Long-Term Serological Analysis and Clinical Follow-Up of Patients with Cat Scratch Disease

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A highly specific enzyme immunoassay (EIA) was recently described for use in the diagnosis of cat scratch disease (CSD). However, data regarding EIA antibody kinetics or its correlation with long-term clinical follow-up data are lacking. The association between antibody kinetics, clinical spectrum, and disease duration were studied in 98 patients with CSD. The median duration of follow-up was 35.3 weeks (range, 2–211.3 weeks). Results of EIA testing for detection of anti–*Bartonella henselae* immunoglobulin M (IgM) antibodies (detected in 53% of the patients) remained positive for ⩽3 months. Therefore, the presence of IgM indicated acute infection. Titers of immunoglobulin G (IgG) also decreased over time; 25% of the patients remained seropositive for >1 year after the onset of CSD. Onset of CSD in patients with an IgG titer with an optical density of ⩾1.0 occurred within the prior 12 months. No association was found between antibody titers or their kinetics and the clinical manifestations or duration of disease. EIA allows for the identification of atypical manifestations of CSD that were unrecognized before the use of serological assays. Complete recovery from these manifestations may take months. Results of this study provide additional data supporting the utility of EIA in the serodiagnosis of CSD.

Cat scratch disease (CSD) is a common cause of infectious regional lymphadenopathy [1]. Despite its self-limited nature, prolonged lymphadenopathy—often accompanied by fever, malaise, fatigue, and night sweats—can simulate a malignant process [2] and, thus, lead to expensive, unnecessary, and often invasive evaluations. In addition, ~10% of patients with CSD will have an atypical disease, such as Parinaud oculoglandular syndrome, neuroretinitis, encephalitis, endocarditis, granulomatous hepatitis, and osteomyelitis, with severe morbidity and a complicated course [1]. Accurate and timely diagnosis is therefore important.

Since its description in 1992 by Regnery et al. [3], the immunofluorescent antibody assay (IFA) for the detection of anti–*Bartonella henselae* IgG has become the most widely used diagnostic test for CSD and has been reported to have a sensitivity of 88% and a specificity of 97%. The major disadvantages of this assay include the need to cultivate *B. henselae* in Vero cells for antigen preparation, the limited data regarding the use of IFA for detection of IgM antibodies, and the bias associated with interobserver variability.

Recently, we described an EIA used for the detection of anti–*B. henselae* IgG and IgM antibodies in patients with CSD [4]. When a positive IgG or IgM test result or both is accepted as diagnostic, the sensitivity of the EIA test is 85%. Applying stringent criteria, this test is highly specific—98% and 99% for IgG and IgM, respectively—as determined by testing healthy control subjects and patients with lymphadenopathy due to defined non-CSD causes.

Little is known about the kinetics of anti–*B. henselae* antibodies, and data are restricted primarily to results of IFA. Information on antibody decay over time is

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important for an improved interpretation of the results of any serological assay and, in particular, for the diagnosis of CSD, because serological testing is often performed weeks after the onset of CSD. The clinical data available today on typical CSD are largely based on the large descriptive studies by Carithers [5] and Moriarty and Margileth [6], who described patients with CSD who received a diagnosis of CSD several decades ago. The impact of the introduction of readily accessible diagnostic tests, and of serological tests, in particular, on our ability to diagnose CSD, especially atypical CSD, is unknown. The major aim of this study was to describe the kinetics of anti–*B. henselae* IgM and IgG antibodies detected by EIA in association with long-term follow-up of the patients with clinical manifestations of CSD.

PATIENTS, MATERIALS, AND METHODS

Specimen and data collection and patient population. Clinical specimens (serum samples for EIA-based serological testing or tissue or pus specimens for PCR analysis) obtained from patients with suspected CSD were sent to the Bernard Pridan Laboratory for Molecular Biology of Infectious Diseases at the Tel Aviv Medical Center (Tel Aviv, Israel). Patients' demographic, epidemiological, and clinical data were collected from the referring physician and/or from the patient (or his/her family). For the purpose of this study, a patient with a clinical presentation that was consistent with CSD and with results of EIA serological testing that were positive for anti–*B. henselae* antibodies (IgM and/or IgG), in the absence of another diagnosis, received a diagnosis of CSD.

