The Clinical Spectrum of Early Lyme Borreliosis in Patients with Culture-confirmed Erythema Migrans

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BACKGROUND: The diagnosis of erythema migrans (EM), the characteristic rash of early Lyme borreliosis, is based primarily on its clinical appearance since it often occurs prior to the development of a specific antibody response. Other skin disorders, however, may be confused with EM.

METHODS: Between June 1991 and September 1993, a prospective study was conducted at the Lyme Disease Diagnostic Center of the Westchester County Medical Center to isolate *Borrelia burgdorferi* systematically from patients with EM, and to characterize the clinical manifestations of patients with culture-documented infection. Skin biopsies and/or needle aspirates of the advancing margin of primary lesions, and blood specimens from adult patients were cultured for *B burgdorferi* in modified Barbour-Stoenner-Kelly medium at 33°C.

RESULTS: *B burgdorferi* was recovered from 79 patients (49 [62%] males) ranging in age from 16 to 76 years old (mean, 43 ± 14 years old). Maximum EM diameter (mean, 16 ± 10 cm; range, 6–73 cm) was a function of EM duration (mean 6.7 ± 6.4 days; range, 1–39 days)(correlation coefficient = 0.7; $P < 0.001$). Twenty (25%) patients had noted a tick bite at the site of the primary lesion a mean of 10 days (range, 1–27 days) before onset. Multiple EM lesions (range, 2–70) were present in 14 (18%) patients. Systemic symptoms were present at the time of culture in 54 patients (68%) including fatigue (54%), arthralgia (44%), myalgia (44%), headache, (42%), fever and/or chills (39%), stiff neck (35%), and anorexia (26%). Thirty-three patients (42%) had at least one objective finding on physical examination in addition to EM, including 18 (23%) with localized lymphadenopathy, 13 (16%) with fever ($T \geq 37.8$°C), seven (9%) with tender neck flexion, six (8%) with joint tenderness, and 1 each with joint swelling, nuchal rigidity, and facial nerve palsy. No patient had new electrocardiogram evidence of atrioventricular block. Liver function assays were abnormally elevated in 37% of patients. Thirty-four percent of patients were seropositive by enzyme-linked immunosorbent assay at presentation. Most others rapidly seroconverted so that 69 of 78 evaluable patients (88%) were seropositive at some point during the first month after diagnosis.

CONCLUSIONS: We describe the largest group of culture-positive patients with EM from the United States to date. Although systemic symptoms were present in most patients, objective evidence of advanced disease was uncommon. Our patients with culture-confirmed EM were less sick than those described in the days before culture confirmation was possible. The ability to isolate *B burgdorferi* from lesional skin of large numbers of patients with EM should make culture-positive patients the standard by which to define manifestations of early Lyme borreliosis associated with this rash. Microbiologic documentation of Lyme borreliosis will help delineate the manifestations of this illness, and should form the framework for research directed at pathophysiology, diagnosis, treatment, and prevention. *Am J Med.* 1996;100:502–508.

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Erythema migrans (EM) was described in Europe almost a century ago[1] and was first reported in the United States 25 years ago.[2] Since these initial accounts, EM has been recognized to be one of the earliest manifestations of a systemic illness, Lyme borreliosis, caused by the spirochete *Borrelia burg-*

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dorferi.[3] EM is a major feature of this disorder, and was noted in 63% of validated cases of Lyme borreliosis reported by states to the Centers for Disease Control and Prevention (CDC) in 1991–1992 (Dr. David Dennis, CDC, personal communication). However, despite the fact that many large series of patients with EM have been described in Europe and the United States,[4–12] relatively few patients (and no entire series) had microbiologic corroboration. The proportion of patients with "EM" who actually had alternative diagnoses is unknown. We describe the largest series to date of culture proven cases of early Lyme borreliosis with EM in the United States.

METHODS

Objective

A prospective study was conducted to isolate *B burgdorferi* systematically from patients with EM, and to characterize the clinical manifestations of patients with culture-documented infection.

