



A RETROSPECTIVE INTERNATIONAL STUDY OF PRIMARY EXTRANODAL MARGINAL ZONE LYMPHOMA OF THE LUNG (BALT-LYMPHOMA)

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1. BACKGROUND

The group of lymphomas classified as low-grade mucosal associated lymphoid tissue (MALT) lymphoma represents marginal zone B-cell lymphomas that can originate in all extra nodal sites, including the lung. Primary lymphoma of the lung is a very rare entity; in fact it represents only 3.6% of all extranodal lymphomas and 0.4% of all Non-Hodgkin's Lymphomas, it is often of MALT type. [1].

Pulmonary parenchyma normally doesn't contain organized lymphoid tissue but some conditions (such as follicular bronchiolitis, pulmonary inflammatory processes and acute infections) can lead to a lymphocytic hyperplasia, which makes up the bronchus mucosa-associated lymphoid tissue (BALT). Additional genetic alterations can lead to the transformation of this reactive lymphoid tissue into a lymphoma.

In gastric MALT lymphoma the pathogenetic role of *Helicobacter pylori* is well recognized, but in non-gastric MALT lymphoma the pathogenetic role of infectious agents is less clearly defined. Previous studies suggest a possible pathogenetic role of *Chlamydia psittaci* and *Borrelia burgdorferi* infections in ocular adnexal lymphoma and skin MALT lymphoma respectively; nevertheless, these results have not been conclusive [2-4]. Several studies have reported high rates of previous hepatitis C virus (HCV) infection in patients with NHL, suggesting a possible pathogenetic link between HCV and certain lymphoma subtype, including marginal zone lymphomas.

The role of chronic infections in BALT lymphoma is still unknown.

Pulmonary involvement by NHL in HIV-positive patients is reported in 14% to 67% of cases [5-7], although most of these lymphomas are high grade, rare cases of low-grade pulmonary lymphomas have been described [8].

BALT lymphoma occurs predominantly but not exclusively in people over 60 years old, past studies documented a slight predominance in men [9]. Most patients are asymptomatic and in these cases the diagnosis was made through routine chest x-rays; nevertheless, non-specific pulmonary symptoms (such as cough, chest pain and dyspnoea) are the most common symptoms in symptomatic patients.

Three chromosome translocations were found to be specific of MALT lymphoma with different incidence depending on primary site of involvement: t (11; 18)(q21; q21), t (14; 18)(q32; q21) and t (1; 14)(p22; q32); they appear to be mutually exclusive.

The t (11; 18)(q21; q21) is the most common, and can be found in approximately 50% of the MALT lymphoma cases presenting an abnormal karyotype [10,11]. Ye H. et al [12] screened 417 cases of MALT lymphoma for t(11; 18); its incidence was the highest in the lung (38%), followed by the stomach (24%), conjunctiva (19%), and orbit (14%). This translocation involves API-2 gene on 11q21 locus that belongs to the family of inhibitors of apoptosis proteins and MALT-1 gene on 18q21 locus that belongs to the paracaspases family. It was demonstrated that the fusion protein is able to activate the Nuclear Factor-kB (NF-kB), leading to antigen-independent B cell proliferation [13,14].

- The t (14; 18)(q32; q21) involves MALT1 gene on 18q21 and IgH gene on 14q32 locus is rare in lung lymphomas; a recent series of 28 cases with primary pulmonary MALT-type lymphoma by Remstein et al. [15]: 75% of them presented with a

cytogenetic abnormalities: one quarter of them had t (11; 18)(q21; q21) without any concomitant aneuploidy, 11% had IgH/MALT1 fusion and trisomy 3 or trisomy 12 also. 39% of patients had only aneuploidy: trisomy 3 and 18 was the most common.

The incidence of t (1; 14)(p22; q32) in MALT lymphomas is very low but it was described in primary lung cases; this chromosome aberration generates a truncated Bcl10 in its carboxyl terminal domain, this mutated protein can activate NF- κ B [14,16,17].

Numeric chromosomal aberrations are common but not specific of MALT lymphoma; a survey, that analyzed 70 archival cases of MALT lymphoma from various site by FISH, included 7 cases of pulmonary primary site and all them had trisomies: 4 had trisomy 3, 3 had trisomy 18 and 2 had trisomy 12 [18].