Study design. The study was approved by the ethics committees of the participating medical centers. Informed consent was obtained from patients or their parents. Patients with serologically confirmed CSD were invited for ⩾1 follow-up visit. Patients with intermediate serological results, as defined previously [4], were excluded from the study. An interview and a questionnaire were used for collection of clinical and epidemiological data, and a physical examination was performed. A blood sample was obtained and centrifuged, and the serum was stored at −80°C until serological analysis was performed. Patients were referred for neurological and/or ophthalmologic evaluation when indicated. The duration of serological analysis or clinical follow-up was defined as the interval between the onset of CSD and the time at which the last serum specimen was obtained or interview was performed. Proportions of seropositive specimens obtained during the follow-up period were calculated by dividing the number of positive samples by the total number of samples available beyond weeks 26, 52, and 104 of the follow-up period.

EIA. All serum specimens obtained from each patient were tested for either anti–*B. henselae* IgG or IgM antibodies in the same EIA plate. EIA was performed as described elsewhere [4]. In brief, antigen was prepared as sarcosyl-insoluble, outer-membrane protein extracts of the agar-derived, *B. henselae* 87–66 strain (ATCC 49793). Plates were coated with 500 ng of protein and were blocked with bovine serum albumin in PBS with Tween 20. Serum specimens (dilution, 1:100) were tested in triplicate. Bound IgM or IgG antibodies were detected by addition of alkaline phosphatase–conjugated goat anti–human IgM or anti–human IgG, respectively (Sigma), followed by the addition of *p*-nitrophenyl phosphate substrate (Sigma). Absorbance was measured at 405 nm using a microplate reader. A serum sample was considered to be positive if the mean optical density (OD) was ⩾3 SDs above the mean OD for serum samples obtained from a blood-donor control group, as described previously [4], which corresponded to ODs of ⩾0.1 and ⩾0.63 for IgM and IgG, respectively.

Statistical analysis. Differences between groups were analyzed with the χ^2 test or Fisher's exact test, for categorical variables, and with Student's *t* test or the Mann-Whitney *U* test, for continuous variables. A 2-sided *P* value of <.05 was considered significant.

RESULTS

Patients. One hundred sixty-three patients with serologically confirmed CSD were eligible for the study, of whom 45 could not be located, 17 refused to participate (mainly because of distance from the participating medical centers), and 3 refused to provide blood specimens. Thus, 98 patients were enrolled in the study. Data on patient characteristics at the time of diagnosis are summarized in table 1. The mean age was 24 years (median, 19 years), and 59% were male; 74% had received antibiotics. None were known to be immunocompromised.

Serological follow-up. A total of 223 serum samples were tested by EIA. Two serum samples were obtained from 76 patients, and ⩾3 samples were obtained from 22. For all patients, the first serum sample was obtained 1–52 weeks (median, 3 weeks) after the onset of disease. Duration of serological follow-up was 2–211.3 weeks (median, 35.3 weeks).

Fifty-two patients (53%) tested positive for anti–*B. henselae* IgM antibody, of whom 46 (88%) had an initial serum sample that tested positive, whereas an additional 6 patients had serum samples that revealed seroconversion within 1–8 weeks after the first serum sampling. Two of these 6 patients had an initial serum sample that was IgG positive, 3 patients had initial serum samples that were IgG negative and remained so on follow-up serological testing, and 1 patient later had seroconversion to IgG positivity. Positive anti–*B. henselae* IgG was identified in 90 patients (92%), of whom 63 (72%) had an initial blood sample that tested positive, whereas 27 initially had negative test results but had seroconversion later.

Table 1. Baseline demographic, epidemiological, and clinical characteristics of 98 patients with cat scratch disease (CSD), compared with data from a study by Carithers [5].

