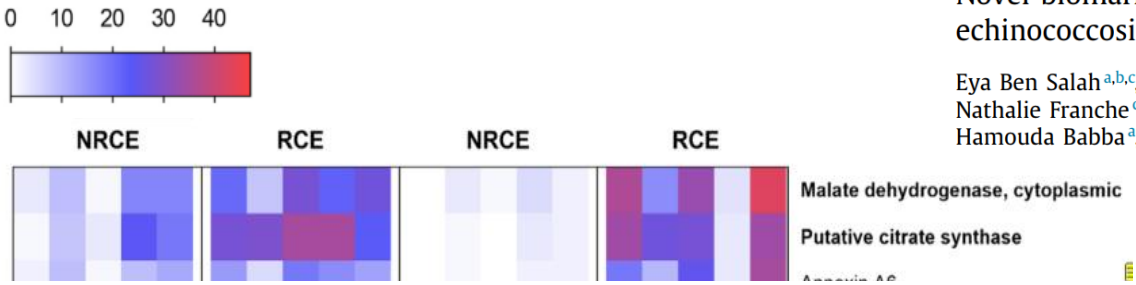
Antibody response	Evolution	NRCE, *n* (%)	RCE, *n* (%)	Probability of being relapse-free (%)
Anti-B2t IgG	Decrease*	25 (64)	6 (30)	81
	Increase	5 (13)	8 (40)	
	Stability	9 (23)	6 (30)	
Anti-2B2t IgG	Decrease	21 (54)	7 (35)	75
	Increase	9 (23)	10 (50)	
	Stability	9 (23)	3 (15)	
Anti-(B2t or 2B2t) IgG	Decrease*	32 (82)	9 (45)	78
	Increase	4 (10)	7 (35)	
	Stability	3 (8)	4 (20)	

NRCE non-relapsed patients, *RCE* relapsed patients

*$p < 0.05$ (chi-square test)

Introduction & objectives

Abundance of immunoreactive-proteins - Comparison strategy



Journal of Infection 84 (2022) 87–93

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Novel biomarkers for the early prediction of pediatric cystic echinococcosis post-surgical outcomes

Eya Ben Salah[a,b,c], Coralie Barrera[b,c], Wahiba Sakly[a], Sana Mosbahi[d], Thierry Balliau[e], Nathalie Franche[c], Bruno Gottstein[f], Sabrine Ben Youssef[d], Mongi Mekki[d], Hamouda Babba[a], Laurence Millon[b,c,*]

Table 1
Evolution of IgG levels between 1 M and 1Y post-surgery in NRCE and RCE patients.

IgG antibody response against	Evolution	NRCE; *n* (%)	RCE; *n* (%)	Chi-square test	Probability of being "relapse-free" at 1Y[a]	Probability of being "relapsed" at 1Y[b]
Eg-cMDH	Decrease	25 (56)	6 (23)	***p* = 0.02**	**83%**	
	Increase	5 (15)	8 (54)	***p* = 0.01**		**64%**
	Stability	9 (29)	6 (23)	Ns		
Eg-CS	Decrease	22 (65)	5 (29)	***p* = 0.01**	**81%**	
	Increase	3 (9)	4 (24)	Ns		57%
	Stability	9 (26)	8 (47)	Ns		
Eg-ANX A6	Decrease	15 (44)	6 (35)	Ns	71%	
	Increase	6 (18)	7 (41)	Ns		54%
	Stability	13 (38)	4 (24)	Ns		
Eg-SVN	Decrease	18 (53)	7 (41)	Ns	72%	
	Increase	4 (12)	2 (12)	Ns		33%
	Stability	12 (35)	8 (47)	Ns		

[a] The probability of being "relapse-free" was calculated as follows: number of NRCE with a decreased level between 1 M and 1Y/number of all patients (NRCE and RCE) with the same status.

[b] The probability of being "relapsed" was calculated as follows: number of RCE with an increased level between 1 M and 1Y/number of all patients (NRCE and RCE) with the same status.

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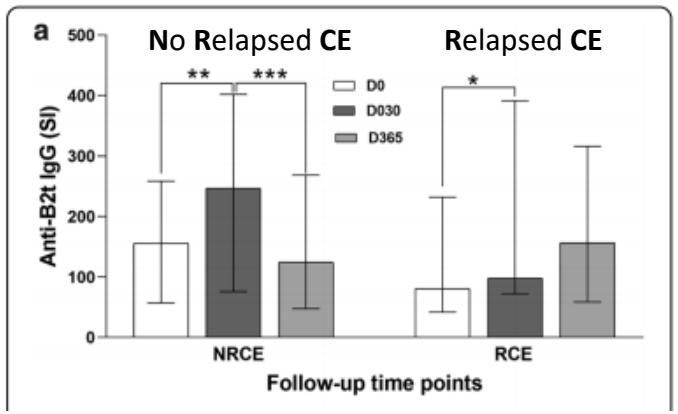
Recombinant Antigens **B2T & 2B2T** : promising biomarkers for follow-up but large number of pre-operatively non-responsive CE cases. (Hernandez-Gonzalez 2018)

