**B-Cell Non-Hodgkin Lymphoma linked to *Coxiella burnetii***

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Key points

- *Coxiella burnetii* is associated with an increased risk of lymphoma, its presence in the tumor microenvironment may favor lymphomagenesis.

- Lymphoma has to be considered in patients with Q fever and lymphoid disorders, especially those with persistent focalized infections.
ABSTRACT

Bacteria can induce human lymphomas, while lymphoproliferative disorders have been described in patients with Q fever. We observed a lymphoma in a patient with Q fever that prompted us to investigate the association between the two diseases. We screened 1,468 consecutive patients of the 2004-2014 French National Referral Center for Q fever database. The standardized incidence ratios (SIR) of diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) were calculated comparatively to the 2012 Francim registry. The presence of Coxiella burnetii was tested using immunofluorescence and fluorescence in situ hybridization using a specific 16S rRNA probe and genomic DNA probe. Seven patients (0.48%) presented mature B-cell lymphoma consisting of 6 DLBCL and 1 FL. An excess risk of DLBCL and FL was found in Q fever patients compared to the general population (SIR [95% confidence interval], 25.4 [11.4-56.4] and 6.7 [0.9-47.9], respectively). C. burnetii was detected in CD68+ macrophages within both lymphoma and lymphadenitis tissues but localization in CD123+ plasmacytoid dendritic cells (pDCs) was found only in lymphoma tissues. Q fever patients with persistent focalized infection were found more at risk of lymphoma (hazard ratio 9.35 [1.10-79.4]). Interleukin-10 overproduction (P = .0003) was found in patients developing lymphoma. These results suggest that C. burnetii should be added to the list of bacteria that promote human B-cell non-Hodgkin lymphoma, possibly by the infection of pDCs and IL10 overproduction. Screening for early lymphoma diagnosis should be considered in the management of patients with Q fever, especially those with persistent focalized infections.
INTRODUCTION

The incidence of non-Hodgkin lymphoma (NHL) is increasing in many regions making it a global health challenge for the coming years and is still associated with significant mortality. Risk factors include mainly infection, immunosuppression (HIV, organ-transplant recipients, high-dose chemotherapy, stem-cell transplantation and inherited immunodeficiency syndromes) or autoimmune diseases. Recent data from the InterLymph consortium reported that some risk factors such as family history of NHL are common among NHL subtypes whereas others such as HIV or hepatitis C seropositivity appeared to be distinct among individuals or a few subtypes. Hereditary factors supporting a genetic predisposition include tumor necrosis factor (TNF) and interleukin-10 (IL10) polymorphisms, suggesting that these pro- or anti-inflammatory cytokines are critical in NHL physiopathology. Besides viruses, bacterial infections play a role in the development of some B-cell NHL, either by inhibition of immune function or by induction of chronic inflammatory response. Helicobacter pylori is associated with gastric B-cell lymphoma, Campylobacter jejuni with immunoproliferative small intestinal disease, Borrelia burgdorferi with cutaneous B-cell lymphoma, and Chlamydia psittaci with ocular adnexal lymphoma.

Q fever is a zoonosis caused by the intracellular bacterium Coxiella burnetii. The primary infection, symptomatic in 10-60% of cases (then called acute Q fever), usually resolves spontaneously in a few weeks. In less than 5% of cases, the infection persists mainly as endocarditis or vascular infection. Persistent lymphadenitis has also been reported with detection of the bacterium in the excised lymph node (supplemental Table 1 available at the Blood Web site). While C. burnetii is known to infect myeloid cells such as monocytes and macrophages, several lymphoid disorders have been reported in the course of Q fever including mononucleosis syndrome, auto-immune manifestations and monoclonal
Moreover, twenty-two cases of lymphoproliferative disease, originating from B cells in all investigated cases, have been associated with Q fever (supplemental Table 2). Lymphoma, however, was previously considered to be a risk factor of persistent Q fever rather than a consequence of the infection.

We previously reported a case of Q fever vascular infection revealed by an aorto-enteric fistula. This patient, while improving under antibiotics, developed lymphadenitis near the site of infection, corresponding to a follicular B-cell lymphoma (FL). \textit{C. burnetii} was identified in the lymphoma tissue sample by fluorescent \textit{in situ} hybridization (FISH). This index case prompted us to i) collect cases of patients developing lymphoma after \textit{C. burnetii} primary infection in order to assess a possible excess risk of B-cell lymphoma in Q fever patients, ii) to test the presence of the bacterium in lymphoma biopsies, iii) to evaluate the IL10 production in Q fever patients with lymphoma and iv) to investigate whether patients with persistent focalized infection were more at risk of lymphoma than acute Q fever patients.