Setting

The Lyme Disease Diagnostic Center (LDDC) at the Westchester County Medical Center is located in central Westchester County, New York, 25 miles north of New York City. Westchester County is highly endemic for Lyme disease, with 1,339 cases (incidence rate = 153 per 100,000 people) reported to the New York State Department of Health for 1994 (Dr. Germaine Jacquette, Westchester County Department of Health, personal communication, May 24, 1995).

Patients

Patients attending the LDDC, who were at least 16 years old, were recruited for the study if they had EM as defined by the Centers for Disease Control and Prevention (CDC) surveillance criteria.[13] During 1991 it was noted that patients who had received antibiotics active against *B burgdorferi* in the days just prior to presentation uniformly had negative cultures.[14] Thus, for the years 1992–3, such patients were excluded. Informed consent was obtained from all patients. The study was approved by the Committee for the Protection of Human Subjects at New York Medical College.

Clinical Specimens and Cultures

Specimens for culture were obtained from either a skin biopsy or a needle aspirate approximately 1 cm inside the advancing margin of the primary EM.[14,15] In cases of multiple lesions, a primary EM site was defined as the lesion located at the site of a prior tick bite (if known), or the lesion that appeared first, was largest, or contained a central eschar consistent with a prior tick bite site. Blood was also obtained for culture using either anticoagulated blood, serum, or fresh whole blood.[16] Cultures were processed as described previously.[14–16] Spirochetes were identified by fluorescence microscopy[14–17] and

TABLE 1
Selected Characteristics of 79 Patients with Culture-confirmed EM*

Patients	
Sex	62% male
Age	43 ± 14 yo (mean) 38 yo (median) 16–76 yo (range)
Erythema Migrans	
Tick bite recalled at primary EM site	25%
Interval from tick bite to EM	10 ± 6.7 days (mean 10 days (median) 1–27 days (range)
Duration of EM at presentation	6.7 ± 6.4 days (mean) 4 days (median) 1–39 days (range)
Larger EM diameter at presentation	16 ± 10 cm (mean) 13 cm (median) 6–73 cm (range)
No. of patients with multiple† EM lesions	14 (18%)
No. of patients with central clearing‡	22 (37%)
No. of patients with central vesiculation‡	4 (7%)
No. of patients with localized pruritus‡	23 (39%)
No. of patients with localized tenderness‡	19 (32%)

* n = 79 unless otherwise noted.
† Range = 2–70 lesions.
‡ EM characteristics were described for 59 of 79 (75%) patients and are not mutually exclusive.

confirmed by polymerase chain reaction (PCR) using primers that specifically direct amplification of a DNA fragment corresponding to nucleotides 689 to 938 of *B burgdorferi* 23S RNA, and in some cases, confirmed using a second set of primers that are targeted at a different region of *B burgdorferi* 23S RNA.[14,15,18]

Serology

Serum samples were tested for antibodies to *B burgdorferi* by a polyvalent (ie, IgM and IgG antibody) enzyme-linked immunosorbent assay (ELISA) using Whittaker Stat® ELISA kits (Whittaker Bioproducts, Inc.; Walkersville, Maryland) according to the manufacturer's instructions. An ELISA was considered positive when the Lyme Index Value (LIV) was >1.09.[19] Acute and convalescent titers were run in parallel on the same plate.

Statistics

Statistical comparisons were performed using either the Fisher's exact test or the Student's *t*-test, both two-tailed. A linear regression was performed to test whether EM duration could be used to predict diameter. A *t*-test was performed to test the relationship between the two variables.

