blood

# To the editor:

## *Coxiella burnetii* antibody seropositivity is not a risk factor for AIDS-related non-Hodgkin lymphoma

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*Coxiella burnetii* is an important human pathogen and the causative agent of Q fever, a disease that can lead to life-threatening endocarditis and other serious conditions. About 3% of the US population has antibody evidence of exposure to this agent, which can persist for months to years after infection. Recently, chronic *C burnetii* infection has been linked to the development of non-Hodgkin B-cell lymphoma (NHL).[1] This study of 1468 patients from the French National Referral Center for Q Fever database found a 25-fold increase in the risk of NHL development among Q fever patients relative to the general population. The link between Q fever and NHL development is believed to be related to plasmacytoid dendritic cell infection within lymphoid tumors and interleukin-10 (IL-10) overproduction.

HIV is also linked to NHL as progression to AIDS puts patients at increased risk for development of NHL subtypes.[2,3] A link between HIV and increased *C burnetii* seroprevalence has been suggested. Two independent studies in France and Brazil found increased *C burnetii* seroprevalence in HIV-positive individuals relative to the general population.[4-6] Conversely, a study in Spain found seroprevalence among HIV-infected IV drug users similar to that of HIV-negative IV drug users.[7] This same study and work in Tanzania found the percentage of HIV-positive individuals within cohorts of acute Q fever patients to be comparable to the general population.[7,8] Whether HIV infection alters antibody responses to *C burnetii* infection or plays a role in increased susceptibility or disease severity is not known.

The finding that HIV and chronic *C burnetii* infections are both risk factors for development of NHL, coupled with the fact that certain groups of HIV-positive individuals demonstrate increased *C burnetii* seropositivity, suggests a potential role for *C burnetii* in AIDS-related NHL. Therefore, we hypothesized that anti–*C burnetii* antibody seroprevalence would be higher in individuals with AIDS-related NHL relative to the general HIV-positive population. To test this, we performed a nested case control study using stored serum and plasma samples from 2 previously published prospective cohort studies, the District of Columbia/New York Gay Men's Cohort (DCG)[9-11] and the AIDS Cancer Cohort Study (ACC).[10-15] Patients with incident (n = 29) and prevalent (n = 39) AIDS-NHL were matched by age, sex, and CD4 count to 67 HIV-positive controls (Table 1). Prelymphoma samples had been collected a median of 1.1 (interquartile range, 0.6-1.5) years prior to NHL diagnosis or from equivalent time points for controls. Institutional review boards at the National Cancer Institute and collaborating institutions approved each study, and all participants gave written informed consent.

**Table 1. Sample set characteristics**

| Characteristics | NHL (n = 68) | Controls (n = 67) |
|---|---|---|
| Mean age, y (SD) | 41 (7) | 41 (7) |
| Males, n | 61 | 60 |
| Median CD4/μL (IQR) | 131 (32-235) | 135 (33-228) |
| **Race/ethnicity, n** | | |
| White | 45 | 43 |
| African American | 21 | 22 |
| Hispanic | 2 | 2 |
| **Study, n** | | |
| ACC | 59 | 59 |
| DCG | 9 | 8 |

IQR, interquartile range; SD, standard deviation.

Samples were analyzed for the presence of phase II anti–*C burnetii* immunoglobulin G antibodies using an enzyme-linked immunosorbent assay (ELISA; Virion/Serion, Wurzburg, Germany) according to the manufacturer's instructions with the following modifications. Samples were diluted 1:100. Optical densities were read at 405 nm using an ELx800 (BioTek). Positive and borderline samples were additionally screened for both phase I and phase II anti–*C burnetii* immunoglobulin G by immunofluorescence assay (IFA), as described previously.[16] Antibody titers ≥1:16 against phase I or phase II antigens were considered positive. Assay reproducibility was excellent based on 16 masked replicates, of which 15 were concordantly ELISA negative and 1 was concordantly borderline with IFA titers within a twofold dilution.

