Chapter 8

No aetiological role for *Bartonella henselae* infection in Henoch-Schönlein purpura

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ABSTRACT

Background and aims: Henoch-Schönlein purpura (HSP) is an acute leukocytoclastic vasculitis of unknown etiology. It has often been associated with infection, with Bartonella henselae as one of the possible infectious agents implicated in the pathogenesis. The aim of this study was to further assess serological evidence for a relation between B. henselae and HSP.

Methods: A prospective case-control study was performed, including Dutch children presenting with the characteristic rash of HSP (n = 45) as well as age-matched controls (n = 90). Anti-B. henselae IgM and IgG titers were determined by immunofluorescence assay (IFA) and streptococcal antibodies were tested. PCR was used to detect B. henselae DNA in the serum samples.

Results: IgM seropositivity was not found in the acute nor in the convalescent phase of HSP. Anti-B. henselae IgG seropositivity was not higher in HSP patients than in controls. Also streptococcal antibodies were not more common in the HSP patients than in controls. No B. henselae DNA was found by PCR in serum during the acute phase of HSP.

Conclusion: A recent or previously experienced B. henselae infection is unlikely to be a contributing factor in the pathogenesis of Henoch-Schönlein purpura.
INTRODUCTION

The cause of Henoch-Schönlein purpura (HSP) is still unknown, with possible associations with infections, food, cold exposure, insect bites and drug allergies [1]. One of these triggers may cause an immunological cascade in patients with a specific genetic background, resulting in a IgA-mediated systemic leucocytoclastic vasculitis [1]. The clinical hallmark is a characteristic cutaneous purpuric rash occurring mainly on the extensor surfaces of the lower limbs and buttocks. HSP is mainly seen in children and is often accompanied by abdominal pain, renal involvement and/or arthritis [2,3].

Despite studies on the role of group A beta-hemolytic streptococci and parvovirus B19 [4,5], convincing evidence for an infectious cause of HSP is still lacking [1,3,6]. Recent serological studies suggested an etiological role for Bartonella (B.) henselae infection, based on IgG seropositivity in HSP patients [7,8]. B. henselae infection causes cat-scratch disease (CSD), that usually presents as a benign lymphadenopathy after traumatic cat contact. In some cases CSD becomes systemic, resulting in a variety of possible symptoms, including vasculitis in rare cases. Two cases of systemic cat-scratch disease associated with HSP have been described in the literature, [9,10] but convincing evidence for an etiological role for B. henselae is still lacking.

The objective of this study is to further assess the role of B. henselae in the etiology of HSP. In a prospective study design, we assessed IgG and IgM serum antibodies for B. henselae in HSP patients in the acute and convalescent phase and compared to age-matched controls. The finding of elevated B. henselae IgM in acute HSP would suggest that CSD plays a role in triggering HSP, whereas elevated IgG with negative IgM in acute HSP could suggest a predisposition for HSP after a B. henselae infection.

MATERIALS AND METHODS

This prospective case-control study was conducted from 2002 until 2007 in the St. Elisabeth Hospital and the Twee Steden Hospital in Tilburg, The Netherlands. Serological testing was performed in the Regional Public Health Laboratory in Tilburg, The Netherlands. The study was approved by the Medical Ethical Committee of the hospital.

Patient selection

The study population consisted of pediatric patients presenting with the characteristic purpura of Henoch-Schönlein at the outpatients or emergency department. After written parental informed consent, routine blood samples were obtained. Patients with thrombocytopenia and/or coagulation disorders were excluded. Paired sera were collected in the convalescent phase only if routine blood tests were deemed necessary by the treating physician. Two controls were matched per case based on age and gender in both the acute...
and convalescent phase. Control sera were collected from patients originally suspected for diagnosis of *Bordetella pertussis* infection but had tested negative. All serum samples were stored at minus 20 °C.

**Diagnostic tests**
The sera of patients and controls were tested for *B. henselae* IgM by a validated in-house prepared immunofluorescence assay (IFA) as described earlier [11,12]. *B. henselae* IgG was tested using commercially obtained IFA slides according to the manufactures recommendations, Focus (IF1300G, Focus Technologies, Cypress, CA, USA) and Euroimmun (Fl 219b-1005G, Euroimmun AG, Lübeck, Germany) Two experienced operators scored all IFA slides blinded and independently. Quantitative detection of streptococcal antibodies, antistreptolysin O (ASO) and antideoxyribonuclease B (anti-DNB) was performed by nephelometry (IMMAGE® Immunochemistry Systems, Beckman Coulter, Galway, Ireland), according to the manufacturer’s instructions. Cut-off rates were 500 IU/mL and 300 IU/mL respectively. A real-time PCR assay targeted at the heat shock protein groEL was used to detect *B. henselae* DNA in the patient’s serum samples, as described earlier [13].