RESULTS

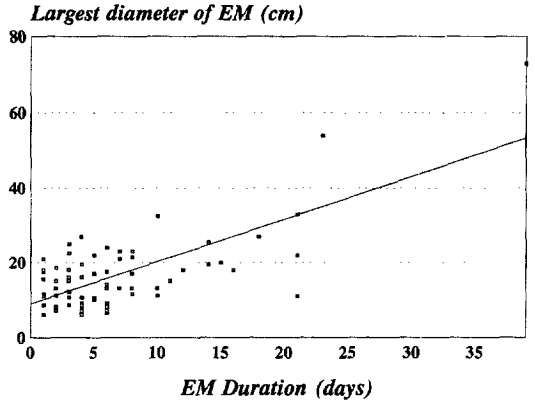
Patient Population

Between June 1991 and September 1993, *B burgdorferi* was isolated from 79 patients with a clinical diagnosis of EM. The source of isolation included 74 skin biopsies, two skin aspirates, one vesicular swab, and eight blood cultures. Seventeen patients (22%) had multiple positive cultures, including 11 with positive skin biopsy and aspirate, 5 with positive skin biopsy and blood culture, and 1 with positive skin biopsy and vesicular fluid. Although 72 of 79 patients had blood cultures obtained, only 8 (11%) were positive for *B burgdorferi*. Patients included 49 (62%) males and ranged in age from 16 to 76 years old (mean, 43 ±14 years old; median, 38 years old) (**Table I**).

EM Characteristics

EM characteristics are summarized in Table I. The largest EM diameter was determined to be a function of lesion duration, varying in linear fashion (correlation coefficient = 0.7; $P < 0.001$ [**Figure**]).

One or more EM characteristics were noted for 59 patients (rash attributes are not mutually exclusive). Lesions were pruritic in 23 of 59 (39%) patients and tender in 19 (32%). However, pruritis and tenderness were almost always mild. Only 22 (37%) patients had rashes with central clearing. Sixteen patients (27%) had rashes that were of uniform color.



Correlation coefficient=0.7 p<.001

Figure. Size of EM (largest diameter in cm [y-axis]) as a function of EM duration (days [x-axis]).

TABLE II
Locations of Primary Erythema Migrans Lesion for 79 Culture-positive Patients

Location	No. (%)	Location	No. (%)
Thigh	14 (18)	Popliteal	5 (6)
Back	12 (15)	Flank	5 (6)
Shoulder	11 (14)	Axilla	4 (5)
Calf	8 (10)	Buttock	4 (5)
Groin	6 (8)	Upper Arm	4 (5)
		Other*	6 (8)

* Chest (2 [2.5%]), abdomen (2 [2.5%]), neck (1 [1%]), ankle (1 [1%]).

Twelve patients (20%) had rashes that were fading by the time of the biopsy. Rashes with central vesiculation were present in 4 patients (7%). Multiple EM lesions (range, 2–70) were present in 14 of 79 patients (18%). The most common sites for solitary and primary EM lesions are shown in **Table II**.

Associated Symptoms and Signs

Twenty-five patients (32%) with EM lacked any associated complaints on the day of their initial visit, or only experienced symptoms localized to the rash or mild fatigue. The remaining 54 (68%) patients experienced systemic symptoms, which are summarized in **Table III**. The most common of these were fatigue (54%), arthralgia (44%), myalgia (44%), headache (42%), fever and/or chills (39%), stiff neck (35%), and anorexia (26%). Thirty-three patients (42%) had at least one objective finding on physical examination in addition to EM (Table III). Lymphadenopathy was localized in 18 (23%) and generalized (more than two separate node groups) in 5 (6%). Fever (T ≥37.8°C) was present in 13 patients (16%). Pain on neck flexion was noted in 7 (9%) patients. One additional patient (1%) had mild nuchal rigidity. Six patients had tender joints (2 with temperomandibular joint tenderness [bilateral in

TABLE III
Presenting Clinical Features in 79 Patients with Culture-confirmed Erythema Migrans

Symptom	No. (%)	Symptom	No. (%)
Fatigue	43 (54)	Anorexia	21 (26)
Arthralgia	35 (44)	Dysesthesia	16 (20)
Myalgia	35 (44)	Dizziness	16 (20)
Headache	33 (42)	Nausea/vomiting	11 (14)
Fever/chills	31 (39)	Difficulty concentrating	7 (9)
Stiff neck	28 (35)	Cough	5 (6)
Sign	**No. (%)**	**Sign**	**No. (%)**
Any objective finding	33 (42)	Tender joint	6 (8)*
Localized lymphadenopathy	18 (23)	Joint swelling	1 (1)†
Generalized lymphadenopathy	5 (6)	Conjunctivitis	3 (4)
Fever (≥37.8°C)	13 (16)	7th nerve palsy	1 (1)
Tender neck flexion	7 (9)	Tender abdomen	1 (1)
Nuchal rigidity	1 (1)	Pharyngeal injection	1 (1)