Variable	Study group, *n/N* (%)[a]	Carithers [5], % of patients
Age, years		
1–10	22/96 (22.9)	72.0
11–20	28/96 (29.2)	18.1[b]
21–30	19/96 (19.8)	9.9[c]
>30	27/96 (28.1)	…
Female sex	40/98 (40.8)	46.1
History of animal contact		
Cat or kitten	86/97 (88.7)	99.1
Dog	3/97 (3.1)	NR
Other	4/97 (4.1)	NR
Lymphadenitis	93/97 (95.9)	100.0
Affected lymph node		
Cervical	15/84 (17.9)	26.1
Preauricular	10/84 (11.9)	6.6
Axillary	27/84 (32.1)	45.0
Epitrochlear	3/84 (3.6)	1.8
Axillary and epitrochlear	9/84 (10.7)	NR
Inguinal	16/84 (19.0)	17.5
Other	4/84 (4.8)	2.3
Primary lesion	44/91 (48.4)	92.6
Fever	51/96 (53.1)	59.0
Malaise	42/96 (43.8)	NR
Atypical manifestation		
Parinaud oculoglandular syndrome	6/95 (6.3)	4.0
Erythema nodosum	4/95 (4.2)	0.4
Arthralgia	5/95 (5.3)	NR
Neuroretinitis	3/95 (3.2)	NR
Granulomatous hepatitis	1/95 (1.1)	NR
Peripheral neuritis	1/95 (1.1)	NR
Encephalitis	1/95 (1.1)	0.3
Osteomyelitis	1/95 (1.1)	0.2
Histopathological findings consistent with CSD		
Necrotizing granulomatous lymphadenitis	3/6 (50.0)	NR
Reactive lymphadenitis	2/6 (33.3)	NR
Granulomatous hepatitis	1/6 (16.7)	NR
Diagnostic tests other than serological tests with positive results		
PCR of pus or tissue specimens obtained from lymph nodes[d]	24/26 (92.3)	ND
Skin test[d]	6/9 (66.7)	99.0

NOTE. The median age of the patients was 19 years (range, 5–70 years). ND, not done; NR, not reported.

[a] No. of observations/no. of patients for whom data were available (%).
[b] Data refer to patients aged 11–17 years.
[c] Data refer to patients aged ⩾18 years.
[d] PCR and skin test were performed as described elsewhere [4].

The kinetics of anti–*B. henselae* IgM and IgG antibodies are presented in figures 1 and 2. A decrease in antibody titers over time was observed for both IgG and IgM. Forty-eight (92%) of the 52 IgM-positive patients became IgM negative within 3 months after the onset of disease. Only 4 specimens from 4 patients remained IgM positive beyond that period. Two hundred eight (93%) of 223 serum samples tested positive for anti–*B. henselae* IgG. Beyond 6, 12, and 24 months, 35%, 25%, and 25% of serum samples (and patients), respectively, remained IgG positive. All patients with CSD and an IgG titer with an OD of ⩾1.0 experienced disease onset within the prior 12 months. There was no association between seropositivity, antibody titer, or duration of seropositivity and the various epidemiological and clinical variables presented in table 1. Lymph node biopsy specimens or aspiration samples were obtained for histopathological examination and PCR analysis for 26 of the 98 patients, suggesting perhaps a more prolonged or severe disease. Of the 24 PCR-positive patients, 83% and 58% were IgG and IgM positive, respectively. No association, however, was found between this group of patients and antibody titer or duration of seropositivity.

Clinical follow-up. Ninety-three patients (95%) had typical CSD, with lymphadenopathy as the main clinical presentation. The axilla was the most common site of lymphadenopathy. The median interval between diagnosis and the end follow-up was 35.3 weeks (range, 2–211.3 weeks). Information on the duration of lymphadenopathy was available for 74 (80%) of the 93 patients with lymphadenopathy, because many patients could not accurately remember its duration. The median duration of lymphadenopathy was 7 weeks (range, 1–78 weeks). Lymphadenopathy was still present in only 1 of the patients available for follow-up ⩾1 year after the onset of disease. The duration and outcome of atypical CSD manifestations are presented in table 2. Of note, 1 of the 3 patients with neuroretinitis still had retinal exudates with impaired visual acuity after almost 2 years, and 1 patient with granulomatous hepatitis still had abnormal results of liver function tests and multiple hepatic lesions demonstrated by ultrasonography after 5 months of follow-up. All other atypical manifestations had resolved by the end of follow-up. None of the patients had recurrent CSD. Only 3 patients reported CSD in another member of their households.

Cat contact. Of the 86 patients with a history of cat contact at the time of diagnosis, 25 (29%) had >1 contact events with stray cats, whereas 61 (71%) reported contact with domestic cats. Of these 61 patients, 30 (49%) were still in contact with the same cat at the end of follow-up. Thirty (31%) of the 98 study patients had acquired new cats.

DISCUSSION

This is the first study to report the kinetics of anti–*B. henselae* IgG and IgM antibodies in patients with CSD, with a follow-up period of up to 4 years, by means of a novel EIA in conjunction with clinical follow-up. The proven high specificity of the EIA, together with a typical clinical presentation complemented by a history of cat contact in 89% of patients, support the accuracy of CSD diagnosis in our study population.

