Prevalence of chlamydial infection in a series of 108 primary cutaneous lymphomas

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MADAM, Several infectious agents have been associated with lymphomagenesis. The identification of new causative agents allows a better understanding of pathophysiology and the development of specific treatments. *Chlamydomphila psittaci*, the causative agent of psittacosis, is a ubiquitous, obligate intracellular bacterium that is transmitted to humans through exposure to infected reservoirs (wild and household animals). This bacterium has been linked to the development of ocular adnexal lymphomas,^{1–3} and bacterial eradication with doxycycline resulted in lymphoma regression in $\approx 50\%$ of patients.^{4,5} Moreover, in a series of 205 consecutive cases of nodal and extranodal lymphomas, chlamydial infection has been detected in some forms of cutaneous lymphomas and in diffuse large B-cell lymphomas (DLBCL) arising in the Waldeyer's ring, suggesting a preferential distribution of this microorganism in lymphomas occurring at extranodal organs considered as 'first barriers' to air-transported antigens.² The limited number of cases investigated did not allow us to draw definitive conclusions, but these intriguing observations prompted us to analyse more extensively the prevalence of chlamydial infection in primary cutaneous B- and T-cell lymphomas.

The presence of *C. psittaci* infection was investigated in DNA from fresh frozen samples of skin biopsies from 108 cases of cutaneous lymphoma of four different histotypes. The samples were reviewed by one expert dermatopathologist and two haemopathologists and categorized according to the European Organisation for Research and Treatment of Cancer/World Health Organization (WHO) classification:⁶ DLBCL ($n = 18$; including six cases of 'leg type' lymphoma), follicular lymphoma ($n = 24$), marginal zone lymphoma (MZL; $n = 31$) and

mycosis fungoides ($n = 35$). Nineteen normal skin samples from surgical quadrantectomies were used as negative controls. The presence of chlamydial DNA was investigated by using three polymerase chain reaction (PCR) protocols allowing the amplification of the 16S rRNA gene and the 16S23S region: touchdown enzyme time release-PCR,¹ outer membrane protein (ompA)⁷ and heat-shock protein 60 (hsp60).⁸ The last protocol allows the distinction between *C. psittaci* and other *Chlamydiae* (*C. abortus*, *C. caviae*, *C. felis*). *Chlamydia trachomatis* and *C. pneumoniae* infections were analysed by amplifying the 16S rRNA gene, and amplification of the β -globin gene was carried out as control of DNA suitability.¹ All PCR products were analysed by agarose gel electrophoresis, fragment size quantification and direct sequencing.

C. psittaci DNA sequences were found in one (5%) of the skin biopsies used as controls. Among the lymphomas, *C. psittaci* DNA was detected in one DLBCL (6%; Fisher exact test $P = 1.00$), in four follicular lymphomas (17%; $P = 0.36$) (Fig. 1) and in one MZL (3%; $P = 1.00$), whereas all cases of mycosis fungoides were negative (0%; $P = 0.35$). One case of follicular lymphoma was positive for *C. pneumoniae*. The different PCR protocols showed a good concordance, with positivity in at least two of the three PCRs in all positive cases (Table 1).

This study showed that the prevalence of chlamydial infections is low in primary cutaneous lymphomas, with similar rates between DLBCL, MZL, mycosis fungoides and normal skin samples, whereas a nonsignificant trend to a higher prevalence of *C. psittaci* infection was observed in follicular lymphomas. This nonsignificant difference may simply be due to the relatively small subgroup size; however, the observation that *C. psittaci* prevalence is significantly higher in follicular lymphoma (four of 24, 17%) in comparison to all the other cutaneous lymphomas as a whole (two of 84, 2%; Fisher exact test $P = 0.02$) suggests that this association deserves to

Fig 1. Examples of some cases assessed by multiplex touchdown enzyme time release-polymerase chain reaction. From the left: lane 1, markers; lane 2, DNA from patient with *Chlamydomphila pneumoniae*-positive cutaneous follicular lymphoma; lane 6, *C. psittaci*-positive specimen; lane 14, negative controls; lane 15, positive controls (*C. psittaci*, *C. pneumoniae*, *Chlamydia trachomatis*).