\section*{METHODS}

\textbf{French National Referral center for Q Fever cohort}

The French National Referral center for Q Fever cohort database includes all patients with a possible or definite \textit{C. burnetii} infection diagnosed since 2004 in our center. Patients are included in the database if they are screened positive for \textit{C. burnetii} in case of specific demand or syndromic diagnosis (blood culture-negative endocarditis, pericarditis, uveitis and central nervous system infection) and systematically on the following biopsies (osteoarticular samples, lymph nodes, skin samples, pharyngeal swabs, cardiac valve and vascular samples). Moreover, \textit{C. burnetii} is also systematically sought for in all sera sent for \textit{Bartonella}, \textit{Rickettsia}, \textit{Francisella}, \textit{Anaplasma} and \textit{Ehrlichia} testing.
Our center receives over 10,000 samples for *C. burnetii* testing each year\textsuperscript{26} from all over France and some other countries. The reasons for *C. burnetii* testing include i) initial screening test, ii) a confirmatory test after a positive initial local test, iii) suspicion of persistent infection or iv) follow-up or discordance between clinical presentation and local serological results (supplemental Table 3). Clinical data are systematically collected for positive patients over the phone and added to our computerized database.

**Diagnosis of *C. burnetii* infection**

Serology and molecular detection of *C. burnetii* have been performed as previously described.\textsuperscript{27,28} Acute Q fever was defined by the association of clinical symptoms (fever, hepatitis and/or pneumonia) with the serological criteria of a phase II IgG titer $\geq 200$ and a phase II IgM titer $\geq 50$\textsuperscript{11} seroconversion or a positive PCR and no endocarditis.\textsuperscript{29} Q fever endocarditis and vascular infection were defined according to recently updated criteria.\textsuperscript{30} Q fever lymphadenitis was defined as lymphadenitis associated with a serology consistent with acute Q fever (acute Q fever lymphadenitis) or with phase I IgG $\geq 800$ or a positive test on lymph node (PCR, culture, immunohistochemistry, FISH).\textsuperscript{12-14} All atypical cases were discussed with an expert (DR) and included in the cohort only if a possible or definite *C. burnetii* infection was retained.

**Diagnosis of lymphoma**

Formalin-fixed and paraffin-embedded biopsy samples were analyzed by an expert hematopathologist (LX) in all cases to confirm the diagnosis of lymphoma. Lymphoma specimens were diagnosed and typed according to World Health Organization criteria\textsuperscript{31} using morphological examination and standard immunohistochemistry. The tissue sections were tested for Epstein Barr virus (EBV) by FISH using the EBER probe (Dakopatts, Glostrup,
Denmark). FISH analysis was performed using BCL2, BCL6 and MYC break-apart probes as recommended by the manufacturer (VYSIS, Abbott, Rungis, France).

**Detection of *C. burnetii* in lymphoma biopsy specimens**

Immunofluorescence (IF), 16S rRNA FISH and genomic DNA FISH were performed as previously reported. For *C. burnetii*-specific FISH, the probes CB-440 (5’- CTTGAGAATTTCTTCCCC -3’) and CB-1348 (5’- CACCGCGACATGCTGATTCGCG -3’) specifically target the *C. burnetii* 16S rRNA sequences. We also used (i) EUB-338, which is complementary to a conserved region of the bacterial 16S rRNA molecule and specific for most Eubacteria, and (ii) non EUB-338 to exclude nonsense hybridization. Probes targeting the genomic sequences of *C. burnetii* were generated by indirect labeling of the DNA with fluorescent dyes (ARES DNA labeling, Molecular Probes, Eugene, Oregon). The slides were blocked in 3% BSA in PBS-0.1% Tween 20 before IF using primary antibodies (supplemental Table 4) followed by goat anti-rabbit IgG or goat anti-mouse IgG conjugated to Alexa Fluor 555 or Alexa Fluor 647. The slides were rinsed with distilled water, air-dried and mounted with the nucleic acid stain 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) from a ready-to-use solution, ProLong Gold Antifade Reagent (Molecular Probes, Cergy-Pontoise, France). The imaging system was driven by Leica MetaMorph® (version 1.6.0; Molecular Devices, Sunnyvale, CA, USA), and confocal analysis was performed using a Leica TCS 4DA confocal microscope. The method used to quantify the immune cell subset infected by *C. burnetii* is detailed in the supplemental data. Twenty-two lymphoma samples from patients without Q fever were included as controls (supplemental Table 5).

**Cytokine measurements**
IL10 amount in sera was determined using specific immunoassays (R&D Systems) according to the manufacturer’s recommendations. IL10 and TNF were measured in supernatants from non-stimulated and C. burnetii stimulated peripheral blood mononuclear cells (PBMC) as previously described.  