In the past, the initial treatment was most commonly a surgical resection, in more recent years less invasive diagnostic and therapeutic approaches are emerging because the indolent nature and slow course of BAL T lymphoma does not justify invasive diagnostic approaches such as wide surgical resection and highly toxic treatments.

In most cases, this indolent form of lymphoma may be managed conservatively with limited resection, low toxicity chemotherapy, and immunotherapy with anti-CD20 or with low dose radiotherapy to obtain symptom control.

Since the 60-70% of patients with BAL T lymphoma presents a localized disease (stage IE-IIIE) and since this lymphoma tends to remain localized for a long time, a local treatment such as radiotherapy on the pulmonary lesion can be indicated but respiratory and cardiac movement-associated limitations and radiation-induced injuries (such as acute or chronic lung injury) can limit this treatment.

High doses chemotherapy and radiotherapy on a large pulmonary field is not practical because these treatments can be more disabling than the primary pathology.

Radical surgery is not always possible and doesn't eliminate the risk of relapse, so recently chemotherapy became the first line of therapy particularly for patients where surgery cannot be curative or possible because of a systemic presentation of lymphoma or patients' performance status. However there is not a standard chemotherapy regimen for this lymphoma.

In a study by Ahmed S. et al on 22 patients with BAL T lymphoma, 12 received chemotherapy and /or Rituximab, 6 surgical treatment, 2 radiotherapy as primary treatment. A complete response was achieved in nine patients and a partial response was obtained in 10 patients; the estimated progression free-survival was 53 months, all patients were alive during the median follow up period of 36 months (range 12-76 months) [19]. This result is in agreement with previous observations by Li G et al, Cordier JF et al and Zinzani PL et al., that show a slow rate of progression and a favorable course in this subtype of lymphoma, including 2-years and 5 years survivals of 100% and 95-84% respectively [9,20,21].

2. STUDY OBJECTIVES

The study aims to answer questions that have not been adequately addressed by the published literature:

- If there is a link between the histological and biological features (immunophenotype and immunohistochemistry, molecular biology) and the clinical pattern (symptoms, stage, response to treatment, survival)
- If the stage of lymphoma should influence the treatment choice

The study will:

- Collect data on all Bronchial Associated Lymphoid Tissue (BALT) lymphomas treated in participating institutions
- Set up a data base with patients characteristics, histological features, diagnostic and treatment modalities, response to treatment, relapse-free survival and overall survival

in order to:

- Define immunophenotypic and immunohistochemistry features
- Study the genetic and molecular characteristics of BALT lymphomas using the PCR (Polymerase Chain Reaction) and FISH (Fluorescent In Situ Hybridization) techniques
- Document treatment outcomes
- Identify some significant prognostic factors
- Generate hypothesis on treatment strategies that later could be evaluated in prospective studies.
- Provide, if possible, management recommendations based on the study results.

3. PATIENT SELECTION CRITERIA

Based on previous IELSG and IIL studies, a series of approximately 100-150 patients with available diagnostic biopsies is expected to be gathered.

Patient will be included in this study if all following criteria are satisfied:

- Paraffin block sent to Dr. Giancarlo Pruneri (Division of Pathology, European Institute of Oncology, Milan) for central pathological review.
- Initial diagnosis of BALT lymphoma (or other indolent lymphoma subtype) between January 1990 and December 2005.
- Primary site in the lungs, either with single or multifocal lesions (including cases with bilateral presentation), with or without lymph node involvement.
- Complete information about clinical characteristics, diagnosis modalities, treatment type and outcome (CRFs to be completed and forwarded to IELSG (Bellinzona, Switzerland)
- Follow-up time of at least one year
- Patients will be eligible regardless of treatment type.

A patient will be excluded if:

- Histological material is not available for central pathological review.
- Pulmonary involvement happens in recurrent or progressive lymphoma, whose origin site was not the lung.
- Incomplete CRFs.

The stage of BALT lymphoma will be based on the Ann Arbor system modified by Ferraro et al. [23], summarized in the following table:

STAGE	DESCRIPTION
STAGE I E	Unilateral or bilateral presentation of the lung
STAGE II 1E	Lung presentation with hilar lymph node involvement
STAGE II 2E	Lung presentation with mediastinal lymph node involvement
STAGE II 2EW	Lung presentation with chest wall or diaphragm involvement
STAGE III E	Lung presentation with abdominal lymph node involvement
STAGE IV E	Lung presentation with extra-lymphatic organs or tissue involvement

4. DATA COLLECTION

Eligible patients' data will be collected in an approved Case Report Form (CRF), completed by each participating individual sites, and will be merged and stored in an appropriate electronic database.