OBJECTIVES : Performed ELISA with both new biomarkers, B2T & 2B2T and the currently used in Besançon lab the *Echinococcus granulosus IgG* ELISA kit from Bordier affinity products (Switzerland)
Evaluated the antibodies kinetics and predictive performance of relapse/no relpase

Methods



Demonstrated on paediatric CE population

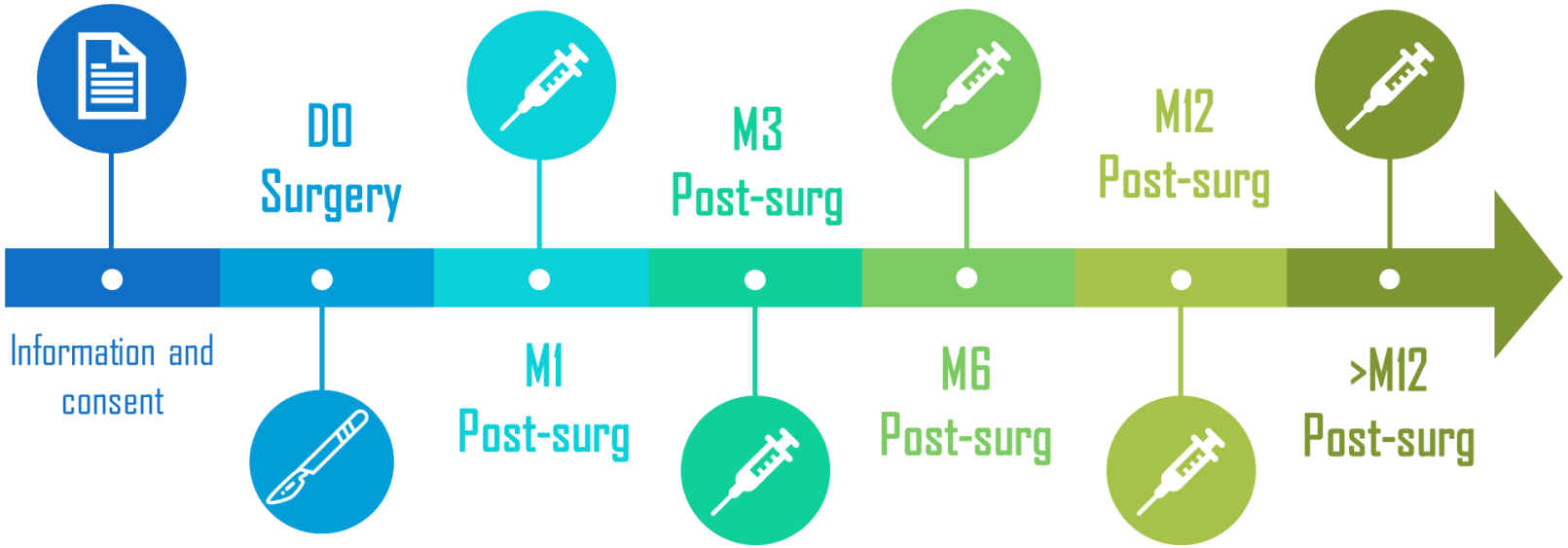
Salah *et al. Parasites Vectors* (2021) 14:180
https://doi.org/10.1186/s13071-021-04679-5

Methods

Recruitment D0 : since january 2022 -> March 2023 = 23 patients

From Algeria

Blood samples collection plan:

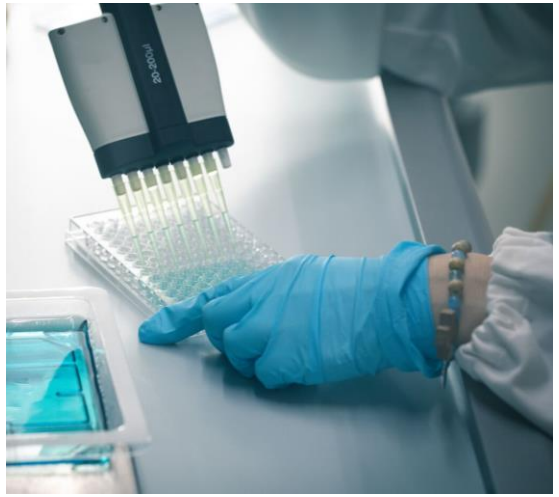


Only 16 patients on 23 have the three samples : D0 + M1 + M12

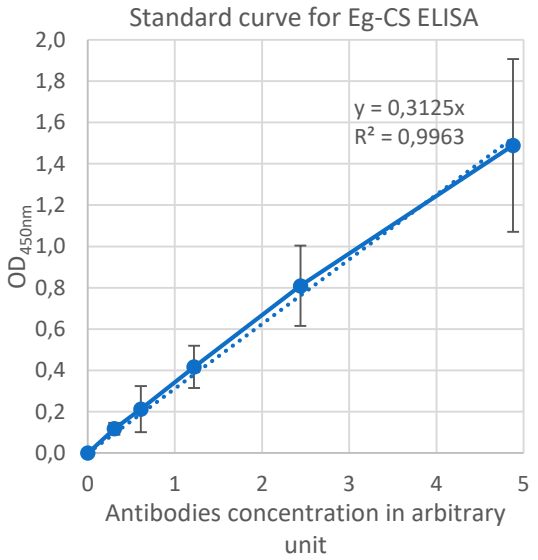
21 patients had the samples D0 + M12

Methods

In-house ELISA with Eg-MDH ; Eg-CS; B2T ; 2B2T



Créateur : sruilk | Crédits : Getty Images/iStockphoto



Controls = polyclonal antibodies from immunized rabbit

Results expressed in Arbitrary Units (AU)