Table 1 Concordance among the three different polymerase chain reaction protocols in positive cases

	TETR	omp-A	Hsp60
FL case 1	Cp and <i>C pne</i>	Cp	Cp
FL case 2	Cp	Neg	Cp
FL case 3	<i>C pne</i>	<i>C pne</i>	Neg
FL case 4	Cp	Cp	Neg
FL case 5	Cp	Cp	Cp
MZL	Cp	Neg	Cp
DLBCL	Cp	Cp	Neg
Control	Cp	Cp	Cp

TETR, touchdown enzyme time release; OMP, outer membrane protein; Hsp, heat-shock protein; FL, follicular lymphoma; MZL, marginal zone lymphoma; DLBCL, diffuse large B-cell lymphoma; Cp, *Chlamydomonas psittaci*; *C pne*, *Chlamydomonas pneumoniae*; Neg, negative.

be further investigated. Importantly, unlike previous studies,⁹ an association between *C. pneumoniae* and mycosis fungoides was not detected. These figures confirm the preliminary results of our previous study.² The main differences between the two studies are follicular lymphoma, a distinct clinicopathological entity recognized by the updated 2008 WHO classification and not included in the previous study, and anaplastic large-cell lymphoma, which was not part of the present series. Overall, both studies suggest that chlamydial infections do not play a major role in the development and growth of mycosis fungoides and the most common forms of cutaneous B-cell lymphomas.

The present study has some limitations. Firstly, skin control samples were not taken from healthy individuals but were collected from patients with breast cancer. However, an interpretation bias can be excluded *bona fide* as histopathological analysis ruled out any neoplastic and inflammatory abnormality, and *C. psittaci* DNA prevalence in these controls (5%) was in line with data from hundreds of controls in previous studies^{1,2} and with *C. psittaci* seroprevalence in the general European population (0–5%).¹⁰ Secondly, DNA samples were collected mostly from single lesions, which does not take into account that cutaneous lymphomas are often multifocal diseases and that a fraction of patients with undetectable *C. psittaci* in the biopsied lesion could actually be infected by *C. psittaci* in other lesions or organs. Unfortunately, serological data and analysis on peripheral blood mononuclear cells (PBMC) were not available. However, the use of these methods would hardly improve the detection rate because of the low specificity of available serological assays,³ and the fact that *C. psittaci* infection in PBMC is present only in some patients with *C. psittaci*-positive lymphoma.¹ Moreover, the superiority of the assessment of *C. psittaci* infection on both tumour tissue and PBMC with respect to those performed exclusively on diagnostic samples in patients with multifocal or disseminated lymphoma remains to be addressed.

Overall, with the intrinsic limitations of retrospective studies, the investigated series is large enough to draw reliable conclusions. Present results do not support an association between chlamydial infections and mycosis fungoides and the most common forms of cutaneous B-cell lymphomas. Alternative infectious agents potentially involved in cutaneous lymphomagenesis and their therapeutic implications should be further explored.

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An exploratory ^1H -nuclear magnetic resonance metabolomics study reveals altered urine spectral profiles in infants with atopic dermatitis

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MADAM, Atopic dermatitis (AD) is a common chronic inflammatory skin disease in children. The prevalence of diagnosed AD in a population of preschool children has been observed to be above 15%,¹ therefore representing a major health impact in paediatrics. The pathogenesis of AD is only partially known,²

and little information is available about potential biomarkers. These facts make an easy and noninvasive test for evaluation of infant AD greatly desired by healthcare workers and parents.

Metabolomics provides a new approach and opportunity to explore the metabolic effects of many conditions in individuals,^{3,4} and recent evidence has demonstrated that it can be usefully employed in the characterization of airway biochemical fingerprints in children with allergic asthma.⁵ Nuclear magnetic resonance (NMR) spectra of biofluids contain signals from hundreds of metabolites. Chemometric approaches, such as principal component analysis (PCA) or cluster analyses, can be used to map the spectra in multivariate space where each sample occupies a position based on its metabolite composition.

We explored the possibility of detecting metabolite-level changes correlated with AD in children by applying a metabolomic analysis of urine samples. Twenty children with AD, 13 boys and seven girls, aged 6–10 months, diagnosed by a staff physician (D.G.P.) according to the U.K. working party's criteria⁶ and referred to the outpatient clinic of the Allergic Diseases Section of the Department of Life and Reproduction Science, were enrolled in the study. Twelve age-matched children, four male and eight female, without any clinical manifestation of AD or other known acute or chronic disease served as controls. The children were recruited in a narrow age range (6–10 months) to minimize biases due to external factors such as diet variability. It was further expected that the

Fig 1. ^1H -nuclear magnetic resonance (NMR) spectra of urine samples showing (a) low-field and (b) high-field regions. In each panel, the lower 20 spectra are measured on atopic dermatitis (AD) samples and the upper 12 spectra are measured on control samples the spectral intensities are not scaled. (c) Standardized global coefficients, or global weights, are reported vs. the original spectral variables (metabolite signals). Positive (negative) values of global coefficients indicate increased levels of the corresponding signals in spectra of the AD (control) group. (d) Same as (c) but refers to a reduced data matrix where the spectral regions (buckets) of strongest signals were removed; significant buckets are labelled by their chemical shift position.