Acta Oncologica, 2008; 47: 355–359

informa
healthcare

ORIGINAL ARTICLE

Assessment of the role of hepatitis C, *Helicobacter pylori* and autoimmunity in MALT lymphoma of the ocular adnexa in 45 Austrian patients

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Abstract

Introduction. A recent series from Italy has suggested a pathogenic link between hepatitis C virus and MALT lymphoma of the ocular adnexa. The hypothesis of our study was to prove this concept in Austrian patients with MALT lymphoma of the ocular adnexa. *Materials and methods.* A total of 45 patients presenting with MALT lymphoma of the ocular adnexa were assessed for the presence of infection with hepatitis A, B and C. Furthermore, extensive staging to evaluate the extent of disease along with analysis of *Helicobacter pylori*-infection, the presence or absence of autoimmune diseases (AD) and assessment of MALT-lymphoma specific genetic changes was performed. *Results.* Only 2/45 (4%) patients were tested positive for hepatitis C, while 10/45 (22%) had an underlying AD and 15/39 (38%) had HP infection. Chromosomal aberrations were detected in 19 (54%) of 35 patients analyzed. Disseminated disease was a significant risk factor for relapse ($p = 0.014$). *Discussion.* Our series suggests that infection with hepatitis C is not a significant contributor to the pathogenesis of ocular adnexal MALT lymphoma in the Austrian population, while a substantial proportion of these patients suffer from autoimmune conditions.

Extranodal marginal zone B-cell lymphoma of the mucosa associated lymphoid tissue (MALT-lymphoma) arises in mucosal lymphoid structures acquired in the context of chronic antigenic stimulation [1,2]. The most impressive proof of this concept was the identification of a causative role of *Helicobacter pylori* (HP) in the development of gastric MALT lymphoma, which has resulted in attempts to use antibiotic eradication of the bacteria to treat gastric MALT lymphoma [3]. These groundbreaking results published by Wotherspoon and co-workers [3] have repeatedly been confirmed by large scale trials defining HP-eradication as the standard management for disease restricted to the stomach [4,5].

Following this initial discovery, different infectious agents have been reported to be related to the development of MALT lymphoma, including *Borrelia burgdorferi* in cutaneous [6], *Chlamydia psitacci* in orbital [7] and *Campylobacter jejuni* [8] in small intestinal lymphoma (IPSID). In addition, we have recently investigated a potential role of HP in extragastric MALT-lymphoma, but could not demonstrate a relation between HP-infection and non-gastric MALT lymphoma [9].

Besides analysing the role of HP, we have also addressed the question whether hepatitis C infection (HCV) might be related to the development of MALT lymphomas, as recent series have found a high rate of HCV in patients with MALT-lymphoma of the salivary glands [8,10]. By contrast, we could not confirm such an association in 26 patients with parotid lymphoma [9] with only one of these patients testing positive for hepatitis C. In this study, also 17 patients with orbital MALT lymphoma were included, two of whom had hepatitis C infection.

In recent series from Italy, Ferreri and co-workers have investigated the clinical implications of hepatitis

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(Received 2 May 2007; accepted 13 August 2007)

ISSN 0284-186X print/ISSN 1651-226X online
DOI: 10.1080/02841860701630283

C virus in 55 patients with ocular adnexal MALT lymphomas [11]. According to their data, HCV seropositivity was present in 13% of patients and was associated with more disseminated disease and aggressive behaviour. In view of the published geographic differences between hepatitis C in parotid lymphoma as well as differences concerning the role of Chlamydia in ocular lymphoma between Italian and Austrian populations, we have expanded our series of MALT lymphoma of the ocular adnexa to further evaluate the suspected positive correlation.

We have retrospectively analyzed 45 patients for hepatitis A, B and C as well as the presence of autoimmune conditions and HP-seropositivity. This was accompanied by investigation of MALT-lymphoma associated genetic changes.