Commercially *E. granulosus* ELISA kit from Bordier

Results expressed in serological indexes

Results

23 CE patients surgically

5 CE patients with a complication form (infection / rupture)
7 CE patients with a history of CE

12 Males / 11 Females
Median age 39y [17 - 78 y]

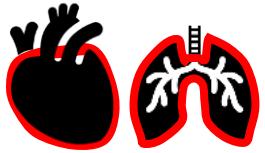
All chemically treated (albendazole)

Lesions location:

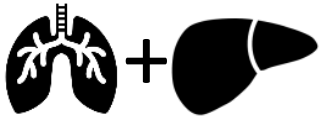
Single location

13 cases

1 case

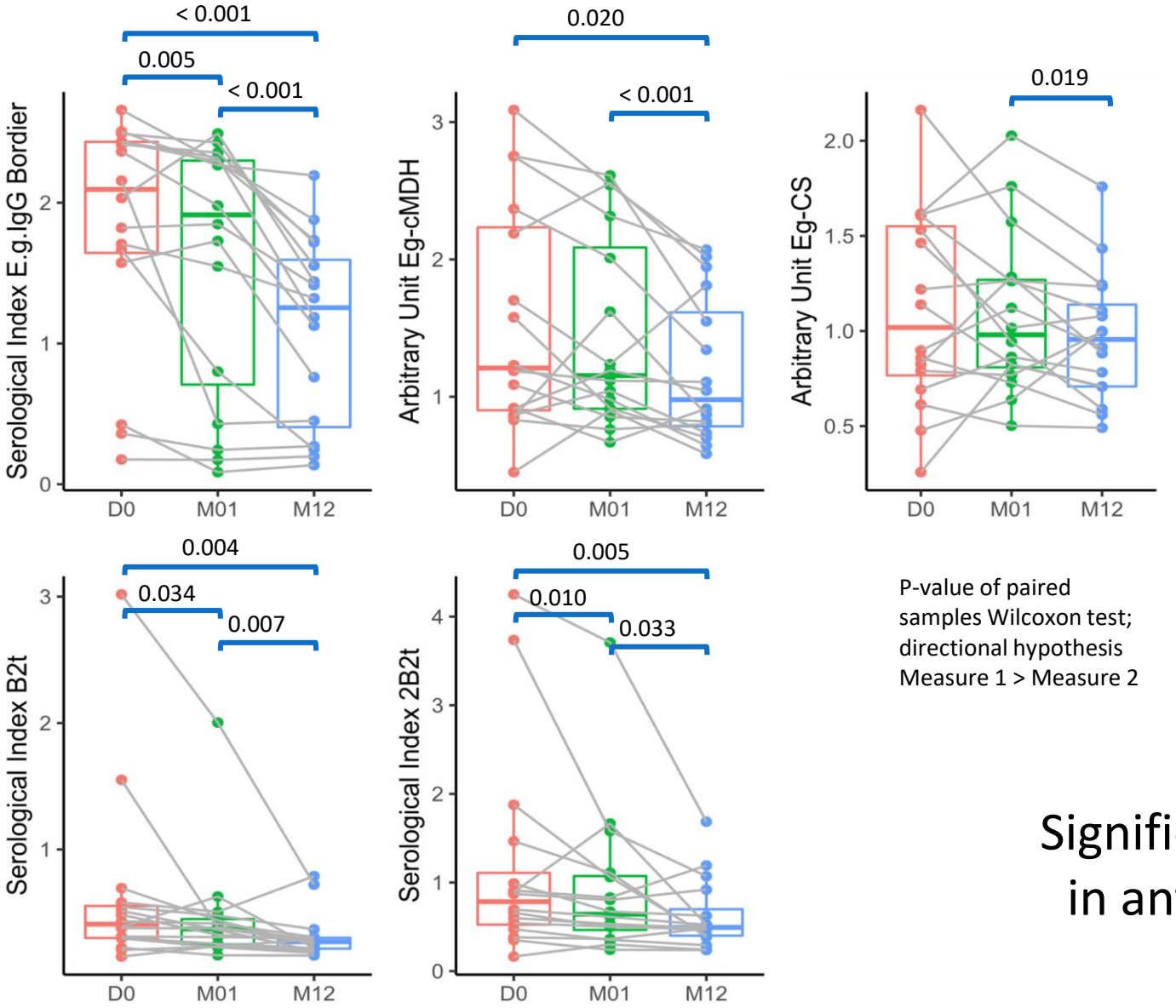
Others
4 cases 

Multiple location

 5 cases

No relapsed patient at M24

Results



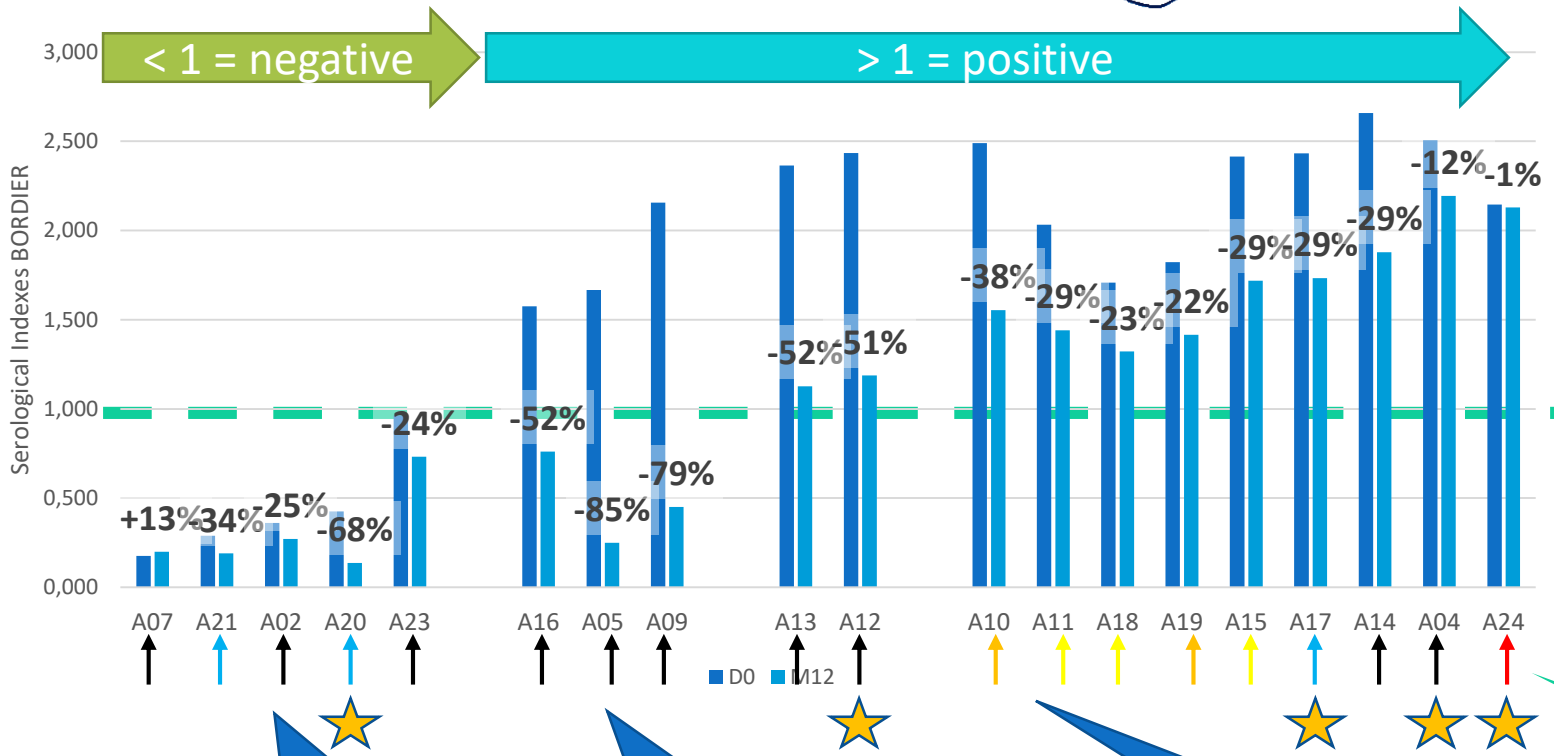
Comparison between
D0 & M12
Except for antigen Eg-CS

P-value of paired samples Wilcoxon test; directional hypothesis Measure 1 > Measure 2

Significant decrease in antibody levels

Results

BORDIER AFFINITY PRODUCTS



One pulmonary cyst completely removed

One cyst (liver or other) completely removed

Two cysts (one liver and one lung) completely removed

Two cysts (one liver and one lung) **NOT** completely removed

Nine pulmonary cysts **NOT** completely removed

History of CE

5 were negative at D0 (26.3%) :

3 positives became negatives after M12 (15.7%)

11 remain positives (58%)