**Statistical analysis**

The incidence rate and its 95% confidence interval (CI) were computed for cases of DLBCL occurring during follow-up in the study population diagnosed between 2004 and 2014 (N=1,468) at the French national reference center of Q fever. Patients were enrolled between January 2004 and December 2014, and they were followed up at most until the end of March 2015, which was used as censoring date. The person-years for the Q fever database were calculated as the total sum of the number of years between Q fever diagnosis of each patient and either his lymphoma diagnosis or his last available follow-up record or the censoring date. To confirm the significant elevation of the risk of DLBCL and FL in patients with Q fever compared with the French general population, we computed the standardized incidence ratios (SIR) and their 95% confidence intervals, taking into account the difference in age-gender distribution between the Q fever cohort and the general population. The data for the general population were extracted from the Francim national registry reporting the estimation of the incidence of cancers in France in 2012. Comorbidities and immunodeficiency were not adjusted for as they were not mentioned in this report. A Cox proportional-hazards regression model was used to estimate the strength of the association between C. burnetii persistent focalized infection and occurrence of lymphoma. A sensitivity analysis was performed by removing from the dataset those patients who experienced both acute Q fever and persistent focalized infections to verify the robustness of the results. The Stata/SE 12.1 software (StataCorp LP, College Station, USA) was used for these analyses.
Ethical considerations

The study was approved by the local ethics committee (Comité de Protection des Personnes Sud Méditerranée 1) under registration number 1355 and by the French National Drugs and Health Products Agency. All patients gave informed consent, in accordance with the Declaration of Helsinki.

RESULTS

Index case

In August 2011, a 78-year-old man presented with Q fever abdominal aortic vascular infection complicated by an aorto-enteric fistula.\(^\text{25}\) *C. burnetii* was isolated from the resected vascular material by culture, polymerase chain reaction (PCR) and genome sequencing. Six months later, F\(^{18}\) fluorodeoxyglucose positron emission tomography identified a latero-aortic mass close to the infected focus with strong hypermetabolic uptake and several mesenteric lymph nodes. One year later, while the infection had improved clinically and serologically, these anomalies worsened (supplemental Figure II,II,III) such that a CT-scan-guided biopsy targeting the latero-aortic mass close to the vascular infected site (supplemental Figure 1III) identified a low-grade follicular B-cell lymphoma with FISH detection of *C. burnetii* in CD68\(^+\) macrophages.

Frequency of lymphoma among Q fever patients

Between 2004 and 2014, 93,166 individuals were tested for *C. burnetii* in our center. 91,628 individuals had negative serology and were not included in our cohort database (Figure 1). The age/gender distribution of these patients assessed but who were determined not to have Q fever was (mean ± standard deviation) 52.3 years (20.8 years) including 48,241 men (52.6%),
respectively. Among the 1,538 individuals from our cohort database, 70 were excluded after detailed medical records analysis (Figure 1) so that 1,468 individuals were considered infected with a mean age of 50.50 ± 17.07 years and involving 998 men (68%). Patients infected were significantly younger (two-tailed t-test, $P < 0.0001$) and more frequently male (two-tailed Chi-squared test, $P < .0001$) than patients assessed but who were determined not to have Q fever and not included in the parent database. Among the 1,468 patients included, 1,028 (70%) had an acute Q fever without progression to persistent focalized infection and 440 (30%) had a persistent focalized infection including 68 (4.6%) with initial acute Q fever. Among the 1,468 Q fever patients, we found seven (0.48%) patients including the index case with a diagnosis of lymphoma after $C. burnetii$ primary infection.

**Increased incidence of DLBCL and FL in Q fever patients compared to the general population**

Overall, DLBCL was found in six out of the 1,468 screened Q fever patients. The crude incidence rate [95% CI] was 280 [126-624] per 100,000 person-years (PY) in Q fever patients, and 274 [103-730] for men and 294 [74-1177] for women; whereas the crude incidence rate/100,000 PY in the general population was 8 for men and 5 for women. The SIR [95% CI] was 25.4 [11.4-56.4] (20.3 [7.6-54.1] for men and 50.6 [12.6-202.2] for women but the difference was not significant). Only one patient included in our cohort was diagnosed with a follicular lymphoma, corresponding to a SIR [95% CI] of 6.7 [0.9-47.9].

**Clinicopathological characteristics**

Among the seven patients analyzed, the mean age (±SD)[range] at the time of Q fever diagnosis was 62.4 ± 11.6 [52-76] years and 5 (71%) patients were males. None of the patients were immunocompromised nor had a risk factor for lymphoma. Among the 7
reported patients, 4 were in the setting of endocarditis, 1 of vascular infection, 1 of acute Q fever and 1 patient presented polyadenopathies. Clinical features including anatomical location and chronological history are summarized in Table 1 and supplemental Figure 2.

All seven patients presented mature B-cell NHL, including six DLBCL (supplemental Figure 3) and one low-grade (1-2) FL. All patients presented elevated LDH and Beta-2 microglobulin at lymphoma diagnosis. Ann Arbor staging at diagnosis was III in 2 patients and IV in 5 patients. Bcl-2 and Bcl-6 translocations were detected in two patients. EBV was negative in all lymphoma tissue samples. Details about DLBCL subtyping, and clinical treatment and outcome are provided in supplemental Methods & supplemental Figure 4, and supplemental Table 6, respectively.