Patients and methods

A total of 45 consecutive patients, seen between 2000 and 2006 at the University Hospital of Vienna, with histologically verified MALT lymphoma of the ocular adnexa were included in this retrospective analysis (for details see Table I). Histologic diagnosis of MALT lymphoma was established by a reference hematopathologist (A.C.) in all cases according to the criteria outlined in the WHO-classification of lymphoid malignancies. Immunologic phenotyping on paraffin sections was done for demonstration of immunoglobulin light-chain restriction and the phenotype CD20+CD5-CD10-cyclinD1- which, in context with the microscopic appearance, is consistent with MALT-lymphoma.

Before initiation of therapy, all patients underwent staging for assessment of extent of disease. Minimum staging for inclusion in this series required gastroscopy with multiple biopsies, endosonography of the upper GI-tract, computed tomography (CT) of thorax and abdomen and a bone marrow biopsy.

Patients also underwent serologic testing for infection with hepatitis A, B and C (HCV). In case of suspected HCV infection, PCR had to be performed for definitive diagnosis. In addition, all patients were screened for the presence of an underlying autoimmune condition, i.e. Sjogren's syndrome (SS) and Hashimoto's thyroiditis (HT). The diagnoses of autoimmune conditions were based on characteristic clinical symptoms and/or positive biopsies and/or serologic changes [12].

The presence or absence of HP was assessed by urease breath test, histology and serologic testing for IgG antibodies against HP.

Paraffin embedded biopsy samples were tested for MALT-lymphoma-associated genetic aberrations including t(11;18)(q21;q21), t(14;18)(q32;q21) involving *IGH* and *MALT1*, t(1;14)(p22;q32), t(3;14)(q14;q32) involving *FOXP1* and *IGH* and trisomies 3 and 18. T(11;18)(q21;q21) involving *API2* and *MALT1* was assessed by reverse transcriptase polymerase chain reaction, t(14;18)(q32;q21) involving *IGH* and *MALT1*, t(1;14)(p22;q32) involving *BCL10* and *IGH*, t(3;14)(q14;q32) involving *FOXP1* and *IGH* and trisomies 3 and 18 were investigated by fluorescence in situ hybridization as previously published [13]. These genetic studies were carried out on representative biopsies if enough material was available.

Statistical analyses were done with the SPSS 12.0 program. The association between the categorical variables were calculated either with the χ^2 test, the Fisher exact test or with contingency tables, additionally univariate analyzes were performed. All tests were used according to the recommendations of Cochran [14].

Results

We have evaluated a total of 45 Austrian patients with ocular adnexal lymphoma. For detailed patients' characteristics and localization of the lymphoma see Table I. The median age of the 22 female and 23 male patients was 64 years (range; 18–94). Primary localization of the MALT lymphoma was the conjunctiva in seven patients, the lacrimal gland in 11 patients and 24 had orbital lymphoma, while one patient had lymphoma involving both orbit and conjunctiva, another one both conjunctiva and lacrimal gland and one patient had an involvement of the lacrimal gland and the orbit. Bilateral manifestations were present in four cases (two patients with bilateral conjunctival lymphoma involvement, and one each with bilateral lacrimal and with orbital lymphoma). Twenty patients had multifocal disease, including four patients with bilateral ocular adnexal lymphoma, two with mulifocal disease in the ocular adnexe of the same eye and 14 patients with involvement of distant organs at diagnosis (see Table I). All sites suggestive of lymphoma had been verified by biopsy.

All 45 patients were tested for HAV, HBV and HCV, but only two (4%) patients were found to be HCV positive. In both patients, the only location of the MALT lymphoma was the orbit, and one patient also had evidence of HP-infection along with HCV, while the other patient was HP-negative. Both patients were negative in terms of AD. HP-status was available for a total of 39 patients with MALT lymphoma of the ocular adnexa, 15 of whom (38%) had evidence of HP-infection.

All patients were screened for the presence of an underlying autoimmune condition. In total, ten

Table I. Patient characteristics.