* Includes painful temperomandibular joint (2 [2%] patients), shoulder (2 [2%] patients), knee (1 [1%] patient), and hip (1 [1%] patient).
† Patient had swollen ankle but had sustained prior trauma to this site.

one patient], 2 with tender shoulders [although one may have been a result of prior trauma], 1 with a tender knee, and 1 with tender hips). The only patient with objective joint swelling had experienced prior trauma to the affected ankle. Three patients (4%) had conjunctivitis. One patient had a facial nerve palsy, 1 had abdominal tenderness, and 1 had an injected pharynx.

In order to determine whether duration of rash correlated with severity of illness, we compared the clinical presentations of patients with EM present for less than 4, 7, 10, or 14 days, with those associated with EM of greater than or equal to each of these respective durations. The clinical manifestations did not differ with varying duration of illness. In addition, the mean interval from onset of EM to presentation was similar for those patients with and without systemic symptoms, fever/chills, fatigue, multiple EM lesions, documented fever (≥37.8°C), and regional lymphadenopathy on physical examination.

Laboratory Findings

Laboratory findings are summarized in **Table IV**. Anemia, abnormal white blood cell count, and thrombocytopenia were rare. One-quarter of all patients had a mildly elevated erythrocyte sedimentation rate. Liver function abnormalities were seen in more than one-third of patients. Although only 1 of 3 patients was initially seropositive, 88% were seropositive within 1 month. No patient had electrocardiographic evidence of heart block.

DISCUSSION

We describe the clinical features of a large group of patients from the United States with culture-confirmed early Lyme borreliosis associated with EM. Most patients had systemic symptoms and experienced viral-like illnesses in which respiratory and gastrointestinal complaints were uncommon. Less than half of patients (42%) had objective physical findings in addition to EM. Lymphadenopathy was the most common abnormality, occurring in 29% of patients. Fever, a hallmark of bacterial infections, was observed in only 16% of patients. Signs of meningitis, cranial nerve palsy, and arthritis were rare, and EKG evidence of new atrioventricular heart block was not noted in any of the 75 patients tested. The paucity of objective findings of advanced (ie, cardiac, neurologic, arthritic) disease in our patients with EM was unlikely to be a result of selection bias, because virtually all patients with EM seen at our center were asked for consent to be cultured regardless of the existence of other underlying conditions or stage of illness.

Leukocytosis, anemia, thrombocytopenia, and a markedly elevated ESR were distinctly uncommon in our patients. Although more than one-third of our patients had at least one abnormal measure of serum liver function, right upper quadrant pain, jaundice, and other clinical manifestations of liver dysfunction were not observed. In addition, anorexia, nausea and/or vomiting were not more common in those patients with abnormal liver function. Muscle damage, as determined by creatine phosphokinase, was infrequent (12%), was not correlated with the presence of myalgia, and may or may not have been due to Lyme borreliosis.

Our patients appeared considerably less ill than the large cohort with EM described by Steere et al in 1983.[4] Overall, our patients reported significantly

TABLE IV

Laboratory Findings of Patients with Culture-confirmed Erythema Migrans

	No. Patients with Abnormal Values* (%)	Abnormal Values (Mean, range)
Hematology		
Anemia†	2 (2.6)‡	11.7 ± 0.7 (11.7–12.7) g/dL
Leukocytosis§	4 (5.3)‡	13.1 ± 1.0 (12.2–14.1) 10^3 cells/mm^3
Leukopenia‖	3 (3.9)‡	3.8 ± 0.6 (3.2–4.4) 10^3 cells/mm^3
Thrombocytopenia¶	1 (1.5)**	132,000/mm^3
Erythrocyte sedimentation rate ≥ twice upper limit of normal††	16 (24)‡‡	47.2 ± 20 (22–85) mm/hr
Liver Function		
Aspartate aminotransferase > 37 U/L	14 (18)	86 ± 82 (41–355) U/L
Alanine aminotransferase > 40 U/L	20 (26)	93 ± 51 (41–238) U/L
Alkaline phosphatase > 117 U/L	13 (17)	164 ± 109 (121–525) U/L
γ-glutamyl transpeptidase > 50 U/L	22 (28)	145 ± 184 (51–869) U/L
≥ one elevated liver function assay	29 (37)	NA§§
Muscle Enzyme		
Creatine phosphokinase > 195 U/L	7 (12)‖‖	474 ± 377 (221–1,271)
Serology		
Positive ELISA¶¶ at baseline	27 (34)***	
Positive ELISA in first month	69 (88)†††	
Electrocardiogram		
Atrioventricular Block	0 (0)‡‡‡	