**C. burnetii** is present in both macrophages and plasmacytoid dendritic cells of lymphoma tumors

Presumably viable *C. burnetii* was detected in four of the seven patients with available tissue samples by all of the three fluorescent methods (IF, FISH targeting *C. burnetii* 16S rRNA and FISH targeting *C. burnetii* genomic DNA (supplemental Figure 5 & supplemental Table 7)). In these four patients, the three fluorescent signals colocalized and demonstrated multiple rounded intracytoplasmic structures around the DAPI-stained nuclei (supplemental Figure 5a). Twenty-two control lymph node biopsies from patients without Q fever and presenting with various types of B-cell, T-cell, or Hodgkin’s lymphoma were treated in parallel experiments, and no specific signals were detected in the FISH/IF assays (supplemental Figure 5b & supplemental Table 5).

We investigated the cellular compartment harboring *C. burnetii* using a combination of double staining and computerized microscopic analysis (supplemental Table 8). Since the distribution of the infected cells within an individual tumor was heterogeneous, the
observation of infected cell subsets was focused on two distinct regions of lymphoma tissue with high content of infected cells. *C. burnetii* was found in 11% and 27% of CD68+ cells of each regions respectively (supplemental Figures 5c and 5d). In addition, *C. burnetii* was present in 100% of CD123+ plasmacytoid dendritic cells (pDCs, supplemental Figure 5e). No *C. burnetii* were detected in CD20+ lymphoma cells, CD3+ T-cells and S-100 protein+ dendritic cells (supplemental Figure 5f). Confocal microscopic analysis confirmed the presence of *C. burnetii* using specific RNA probes inside the cytoplasmic vacuoles of infected cells (Videos 1, 2 & 3). Among the 4 FISH-positive biopsies, PCR was negative in 2, immunohistochemistry in 4 and culture in 2. *C. burnetii* was not detected by PCR (1 sample) or immunohistochemistry (4 samples) in the 3 FISH-negative samples.

As a control, we tested 3 patients with Q fever lymphadenitis but without lymphoma. *C. burnetii* was detected in several CD68+ cells in 2 patients. In contrast to lymphoma samples, CD123+ pDCs cells were rare and none of them were infected (supplemental Figure 6). Altogether these findings suggest a specific pDCs localization of *C. burnetii* in the microenvironment of lymphomas occurring in the setting of *C. burnetii* infection.

**Interleukin-10 production in Q fever patients with lymphoma**

Interleukin-10 (IL10) was tested as a putative instrumental cytokine in 386 sera from 79 patients and was found to be highly elevated in patients with lymphoma (median 25.3 pg/ml [interquartile range 17.7-77.1]) compared to patients with lymphadenitis (15.5 [8.8-34.8]), patients with acute Q fever without valvulopathy and no progression to persistent focalized infection (10.4 [7.3-23.0]) or healthy controls (6.1 [5.1-8.9], *P* < .05, two-tailed Kruskal-Wallis test with Dunn’s multiple comparisons test, Figure 2 & supplemental Figure 7). Among Q fever patients, those with lymphoma have very significantly higher IL10 levels than
those without lymphoma (25.3 [17.7-77.1] vs. 12.7 [8.1-32.9], \( P = .0003 \), two-tailed Mann-Whitney test). IL10 production by non-stimulated PBMC was highly increased in patients with lymphoma while TNF production was very low even after stimulation by \( C.\ burnetii \) (\( p < 0.05 \), supplemental Figure 8).

**Risk factors of lymphoma among Q fever patients**

Comparing Q fever patients with lymphoma, patients with lymphadenitis who did not develop lymphoma and other Q fever patients (Table 2), we found that persistent focalized infection was a risk factor for lymphadenitis (Odds ratio, 1.78; 95% CI, 1.04-3.03; \( P = .047 \)) but to a lesser extent than for lymphoma (OR, 14.54; 95% CI, 2.14-337.7; \( P = .007 \)). Indeed, patients with persistent focalized infections were more likely to develop lymphoma (6/440 (1.4%)) than acute Q fever patients without known progression to persistent focalized infection (1/1028 (0.1%), two-tailed Fisher exact test, \( P = .007 \)). This was confirmed by a Cox model (hazard ratio (HR), 9.35; 95% CI, 1.10-79.4, \( P = .041 \)) and after the sensitivity analysis excluding patients with acute Q fever progressing to a persistent focalized infection (HR, 9.41; 95% CI, 1.07-82.9, \( P = .043 \)).