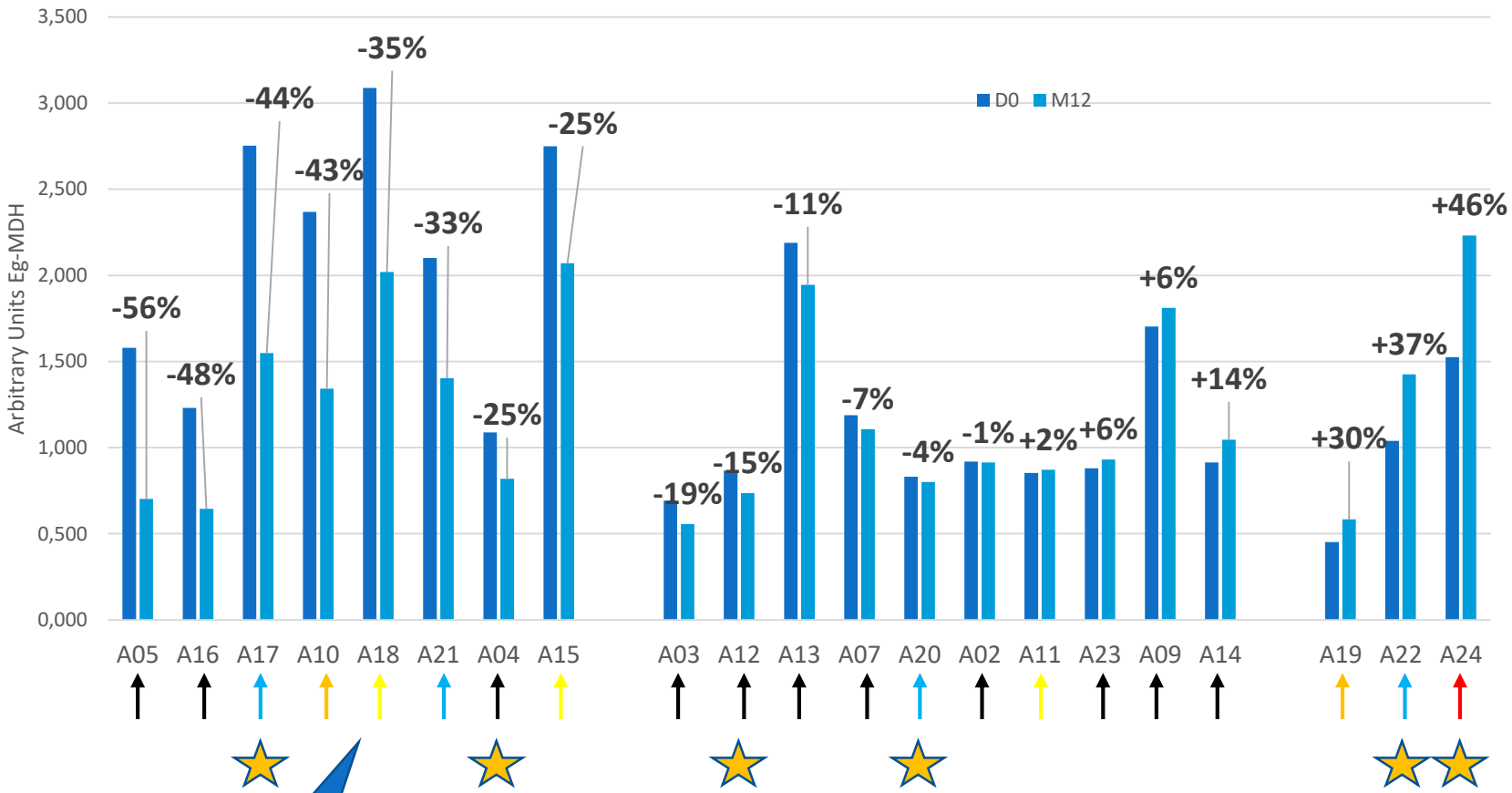
A12 and A13 with decrease <-50% :

16 decrease at least of -20% (84%)
3 kept stables [-20% : +20%] (16%)

Weak antibody levels or important decrease =
Relatively simple CE cases
Clinical board consistent with serological findings

