Retrospective study of Borrelia Elispot INF-g before and after antibiotic treatment in tick-borne diseases patients.

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The Borrelia Elispot INF-g, an IGRA biological test, explores the cellular immune response to a Borrelia infection. It detects, in the blood, the presence of T effectors lymphocytes specific to Borrelia that are able to secrete INF-g, in vitro, during incubation with Borrelia antigens. A positive result of Borrelia Elispot INF-g means a recently or currently Borrelia active phase. A negative result of Borrelia Elispot INF-g means, a priori, an absence of a recently or currently Borrelia active phase[1,2]. The borrelia serology explores the humoral immune response and is a contact indicator with the borrelia bacteria, but don't mean a recently or currently Borrelia active infection. Serology isn't an efficient tool to monitoring the antibiotic treatment[3]. The antibiotic monitoring is primarily based on the symptoms but is sometimes not so easy.

The aim of this study was to evaluate the contribution of the Borrelia Elispot INF-g in the evaluation of clinical improvement to monitoring the antibiotic treatment efficiency, particularly in the late systemic stages of borreliosis.

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KEYWORDS: IGRA (INFg Release Assay)**, Elispot Borrelia, SPPT** (Persistent polymorphic symptoms after tick bite)

Method and technics:

Abbreviations: IGRA: INFg Release Assay, PBMC: Peripheral Blood Mononuclear Cells, HCSP: Haut conseil santé publique, SPPT: Persistent polymorphic symptoms after tick bites, PWM: Pokeweed Mitogen, EM: erythema migrans.

Méthod: Ten adult patients were included retrospectively, by their positive result of Borrelia Elispot INF-g, without any antibiotic treatment. They were 5 men and 5 women 41 years old (from 24 to 61 years old). They completed a symptoms survey: tick bite noted (figure 1), erythema migrans observed, results of Borrelia serology (Elisa and Immunoblot), date of symptoms, details of symptoms (diagnostic grid of SPPT (Polymorph Persistent Symptoms after Tick bite) - HCSP report, 2014[4]), antibiotic treatment received (figure 2), clinical course after treatment. Three months after the end of antibiotic treatment, a second Borrelia Elispot INF-g was analyzed. The clinical course was classified into 3 types: no clinical improvement, moderate clinical improvement and significant clinical improvement.

Technics :
In our laboratory, the screening serology (immuno-enzymatic technique) used to the Liaison IgG and IgM kit from Diasorin. The confirmation serology (immunoblot technique) used to the Lymecheck IgG and IgM from Mikrogen. The Borrelia Elispot INFg used to the LymeSpot Borrelia from AID Diagnostica (figure 3).

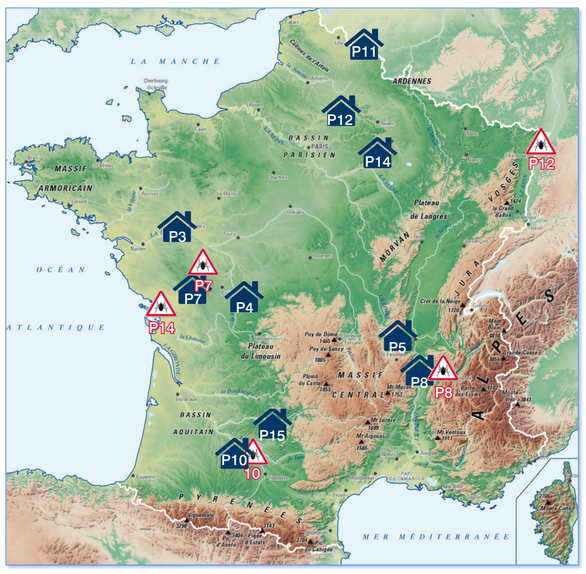

Figure 1 :
Geographical origin of the patients and place of noted tick bite.

					cures of ATB	
P3	♀	26	×	>6	Ceftriaxone/ Flagyl/ Tetralysal/ Plaquenil/Tinidazol	<3
P4	♂	31	×	>6	Azythromycine/ tetralysal/ ciprofloxacine/doxycycline/rifadine/bactrim	3
P5	♂	36	×	>6	fluvermal/doxycycline/clarythromycine/ fluconazole	4
P7	♂	61	·	>6	antibiotiques/antifongiques/antiparasitaires/HE	9
P8	♂	46	·	>6	josamycine/cefuroxime/doxycycline puis ceftriaxone/doxycycline/fungizone	27
P10	♂	61	◎	>6	doxycycline/flagyl/azythromycine	7
P11	♀	31	×	<6	oxycycline/azythromycine/tinidazol/fluconazol/	14
P12	♀	34	◎	>6	azythromycine/doxycycline/fluconazol/ tinidazol	11
P14	♀	34	·	>6	lagyl/azythromycine/fluconazol puis ceftriaxone/doxycycline puis roxythromycine/ plaquenil/cefpodoxime puis fluvermal	12
P15	♂	50	×	>6	tetracycline/plaquenil	3

P12 identification of the patient · ♀ man · ♂ woman · 34 Age of the patient in years · >6 datation of Symptoms in months · · bite without EM · × absence of bite · ◎ bite with EM · 6 duration of treatment in months

Figure 2 :
duration of treatment (months); no noted tick bite; tick bite without erythema migrans; tick bite with erythema migrans; age.