**DISCUSSION**

In this study, we found 7 occurrences of B-cell NHL after \( C.\ burnetii \) primary infection in patients included in the 2004-2014 French National referral center for Q fever cohort database. The strength of this association was suggested by a 25-fold excess risk of DLBCL in Q fever patients compared to the general population in France. The inclusion period of our study (2004-2014) was consistent with that of the French registry used for comparison (2012). This size effect is unlikely to be attributable to plausible confounding as it was adjusted for age and sex, and is far greater than the mean annual increase incidence.
(3%) in the French general population between 2004 and 2014. This excess risk was consistent with other bacterium-related cancers.  

The detection of the bacterium in lymphoma and lymphadenitis biopsies was ascertained by three different techniques (16S rRNA FISH, genomic DNA FISH and IF) with confocal microscopy validation. Several negative controls confirmed the specificity of our technique (supplemental Table 5). Moreover, presumably viable *C. burnetii* were observed in the cytoplasm of macrophages in multiple rounded structures consistent with intracytoplasmic vacuoles typical of the *C. burnetii* infection. The very high sensitivity of fluorescence-based techniques (IF and FISH) was critical in the detection of the bacterium as all other techniques were negative. The use of a universal eubacterial probe for FISH detection (Eub-338) allowed us to exclude other infectious etiologies in biopsy samples, such as *H. pylori, C. jejuni* or *B. burgdorferi*, since all Eub-338 positive signals colocalized with *C. burnetii* specific FISH. 

The specific pDC infection and increased IL10 levels in patients with Q fever-associated lymphoma suggest an alteration of the immune signals within the lymphoma microenvironment. IL10 was previously shown to be a B-cell growth factor with immunosuppressive properties that up-regulates bcl-2 expression. Viral IL10 is critical for B-cell transformation and proliferation by EBV. High IL10 levels were found in the sera of NHL patients and were associated with poor prognosis. In Q fever, overproduction of IL10 by infected monocytes is critical in sustaining replication of the bacterium, and is associated with an inhibition of the microbicidal activity of macrophages. In acute Q fever patients with valvulopathy and in endocarditis, and persistent elevated IL10 levels are predictive of relapse. A putative scenario could be proposed in which *C. burnetii*-infected monocytes and pDCs induce impairment of the immune system favoring lymphoma occurrence. In primary or
persistent Q fever, infected monocytes migrate via lymphatic vessels to the lymph nodes. The influence of the lymph node microenvironment, including apoptotic lymphocytes from germinal centers, may induce a specific polarization of the monocytes/macrophages towards an M2 profile, characterized by a higher production of anti-inflammatory cytokines and IL10. Our observation of intermediate IL10 levels in \textit{C. burnetii} related lymphadenitis, which occurs more frequently in the setting of persistent infection, suggests that it might represent a pre-lymphoma condition. Infection of pDCs may represent a critical step towards lymphomagenesis as these cells were not infected in patients with lymphadenitis. Infected pDCs can also induce an increase in IL10 secretion via type 1 interferon production and upregulation of inducible co-stimulating ligand expression. Thus, monocytes and plasmacytoid dendritic cells are probably both responsible for the impairment of the immune system. IL10 in turn stimulates the replication of \textit{C. burnetii} in monocytes and prevents apoptosis of germinal center B cells by overexpression of the Bcl-2 protein. IL10-mediated immune impairment may be favorable to both \textit{C. burnetii} replication and lymphoma growth. Our observation of bcl-2 and bcl-6 rearrangements suggests that the early steps of lymphomagenesis may be shared in Q fever patients and in the general population. This hypothesis is also consistent with the localization of \textit{C. burnetii} in the micro-environment but not in the neoplastic cells. Of note, the presence of \textit{C. burnetii} in lymphoma tissues cannot be explained by a clinical setting of immunosuppression in our patients. The absence of EBV in \textit{C. burnetii} infected tumors also argues against a pre-existing immunosuppression that could have favored \textit{C. burnetii} persistence in the tumor microenvironment.

The InterLymph consortium reported that risk factors of lymphoid neoplasms are related to either specific NHL subtypes or to virtually all lymphomas. Considering the 7 included cases (Table 1) and 4 additional cases from France, Spain and Israel (supplemental Table 6), we found 6 DLBCL, 2 FL, 2 marginal zone lymphomas and 1 lymphoplasmocytic
lymphoma. In addition, at least 5 hairy cell leukemias have been reported in the literature\(^1\)\(^9\),\(^2\)\(^1\)(supplemental Table 2). Altogether, the 16 clinical cases of Q fever associated lymphoproliferative lesions reported in the present work and in the literature correspond to mature B-cell neoplasms.\(^3\)\(^7\) Larger series are needed to confirm that the risk of lymphoma occurrence in Q fever patients does not involve precursor lymphoid neoplasms nor T-cell lymphomas.

