Seroprevalence of *Bartonella* spp. infection in HIV patients in Catalonia, Spain

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**Abstract**

**Background:** Although the first clinical descriptions of *Bartonella* infection were associated with immunocompromised patient with bacillary angiomatosis, we currently know that this organism is directly involved in diseases affecting a large number of patients, regardless of their immune status. Cat scratch disease, hepatic peliosis, and some cases of bacteraemia and endocarditis, are directly caused by some species of the genus *Bartonella*. The purpose of this study was to determine the prevalence of IgG antibodies against *Bartonella henselae* and *B. quintana* in HIV patients and to identify the epidemiological factors involved.

**Methods:** Serum samples were collected from HIV patients treated at Hospital de Sabadell. Antibodies to *B. henselae* and *B. quintana* from 340 patients were examined by indirect immunofluorescence assay (IFA). Significance levels for univariate statistical test were determined by the Mann-Whitney U test and $\chi^2$ test.

**Results:** Of 340 patients, 82 were women and 258 men, with a median age of 42.21 ± 10.35 years (range 16–86 years). Seventy-six (22.3%) patients reacted with one or more *Bartonella* antigens. Of all the factors concerning the seroprevalence rate being studied (age, sex, intravenous drugs use, alcohol consumption, CD4 levels, AIDS, HCV, HBV, residential area), only age was statistically significant.

**Conclusion:** A high percentage of HIV patients presents antibodies to *Bartonella* and is increasing with age.

**Background**

The spectrum of *Bartonella* infections has expanded rapidly since the first HIV-infected patient with unusual, vascular proliferative lesions of bacillary angiomatosis (BA) was described in 1983 [1]. Of the 19 species of *Bartonella* described until now, only 10 were acknowledged as human pathogen species; *B. bacilliformis, B. quintana*, and *B. henselae*, are the most frequently described species [2-4], while *B. elizabethae, B. winsonii, B. washoensis, B. grahamsii, B. claridgeiae, B. koehlerae and B. alsatica* were...
recently identified as responsible for a few cases of human infections [5-10]. Cat scratch disease (CSD), hepatic peliosis and some cases of bacteraemia and endocarditis are directly caused by some species of the genus Bartonella [11-14]. To determine the real incidence of Bartonella infections, we must study the seroprevalence in the general population and the principal reservoirs and vectors of infection transmission. The results yielded by different studies on seroprevalence vary depending on the type of population under study; thus, the research conducted on collective groups that present special characteristics or associated risk factors present a higher prevalence than that found in studies carried out on the normal population.

In patients with addiction to parenteral drugs, it ranged from 15% to 47.5% [15,16]; in patients with HIV infection, it varied between 17.3% and 40% [17,18]. The objective of this study was to evaluate the prevalence of Bartonella infections in patients with HIV in our catchment area and to assess related factors.

Methods

Geographical area
The study was undertaken in Vallès Occidental (Catalonia), a predominantly urban area near the coast in the northeast of Spain.

Samples
The collection of samples took place over a 10-month period, from October 2004 to July 2005. The sample included adults and children treated at Hospital de Sabadell (Vallès Occidental, an area near Barcelona, Spain), where most of the patients diagnosed with HIV from the region are treated (cathment area 407,763 inhabitants). They were attended in periodical CD4 follow-up. Serum samples were collected from these patients at their scheduled follow up visits. The residential area was determined considering the number of inhabitants who lived in the municipalities. In fact, municipalities with < 50,000 inhabitants were considered semirural areas, and >50,000 were regarded as urban areas. Demographic information was obtained from computerized clinic record. Information about alcoholism, drug use, CD4 levels, HCV and HBV serology, was available from computerized records for patients who regularly received care at the clinic and was obtained by chart review for some patients. Informed consent was obtained from adult participants and from the parents of minors.

Sero logical technique
The sample of heparinized blood was sedimented (centrifugation of 5 ml samples of blood at 1,500 rpm for 10 minutes), and the supernatant was collected and stored at -80°C until used. Human serum samples were evaluated by indirect immunofluorescence assay (IFA). We used commercial slides (Bartonella IFA IgG. Focus Technologies, Inc., Herndon, VA) to determine antibodies to Bartonella spp. The kit for detecting IgG antibodies that employees Vero cells infected with either B. henselae or B. quintana was used according to the manufacturer’s instructions. The serum samples were initially diluted 1/64. Any serum samples found to be positive at the initial dilution were further titrated. Positive and negative controls were included in each test. We considered specimens showing no fluorescence at IgG titers of 1/64 as negatives and specimens with bright fluorescence in a dilution of 1/64 or greater as positive. The intensity of each specific fluorescent test was subjectively evaluated and independently graded by two of the authors [19].

Data analysis
Statistical analysis was performed using statistical package SPSS for Windows, Release 13.0.1 (standard version; SPSS, Inc., Chicago, IL). Significance levels for univariate statistical test were determined by the Mann-Whitney U test, \( \chi^2 \) test and Fisher exact test. A \( p \) value of 0.05 or less was considered to be significant in all statistical tests used.

