Re: Evidence for an Association Between *Chlamydia psittaci* and Ocular Adnexal Lymphomas

Ferreri et al. *(1)* recently reported that patients with ocular adnexal lymphoma had a high prevalence of *Chlamydia psittaci* infection in both tumor tissues and peripheral blood mononuclear cells. More recently, one study reported a similar prevalence of the infection in a Korean patient series *(2)*, whereas three other studies did not observe such an association *(3–5)*.

To investigate the reasons for these different results, our two laboratories, one in Milan (which produced the Ferrari et al. manuscript) and one in Paris, investigated both in independent blinded duplicate the presence of *C. psittaci*, *C. trachomatis*, and *C. pneumoniae* DNA in tumor samples from ophthalmologic biopsies obtained from 16 French patients with ocular adnexal lymphoma and from tissue of two control subjects with other types of lymphoproliferative disease. All 16 patients had histologically proven non-Hodgkin lymphoma of the ocular adnexa (10 at conjunctival sites and six at intraorbital sites). Ten samples from

tumor lymph node biopsies from patients diagnosed with marginal zone B-cell lymphoma ($n = 8$), splenic-type marginal zone B-cell lymphoma ($n = 1$), and follicular lymphoma ($n = 1$) were also obtained; 10 nodal biopsy samples from patients diagnosed with reactive lymphoid hyperplasia were also collected.

DNA was extracted from three 15-μm thick sections of paraffin-embedded biopsies that had been fixed in alcohol, formalin, and acetic acid, using the QiampDNA mini kit (Quiagen, Courtaboeuf, France). A multiplex touchdown enzyme time-release polymerase chain reaction (PCR) was performed that was designed to simultaneously detect the DNA sequences of *C. psittaci*, *C. pneumoniae*, and *C. trachomatis*. Briefly, 10 mL of template DNA were used in 40 mL of PCR mix that included 25 pmol of each primer *(1,6)*, 0.25 mM deoxynucleotide triphosphates, and 2 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) in a 2.5 mM $MgCl_2$ buffer. Blank reactions were inserted between samples to rule out contamination between samples. A mix of DNA extracted from cells infected by each of the three chlamydial species was included as a positive control. To determine the limit of detection of the PCR, we cloned each PCR amplicon into a plasmid that we used as a template. We were able to detect as few as 10 copies of template per sample. A second set of PCRs, using a human reference gene (GAPDH) *(6)*, was used to ensure the quality of the PCR and to prove that no PCR inhibitors were present in any sample. PCR analyses were performed in duplicate in our two independent laboratories.

C. psittaci DNA was detected in the tumor tissue of only one patient with follicular ocular adnexal lymphoma. No *C. psittaci*, *C. pneumoniae*, or *C. trachomatis* DNA sequences were detected in any of the tumor samples obtained from other patients diagnosed with ocular adnexal lymphoma, nodal lymphoma, or lymphoid hyperplasia.

In conclusion, the prevalence of *C. psittaci* infection in this series of ocular adnexal lymphoma patients was considerably lower than that reported by Ferreri et al. *(1)*. The identical results obtained by our two laboratories indicate that this finding is not due to different experimental conditions. It could be explained by a heterogeneous distribution of the bacterial infection. However, objective responses observed after *C. psittaci* eradication with doxycycline suggest the role of this microorganism in the maintenance of ocular adnexal lymphoma *(7)*.

Patricia de Cremoux
Agathe Subtil
Andrés J. M. Ferreri
Anne Vincent-Salomon
Maruilio Ponzoni
Driss Chaoui
Philippe Arnaud
Livia Lumbroso-Le Rouic
Federico Sacchetti
Rémi Dendale
Martine Thioux
Marie-Christine Escande
Marc-Henri Stern
Riccardo Dolcetti
Didier Decaudin

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Notes

Affiliations of authors: Department of Tumor Biology (PD, AV-S, MT, M-CE), Department of Clinical Hematology (DC, DD), Department of Ophthalmology (PA, LL-LR), Department of Radiation Oncology (R. Dolcetti), Institut Curie, Paris, France; Unité de Biologie des Interactions cellulaires, URA CNRS 2582, Institut Pasteur, Paris, France (AS); Department of Oncology (AJMF), Department of Pathology (MP), San Raffaele H Scientific Institute, Milan, Italy; Immunovirology and Biotherapy Unit, Department of Pre-clinical and Epidemiological Research, Centro di Riferimento Oncologico IRCCS National Cancer Institute, Aviano, Italy (FS, R. Dendale); Inserm/Institut Curie U 509, Paris, France (M-HS).

Correspondence to: Didier Decaudin, MD, PhD, Service d'Hématologie Clinique, Institut Curie, 26 rue d'Ulm, 75.248 Paris cedex 05, France (e-mail: didier.decaudin@curie.net).

DOI: 10.1093/jnci/djj079