**Table 1.**
Patient characteristics of 45 Henoch-Schönlein purpura patients at initial presentation.

<table>
<thead>
<tr>
<th>Patient data</th>
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<tbody>
<tr>
<td>Age years*</td>
<td>5.2</td>
</tr>
<tr>
<td>Male gender</td>
<td>51%</td>
</tr>
<tr>
<td>History of cat contact†</td>
<td>21%</td>
</tr>
<tr>
<td>History of cat-scratch†</td>
<td>2.4%</td>
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</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th></th>
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<tbody>
<tr>
<td>Palpable purpura</td>
<td>100%</td>
</tr>
<tr>
<td>Arthritis/arthralgia</td>
<td>53%</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>8.9%</td>
</tr>
<tr>
<td>Abdominal symptoms</td>
<td>84%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory results</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>CRP, mg/dl*</td>
<td>7 (0-182)</td>
</tr>
<tr>
<td>ESR, mm/hr*</td>
<td>14 (2-98)</td>
</tr>
<tr>
<td>Haemoglobin, mg/l†</td>
<td>75 (0.6)</td>
</tr>
<tr>
<td>Platelets, x10⁹/l†</td>
<td>425 (108)</td>
</tr>
<tr>
<td>White blood count, x10⁹/l†</td>
<td>11 (4.2)</td>
</tr>
<tr>
<td>APTT, s†</td>
<td>31 (76)</td>
</tr>
<tr>
<td>PTT, s†</td>
<td>14 (6.4)</td>
</tr>
</tbody>
</table>

*Indicated is median (range); †data on 3 patients missing; ‡ mean (standard deviation); CRP indicates C-reactive protein; ESR, erythrocyte sedimentation rate; APTT, activated partial thromboplastin time; PTT, partial thromboplastin time.
Statistical analysis
SPSS 11.0 statistical package was used, applying the Pearson’s Chi-square Test and, if necessary, 2-sided Fisher’s Exact Tests for comparison of the proportion of seropositivity in the cases and controls. The McNemar Test for matched pairs was applied for comparison of the ‘early samples’, collected in the acute phase, versus the ‘late samples’ in the convalescent phase. The two-sample t-test was applied for comparison of continuous data. P-values of 0.05 or lower were considered to be statistically significant.

RESULTS

Subjects and samples
Forty-five HSP patients were enrolled in the study, all presented with the characteristic palpable purpura of HSP without thrombocytopenia or prolonged coagulation time (Table 1). Nine patients reported contact with cats, with one recalling a cat scratch. Data on cat contact were not available for 3 patients. Four patients had mild lymphadenopathy during the course of HSP, resolving spontaneously in all cases. The acute phase blood samples (‘early samples’) were obtained within 1-9 days of the onset of the illness (median 1 day). In 15 cases convalescent-phase serum (‘late samples’) for serological analysis was obtained, with a median time interval of 4.7 weeks (range 2.3-10 weeks) between early and late sample taking. All patients were followed until clinical signs of HSP had disappeared.

Serological data
In Table 2 the results of the serological tests are summarized. All HSP patient samples were negative for anti-\textit{Bartonella henselae} IgM in both the early and late samples of the HSP patients, which was not significantly different from the 2% positive samples among the controls (p = 0.6).

No significant differences were found between anti-\textit{B. henselae} IgG positivity in the HSP patients (16% and 19% by Focus and Euroimmun IFA assays respectively) and controls (12% and 24% respectively, p > 0.5). In addition, the late samples showed no significant increase in IgG positivity (p = 0.5).

Seroprevalence for ASO and anti-DNB was not significantly higher in HSP cases than in controls in both the early and late samples.

No \textit{B. henselae} DNA was detected by PCR in 31 early serum samples of HSP patients. One of the 13 late samples tested positive for \textit{B. henselae} DNA, with Ct-value 39.2 (cut-off value for positivity < 40).
**DISCUSSION**

This is the first prospective study to detect anti-*B. henselae* antibodies in HSP patients. Anti-*B. henselae* IgM was undetectable in all HSP cases. We also found that the frequency of anti-*B. henselae* IgG positivity was not significantly higher in HSP patients than in the age-matched controls, in the acute phase of HSP nor at follow up. These results do not support an etiologic role for CSD in HSP. Our results are in contrast to earlier reports on the role of *B. henselae* infection in HSP patients. In 2002, Ayoub et al. reported elevated *B. henselae* IgG titers in 67% of 18 American HSP patients compared to 14% in 57 controls [8]. Based on their results, the authors claimed that antecedent *B. henselae* infection is associated with HSP. Later, Robinson et al. found elevated *B. henselae* IgG titers in 61% of 28 Canadian children with HSP compared to 21% of controls. However, detection of *B. henselae* DNA in blood was negative in the acute phase of HSP [7].