General farm worker was identified by the InterLymph consortium as an independent risk factor affecting overall NHL risk\(^4\) and particularly hairy cell leukemia.\(^5\)\(^8\) On the other hand, occupation as a farmer is the main risk factor for Q fever, associated with hairy cell leukemia in the literature.\(^1\)\(^9\),\(^2\)\(^1\),\(^2\)\(^2\) Altogether these findings suggest that \textit{C. burnetii} infection could be responsible, at least in part, for the excess risk of NHL in farm workers. While our data are supportive of an association between \textit{C. burnetii} and B-cell NHL, other lymphoma risk factors could not be excluded. Indeed, this was a retrospective study and some factors like family history of hematologic malignancy were not collected.\(^4\) Future prospective studies are needed to confirm the accurate incidence rate, as our cohort possibly suffered from center bias that probably tends to overestimate incidence. Conversely, the incidence may have been underestimated as the Q fever-lymphoma association was unknown, so that some lymphoma cases possibly diagnosed several months to years after Q fever may have been missed.

For the first time in the literature, we provide evidence that \textit{C. burnetii} may be a lymphoma cofactor. This confirms other abundant data linking this bacterium to lymphoproliferative disorders (supplemental Table 2) including very recent reports from the Dutch outbreak.\(^2\)\(^2\) Although we cannot conclude that Q fever directly causes lymphoma, our results are unlikely to be due to chance since several criteria for causation are fulfilled.\(^3\)\(^8\),\(^5\)\(^9\) Additional reports of cases treated by antibiotics alone would provide greater support for the
purported association. Disregarding the causality issue, the link between Q fever and lymphoma that we evidence herein should not been neglected since early diagnosis of lymphoma would result in improved outcomes of Q fever patients.\textsuperscript{1} Moreover, the management of patients with B-cell NHL would be improved by the detection of \textit{C. burnetii} infection in endemic areas.
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Author contributions

CM and MM wrote the article. GA performed the FISH analysis and wrote the article. AG, HD, MD and AM provided clinical information. GR made the challenging diagnostic biopsy of the index case (13mm from the aortic wall!). SC performed the PET scan of the index patient. MPC and CP performed the statistical analysis. PR determined the genetic signatures of the lymphoma biopsy samples. HL performed immunohistochemistry on the lymphoma biopsy samples. BN participated in the discussion. JLM participated in dendritic cell investigation, analysis of the results and in the discussion. LX performed histopathological analysis of all biopsy samples, wrote the article and participated in the discussion. DR designed and supervised the study. CM and MM had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of its analysis.
Conflicts of interest

The authors have no conflicts of interest to declare. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.
References


<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (Q fever)/Sex</th>
<th>Country (region)</th>
<th>Type of Coxiella burnetii infection (score)\textsuperscript{a}</th>
<th>First lymphadenopathy or mass/anatomical proximity with Q fever</th>
<th>Delay between Q fever and lymphoma (months)/chronology consistent with temporal criterium\textsuperscript{c}</th>
<th>Histology/Ann Arbor stage</th>
<th>Detection of C. burnetii in the lymphoma biopsy specimen</th>
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<td>France (Provence-Alpes-Côte-d’Azur)</td>
<td>Definite vascular infection\textsuperscript{d} (A1B2C2)</td>
<td>Latero-aortic\textsuperscript{d}/Yes (see suppl Fig. 1)</td>
<td>20m/Yes</td>
<td>Low grade Follicular B cell lymphoma/III</td>
<td>Yes</td>
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<td>Patient 2</td>
<td>65/M</td>
<td>France (Alsace)</td>
<td>Definite endocarditis (A0B2C3)\textsuperscript{e}</td>
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<td>Splenic/Unknown</td>
<td>0.5m/Yes</td>
<td>Diffuse Large B cell lymphoma IV</td>
<td>Yes</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Definite endocarditis on prosthetic mitral valve</td>
<td>Mediastinal/Yes</td>
<td>1m/Yes</td>
<td>Diffuse large B cell lymphoma IV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>70/M</td>
<td>France (Provence-Alpes-Côte-d’Azur) 1600, 0, 0/3200, 0, 0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Patient 5</td>
<td>Chronic Q fever lymphadenitis</td>
<td>Cervical/Yes</td>
<td>0.5m/Yes</td>
<td>Diffuse Large B cell lymphoma IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52/M</td>
<td>France (Pays de la Loire) 800, 0, 0/1600, 0, 0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Patient 6</td>
<td>Definite endocarditis of prosthetic aortic valve contiguous to left atrial appendage</td>
<td>Mediastinal mass</td>
<td>-4m/Yes ++</td>
<td>Diffuse Large B cell lymphoma IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58/M</td>
<td>France (Rhône-Alpes) (A0B1C3) 800, 0, 400/1600, 0, 800</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Patient 7</td>
<td>Acute Q fever valvulopathy progressing to possible endocarditis of native mitral valve</td>
<td>Mediastinal and right lung tumor</td>
<td>4m after acute Q fever and 1m after endocarditis diagnosis/Yes</td>
<td>Diffuse Large B cell lymphoma IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72/F</td>
<td>France (Provence-Alpes-Côte-d’Azur) (100,400,0/200,800,0)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
(A0B0C3)