Results

ELISA results – Malate dehydrogenase



8 patients (38%) with decrease < -20%

10 patients (48%) with stability

3 patients (14%) with increase > +20%

One pulmonary cyst completely removed

One cyst (liver or other) completely removed

Two cysts (one liver and one lung) completely removed

Two cysts (one liver and one lung) **NOT** completely removed

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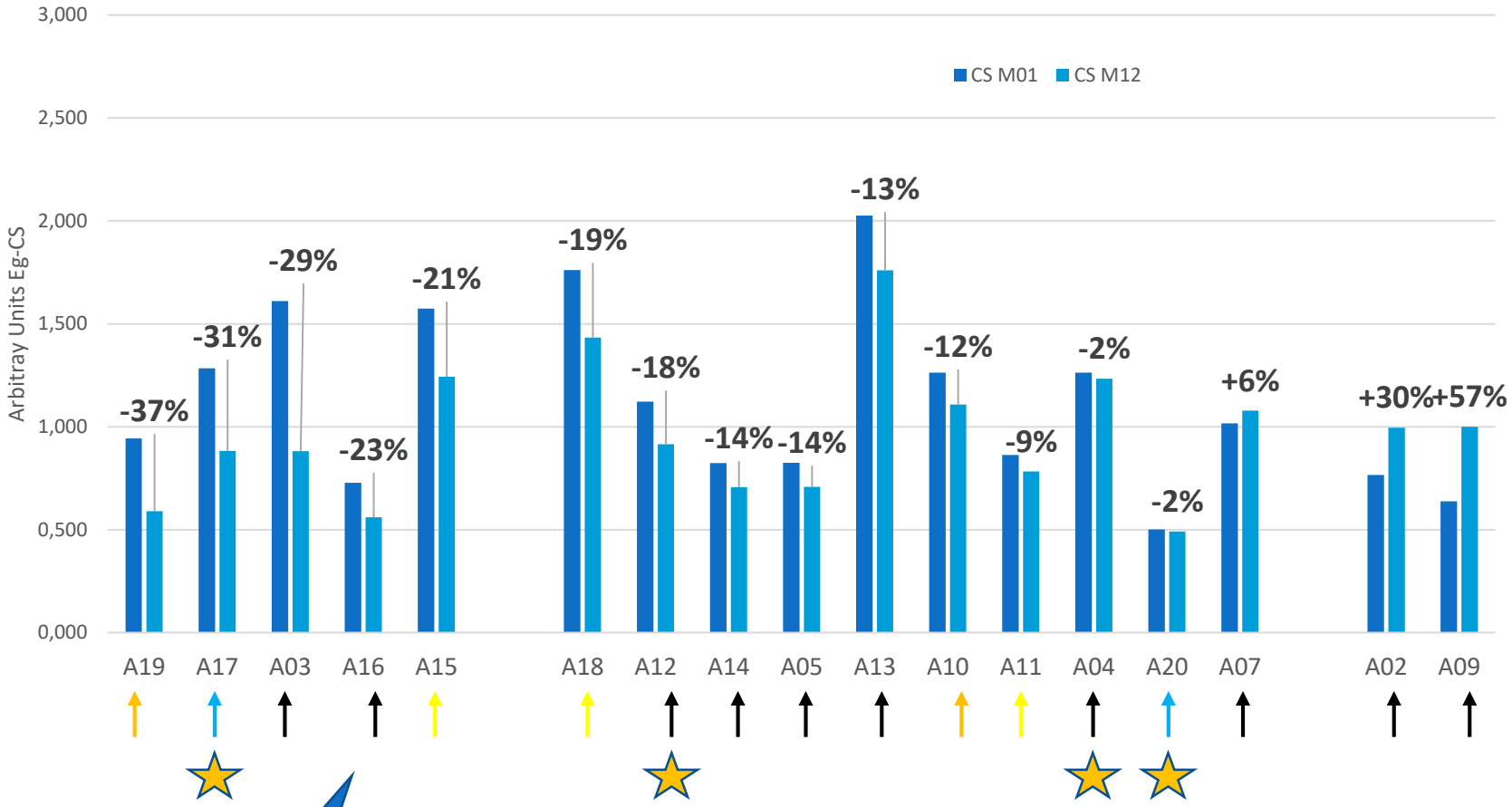
History of CE