Results
Of the 340 subjects, 82 (24.1%) were women and 258 (75.9%) men. Mean age was 42.21 ± 10.35 years (range 16–86 years). One hundred and ninety four (57%) and 146 (43%) patients lived in urban and semirural- rural areas, respectively. One hundred and ninety-six (57.6%) patients consumed or had consumed intravenous drugs, whilst 97 (28.5%) presented an excessive intake of alcohol. 13.8% of patients presented CD4 levels below or equal to 200 cell/ml at the time of the study. Of all the patients being followed-up, 102 (30%) had been diagnosed with AIDS. 50% of the patients were infected with the hepatitis C virus, whereas only 5% was infected with the hepatitis B virus (Table 1). None of the patients enrolled in this study presented neither a past history of diseases caused by exposure to Bartonella spp nor bartonellosis symptoms. Antibodies to Bartonella species were highly prevalent in this group of HIV patients; 76 (22.3%) of the samples reacted with at least 1 Bartonella antigen, 32 (42.1%) of the positive samples reacted with only B. henselae antigen, one sample (1.3%) reacted with only B. quintana, and 43 (56.6%) with both. In 34 serum samples, the titers obtained did not allow for differentiation between the 2 Bartonella species, as the specimens presented the same titer or one dilution as difference only. In the 9 remaining serum samples, the titers obtained for B. henselae were clearly superior (2 or more dilutions as difference) (Table 2). Fifty-seven patients (75%) were positive at a titer of 1/64, 10 (13.1%) at 1/128, 5 (6.6%) at 1/256, 2 patients (2.6%) at 1/512 and 2 patients (2.6%) were positive at a titer of 1/1024. The antibody titer was
specific for \textit{B. quintana} in one case only, with a titer of 1/128.

24.4% of women and 21.7% of men presented antibodies to \textit{Bartonella} spp. A statistically significant increase of seropositivity against \textit{Bartonella} spp. was observed as patient age increased (p < 0.05). Twenty-three (30.2%) patients with positive serology for \textit{Bartonella} presented a past history of alcohol abuse. Of the 76 patients with positive serology, 47 (61.8%) were addicted to parenteral drugs and 22 (28.9%) had, at some time, being diagnosed with AIDS. Thirty-nine patients presented co-infection between \textit{Bartonella} and HCV, whereas in 3 patients it was between \textit{Bartonella} and HBV. No differences were found regarding the way of transmission of the human immunodeficiency syndrome.

### Discussion

Preliminary estimates of the prevalence of antibody to \textit{Bartonella} among apparently healthy humans range from 5.88% to 24.7% [17,20]. In a study carried out on a healthy population sample from the same area (83 were men and 78 women, and the mean age with positive serology was 45.18 ± 14.26 years), the seroprevalence was 10.6% [21], a figure that is very similar to that reported in other studies. The incidence of \textit{Bartonella}-associated disease among HIV-infected people is less known. The studies carried out on a healthy population sample from the same area (83 were men and 78 women, and the mean age with positive serology was 45.18 ± 14.26 years), the seroprevalence was 10.6% [21], a figure that is very similar to that reported in other studies. The incidence of \textit{Bartonella}-associated disease among HIV-infected people is less known.

### Table 1: HIV patients description and serologic results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N total (%) (340 patients)</th>
<th>Positive to Bartonella N (%) (76 patients)</th>
<th>Negative to Bartonella N (%) (264 patients)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>82 (24.1)</td>
<td>20 (26.3)</td>
<td>62 (24.4)</td>
<td>0.611</td>
</tr>
<tr>
<td>Male</td>
<td>258 (75.9)</td>
<td>56 (73.7)</td>
<td>202 (78.3)</td>
<td></td>
</tr>
<tr>
<td>Immunitary state</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 200 cell/ml</td>
<td>47 (13.8)</td>
<td>7 (9.2)</td>
<td>40 (15.2)</td>
<td>0.368</td>
</tr>
<tr>
<td>&gt; 200 cell/ml</td>
<td>293 (86.2)</td>
<td>69 (90.8)</td>
<td>224 (84.8)</td>
<td></td>
</tr>
<tr>
<td>AIDS ¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>102 (30)</td>
<td>22 (28.9)</td>
<td>80 (30.3)</td>
<td>0.820</td>
</tr>
<tr>
<td>No</td>
<td>238 (70)</td>
<td>54 (71.1)</td>
<td>189 (69.7)</td>
<td></td>
</tr>
<tr>
<td>Drugs abuse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>196 (57.6)</td>
<td>47 (61.8)</td>
<td>149 (56.4)</td>
<td>0.401</td>
</tr>
<tr>
<td>No</td>
<td>144 (42.4)</td>
<td>29 (38.2)</td>
<td>115 (43.6)</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>97 (28.5)</td>
<td>23 (30.2)</td>
<td>74 (28.0)</td>
<td>0.704</td>
</tr>
<tr>
<td>No</td>
<td>243 (71.5)</td>
<td>53 (69.7)</td>
<td>190 (72)</td>
<td></td>
</tr>
<tr>
<td>HBV ²,³</td>
<td>17 (5)</td>
<td>3 (3.94)</td>
<td>14 (5.3)</td>
<td>0.449</td>
</tr>
<tr>
<td>HCV ⁴</td>
<td>170 (50)</td>
<td>39 (51.3)</td>
<td>37 (48.7)</td>
<td>0.795</td>
</tr>
<tr>
<td>Municipality (inhab)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50000</td>
<td>194 (57.0)</td>
<td>45 (59.2)</td>
<td>149 (56.4)</td>
<td>0.667</td>
</tr>
<tr>
<td>&lt;50000</td>
<td>146 (43.0)</td>
<td>31 (40.8)</td>
<td>115 (43.6)</td>
<td></td>
</tr>
<tr>
<td>Transmission group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual</td>
<td>62 (18.2)</td>
<td>11 (14.5)</td>
<td>51 (19.3)</td>
<td>0.390</td>
</tr>
<tr>
<td>Homosexual</td>
<td>15 (4.4)</td>
<td>1 (1.3)</td>
<td>14 (5.3)</td>
<td></td>
</tr>
<tr>
<td>IDU ⁵</td>
<td>194 (57.1)</td>
<td>47 (61.8)</td>
<td>147 (55.7)</td>
<td></td>
</tr>
<tr>
<td>Bisexual</td>
<td>5 (1.5)</td>
<td>2 (2.6)</td>
<td>3 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td>3 (0.9)</td>
<td>0 (0)</td>
<td>3 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>61 (18)</td>
<td>15 (19.7)</td>
<td>46 (17.4)</td>
<td>0.795</td>
</tr>
</tbody>
</table>