* n = 78 unless otherwise indicated (one patient only had serology performed at baseline).
† Hemoglobin < 11.6 g/dL (females) or <12.8 g/dL (males).
‡ n = 76.
§ WBC > 12×10^3 cells/mm^3.
‖ WBC < 4.5×10^3 cells/mm^3.
¶ Platelets < 150,000/mm^3.
** n = 66.
†† Westergren method: ≥40 mm/hr (females) or ≥20 mm/hr (males).
‡‡ n = 67.
§§ NA = not applicable.
‖‖ n = 58.
¶¶ Enzyme-linked immunosorbent assay (polyvalent).
*** n = 79.
††† n = 78 (one patient did not have a repeat ELISA performed).
‡‡‡ Excludes one patient with pre-existing (ie, unrelated) 1° (PR > 200 msec) atrioventricular block.

less systemic complaints, were less likely to have multiple EM lesions, and had less frequent objective findings on physical examination (**Table V**). However, our patients were significantly more likely to report dysesthesia and dizziness.

There are a number of possible explanations for the different findings in the two studies. One factor may be that our patients' signs and symptoms were limited to those described at the initial physician encounter (ie, not prior or after). In addition, unlike Steere, we did not enroll children. The older age of our patients (median, 38 years old versus 28 years old for Steere's cohort) could have affected symptomatology. For example, in a separate study conducted in Westchester County, children with Lyme disease were more likely than adults to have fever and joint complaints.[20]

Our patients may have been evaluated earlier after onset of EM than patients seen by Steere, although duration of illness was not stated in his report.[4] The smaller maximum median diameter of EM lesions in our patients compared with those of Steere (13 cm versus 15 cm)[4] is consistent with our patients seeking medical attention earlier. In a report describing 237 patients with EM, Berger found, as did we (Figure 1), that the median size of lesions tended to increase with duration.[6] However, despite the possibility that our patients were seen earlier, we were unable to demonstrate a correlation between the severity and duration of illness. It is possible that we had too small a sample to determine true differences among patient subgroups. Alternatively, there may not be a correlation between the duration of EM and severity of illness. Systemic symptoms often develop independently (ie, before, concomitant with, or after the spontaneous resolution of EM).[4,5,20]

Strain variability could have resulted in different clinical presentations. There is evidence, at least from Europe, that certain strains of Borrelia may be associated with specific disease manifestations.[21] For example, 57 of 58 strains obtained from the skin of Dutch patients with EM alone, and all eight strains

TABLE V
Comparison of Selected Clinical Features in Two Groups of Patients with Culture-confirmed Erythema Migrans

Symptom	Present Report (n = 79) No. (%)	Steere's Series* (n = 314) No. (%)	p value†
Any systemic complaint	54 (68)	≥252 (≥80)	.03
Fatigue	43 (54)	251 (80)	.000009
Headache	33 (41)	200 (64)	.0003
Stiff neck	28 (35)	151 (48)	.06
Fever	31 (39)	185 (59)	.002
Dizziness	16 (20)	15 (5)	.00004
Dysesthesia	16 (20)	35 (11)	.04
Physical Finding			
Multiple EM	14 (18)	150 (48)	.0000008
Localized lymphadenopathy	21 (27)	128 (41)	.02
Generalized lymphadenopathy	4 (5)	63 (20)	.0007
Conjunctivitis	3 (4)	35 (11)	.05
Splenomegaly	0 (0)	18 (6)	.03
Hepatomegaly	0 (0)	16 (5)	.05
Right upper quadrant tenderness	0 (0)	24 (8)	.007