1600, 50, 50/3200, 100,

100

F: Female, M: Male, D: Doxycycline, OHCQ: hydroxychloroquine, R: Rituximab, R-CHOP: rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine and prednisolone, NA: not available. "Endocarditis and vascular infection were defined according to recently updated criteria."\textsuperscript{30} \( \text{b} \)Phase I IgG, IgM, IgA and phase II IgG, IgM and IgA. \( \text{c} \)A temporal succession was considered when \( C. \ burnetii \) primary-infection preceded lymphadenitis and lymphoma. \( \text{d} \)Index case with positive \( C. \ burnetii \) culture and PCR on surgical blood clot. \( \text{e} \)Positive \( C. \ burnetii \) PCR on resected valve. \( \text{f} \)Positive \( C. \ burnetii \) PCR on blood. \( \text{g} \)As Q fever endocarditis can last for years, a diagnosis of lymphoma 4 months before Q fever endocarditis diagnosis is consistent with the temporality criteria.
Table 2. Comparisons of Q fever patients according to lymphoma or benign lymphadenitis diagnoses

<table>
<thead>
<tr>
<th></th>
<th>All (n = 1,468)</th>
<th>Lymphoma (A&lt;sup&gt;a&lt;/sup&gt;) (n = 7)</th>
<th>Lymphadenitis (B&lt;sup&gt;b&lt;/sup&gt;) (n = 59)</th>
<th>Q fever without lymphoma nor lymphadenitis (C&lt;sup&gt;c&lt;/sup&gt;) (n = 1402)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.5 ± 17.1</td>
<td>62.7 ± 12.0</td>
<td>50.0 ± 20.1</td>
<td>50.4 ± 16.9</td>
<td>0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male gender</td>
<td>998 (68)</td>
<td>5 (71)</td>
<td>50 (85)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>943 (67)&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.018&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immunodepression</td>
<td>159 (11)</td>
<td>0 (0)</td>
<td>10 (17)</td>
<td>149 (11)</td>
<td>0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>History of valvulopathy</td>
<td>361 (25)</td>
<td>4 (57)</td>
<td>13 (22)</td>
<td>344 (25)</td>
<td>0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Form of <em>Coxiella burnetii</em> infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute Q fever</td>
<td>1096 (75)</td>
<td>2 (29)</td>
<td>41 (70)</td>
<td>1053 (75)</td>
<td>0.012&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acute Q fever without known progression to persistent focalized infection</td>
<td>1028 (70)</td>
<td>1 (14)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34 (58)</td>
<td>993 (71)&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acute Q fever with progression to persistent focalized infection</td>
<td>68 (5)</td>
<td>1 (14)</td>
<td>7 (12)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60 (4)&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.012&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Persistent focalized infection</td>
<td>440 (30)</td>
<td>6 (86)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25 (42)</td>
<td>409 (29)&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>331 (23)</td>
<td>4 (57)</td>
<td>11 (19)</td>
<td>316 (22)</td>
<td>0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vascular infection</td>
<td>68 (5)</td>
<td>1 (14)</td>
<td>2 (3)</td>
<td>65 (5)</td>
<td>0.43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Condition</td>
<td>Column 1</td>
<td>Column 2</td>
<td>Column 3</td>
<td>Column 4</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
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<td>---------</td>
</tr>
<tr>
<td>Osteoarticular infections</td>
<td>24 (1.6)</td>
<td>0 (0)</td>
<td>4 (7)</td>
<td>20 (1.5)</td>
<td>0.006c</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>8 (0.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>8 (0.6)</td>
<td>0.82c</td>
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<tr>
<td>Pregnancy</td>
<td>13 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (1)</td>
<td>0.73c</td>
</tr>
<tr>
<td>Isolated lymphadenitis</td>
<td>10 (0.7)</td>
<td>1 (14)</td>
<td>9 (15)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Localization of lymphadenopathies**

<table>
<thead>
<tr>
<th>Location</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediastinal</td>
<td>31 (2)</td>
<td>5 (71)</td>
<td>26 (44)</td>
<td>-</td>
<td>0.33d</td>
</tr>
<tr>
<td>Cervical</td>
<td>15 (1)</td>
<td>1 (14)</td>
<td>14 (24)</td>
<td>-</td>
<td>0.99d</td>
</tr>
<tr>
<td>Axillar</td>
<td>10 (1)</td>
<td>1 (14)</td>
<td>9 (15)</td>
<td>-</td>
<td>0.99d</td>
</tr>
<tr>
<td>Abdominal</td>
<td>20 (1)</td>
<td>4 (57)</td>
<td>16 (27)</td>
<td>-</td>
<td>0.23d</td>
</tr>
<tr>
<td>Inguinal</td>
<td>7 (0.5)</td>
<td>1 (14)</td>
<td>6 (10)</td>
<td>-</td>
<td>0.99d</td>
</tr>
</tbody>
</table>