Results

ELISA results – Citrate synthase



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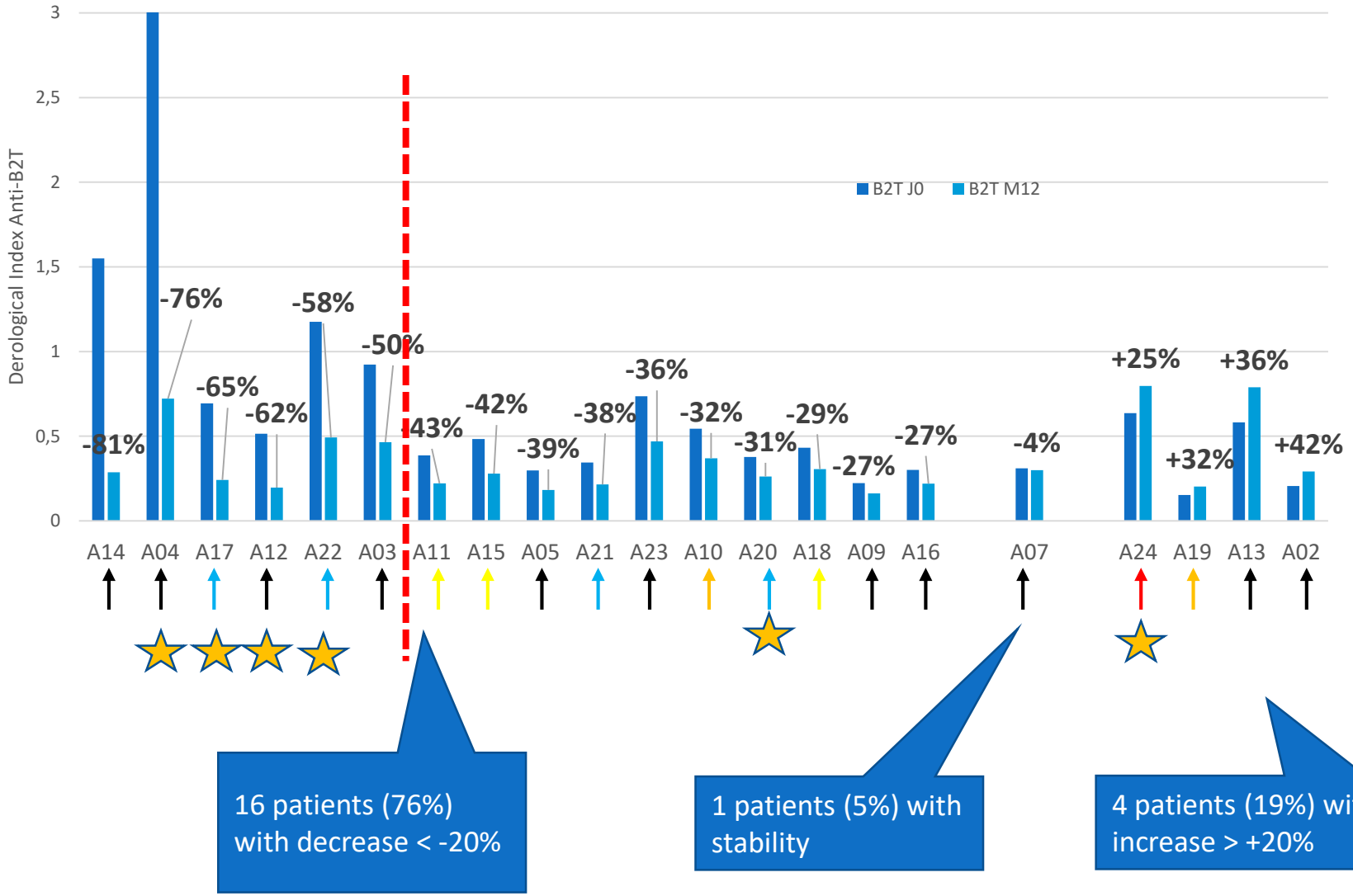
5 patients (29%) with decrease < -20%

10 patients (59%) with stability

2 patients (12%) with increase > +20%

Results

ELISA results – B2T



One pulmonary cyst completely removed

One cyst (liver or other) completely removed

Two cysts (one liver and one lung) completely removed

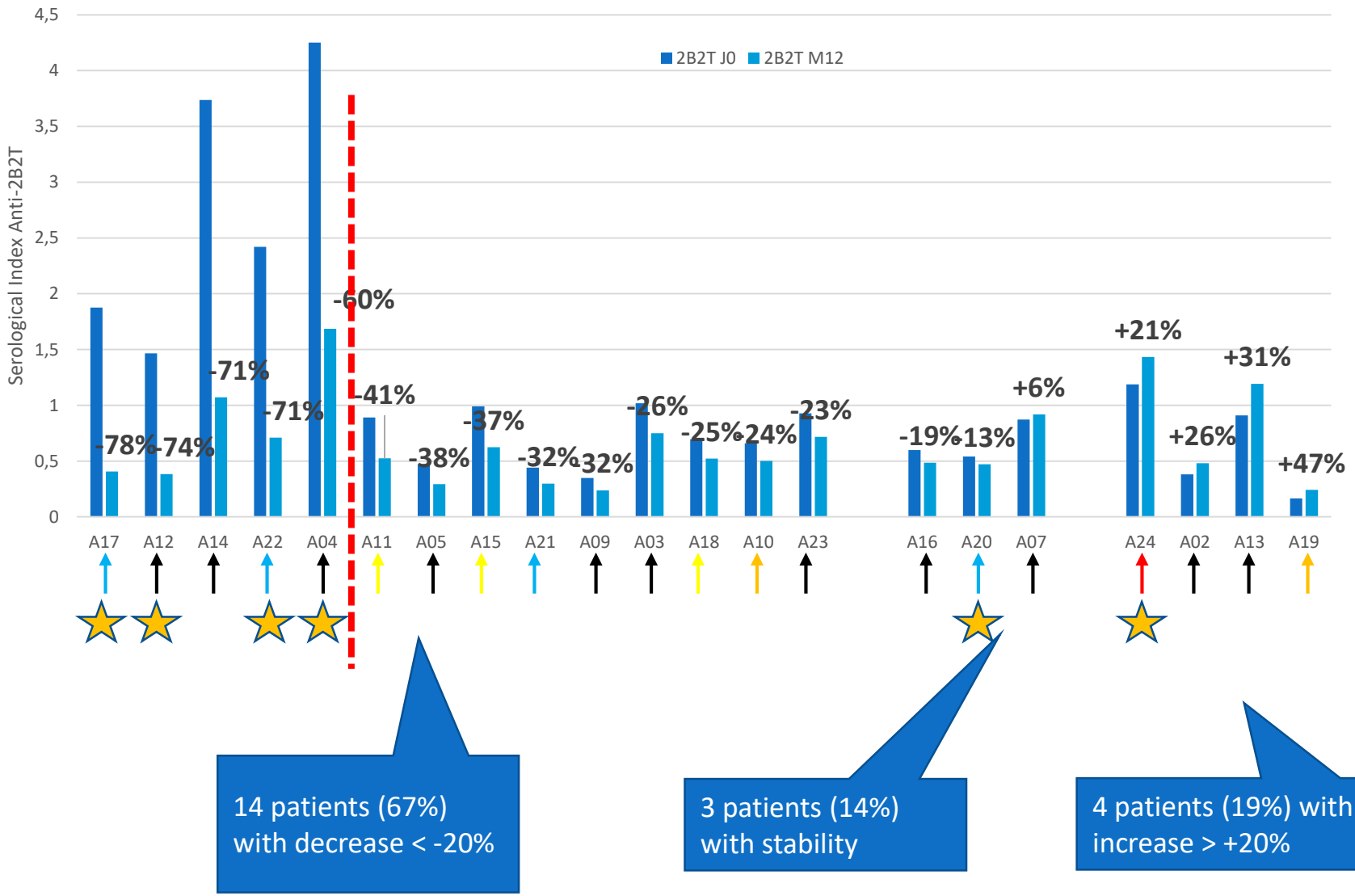
Two cysts (one liver and one lung) **NOT** completely removed