Data to be collected include:

- Epidemiological data
- Clinical characteristics: symptoms, stage of disease
- Diagnostic modalities and their adequacy
- Biochemical parameters
- Serology for *Mycoplasma pneumoniae*, HIV and HCV test, only if previously done.
- Histological diagnosis (morphological, immunophenotypic and immunohistochemical analysis, molecular biology with PCR and FISH technique)
- Treatment modality (type of surgery; radiotherapy dose, fractionation and fields; chemotherapy dose, agents used, number of cycles)
- Imaging and laboratory evaluation during follow up
- Response to treatment
- Site and therapy of relapse
- Treatment outcome and survival

5. ENDPOINTS

The study will provide an overview of BALT lymphoma's histological characteristics to find if there's a link between pathological pattern and clinical characteristics (such as symptoms, biochemical parameters, disease stage, response to treatment, OS, DFS). The specific questions, contained in the Objectives section will be examined.

The results could suggest changes to current management practices that could be incorporated into a following prospective study.

5.1 Primary Endpoints:

- The incidence of recurrent chromosome alteration will be studied.
Translocation of the 18q21 locus, involving the MALT1 gene will be searched on paraffin-sections by FISH. In the cases with 18q21 translocation the chromosome partner will be further characterized testing the 11q21 and 14q32 loci.
- The link between the presence of chromosome alterations detected by FISH and the clinical characteristics (such as symptoms, biochemical parameters, stage, response to treatment, survival) will be studied.
The presence of statistically significant associations will be tested using standard statistical methods (Mantel-Haenszel test, Log rank or other tests as appropriate, see the statistics section).

5.2 Secondary Endpoints

- Response to treatment
- Time-to relapse, Progression-free survival and overall survival

Response will be assessed one month after the end of planned initial therapy, taking into account the following guidelines:

- The patient, who has not achieved CR after first line therapy, would undergo second line therapy and may subsequently achieve CR. Details of subsequent therapy should be recorded.
- A clinical/radiological residual mass in the lung at the end of treatment, which is stable or continuously regressing at repeat CT scan is considered as a CRu. A residual mass, which is negative on functional imaging, should be recorded as CRu.
- If there is a residual mass at the end of treatment, the response category should be recorded as PR or SD, according to World Health Organisation criteria for response [22].

- If there' s an increment or appearance of disease in a new site, this will be considered as PD.
- Complete response (CR): complete disappearance of all disease clinically and on imaging after at least four weeks from the end of therapy.
- Partial response (PR): reduction of at least 50% of the TAC-documented bigger lesion after at least 4 weeks from the end of therapy.
- Stable disease (SD): less than 50% reduction or less than 25% increase of the largest lesion on CT scan, after at least 4 weeks from the end of therapy.
- Progressive disease (PD): increase of 25% or more of the CT-measured largest lesion.
- Dates and sites of first and subsequent relapse/progression will be recorded, as well as the date and cause of death.

5.3 Definition of time-to-event variables

The potential follow-up time for each patient will be measured from the time from treatment beginning to the close-out date (for patients who died or are still followed up) or to the last date of follow-up (for patients lost to follow-up). At least one-year follow-up will be required for inclusion in the primary analyses.

The time-to-event variables to be studied will de defined as follows:

TYPE	PATIENT	FROM*	TO	CENSORED BY
Time to local-regional relapse	All patients	Diagnosis	Local-regional relapse	Close-out date Loss to follow up Death
Time to any relapse	All patients	Diagnosis	Relapse at any site	Close-out date Loss to follow up Death
Relapse-free survival	All patients	Diagnosis	Relapse or death	Close-out date Loss to follow up
Overall survival	All patients	Diagnosis	Death (any cause)	Close-out date Loss to follow up

* All time will be measured from the treatment beginning for the BALT lymphoma.

6. STATISTICAL ANALYSIS

Demographic characteristics and other baseline characteristics of 100-150 patients included will be tabulated and analyzed by appropriate descriptive statistics. Summary statistics and frequency tables will be provided for all relevant continuous and categorical variables.

