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Original Article

Diagnostic use of the lymphocyte transformation test-memory lymphocyte immunostimulation assay in confirming active Lyme borreliosis in clinically and serologically ambiguous cases

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Abstract: The aim of this study was to carry out an independent evaluation of the proposition that the lymphocyte transformation test-memory lymphocyte immunostimulation assay (LTT-MELISA) may be diagnostically useful in the confirmation of active Lyme borreliosis in clinically and serologically ambiguous cases. Blood samples from 54 patients consecutively presenting to a British center with clinical suspicion of Lyme borreliosis were tested for this disease by immunoglobulin M (IgM) and immunoglobulin G (IgG) Western blots and by LTT-MELISA. Forty-five of these patients had Western blot results which were negative for both IgM and IgG by the criteria of the Centers for Disease Control and Prevention (CDC); of these patients, 19 (42%) were LTT-MELISA-positive. Two of the patients who had IgM positive results by the CDC criteria were LTT-MELISA-negative. It is concluded that, for putative European-acquired Lyme borreliosis infections, it would be sensible to carry out both the LTT-MELISA and Western blot assay.

Keywords: Lyme borreliosis, lymphocyte transformation test, MELISA

Introduction

The multi-systemic disorder Lyme borreliosis (Lyme disease) is an arthropod-borne zoonosis which can give rise to dermatological, joint, cardiac, and neurological involvement in affected humans [1]. While the causative spirochetal species in the United States tends to be *Borrelia burgdorferi* sensu lato [2], in Europe the causative species are more varied and most likely to include *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia burgdorferi* sensu lato [3], but may also include *Borrelia valaisiana*, *Borrelia lusitaniae*, and *Borrelia spielmanii* [4, 5]. Therefore, it has been suggested that the two-tiered serodiagnostic algorithm currently recommended by the Centers for Disease Control and Prevention (CDC) [6], whereby an initial enzyme immunoassay or immunofluorescence assay is followed, in positive or equivocal cases, by immunoglobulin G (IgG) plus or minus immunoglobulin M (IgM) Western blots (immunoblots) in which the antigen used is usually derived from *Borrelia burgdorferi*, is inappropriate for suspected cases of Lyme borreliosis acquired in Europe [7-9]. (According to the CDC recommendations, the IgM Western blot should not be carried out if the patient has been ill for longer than 30 days.) Other factors which may be associated with serodiagnostic ambiguity include cross-reactivity with antigenically-related microorganisms such as *Treponema pallidum* and Epstein-Barr virus, delayed or failed antibody production, and IgM persistence.

Valentine-Thon and colleagues have suggested that the lymphocyte transformation test-memory lymphocyte immunostimulation assay (LTT-MELISA) may be diagnostically useful in the confirmation of active Lyme borreliosis in clinically and serologically ambiguous cases, with most of the LTT-MELISA-reactive patients tested in their study being seropositive [10]. The LTT-MELISA is a modified LTT in which the T-cell

Table 1. Comparison of the results by the CDC criteria (for both IgM and IgG) and the LTT-MELISA

		CDC criteria		Total
		Positive	Negative	
LTT-MELISA	Positive	7	19	26
	Negative	2	26	28
Total		9	45	54

immune response (lymphoblast transformation and proliferation) to recombinant *Borrelia*-specific antigens can be detected (by ^{3}H-thymidine incorporation) and evaluated [10].

The aim of the present study was to carry out the first independent evaluation of the value of the LTT-MELISA in serologically and clinically ambiguous cases of Lyme borreliosis in which Western blot IgG and IgM results were negative.

Materials and methods

Patient samples

Blood samples were taken from 54 patients consecutively presenting to a British center (Breakspear Medical Group) with clinical suspicion of Lyme borreliosis. Following centrifugation, serum samples were sent to IgeneX Inc. (Palo Alto, CA, USA) for IgG and IgM Western blot assays for Lyme borreliosis, while whole blood samples were sent to the MELISA (LTT) Center Laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e. V.) (Bremen, Germany) for LTT-MELISA evaluations for Lyme borreliosis. There was no communication between the two laboratories regarding the results of each patient.

Western blots for Lyme borreliosis

For each sample, an aliquot of 10 µL of serum was tested with Western blot strips prepared from a mixture of two strains of *Borrelia burgdorferi*, 297 and B31, as described by Shah and colleagues [11]. The IgM bands tested included those required to determine immunoblot positivity according to the CDC criteria, namely 23-25 kDa, 39 kDa, and 41 kDa. Similarly, the IgG bands tested included those required by the CDC criteria, namely 18 kDa, 23-25 kDa, 28 kDa, 30 kDa, 39 kDa, 41 kDa, 45 kDa, 58 kDa, 66 kDa and 83-93 kDa.

LTT-MELISA for Lyme borreliosis

The LTT-MELISA was carried out as described by Valentine-Thon and colleagues [10, 12]. One million peripheral blood mononuclear cells in 1 mL 10% medium were incubated, together with controls, in a multi-welled culture plate coated with recombinant *Borrelia* antigens at three dilutions for five days at 37°C with 5% carbon dioxide gas. Following exposure to 3 µC methyl-^{3}H-thymidine (Amersham Buchler, Brunswick, Germany; specific activity, 185 GBq mmol^{-1}) for five hours, the uptake of the tritiated thymidine was measured in counts per minute using a liquid scintillation counter (1450 Microbeta Trilux; Wallac Distribution, Freiburg, Germany). The ratio of the radioactive count from a given test well to the average count from three negative control wells was defined as the stimulation index. A stimulation index of at least three, in at least one well, was defined as being a positive result for a given patient.

Results

The main results are shown in **Table 1**. Forty-five (83%) of the patients had Western blot results which were negative by the CDC criteria for both IgM (which require at least two IgM bands among 24 kDa or 21 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla)) and IgG (requiring at least five IgG bands among 18 kDa, 24 kDa or 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa). Of these 45 seronegative patients, 19 (42%) were LTT-MELISA-positive.

Two of the patients who had IgM positive results by the CDC criteria were LTT-MELISA-negative.

Discussion

This first independent study of LTT-MELISA in patients clinically suspected of suffering from Lyme borreliosis confirms the diagnostic value of this investigation. There are at least two possible reasons why we found a relatively high (42%) level of LTT-MELISA-positive results among our seronegative patients.

Bearing in mind that our study was from a British center, one possibility is the greater *Borrelia* species heterogeneity in Europe compared with the United States, so that the CDC criteria may be inappropriate for European-acquired infections. In line with this possibility,

it is noteworthy that a recent study of 64 Lyme borreliosis patients from Slovenia also demonstrated the inadequacy of these CDC criteria [7, 13].

A second possibility relates to one of the ways in which the LTT-MELISA differs from serological tests such as Western blotting in that, in the former, the degree of lymphocytic reaction to the bacterial antigen is assessed. Thus, LTT-MELISA can measure disease activity in those infected patients who have not mounted an adequate antibody response.

Finally, it is noteworthy that two of the patients who were IgM positive failed to give LTT-MELISA-positive results. This would argue against replacing the Western blot assay with the LTT-MELISA in those who have possibly been infected in Europe. On the contrary, the results of the present study suggest that, in such a patient, it would be sensible to carry out both the LTT-MELISA and Western blot assay.

Disclosure of conflict of interest

None.

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