Nine percent (6 of 67) of HIV-positive controls were positive for anti–*C burnetii* antibodies, as compared with 7% (2 of 29) of incident and 8% (3 of 39) of prevalent AIDS-NHL cases. We used multinomial logistic regression to determine the associations between *C burnetii* seropositivity and incident or prevalent AIDS-related NHL, as well as binary logistic regression for both NHL outcomes combined. All regression models were adjusted for age at blood collection, sex, race/ethnicity (ie, white, African American, or Hispanic), CD4 count per μL and cohort (ie, DCG or ACC). Two-sided $P$ values $< 0.05$ were considered statistically significant. All analyses were performed by using Stata version 13 (Stata Corp, College Station, TX). The adjusted odds ratios (ORs) were not statistically significant: 0.85 and 1.04 for incident and prevalent AIDS-NHL, respectively (Table 2). The overall seroprevalence combining incident and prevalent cases was 7% (5 of 68) for a nonsignificant OR of 0.82. These serologic findings indicate that the development of AIDS-associated NHL is not associated with exposure to *C burnetii*.

**Table 2. *C burnetii* seropositivity in AIDS-NHL cases and HIV-infected controls**

| | ELISA | | | | IFA | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Patient group** | **(–)** | **B** | **(+)** | **Total** | **(–)** | **(+)*** | **Total†** | **Percent positive‡** | **Adjusted OR§ (95% confidence interval)** |
| HIV-infected controls | 61 | 4 | 2 | 67 | 0 | 6 | 6 | 9 | |
| **AIDS-NHL cases** | | | | | | | | | |
| Incident | 27 | 1 | 1 | 29 | 0 | 2 | 2 | 7 | 0.85 (0.15-4.66) |
| Prevalent | 36 | 2 | 1 | 39 | 0 | 3 | 3 | 8 | 1.04 (0.23-4.70) |
| Incident + prevalent | 63 | 3 | 2 | 68 | 0 | 5 | 5 | 7 | 0.82 (0.24-2.85) |

B, borderline.

*Titers ≥1:16 were considered positive.

†Only samples with borderline or positive ELISA results were validated by IFA.

‡Calculated as number of IFA-validated positive samples divided by total samples.

§ORs for *C burnetii* seropositivity adjusted for age at blood collection, sex, race/ethnicity, CD4 counts, and cohort.

*C burnetii* infection of plasmacytoid dendritic cells within lymphoid tissues and IL-10 overproduction support the development of NHL.[1] HIV infection also leads to significantly higher IL-10 serum levels.[17] As such, it is possible that IL-10 overproduction as a result of *C burnetii* infection in HIV-positive individuals is negligible relative to the HIV-induced cytokine response. Furthermore, HIV infection leads to depletion of plasmacytoid dendritic cells,[18] perhaps impeding *C burnetii* infection of these cells and hindering *C burnetii*–related NHL development. Perhaps *C burnetii* is unable to elicit a comparable cytokine response in HIV-positive individuals relative to healthy hosts owing to HIV infection–related immune system dysfunction. As such, the disease characteristics that lead to NHL in healthy hosts with Q fever may be altered in HIV-positive individuals. The possibility exists that NHL-related B-cell dysfunction may lead to diminished antibody titers against *C burnetii*. However, previously published vaccine studies suggest that although antibody responses may be less robust than in healthy individuals, NHL patients are still able to produce antibodies to influenza.[19,20] We anticipate that measuring the presence of *C burnetii* antibodies rather than comparing antibody titers reduces the possibility that NHL-related B-cell dysfunction influenced our results.

Our findings suggest a potential for increased prevalence of anti–*C burnetii* antibodies among HIV-positive individuals in the United States compared with the general population. Of the 135 samples tested, 11 (8.1%) were positive for anti–*C burnetii* antibodies, which is higher than the 3.1% among adults in the National Health and Nutrition Examination Survey in the United States during 2003 to 2004.[21] These findings support the French and Brazilian studies, which found the presence of anti–*C burnetii* antibodies to be higher in HIV-positive individuals.[4-6] Collectively, our findings suggest that rates of infection with *C burnetii* are similar among HIV patients with and without NHL, indicating that *C burnetii* infection is not a risk factor for NHL in this setting.

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**Contribution:** M.C.C., C.S.R., and G.J.K. designed the study; J.J.G., C.A.W., and C.S.R. provided blood samples; H.K.M. performed the experiments; H.K.M., L.S., M.C.C., and C.S.R. analyzed the data; H.K.M., G.J.K., and C.S.R. drafted the paper; and all authors reviewed and approved the final manuscript.

