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The use of *Bartonella henselae*-specific age dependent IgG and IgM in diagnostic models to discriminate diseased from non-diseased in Cat Scratch Disease serology

M. Herremans [a,*], M.J. Vermeulen [b], J. Van de Kassteele [c], J. Bakker [a], J.F.P. Schellekens [d], M.P.G. Koopmans [a]

[a] *National Institute for Public Health and the Environment (RIVM). Diagnostic Laboratory for infectious diseases and perinatal screening (LIS), Bilthoven, The Netherlands*
[b] *VU Medical Centre, Amsterdam, The Netherlands*
[c] *RIVM. Expertise Centre for Methods and Information, Bilthoven, The Netherlands*
[d] *Laboratory for Infectious Diseases, Groningen, The Netherlands*

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Abstract

Cat Scratch Disease (CSD) is caused by *Bartonella henselae* infection and is a common cause of regional lymphadenopathy. The diagnosis of CSD largely depends on serology, but is hampered by both low sensitivity and specificity of the applied IgG and IgM assays. Using an in-house ELISA, we detected a significant age-dependent increase in the IgG levels in the general population compared to CSD patients. With this knowledge, we developed diagnostic models to differentiate diseased from non-diseased persons. Evaluation of these models using samples from PCR-positive patients ($n=155$) and age-matched controls ($n=244$) showed an important increase in the assay performance if the combination of the IgG and IgM results were taken into account. If the specificity was set at 98% the sensitivity was only 45% and 32% for the IgM and IgG ELISA, respectively but increased to 59% when these results were combined. Also the use of age-dependent factors further improved the clinical relevance of the outcome raising the sensitivity to 64%. Although the sensitivity of the ELISA remains low we conclude that the use of models using the combination of both IgM and IgG test results and age-depending factors can be a useful diagnostic tool in the serodiagnosis of CSD.

Keywords: Age-dependent; *Bartonella henselae*; Cat Scratch Disease; IgG; IgM; Modelling

1. Introduction

Although the clinical profile of Cat Scratch Disease (CSD) was described in 1950, the pathogen that causes it – *Bartonella henselae* – was not identified until 1993 (Regnery et al., 1992). Currently the domestic cat is the only known reservoir of *B. henselae* with a confirmed link to disease in humans (Koehler et al., 1994). The majority (60%) of patients with CSD are children under the age of 20 and the predominant clinical feature of CSD is a regional lymphadenopathy that in most cases develops two to three weeks after the exposure (range 7-50 days) with a possible long duration of the clinical symptoms up to several months (Margileth, 1993). Although the lymph node swellings resolve spontaneously in CSD without leaving residual symptoms, identifying the causative agent is important because of the non-specific nature of the presentation (lymfadenopathy). For this reason a wide differential diagnosis will be considered in most cases.

An important diagnostic tool in the detection of *B. henselae* is the examination of lymph node biopsy specimens or pus by PCR, with a reported sensitivity between 58 and 96% (Bergmans et al., 1995; Sander et al., 1999). However, pus can only be collected

* Corresponding author. PO box 1 3720BA Bilthoven, The Netherlands. Tel.: +31 30 2743705; fax: +31 30 2744418.
E-mail address: Tineke.Herremans@rivm.nl (M. Herremans).

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from approximately 15% of patients without performing puncture. The collection of a biopt from an affected lymph node is not always feasible or advisable if possible malignancy can not be excluded. For this reason, the first choice for laboratory diagnosis is the detection of *B. henselae*-specific antibodies.

Both IFA and ELISA's have been described for the detection of IgM and IgG antibodies (Bergmans et al., 1997; Regnery et al., 1992; Sander et al., 1998). The evaluation of serological tests in earlier studies reported various specificities (95-100%) and sensitivities (40-100%) (Bergmans et al., 1997; Giladi et al., 2001; Regnery et al., 1992; Sander et al., 1998; Zangwill et al., 1993). Especially the detection of *B. henselae*-specific IgM is thought to be a marker for recent infections in patients. However the clinical use of IgM detection is hampered by the low sensitivity (60-74%). This low sensitivity will be difficult to increase due to the specifics of *B. henselae* infections: CSD patients develop symptoms relatively late, often more than eight weeks after traumatic cat contact (Loutit, 1997). With large inter-individual differences, IgM stays present in the serum for approximately three months (Bergmans et al., 1997; Metzkor-Cotter et al., 2003), and therefore IgM levels are already declining in many patients at the time of their first physician contact. In patients with a clear IgG response in the absence of IgM, it can not be concluded with certainty whether the IgG response reflects past or recent contact with *B. henselae* since patients can remain positive for extended periods of time.