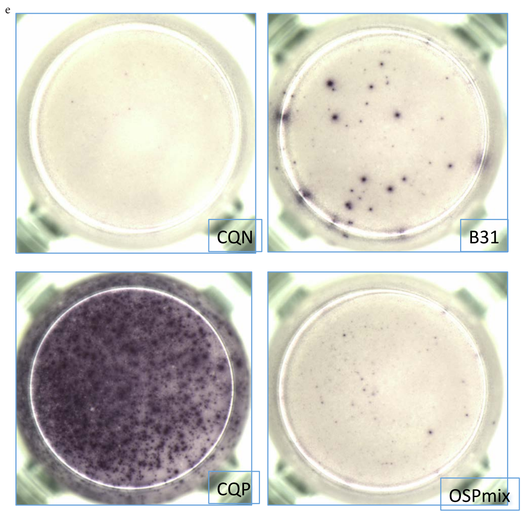

Figure 3: Elispot Borellia reaction

Elispot Borellia reaction

The lymphocytes are drop in an anti-INFg coated plate, in duplicate. Well negative control: lymphocytes only, well positive control: lymphocytes and PWM, wells with a specific Borrelia antigen: B31 (B31 lysat, B. burgdorferi sensu stricto), OSPmix (mix of recombinant Borrelia proteins from B. garinii, B. afzelii, B. spielmanii).

The INFg released is revealed with the immunoenzymatic reaction, seen with a spot.

One spot is one activated lymphocyte.

Results are formulated with a stimulated index (SI).

For B31, result is limit when SI is between 2 and 4, and become positive when SI > 4. For OSPmix, result is positive when SI > 2.

BIOLOGICAL RESULTS

4 patients had a positive screening Borrelia serology and only two had a positive confirmed Borrelia Immunoblot. One patient (P15) communicated a positive Immunoblot in his medical history. Before antibiotic treatment, all patients had a positive Borrelia Elispot. After treatment, Borrelia Elispot became negative for seven patients (figures 4, 5a et 5b). Among the three remaining positive Elispot after treatment (P10, P12, P14), two patients had a moderate clinical improvement (P10, P12) and one patient (P14) had a significant clinical improvement (figure 5c).

patient	screening Elisa serology		confirmation Immunoblot serology		first ElISpot			second ElISpot			improvement
	IgM	IgG	IgA	IgG	date	résultat B1	résultat Ospmix	date	résultat B1	résultat Ospmix	
P3	Neg	Neg	Neg	Neg	08/04/15	11	10,33	31/08/15	1	1	*
P4	Neg	Neg	Neg	Neg	07/07/15	2,86	5,29	06/07/16	1	1	*
P5	Neg	Neg	Neg	Neg	04/11/15	4,24	3,59	05/07/16	1	1	***
P7	/	Pos*	/	Pos*	27/05/15	7,9	4,5	13/04/16	1	1	*
P8	Neg	Pos 69	Neg	Neg 5, VlsE pos	21/04/15	3,75	3,75	19/04/16	1	1	***
P10	/	/	Neg	Neg	27/04/16	15	6,5	28/03/17	19	9,5	*
P11	Pos 2,45	Neg	Neg	Neg	19/04/16	24	7	07/02/17	1	1	***
P12	Neg	Neg	/	/	24/05/16	23	7	01/02/17	13	10	*
P14	Pos 1,55	Neg	Pos* p41 OspC	Neg fev 2016 Pos* 2014	10/08/16	82	5,5	10/01/17	43	2	*
P15	/	/	Neg	Neg fev16 Pos 2014	09/08/16	21,5	19,5		1	1	***

Figure 4: biological results of serology and Elispot.
Moderate clinical improvement = * Significant clinical improvement = ***
* Positive declarative results, not available.