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# To the editor:

## Whole-genome noncoding sequence analysis in T-cell acute lymphoblastic leukemia identifies oncogene enhancer mutations

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Our understanding of acute lymphoblastic leukemia (ALL) has expanded tremendously in the past few years because of large-scale genomic studies.[1,2] ALL is characterized by a relatively small number of genomic alterations that disproportionally affect hematopoietic transcriptional factors.[1,3] ALL that arises from the T-cell lineage (T-ALL) vs B-cell lineage has distinctive immunophenotypes and unique genomic abnormalities.[4,5] However, the vast majority of genome sequencing and subsequent mechanistic studies of ALL singularly focus on coding regions and the role of noncoding genomic aberrations in leukemogenesis remains mostly uncharacterized with few exceptions.[6,7] There is growing evidence implicating noncoding genetic variation in human diseases, either by modulating transcriptional regulatory elements of protein-coding genes or by altering noncoding RNAs themselves.[8,9-11] Somatically acquired promoter and/or enhancer mutations have also been identified that profoundly change oncogene expression and drive tumorigenesis (eg, *TAL1* enhancer mutation in T-ALL).[6] In this study, we systematically searched for functional noncoding genomic alterations in T-ALL by whole-genome and transcriptome sequencing.

Whole-genome sequencing was performed in germ line and tumor samples from 31 Chinese children with T-ALL, 30 of whom were also profiled by transcriptome sequencing (see supplemental Materials and methods, available on the *Blood* Web site). On average, we observed 6.4 coding mutations per patient, with 9.9 and 3.2 copy number losses and gains, respectively. Consistent with previous reports,[12] coding mutations were most frequently observed in *NOTCH1*, followed by *FBXW7*, *USP7*, and *PTEN* (supplemental Figure 1; supplemental Table 1).

The majority of sequence mutations arose in noncoding region of the genome (median, 979 per patient [range, 157 to 3143]; supplemental Table 2). We used 3 parallel approaches to systematically identify potential functional noncoding mutations: (1) "hotspot analysis" to identify tight clusters of mutations in small focal regions of the DNA (21 bp), (2) "regional recurrence analysis" to identify annotated regulatory regions (eg, promoter) with significant enrichment of noncoding mutations, and (3) "transcription factor analysis" to identify mutations that potentially result in gain or loss of transcription factor binding sites. Remarkably, 3 known T-ALL oncogenes, *LMO1*, *LMO2*, and *TAL1,* were among loci with the most significant enrichment for noncoding mutations (Figure 1A). When tested for their effects on the transcription of adjacent genes, only the *TAL1* and *LMO1* mutations were significantly associated with *TAL1* and *LMO1* expression, respectively, in support of *cis*-regulating effects (Figure 1B-C; supplemental Figures 2 and 3). At the *LMO1* locus, 3 patients (9.7%) showed an identical point mutation (chr11:8289481G>A) proximal to the transcription start site (supplemental Figure 4; supplemental Table 3) that resulted in the gain of a canonical MYB binding site (AACGG), with ~120-fold increase in *LMO1* transcription in an allele-specific fashion and no effect on other adjacent genes (supplemental Figures 5 and 6). Recurring *LMO2* intronic mutations were identified in 4 patients, creating a potential binding site of the ETS factors (CATCC). Within the same intron, a fifth patient had concomitant short deletion and point mutation; however, these *LMO2* noncoding mutations were not associated with *LMO2* gene expression or alternative splicing (supplemental Figure 3). Of 15 cases with *TAL1* overexpression, 11 were positive for the *STIL-TAL1* fusion (Figure 1C) and 4 had a somatic insertion that created an MYB-mediated super enhancer (supplemental Figure 7), consistent with recent reports.[6,13]

In the independent validation cohort of 26 children with T-ALL, *LMO1* was minimally expressed in all but 3 patients, 2 of whom had the chr11:8289481 G>A enhancer mutation (supplemental Figures 8A and 9A). In the third patient, we identified an intrachromosomal inversion event that juxtaposed the active promoter of the *MED17* gene with coding sequence of *LMO1*, leading to constitutive expression of *LMO1* (supplemental Figures 8B-E, 9B, and 10). The *LMO2* noncoding mutation was observed in 3 patients, but again was not significantly associated with *LMO2* expression (supplemental Figure 11).