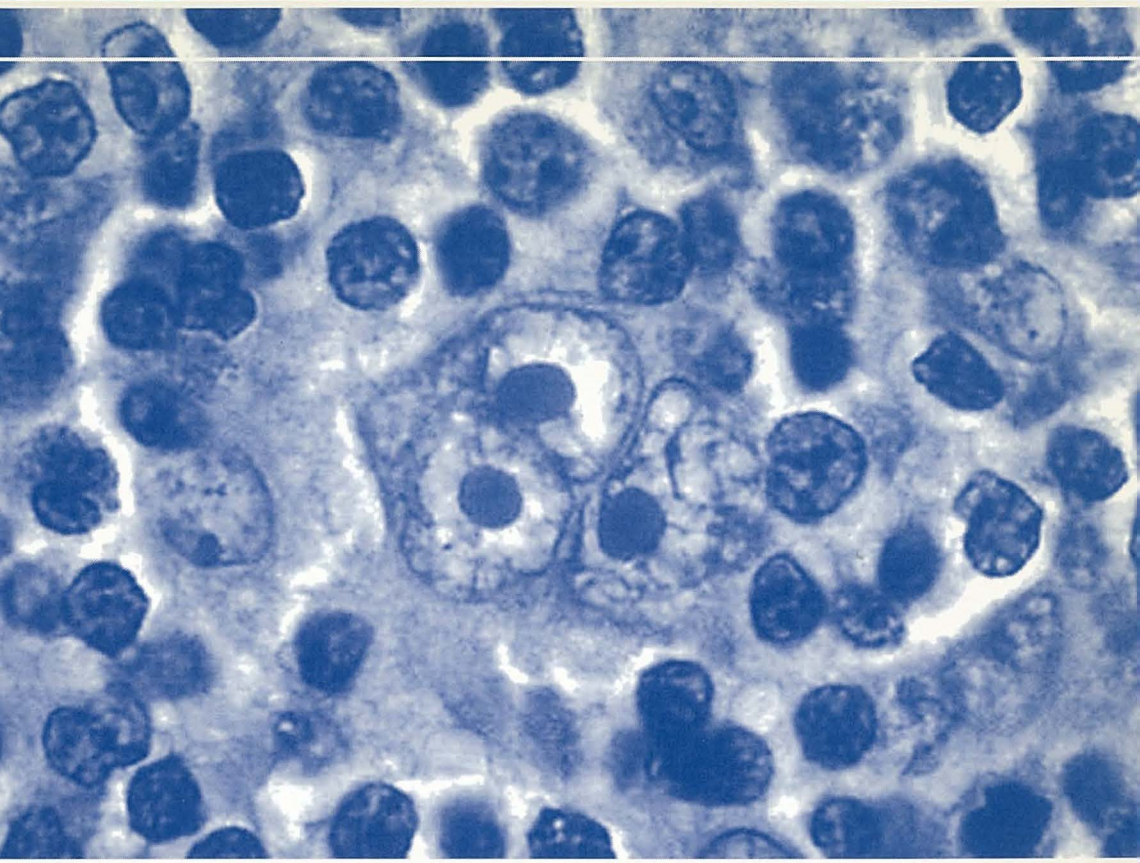

AN UPDATE OF DIAGNOSTIC APPROACH TO MALIGNANT LYMPHOMAS

XXXIII Memorial Meeting for Professor Janez Plečnik with International Participation

Proceedings

Edited by M. Bračko, J. Jančar, A. Zidar

Institute of Pathology, Faculty of Medicine
University of Ljubljana
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AN UPDATE OF DIAGNOSTIC APPROACH TO MALIGNANT LYMPHOMAS

PROCEEDINGS

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with International Participation

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PREFACE

The advent of hybridoma technology and development of sophisticated molecular techniques extended tremendously our understanding of the relationship of lymphoid neoplasms to their normal counterparts and the genetic changes associated with malignant transformation. The combination of clinical, morphological, phenotypic and molecular evaluation of these neoplasms represent new diagnostic approach to disease definition, prediction of prognosis and response to treatment. Due to their complex biology and the complexity of diagnostic approach, lymphoid neoplasms are considered as one of the major problems in pathology. As to its objectives, the meeting is primarily conceived for those general or surgical pathologists who are at an appropriate level of their routine work involved also in working out numerous hematopathological cases and are, therefore, highly motivated to upgrade their knowledge and extend their diagnostic experience. However, the topics to be discussed at the meeting may represent a challenge to young pathologists and even clinicians, encouraging them to take part in the development of this particular field of medicine. We therefore believe that the discussion of this subject at the XXXIII Memorial Meeting for Professor Janez Plečnik is the opportunity to spread the recent concepts and modern diagnostic approach in the region. The workshop that includes the presentation of 17 selected cases with theoretical background, discussion on differential diagnosis and diagnostic value of the methods used, reflects applicative orientation of the meeting.