Our study indicates that IgM seropositivity lasts for ~3 months, with only 4% of the study population remaining IgM positive for >3 months. Thus, IgM seropositivity in a typical patient with CSD is indicative of acute disease. This finding is particularly important in patients with regional lymphadenopathy thought to be (possibly) malignant. Because only one-half of the patients were positive for anti–*B. henselae* IgM antibodies, improving the sensitivity of the IgM EIA is desired.

Titers of IgG may remain positive for ⩾2 years after disease onset. Although the level of IgG antibodies decreases over time, the rate of the decay is usually too slow to allow detection of a significant decrease over intervals of 2–3 weeks, as is often recommended for serodiagnosis of infectious diseases. This finding is in accordance with the findings of a previous report that also showed lack of significant change in anti–*B. henselae* IgG titers tested at 6-week intervals using an IFA [7]. Also, the lack of association between antibody kinetics or titers and CSD manifestations (typical and atypical) or duration precludes this assay's utility as a tool for monitoring the disease course. However, an IgG titer with an OD of >1.0 is characteristic of disease onset that occurred within the previous 12 months and, therefore, supports the diagnosis of recent CSD. Seventy-five percent of IgG-positive patients with CSD became seronegative 1 year after the onset of disease. We have previously shown that results of EIA that are positive for IgG antibodies, even those with an OD of <1.0, are highly specific in patients with typical CSD

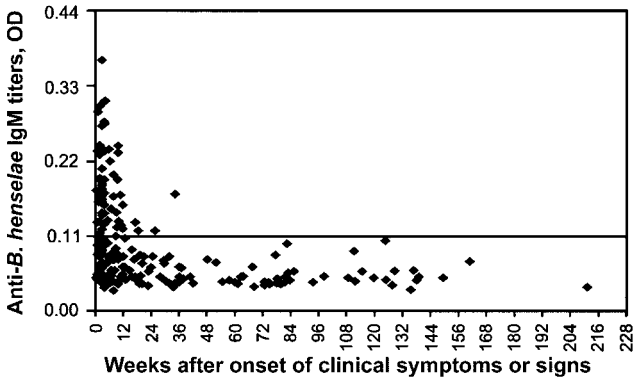

Figure 1. Distribution of anti–*Bartonella henselae* IgM antibody titers over time, expressed as optical density (OD) values. *Horizontal line,* cutoff point for a positive test result.

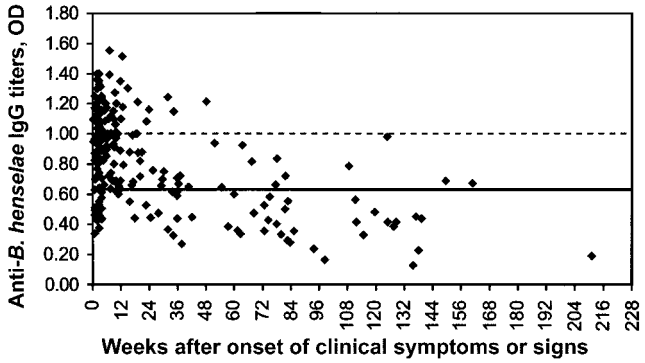

Figure 2. Distribution of anti–*Bartonella henselae* IgG antibody titers over time, expressed as optical density (OD) values. *Solid horizontal line,* cutoff point for a positive test result; *dashed line,* titer with an OD of 1.

[4]. However, an IgG titer with an OD of <1.0, in the absence of a positive IgM titer and in association with an unusual clinical course, must be interpreted with caution. Such a prudent approach is particularly important when evaluating cat owners (as opposed to patients with a history of a single cat contact), because, as shown in this study, patients with CSD who own cats tend to keep their cats or acquire new ones. Establishing an association between a cat scratch or bite and the development of lymphadenopathy might be more difficult in this group of patients. When in doubt, PCR analysis, which has been shown to be highly sensitive and specific, can be performed on tissue specimens obtained from the affected lymph node and/or primary lesion [8, 9].