Our laboratory has implemented an in-house ELISA for the serodiagnosis of *B. henselae* since 1995 (Bergmans et al., 1997). Using this ELISA, we detected a significant age-depended increase in the IgG levels in the general population. Since the average age of CSD patients is relatively low, we investigated if the use of diagnostic models that take age into consideration provide a better discrimination between patients and controls. The developed models were evaluated for application in serodiagnosing CSD.

2. Materials and methods

2.1. Clinical samples

The performance of the *B. henselae*-specific IgM and IgG ELISA's assays was determined using a panel of sera collected from CSD patients with a positive PCR result in material of the affected nodes ($n=161$). The average age in this patient group was 26 years (range 3 to 74 years). A total of 275 controls were used consisting of 126 age-matched healthy controls to the PCR positive patients and 149 children (ages 0 to 10) suspected of *Bordetella pertussis* infection. This last group was added as an extra control group for the younger age groups that are usually affected by *B. henselae* infections.

2.2. PCR

Clinical material for PCR detection of *B. henselae* DNA consisted of pus aspirates and biopsy specimens from lymph nodes. The extraction of DNA and subsequent PCR detection were performed as described earlier (Bergmans et al., 1995).

2.3. B. henselae-specific IgM and IgG ELISA

The IgM and IgG ELISA's were performed based on methods previously described with several modifications to improve the sensitivity (Barka et al., 1993; Bergmans et al., 1997). The *B. henselae* strain ATCC 49882 (genotype I) was grown on Columbia agar plates containing 5% sheep blood for 7-10 days at 35 °C in an atmosphere containing 5% CO_2. Colonies were scraped from the plates, suspended in Phosphate Buffered Saline (PBS) and sonificated for 30 minutes, and stored at -20 °C. Wells of polysorb microtiter plates (Nunc, Copenhagen, Denmark) were coated overnight at +4 °C with 100 µl of an optimal dilution of the prepared *B. henselae* antigen in PBS. The optimal dilution of the antigen was defined as the concentration that gave comparable and reproducible results with a test panel (a selection of negative and high and low positive sera). Parallel wells were coated with PBS alone to control for the non-specific binding of serum components. After incubation, the plates were washed two times with PBS with 0.05% Tween 20 and blocked with blocking reagent (Boehringer, Mannheim, Germany) according to the manufacturer's instructions. Serum dilutions (1:100) were added and were incubated for 1 hour at 37 °C. Plates were washed four times between each incubation step. Prior to testing in the IgM assay, sera were depleted of IgG with IgG-RF sorbent (Biotest, Soest, The Netherlands) according to the manufacturer's instructions to prevent possible inter-isotype competition. Bound antibody was detected by horseradish-peroxidase-labelled goat anti-human IgM (Biorad, Veenendaal, The Netherlands) or rabbit anti-human IgG (Dako, Glostrup, Denmark) (1 hour, 37 °C). Tetramethylbenzidine was used as substrate, and colour development was stopped after 10 minutes by the addition of 2 M H_2SO_4. The plates were read at 450 nm (second filter 690 nm). A high positive, low positive and negative control serum was included in each assay. For each sample the own background was deducted from its reaction with the *B. henselae* antigen to correct for non-specific binding. The prediction of diseased (positive) from not-diseased (negative) was done according to the developed diagnostic models (for description see below and Table 1).

Table 1
The six diagnostic models for the serodiagnosis of CSD and their Akaike's Information Criterion (AIC)

		log(odds)=	AIC
Model 1	IgM	4.014+1.470 IgM	341.3
Model 2	IgG	2.025+1.840 IgG	382.1
Model 3	IgM&IgG	4.9800+1.0169 IgM+0.7300 IgG − 0.3317 IgM*IgG	258.9
Model 4	IgM+age	−1.731+1.403 IgM+14.867 age − 11.235 age^2+2.731 age^3	285.2
Model 5	IgG+age	−0.8797+1.6765 IgG+5.5407 age − 2.2790 age^2	354.6
Model 6	IgM&IgG+age	3.2275+0.9415 IgM+1.2601 IgG+3.8662 age − 1.8654 age^2 − 0.8031 IgG*age − 0.4138 IgM*IgG	241.4

IgM=log(IgM), IgG=log(IgG) and age=log(1+age/10).