figures 5: Borrelia Elispot INFg before/after antibiotic treatment

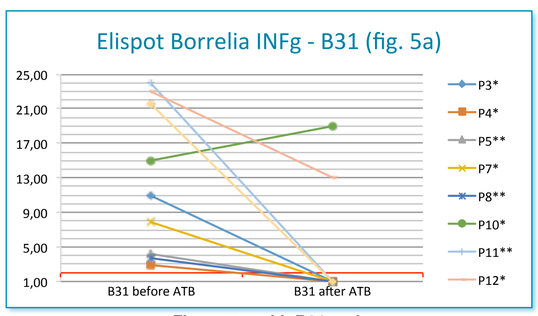

Figure 5a: with B31 antigen

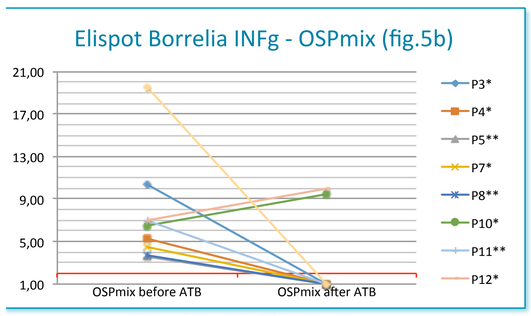

Figure 5b: with OSPmix antigen

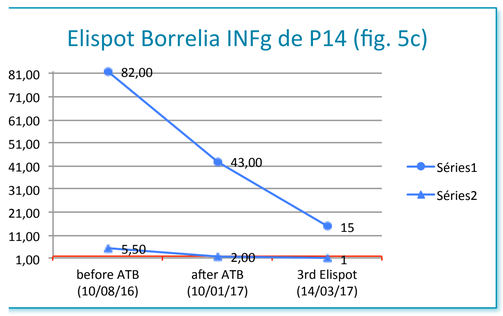

Figure 5c: Particular case of patient P14. He had a significant clinical improvement but his Elispot was still positive. Three months after the end of antibiotics a third Elispot was analyzed: it became partially negative with a constant clinical improvement.

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CLINICAL RESULTS:

The collected data with the symptoms survey gave the symptoms prevalence before treatment (figure 6). The expressed clinical improvements were classified into 3 types detailed in figure 7. After antibiotic treatment, 5 patients had a significant clinical improvement (P5, P8, P11, P14, P15) and 5 patients had a moderate clinical improvement (P3, P4, P7, P10, P12).

Diagnostic grid of SPPT (HCSP 2014, CHRONIMED)		prevalence
fatigue not reactive to a painful psychological event		
Fatigue > 6 months: physical (massive fatigue, need of nap, athletical fatigue)		90,00%
tigue > 6 months: psychological (anxiety, depression, dissatisfaction)		90,00%
Fatigue > 6 months: intellectual (brain fog, memory disorder, drop of school results)		90,00%
Fatigue > 6 months : sleep disorders		90,00%
Muscular criteria		
1	Muscles: night cramps or at rest	70,00%
2	Muscles: myoclonies, sursauts d'endormissement (impression de tomber)	60,00%
3	Muscles: restless legs, diurnal, in seated position	40,00%
4	Muscles: distressful sensation need to inhale or exhaledeeply, effort dyspnea)	70,00%
5	Muscles: eyelid fasciculation	60,00%
Vascular criteria		
6	Vessels: spontaneous bruise or after a light impact	10,00%
7	Vessels: escessive night sweat, rash/flush (face or bust)	80,00%
8	Vessels: atypical intermittent visual disturbances (visual blur, lateral transiet shadows, pseudo-hallucinations, tasks, excessive photophobia)	90,00%
9	Vessels: palpitations	70,00%
10	Vessels: positional faintness	40,00%
11	Vessels: sensitive to the cold, S Raynaud	100,00%
12	Vessels: dysesthesia / let go of items/ indistinct gesture	20,00%
13	Vessels: unilateral tinnitus	70,00%
14	Vessels: afternoon heavy legs	20,00%
Irritation criteria (each days)		
15	Irritation: pruritus, flush, rash	70,00%
16	Irritation: arthralgia / myalgia / tendinitis / migrant/ headaches / cramp / lombalgia / dorsalgia / neck cracking	70,00%
17	Irritation: ophtalmic irritation dry eyes)	30,00%
18	Irritation: pharyngeal irritation, perennial rhinitis without allergy, snoring, sugar desire	70,00%
19	Irritation: gastralgia, transit disorders, intestinal disorders, nausea, foaming urine	90,00%

Figure 6: symptoms prevalence according to the answers of the SPPT clinical survey (date of symptoms > 6 months).

Patient	score before ATB	score after ATB	clinical evolution
P3	19	14	*
P4	17	10	*
P5	8	2	***
P7	10	6,5	*
P8	13	3,5	***
P10	13	7,5	*
P11	7	2	***
P12	14	10,5	*
P14	15	6	***
P15	15	0	***

Figure 7: expressed clinical improvement
0 = no clinical improvement (< 20% of improved symptoms),
* = moderate clinical improvement (20- 50%)
*** = significant clinical improvement (50-80%).

CONCLUSION

In our retrospective study of ten patients, the Borrelia Elispot INF-g correlates with the clinical course after antibiotic treatment: it becomes negative in case of significant clinical improvement and remains positive in case of moderate clinical improvement. Considering these results, the Borrelia Elispot INF-g could be used as a tool for the physician to help him in the evaluation of the clinical course in tick borne diseases.
It will be necessary to study a larger number of patients to confirm these observations.

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