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Evaluation of the C6 enzyme-linked immunoadsorbent Assay for the serodiagnosis of Lyme borreliosis in north-eastern Italy

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SUMMARY

A novel antigen preparation - the synthetic C6 peptide, a conserved portion of the variable VlsE antigens of *Borrelia burgdorferi* - has been evaluated for serodiagnosis of Lyme borreliosis (LB) by an ELISA procedure. Serum specimens were from early and late LB patients, all resident in an endemic area in north-eastern Italy. The specificity of the test approached the 100% and sensitivity was in the order of 63% (early LB) and 100% (late LB); this performance is superior to the preceding generation of Lyme disease tests.

KEY WORDS: *Borrelia burgdorferi*, serodiagnosis, VlsE

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INTRODUCTION

Lyme borreliosis (LB) continues to be endemic in the Friuli Venezia Giulia Region (FVG), north-eastern Italy, with an incidence of notified cases up to 13.5% (x 100.000 inhabitants). Though *Borrelia afzelii* is the most prevalent genospecies (Ciceroni *et al.,* 2000), *Borrelia garinii* and *Borrelia burgdorferi* sensu stricto (Cinco, 2001) were also isolated from human patients. Although it is recommended that Lyme disease diagnosis be based on the patient's clinical history, serology is the most frequently used approach to confirm clinical diagnosis. ELISA (Enzyme-linked Immunoadsorbent Assay) and Immunoblot are currently the most commonly used serological techniques both in the USA and in Europe, and a two-tiered approach composed of an initial ELISA of a relatively high sensitivity but low specificity, followed by an Immunoblot that incorporates the EUCALB (European Concerted Action on Lyme borreliosis) band criteria has been recommended) (Hausr *et al.,* 1999). This approach enhances the specificity of serology up to 98%.

However the need to include the Immunoblot increases the cost of serodiagnosis and makes inter-laboratory discrepancies more frequent (information found at the EUCALB website http://vie.dis.strath.ac.uk/vie/LymeEU/). Antigens, which have been used up to now, though species-specific tend to cross-react with non-Lyme disease conditions, thus complicating the interpretation of the data in Europe, where the heterogeneity of the *Borrelia burgdorferi* (*B.b.*) infection is significant.

A recently developed ELISA based on the use of a synthetic peptide, the C6 peptide, derived from a conserved region of VlsE protein, has been shown to be sensitive and highly specific to detect *B. burgdorferi* antibodies both in US and European LB patients (Liang *et al.,* 1999; Liang

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et al., 2000). The C6 peptide ELISA reached 74, 85 and 100% sensitivity with acute, convalescent and late-phase specimens respectively, and an excellent level (99%) of specificity (Liang *et al.,* 1999; Liang *et al.,* 2000); these characteristics of the test together with its capacity to detect anti-*B.burgdorferi* antibodies elicited by the three pathogenic genospecies *B.burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*, would indicate that the C6 peptide has the potential for a universal assay for Lyme borreliosis in Europe.

In the present study we evaluated the ability of the C6 ELISA to detect *B. burgdorferi* antibodies in a human population resident in the FVG region and with different LB stages. Serum specimens were collected from 76 LB patients: 54 patients with ECM confirmed by culture, 15 with arthritis, 6 with neuroborreliosis, both categories in the late stage of infection. As negative control serum specimens were also taken from blood donors (BD), 26 living in a LB non-endemic area (Sicily) and 33 living in an endemic region (FVG). None of these had received a LB vaccine. Serum samples were tested with the Enzignost ELISA (Behring) and with the C6 peptide ELISA Kit (Immunetics, Inc. Cambridge, Mass.), performed according to the manufacturer's instructions. BD serum specimens positive for C6 were re-tested with an Immunoblot assay for *B. burgdorferi* IgM and IgG according to Hauser et al. 1999. The results are reported in Table 1. All of the serum specimens from the 22 late LB patients were positive with both the Enzygnost and the C6 Immunetics ELISAS tests (100% sensitivity), whereas 62.96% of the early LB specimens reacted with C6 ELISA and only 50% with the Enzygnost ELISA.

The 100% sensitivity obtained with late LB sera agrees with the data reported by others (Levin, A., T. Talaska, and V. Kovalenko Intern. Conf. on Lyme-Borreliosis, abstr° 114, 2002) on the same type of patient group (arthritis and neuroborreliosis); concerning the early LB specimens clearly the level of 62.96% sensitivity is superior to that of the Enzygnost test (50%), but inferior to the 74% and 87% sensitivity level reported for US and European ECM patients (Liang *et al.,* 1999; Liang *et al.,* 2000). Concerning the specificity of the C6 ELISA, all the BD serum samples taken from the non endemic locality were negative; one of the BD serum samples taken from a subject of the endemic FVG region was strongly positive and three of such samples were weakly positive (borderline). Only the strongly C6-positive serum gave significant bands when tested with Immunoblot, confirming the presence of anti-*B. burgdorferi* specific antibodies, presumably due to asymptomatic *B. burgdorferi* infection. Borderline optical-density readings obtained from healthy people by the C6 ELISA should interpreted with caution and checked with Immunoblot as we did in this study. The seropositivity detected on BD sera from the endemic area was expected: in fact a level of 13.3-14.5% seropositivity was reported in a BD group of the same area (Cinco *et al.,* 1993) in past investigations, employing a conventional ELISA.

Though based on a small number of serum samples, our data confirm that the C6 ELISA is very

TABLE 1 - *Antibody responses measured by the C6 ELISA and Enzygnost ELISA.*

Study group	*N° samples positive*	*N° samples tested*
	C6 peptide ELISA	*Enzygnost*
Early LB (ECM)	34/54 (62.96%)	27/54 (50%)
Late LB		
arthritis	16/16 (100%)	16/16 (100%)
neuroborreliosis	6/6 (100%)	6/6 (100%)
Blood donors		
Non endemic	0/24 (0%)	ND**
Endemic	4*/33	ND

*One serum was clearly positive. The other three were equivocal. **Not in the aim of this study.

specific for *B.burgdorferi* and has a sensitivity superior in early LB sera to that of the Enzygnost ELISA commonly used in FVG for LB serology. Data previously published (Liang *et al.,* 2000) reported that the C6 ELISA assayed on two panels of European (Austrian and Italian) ECM sera indicate that this test antigen performed better than the Immunoblot alone or ELISA + Immunoblot, in terms of sensitivity. Taking into account all of these data and the high cost of the recommended two-tiered procedure, we believe that the introduction of C6 ELISA as a routine single test will be very helpful for LB serology.

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