Sex	Age	Localisation	HP	HCV	Autoimmun Disease	Genetic Findings
M	48	orbit	neg	pos		+3, +18
F	51	conjunctiva bil	pos	neg		Normal
M	77	orbit bil	neg	neg	Rheumatoid arthritis	Normal
M	65	orbit, pharynx	pos	neg		+3,+18
F	62	lacrimal	neg	neg		n.d.
F	52	orbit, conjunctiva	neg	neg	Sjogren's syndrome	Normal
M	92	lacrimal, lung	pos	neg		+3,+18
F	60	lacrimal, breast	neg	neg	Sjogren's syndrome	+3,+18
F	74	orbita, colon	pos	neg		IGH-MALT1
M	94	orbit	neg	pos		n.d.
M	74	lacrimal bil, lung	pos	neg		n.d.
M	66	orbit	pos	neg		IGH-FOXP1
M	85	lacrimal, skin	neg	neg		Normal
F	43	lacrimal, parotid	pos	neg		+3
M	73	conjunctiva, parotid	pos	neg		n.d.
F	91	conjuctiva, skin	pos	neg	Ank. sponyloarthritis	n.d.
F	78	lacrimal	neg	neg		Normal
F	83	lacrimal	neg	neg	Sjogren's syndrome	+18
F	63	orbit, breast	neg	neg		Normal
M	40	conjunctiva bilat	neg	neg		Normal
F	80	lacrimal	pos	neg		IGH-MALT1
M	83	lacrimal bil	neg	neg		+3, IGH-MALT1
M	82	lacrimal, orbit	neg	neg		+3
M	55	orbit	neg	neg		Normal
M	49	conjunctiva	neg	neg		n.d.
M	65	orbit, parotid, kidney	neg	neg		+3,+18
F	41	conjunctiva	neg	neg		n.d
M	85	orbit	pos	neg		+3, IGH-MALT1
F	60	orbit	n.d.	neg		Normal
F	54	orbit	neg	neg	Lupus erythematosus	Normal
M	18	orbit	neg	neg		+3
F	61	orbit	pos	neg	Sjogren's syndrome	IGH-MALT1
F	78	orbit	n.d.	neg		+18
F	64	orbit	n.d	neg	Hashimoto thyroiditis	Normal
F	32	orbit	pos	neg		n.d.
F	77	orbit	neg	neg		n.d.
M	36	orbit	neg	neg		Normal
M	91	orbit, cervikal lymph nodes	pos	neg	Rheumatoid arthritis	Normal
M	68	orbit	n.d	neg		+3
F	56	orbit	neg	neg	Sjogren's syndrome	Normal
M	55	orbit	n.d.	neg		Normal
M	52	orbit, lung	pos	neg		+3, IGH-MALT1
M	81	conjunctiva, lacrimal, stomach	neg	neg		Normal
F	42	lacrimal	neg	neg		+3, +18
F	45	conjunctiva	n.d.	neg		n.d.

Abbreviations: HP, *Helicobacter pylori*; neg, negative; pos, positive; nd, no data; bil, bilateral; HCV hepatitis C virus; M, male; F, female.

(22%) patients were found to suffer from an underlying autoimmune condition. Five (11%) patients had SS, two patients with orbital lymphoma had long-standing severe chronic polyarthritis, whereas one patient with lymphoma of the orbit had Hashimoto's thyroiditis, one with orbital lymphoma suffered from Lupus erythematosus, and one patient suffered from ankylosing spondyloarthritis (for details see Table I). Of these ten cases, three had disseminated disease.

Sufficient material for analysis of chromosomal aberrations was available from 35 patients (for details see Table I). The majority of patients had numerical aberrations including trisomies 3 (n = 4) and 18 (n = 2) or a combination thereof (n = 6), while six patients (13%) were positive for t(14;18)(q32;q21) involving IGH/MALT1, three of them along with trisomy 3. One patient had t(3;14)(q14;q32) involving *FOXP1* and *IGH* and none had evidence of t(11;18)(q21;q21). No correlation between genetic aberration and extent of disease or recurrence rate was detected.