* Reference 4.
† Fisher's exact test, two-tailed.

recovered from the skin of patients with acrodermatitis chronica atrophicans (ACA) were *B afzelii*, whereas all seven isolates from patients with neuroborreliosis were *B garinii*. In addition, the median duration of EM was considerably shorter in patients infected with *B garinii* (14 days) than in those from whom *B burgdorferi* sensu stricto was recovered (52 days). However, North American strains of *B burgdorferi* have not been shown to vary as much as those in Europe,[22] and there have been no published data to date to support the existence of strain-specific disease manifestations in the United States.

Only 34% of our patients were seropositive by polyvalent ELISA at presentation. This is consistent with the findings of Shrestha et al in 31 patients with EM, only 26% of whom were initially seropositive by IgM ELISA, with 16% positive by IgG ELISA.[23] Massarotti et al had a slightly higher initial seropositivity rate in a series of 57 patients with early disease (including 55 with EM).[9] Thirty-nine percent were initially seropositive by IgM, 26% by IgG, and 46% by either one of the assays. Berardi et al reported that the use of a capture ELISA could improve the yield of seropositivity in patients with EM to 67%.[24] However, this may have been partly attributable to an increased duration of symptoms (mean of 11.3 days) at the time of presentation. There is compelling evidence that initial seropositivity in patients with EM directly correlates with duration of illness.[19,24]

Most of our patients who initially lacked antibodies to *B burgdorferi* seroconverted within 1 month despite treatment. The vast majority (88%) of our 78 evaluable patients (ie, those with repeat serologic testing) were seropositive by polyvalent ELISA at some point during the first month after diagnosis. This is higher than the seropositivity rate observed by Shrestha et al[23] (69 of 78 [88%] versus 14 of 22 [64%]; $P = 0.01$), and Massarotti et al[9] (69 of 78 [88%] versus 41 of 57 patients [72%]; $P = 0.02$), and is similar to the rate (28 of 30 [93%]; $P = 0.7$) reported by Berardi et al,[24] using a capture ELISA on specimens obtained at presentation and at convalescence, a mean of 27.2 days after onset of illness.

The clinical basis for recognition of EM leaves open the not inconsiderable possibility of diagnostic error. Bacterial cellulitis and vesicular eruptions may be difficult to distinguish from EM, even for an experienced clinician.[25] In addition, the "pathognomonic" association of EM with *B burgdorferi* infection has recently come into question. EM-like rashes have been observed in large numbers of patients in certain areas of the United States, where attempts to isolate *B burgdorferi* from suspected EM lesions have been unsuccessful, and reports of rare isolation from ticks have been unconfirmed.[26] Culture confirmation may be useful in the definitive diagnosis of patients with atypical rashes or in those patients who acquire EM-like rashes in areas not known to be endemic for Lyme borreliosis.

The full spectrum of Lyme borreliosis cannot yet be defined in culture positive patients since *B burgdorferi* is not easily recovered from patients with late disease (ie, arthritis or neurologic disease), or from specimens other than skin (ie, in patients without EM). However, the ability to isolate this spirochete readily from lesional skin in early disease should make culture positivity the standard by which to delineate the clinical features of patients with EM who account for a substantial proportion of cases of Lyme borreliosis. The short- and long-term outcomes of this group will be important to follow.

The isolation of *B burgdorferi* from clinical specimens may also be valuable in determining optimal antimicrobial therapy for Lyme borreliosis and in standardizing clinical treatment trials. Clinical isolates will be essential to the understanding of the relationship between specific clinical manifestations and particular strains of *B burgdorferi* sensu lato.[21] The categorization of isolates will be useful in understanding the immune response to this infection, and developing an effective vaccine. Perhaps most important in the immediate future, clinical specimens and isolates of *B burgdorferi* from culture-confirmed "pedigree" cases of Lyme borreliosis will be

invaluable in the development of sensitive and specific diagnostic tests for this illness.

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