Cochran-Mantel-Haenszel Test, which adjusts for the effects of multiple study centers, will be a statistical test used to compare groups with and without chromosome alteration, and between treatments groups with respect to symptoms, biochemical parameters, disease stage and response rates. Response rates will be displayed separately by study center and some extreme or opposite results among centers will be evaluated.

Estimate of response rates (CR, PR, SD, PD) and their 95% confidence intervals will be computed. Response rates will be displayed separately for different values of baseline characteristics of the patients, for presence of chromosome alteration and clinical characteristics.

Disease free, progression free and overall survival curves will be computed using the Kaplan-Meier technique.

The log rank 2-sided test will be used for the comparison of the treatment outcome, clinical characteristics and presence of chromosome alteration.

The Cox Proportional Hazards Model will be used to determine the independent prognostic factors to adjust the treatment effect comparison and differences for chromosome alteration by possible confounding factors, and to obtain estimates of the hazard ratios and the corresponding 95% confidence intervals, whenever all assumptions will be satisfied.

Sensitivity, specificity, negative predictive value, and positive predictive value will be calculated for the assessment of accuracy of diagnostic modalities.

7. STUDY MANAGEMENT

7.1 Case Report Forms (CRF)

Data collected about each patient with BALT lymphoma diagnosis should be recorded on the approved CRF. The site investigator or data manager may complete CRFs. The site investigator is responsible for ensuring that all blank data spaces on each form are completed. The site investigator for reference should retain a copy of the completed CRF, and the original paper copy sent to IELSG headquarters.

All data will be recorded in central database and merged for analysis.

CRFs may be sent in batches rather than individually.

7.2 Pathology review

Each case included in the study should be subjected to a central pathological review, which will be performed by Prof Stefano Pileri (Division pathology of Seragnoli Haematology and Oncology Institute, Bologna) and Dr. Giancarlo Pruneri (European Institute of Oncology).

A paraffin block representative of the BALT lymphoma should be sent to Dr. G. Pruneri (Division of pathology, European Institute of Oncology, via Ripamonti 435, 20141 Milan, Italy). The phenotype of the tumour will be evaluated by immunohistochemistry with anti- CD3, CD5, CD10, CD20, CD 43, Ki67, Bcl-10, Bcl-6, IFR4/MUM1 and IRTA antibodies.

The pattern of rearrangement of the immunoglobulin heavy chain gene will be analyzed by PCR (polymerase Chain Reaction) using FR2 and FR3A primers (Biomed).

The translocation of the 18q21 locus, involving the MALT-1 gene, will be analyzed by FISH (Fluorescence In Situ Hybridization) in interphase nuclei obtained by paraffin sections in the all cases fulfilling the morphological and phenotypical diagnostic criteria of marginal zone lymphoma.

In the cases bearing the translocation of the locus 18q21, involving MALT-1 gene, the chromosome partner will be further characterized by using probes specific for the t (11; 18)(q21; q21) and t (14; 18)(q32; q21) translocation.

As soon as the results of the central revision are available, they will be sent to the local pathologist.

8. ETHICAL CONSIDERATIONS

Patient privacy and confidentiality will be respected. All information provided will be confined to the personnel involved in conducting the study. No identification of individual patients will be made in any presentation or publication.

The study will have to be approved by the Ethical Committee and/or IRB of each participating institution, according to local guidelines.

9. SHIPMENT OF STUDY MATERIAL AND CRFs

For the pathological review (with morphological, immunohistochemical and molecular biology analysis), paraffin blocks with the pathologic material of the initial diagnosis of BALT lymphoma or any other indolent subtype of primary pulmonary lymphoma (performed from January 1990 to December 2005) will have to be sent by each participant site to:

Dr. Giancarlo Pruneri
Division of Pathology
European Institute of Oncology
via Ripamonti 435, 20141 Milan, Italy

Complete CRFs should be sent by each participant site to the:

IELSG Operation Office
c/o Oncology Institute of Southern Switzerland
Ospedale San Giovanni
CH 6500 Bellinzona, Switzerland

10. PUBLICATION OF RESULTS

- It is intended that a manuscript will be prepared for submission in an appropriate journal.
- Authorship on publications and presentations will comprise the study personnel who made a significant contribution to the scientific content of the study, have reviewed the manuscript and approved the final version.
- The principal investigators will be the first and last author on publications reporting the main aims of the study.
- Presentations and publications will not be made without the knowledge and consent of all authors.

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