After a median follow-up of 33.5 months, 37 (82%) patients are still alive. A recurrence was

detected in 15 (33%) patients with the median time to relapse being 26 months (range; 7–72).

In our statistical analyses, we found no correlation between extent of disease and HP-infection, autoimmune conditions or HCV infection. In addition, none of these factors was predictive of relapse. A highly significant correlation was found between extent of disease at initial diagnosis and relapse ($p = 0.014$).

Discussion

There are several reports about the association of hepatitis C virus infection and non-Hodgkin lymphomas [10,15,16]. Recent data by Ferreri and coworkers [11] have shown clinical implications of hepatitis C virus infection in MALT-type lymphoma of the ocular adnexa. Of 55 patients, 13% tested positive for HCV. The concomitant HCV infection was associated with more disseminated disease and aggressive behaviour in ocular adnexal lymphoma.

By contrast, only two (4%) of our 45 patients had a concomitant HCV infection. Both patients had stage I disease with the only location of both patients being the orbit. Accordingly, no influence of HCV on clinical course and risk of relapse could be found in our analysis. This finding again underscores the importance of putting reports of a possible causative role of an infectious agent in extragastric MALT lymphoma into a geographic perspective. In analogy, the role of *Chlamydia psittaci* in ocular adnexal lymphoma appears to be especially pronounced in Italy [7,17], while widely varying rates of infection could be found in different geographic regions [18], including Austria, where treatment with doxycycline was not effective in 11 patients investigated [19]. The difference of HCV seropositivity between our study and the report by Ferreri [11] may again be explained by geographic differences in HCV prevalence. In addition, there is not only a difference in HCV incidence between Italy and Austria but also, as an Italian multicenter study has demonstrated, within Italy itself [16]. The explanation of geographic differences appears to be plausible in view of the fact that similar differences have been shown in parotid MALT lymphoma, where HCV was apparently associated with the disease in Italian reports [10,11], but not in a recent study from our institution [9]. Thus, the suggestion put forward by Zucca and Bertoni [20] that geographic differences might exist in terms of causative agents and MALT lymphoma is again underscored by our findings.

The presence of AD has repeatedly been linked to MALT lymphoma of the parotid in Sjogren's syndrome and thyroidal MALT lymphoma in Hashimoto's thyroiditis. In addition, a small series from our institution has demonstrated the presence of AD as an adverse prognostic factor in terms of response to HP eradication in gastric MALT lymphoma [21]. To date, no such association has been reported for ocular adnexal MALT lymphomas. In our series 10/45 patients (22%) suffered from an underlying AD. While no association with clinical behaviour was found, this has to be interpreted with some caution, as the number is relatively small. To our knowledge, this is the first series to report such a high rate of AD in patients with ocular adnexal MALT lymphomas.

We have analyzed material for chromosomal aberrations in a total of 35 patients and found that 54% of patients analyzed had MALT-lymphoma-associated genetic aberrations (for details see Table I). No correlation between genetic aberration and extent of disease or recurrence rate was detected.

In an extension of a prior series, we have also assessed the HP status in our patients, with 15 (38%) of 39 analyzed patients having evidence of HP-infection. This percentage corresponds to the rate of infection found in a healthy Austrian population in a previous study performed at our institution [22] and is in keeping with results of a prior study including 17 of these patients [9].

In our study, no correlation was found between extent of disease and HP-infection, autoimmune conditions, HCV infection or genetic aberrations. Interestingly, this was also true for the presence of AD, which did not influence clinical outcome. A highly significant correlation, however, was demonstrated between dissemination at diagnosis and relapse ($p = 0.014$).

Taken together, our findings suggest that HCV infection does not play a major role in the development of ocular adnexa lymphoma in the Austrian population. By contrast, a high rate of AD was found in our patients, which nevertheless did not appear to influence clinical presentation and outcome.

Conflict of interest

The authors indicated no potential conflicts of interest.

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