Nine pulmonary cysts **NOT** completely removed

History of CE

Results

ELISA results – 2B2T



14 patients (67%) with decrease < -20%

3 patients (14%) with stability

4 patients (19%) with increase > +20%

One pulmonary cyst completely removed

One cyst (liver or other) completely removed

Two cysts (one liver and one lung) completely removed

Two cysts (one liver and one lung) **NOT** completely removed

Nine pulmonary cysts **NOT** completely removed

History of CE

Results

Correspondance B2T / 2B2T and Bordier kit

Patients	ELISA Bordier	anti-MDH	anti-CS	anti-B2T	anti-2B2T	Concordance
A17	-29%	-44%	-31%	**-65%**	**-78%**	*****
A15	-29%	-25%	-21%	-42%	-37%	*****
A18	-23%	-35%	-19%	-29%	-25%	*****
A16	**-52%**	-48%	-23%	-27%	-19%	*****
A12	**-51%**	-15%	-18%	**-62%**	**-74%**	****
A14	-29%	+14%	-14%	**-81%**	**-71%**	****
A05	**-85%**	-56%	-14%	-39%	-38%	****
A21	-34%	-33%	NT	-38%	-32%	****
A03	NT	-19%	-29%	**-50%**	-26%	****
A10	-38%	-43%	-12%	-32%	-24%	****
A04	-12%	-25%	-2%	**-76%**	**-60%**	***
A11	-29%	+2%	-9%	-43%	-41%	***
A23	-24%	+6%	NT	-36%	-23%	***
A07	+13%	-7%	+6%	-4%	+6%	Stability
A24	-1%	+46%	NT	+25%	+21%	Increase
A22	NT	+37%	NT	**-58%**	**-71%**	
A09	**-79%**	+6%	+57%	-27%	-32%	
A20	**-68%**	-4%	-2%	-31%	-13%	
A02	-25%	-1%	+30%	+42%	+26%	
A13	**-52%**	-11%	-13%	+36%	+31%	
A19	-22%	+30%	-37%	+32%	+47%	

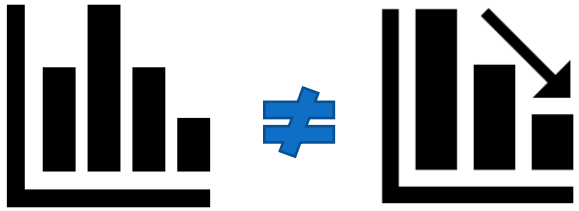
Good correlation / important decrease

Good correlation

✓ 12/21

Discussion & Conclusions

No peak in immunoreactivity at M1 for Bordier & B2T/2B2T



Majority of patients with :
-a **decrease** of Ab levels for Bordier & B2T/2B2T
-a **stability** of Ab levels for Malate dehydrogenase and Citrate synthase.

12/21 (57%) = good match btw the 5 tests.
Particularly Bordier and tests with B2T and 2B2T

Strategy to identified MDH & CS ≠ Bordier & B2T/2B2T

MDH & CS = initially developed for paediatric population (no chemical treatment)

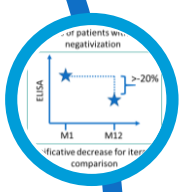
≠

- Immunoreactivity in adult population
- all treated with albendazole

No relapsed patients among the 23, no evaluation of probability of being « relapse free » vs « relapsed ».

Perspectives

Follow-up at 3 years post-surgery; in process…



Iterative comparison best strategy than status positive/negative.
Samples at DO and M1 post-surgery necessary

ELISA kit commercialized by Bordier seems to be informative.

Test other samples retrospectively collected from
plasma collection CNR-Echinococcoses (Besançon, France)

Thank you

Thanks to
Maria Mar SILES LUCA
For providing us
B2T and 2B2T antigens

Thanks to
Bordier Affinity products
For providing us
E.g ELISA kit

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