The results of the two earlier reports, approximately three-fold higher *B. henselae* IgG seroprevalence in HSP patients than in controls, is in contrast with the results of the present study. All three studies used IFA techniques, using the same *Bartonella* strain (ATCC 49882). In all studies IgG seroprevalence in the controls groups was roughly similar. To exclude the possibility of insufficient sensitivity of the technique used in the present study, we tested all serum samples by two different commercially available IFA tests (Focus and Euroimmun), that both gave similar results. To exclude the possibility of inadequate diagnostic performance
of the tests used in the present study, a recent validation study of our anti-\textit{B. henselae} IgM test was undertaken. The in-house IgM IFA showed a sensitivity of 50\% with a negative predictive value in patients highly suspected of CSD of 68\% (unpublished data), suggesting that the negative results are not caused by unreliable testing. The sensitivity of the two commercial IFA IgG tests was found to be high in our validation study (88\% and 98\% respectively, unpublished data). Selection bias was limited in the present study by prospectively including HSP patients at first presentation of current HSP. In addition, the control group was age and gender matched to the cases and originated from the same geographic region as the HSP patients.

To explain the conflicting results with the previous studies, we speculate that severity of HSP is different among the described HSP populations. For example, Ayoub et al. retrospectively selected serum samples of 18 cases within 4 months after onset of HSP symptoms [8]. Robinson et al. included 6 cases of ‘current HSP’, defined as 7-34 days post onset of symptoms and 22 cases of ‘remote HSP’, defined as 42 or more days post onset of symptoms, with half of them 1-8 years post onset [7]. In contrast, our study included blood samples that were obtained from HSP patients within 1-9 days of the onset of the illness. Therefore, the serum samples in the previous studies might represent a selection of patients with more severe or complex disease. A second possible explanation for the conflicting results could be the pitfall of cross-reactivity in \textit{B. henselae} serology. A third explanation may be the increased ability of some American or Canadian \textit{B. henselae} strains for inducing HSP as compared to Dutch strains. Another explanation may be that it is not a previous infection but an acute \textit{B. henselae} infection that is related to HSP. As CSD can present atypically with vasculitis in rare cases, one could speculate that HSP is a clinical presentation of an atypical form of CSD. This is, however, unlikely as anti-\textit{B. henselae} IgM and IgG was undetected in 45 acute phase HSP patients and remained undetected in the convalescent phase, while seropositivity is expected in about 70\% of CSD cases within 4-6 weeks [11]. In conclusion, we found no satisfying explanation for the conflicting results.

Consistent with the serological findings, we detected no \textit{B. henselae} DNA in the acute phase serum samples. One of the late samples tested repeatedly ‘weak positive’, with Ct-values (39.2 and 38.9) close to the cut-off value (< 40). This sample was taken 6 weeks after first HSP symptoms in a 6 year old girl, that showed no IgM nor IgG seroconversion. We speculate that this patient was coincidentally infected with \textit{B. henselae} after her HSP episode. However, no cat contact was reported and no lymphadenopathy or other symptoms consistent with CSD were found. Contamination of the blood sample seems unlikely, as all samples were treated following strict laboratory regulations.

We found no serological evidence for a significant role of acute or remote \textit{B. henselae} infection in the etiology of HSP. Our findings are supported by the two largest studies on the clinical spectrum of CSD describing over 1800 CSD patients. They report on many atypical presentations of CSD including skin disorders, but do not mention HSP or similar vasculitis [14-16]. A link between HSP or other types of vasculitis has only been reported anecdotally.
Although no serological role for *B. henselae* could be found, epidemiologic data do support an association between infection and HSP [1,2]. Most widely studied is the role of antecedent respiratory infections by group A beta-hemolytic streptococci. In 20-50% of HSP patients positive antibodies or bacterial cultures have been reported [1]. The present study does not support an etiological role for streptococcal infections as seroprevalence for streptococcal antibodies was not higher in HSP cases than in age and sex-matched controls. In conclusion this study found no evidence for a significant role of acute or previously experienced *B. henselae* infections in the etiology of Henoch-Schönlein purpura.

**ACKNOWLEDGEMENTS**

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