Only a few reports have studied the kinetics of anti–*B. henselae* antibodies in patients with CSD. Szelc-Kelly et al. [7] used ELISA to test paired serum samples obtained 6.6 weeks apart from 63 patients with clinical CSD, of whom 43 had a positive skin test result. Although IgM titers decreased significantly, the conclusions regarding IgG kinetics are limited because of the short follow-up period and the poor sensitivity (18%–35%) of the IgG assay. Not et al. [10] showed, in a study of 34 patients, that IgM and IgG titers measured by ELISA decreased between the time of CSD diagnosis and recovery; however, detailed description of the antibody kinetics is lacking in their study. Two studies provide data on the kinetics of anti–*B. henselae* antibodies detected by IFA. Dalton et al. [11] reported that the geometric mean antibody titer generally increased during the first 8 weeks, followed by a slow decrease during the next 12 months. Bergmans et al. [12] studied antibody kinetics in 18 patients with CSD and demonstrated that IgM was produced earlier than IgG and disappeared within 100 days, whereas the titer of IgG remained high in some patients at the end of 100 days of follow-up.

The clinical presentation and the duration of symptoms and signs in our patients were similar in most aspects to the data collected by Carithers [5] and Moriarty and Margileth [6], who described the classical clinical presentations of CSD in study groups that were followed between 1955–1985 and 1975–1987, respectively. All patients in these studies had lymphadenitis. Similarly, 96% of our patients had lymphadenitis in an anatomical distribution nearly identical to the distribution described in these previous reports. However, 4 of our patients had atypical manifestations (neuroretinitis, granulomatous hepatitis, erythema nodosum, and osteomyelitis) without lymphadenitis. These patients might have received an incorrect diagnosis in previous years, when extranodal CSD in the absence of lymphadenopathy was not well recognized. In fact,

Table 2. Clinical follow-up data for patients with cat scratch disease, compared with data from a study by Moriarty and Margileth.

Clinical manifestation	Complete resolution, *n/N* (%)[a]	Duration of follow-up, median months (range)[b]	Time to resolution, months	
			Current study, median (range)[b]	Moriarty and Margileth [6], range
Lymphadenitis	74/74 (100.0)	8.0 (0.5–47)	1.6 (0.25–17)	0.5–12
Parinaud oculoglandular syndrome	6/6 (100.0)	2.5 (1–18)	1.2 (1–6)	0.75–7
Neuroretinitis	2/3 (66.0)	23.0 (2–25)	13 (1–25)	NR
Erythema nodosum	4/4 (100.0)	22.5 (8–33)	1.1 (0.25–2)	0.1–1.4
Arthralgia	4/4 (100.0)	17.5 (2–23)	4.9 (4–5)	0.1–2.8
Granulomatous hepatitis	0/1 (0.0)	5.0 (NA)	NA (NA)	NR
Peripheral neuritis	1/1 (100.0)	12.0 (NA)	1.0 (NA)	NR
Encephalitis	1/1 (100.0)	1.5 (NA)	1.0 (NA)	0.25–8
Osteomyelitis	1/1 (100.0)	27.0 (NA)	12.0 (NA)	1.0–9

NOTE. NA, not applicable; NR, not reported.

[a] No. of patients for whom the clinical manifestation cleared/no. of patients with the given clinical manifestation (%).

[b] When data for only 1 patient are available, the actual duration in months in noted.

CSD neuroretinitis (first reported in 1984 [13]), granulomatous hepatitis (reported in 1985 [14]), and peripheral neuritis (reported in 1998 [15]) were not reported in these aforementioned studies. The introduction of newer diagnostic tests—serological testing and PCR, in particular—that have essentially replaced the skin test used in the studies of Carithers [5] and Moriarty and Margileth [6] has permitted the recognition of a broader clinical spectrum of CSD and has allowed for the association of new clinical entities with *B. henselae* infection. This might also explain the relatively higher rates of CSD-associated erythema nodosum (4%) and arthralgia/arthritis (5%) in our study, compared with those reported by Carithers [5] and Moriarty and Margileth [6] (<1%).

In conclusion, positive anti–*B. henselae* IgM results detected by EIA indicate an acute disease with a duration of ⩽3 months. IgG titers decrease over a longer period of time. Titers with an OD of >1.0 are indicative of a disease with a recent (⩽1 year) onset. These data are important for a better interpretation of EIA results. The clinical manifestations in patients with CSD that was diagnosed with the currently used EIA are similar to the manifestations in patients who received a diagnosis of CSD in previous years on the basis of skin test results. Serological testing, however allows for the identification of previously underdiagnosed or unknown manifestations of CSD, particularly those that are unaccompanied by lymphadenopathy.

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