1: AIDS criteria: Centers for Disease Control and Prevention (CDC) category C; 2: Hepatitis B virus infection; 3: Fisher Exact test; 4: Hepatitis C virus infection; 5: Injection drug user.

### Table 2: Pattern of IgG antibody titers to \textit{B. henselae} and \textit{B. quintana} in the study population (N= 340)

<table>
<thead>
<tr>
<th>B. henselae titers</th>
<th>B. quintana titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1/64</td>
<td>264 0 1 0</td>
</tr>
<tr>
<td>1/64</td>
<td>28  29  0  0</td>
</tr>
<tr>
<td>1/128</td>
<td>4   6  0  0</td>
</tr>
<tr>
<td>1/256</td>
<td>1   5  0  0</td>
</tr>
<tr>
<td>1/512</td>
<td>0   1  1  0</td>
</tr>
<tr>
<td>1/1024</td>
<td>0   0  1  1</td>
</tr>
</tbody>
</table>
been observed between both); however, there are studies that have a direct impact on increased infection can last longer or that these patients might become in contact more easily with some of the factors which is superior to that observed in the healthy population [13,19,25], with the exception of that described in some other studies [25]. Some authors consider HIV patients as a risk group for Bartonella infection [34,35] (characteristic personal and hygienic habits); however, other authors do not consider this infection risk to be greater [20,34].

In our experience, a previous study carried out on a healthy population sample from the same area show a seroprevalence of 22.4% to Bartonella species in HIV patients, which is superior to that observed in the healthy population [13,19,25], with the exception of that described in some other studies [25]. Some authors consider HIV patients as a risk group for Bartonella infection [34,35] (characteristic personal and hygienic habits); however, other authors do not consider this infection risk to be greater [20,34].

Our study highlights in the first place an antibody seroprevalence of 22.4% to Bartonella species in HIV patients, which is superior to that observed in the healthy population [13,19,25], with the exception of that described in some other studies [25]. Some authors consider HIV patients as a risk group for Bartonella infection [34,35] (characteristic personal and hygienic habits); however, other authors do not consider this infection risk to be greater [20,34].

In our experience, a previous study carried out on a healthy population sample from the same area show a seroprevalence of 22.4% to Bartonella species in HIV patients, which is superior to that observed in the healthy population [13,19,25], with the exception of that described in some other studies [25]. Some authors consider HIV patients as a risk group for Bartonella infection [34,35] (characteristic personal and hygienic habits); however, other authors do not consider this infection risk to be greater [20,34].

In conclusion, the seroprevalence of Bartonella spp. among HIV infected patients is greater than that of the healthy population of the same area and thus Bartonella infection should be considered in HIV patients.
Competing interests
Immaculada Pons, Isabel Sanfeliu, María Mercedes Nogueras, Montserrat Sala, Manuel Cervantes, M José Amengual and Ferran Segura: The authors declare that they have no competing interests.

Authors' contributions
IP carried out the analysis and interpretation of data, serological technique and the preparation and revision of manuscript.

IS participated in the study concept and design, serological technique and revision of manuscript.

MMN participated in the analysis of data and revision of manuscript.

MS participated in acquisition of epidemiological and clinical data.

MC participated in acquisition of epidemiological and clinical data.

MIA participated in the acquisition of data from laboratory.

FS participated in the study concept and design, and revision of manuscript.

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References

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