*a Cells with a significant difference compared to another column are represented with the letter of the comparison column(s) with which a significant difference was found (z-test comparing column proportion with Dunn’s test for multiple comparisons). b One-way ANOVA test with Tukey’s multiple comparison test. c Chi-squared test for 3 groups. d Fisher exact test.*
**Figure legends**

**Figure 1. Study Flow chart**

*A* A patient with unexplained elevated serology was considered unexplored if he did not have transthoracic echocardiography (TTE), transeosophageal echocardiography (TEE) and PET scan. *b* 76-year old man with Phase I IgG at 25600 two years after an acute Q fever living in an endemic area (La Rochelle, region of Poitou Charente). TTE and TEE were normal. PET scan was considered as normal by local practitioner. Second look by SC at our center found hyperfixation of subclavian arteries predominant right. Combination therapy with doxycycline and hydroxychloroquine resulted in a dramatic serology decrease. *c* Osteomyelitis of the pubic symphysis. *d* Lung fibrosis (n = 5), lung pseudotumor, giant cell arteritis, chorioretinitis and chronic hepatitis (one case each).

**Figure 2. Interleukin 10 levels in Q fever patients**

Interleukin-10 was evaluated in 397 sera including 24 sera from 5 patients with lymphoma, 250 sera from 48 patients with lymphadenitis, 112 sera from 26 patients with acute Q fever without valvulopathy and without progression to persistent focalized infection and 11 healthy controls. Median and interquartile range. The horizontal line corresponds to the maximum level observed in 11 healthy controls (12pg/ml). *P < .05, ***P < .0005, ****P < .00005. Bilateral Mann-Whitney test. A gradient was observed suggesting that patients with lymphadenitis without lymphoma may represent a pre-lymphoma condition.
93,166 patients tested for *Coxiella burnetii* at the French National Referral Center (NRC) for Q Fever between 2004 and 2014

1,538 record files in the French NRC for Q Fever 2004-2014 cohort database

91,628 individuals assessed but determined not to have Q Fever

Excluded (n = 70)
- Unproven infection (n = 62)
  - Unexplored elevated serology/unavailable data (n = 39)
  - Finally considered to have past infection (n = 18)
  - Unexplained elevated serology despite TTE, TEE & PET scan? (n = 1)
  - Positive decreasing serology in babies from infected mothers (n = 2)
  - Positive serology in a patient with gut colonisation (n = 1)
  - Serology of primary infection without consistent symptoms? (n = 1)
- Negative serology (n = 4)
- Duplicated patients (n = 3)
- No sample (n = 1)

1028 acute Q fever without progression to persistent focalized infection (70%)
- 935 with good serological outcome or only one serology
- 93 with unexplored/unexplained elevated serology

440 persistent focalized infections (30%)
- 68 acute Q fever progressing to persistent focalized infection including 50 cardiovascular infections
- 372 persistent focalized infections at the first diagnostic serology (17%)
  - 329 cardiovascular infections
  - 16 osteo-articular infections
  - 10 Q fever during pregnancy
  - 4 isolated lymphadenitis
  - 4 pericarditis
  - 9 exceptional forms

79 patients with cancer or lymphoid disorder

Excluded patients (n = 7)
- Lack of data (n = 4)
- Other cancer (n = 1, pancreatic adenocarcinoma)
- History of lymphoma < 3 months prior to *Coxiella burnetii* primary infection (n = 2)
  - Caecal lymphoma (1)
  - Follicular lymphoma 6 years before (1)

72 patients with lymphoid disorders (5%)

Lymphoid disorders without lymphoma (n = 65)
- Lymphadenitis without lymphoma (59)
- Lymphopenia (3)
- Hyperlymphocytosis (2)
- Mononucleosis syndrome (1)

7 patients with lymphoma after *Coxiella burnetii* primary infection (0.5%)
- 1 with acute Q fever
- 2 endocarditis on native valve
- 2 endocarditis on prosthetic valve
- 1 vascular infection
- 1 persistent lymphadenitis

Figure 1
Figure 2

IL10 (pg/ml)

Lymphoma  
Lymphadenitis  
Acute Q fever  
Controls

****  
**
***
*

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B-cell non-Hodgkin lymphoma linked to *Coxiella burnetii*

Cléa Melenotte, Matthieu Million, Gilles Audoly, Audrey Gorse, Hervé Dutronc, Gauthier Roland, Michel Dekel, Asuncion Moreno, Serge Cammilleri, Maria Patrizia Carrieri, Camelia Protopopescu, Philippe Ruminy, Hubert Lepidi, Bertrand Nadel, Jean-Louis Mege, Luc Xerri and Didier Raoult