Janez Jančar
Nina Gale

WHO CLASSIFICATION OF LYMPHOID NEOPLASMS - BASIC PRINCIPLES

Hans Konrad Müller-Hermelink

Institute of Pathology, University of Würzburg, Germany

History

The history of recognition and classification of tumors that today are called malignant lymphomas is long, controversial, and complicated. In 1832, Sir Thomas Hodgkin first identified malignant tumors of lymph nodes. Among those were cases that we would now classify as Hodgkin lymphoma. The definition of "leukemia" followed in 1845 by Rudolf Virchow and in 1863, he laid down the concepts of lymphosarcoma and lymphoma. At the turning of the century, Sternberg and Reed, 1898 and 1902, defined morphologically the tumor cells of Hodgkin's disease. 25 years later, Brill and Symmers described follicular lymphoma and in 1948 Burkitt and in 1960 O'Connor and Davis described African lymphoma, now called Burkitt's lymphoma. Within this short list, of course, only historical milestones have been cited constituting only those steps that still remain valuable at the turning of the Millennium.

Since 1960, modern immunology has severely influenced our knowledge of lymphoid tumors. The biology of lymphoma cells, reflecting the immunological activities of their normal counterparts, has been clarified to a great extent, and has enabled the establishment of a biological basis for new and more comprehensive classification systems of malignant lymphomas. Two classification systems have been widely used until recently, the Kiel classification of non-Hodgkin's Lymphomas and the Working Formulation for Clinical Usage. The Kiel classification was based on the exact morphological description and immunological identification of the normal cellular counterparts of tumor cells and was updated several times introducing new findings and more comprehensive knowledge. The so-called Working Formulation (WF), on the other hand, was based on historical clinical survival data and, therefore, was not updated, although its usage was also adapted to modern findings. Of importance, there was a geographic split of categories in the diagnosis of lymphoid tumors, the Kiel classification in Europe and the WF in the USA.

An international group of experienced hematopathologists, the "International Lymphoma Study Group" (ILSG), formulated a new proposal for a modern lymphoma classification and published it as the so-called "Revised European American Lymphoma (REAL)" Classification. In the following years, this proposal was tested for its applicability and reliability and then accepted worldwide. It is, therefore, largely identical (with some minor corrections and additions) to the now proposed WHO classification.

Basic taxonomic principles

The taxonomic unit of the new WHO classification is the disease entity. Distinct entities in malignant lymphomas can be recognized by pathologists and, ideally, are of clinical relevance. For each disease entity, a combination of morphologic, immunophenotypic, genetic, and clinical features is needed for its definition. The relative value of each of these features may vary among different disease entities. Variations in grade and aggressiveness, that may exist within a given disease entity, and may be related to patients' survival and treatment response, must be distinguished from "different diseases".

The basic rules for the definition of disease entities in this proposal are comparable to the

general rules of tumor classification as used in many other organs and organ systems. A given lymphoma entity is defined in first line by recognition of the predominant differentiated cell type using morphological and immunological features, a principle that follows the rules of the Kiel classification. In addition, the importance of the primary site of involvement which is not only a feature of staging, but also an easy, clinically relevant, and important biological distinction, has been explicitly stated.

Second line principles of classification are important for some entities. These are etiological features, such as the association of certain infectious agents, like the Epstein-Barr virus, Helicobacter pylori or HTLV I, primary cytogenetic abnormalities or specific clinical features. The daily clinical and pathological experience shows minor or more evident exceptions from the proposed rules, leading to the well recognized heterogeneity of each type of lymphoid neoplasia. Therefore, within many entities, specific morphological or clinical subtypes are mentioned which are of clinical importance. Morphological variants reflect the diagnostic spectrum of a disease, which is important to be recognized in order to establish a correct differential diagnosis.

Table 1 lists the recent WHO classification of B and T cell lymphomas and of Hodgkin lymphoma.

The non-Hodgkin's lymphomas are divided primarily into those of the B and the T cell system. In both lineages, there is a primary distinction of lymphomas that arise from precursor cells (the lymphoblastic lymphomas or acute lymphoid leukemias) and from peripheral cells.

Clinical relevance

The clinical relevance of the WHO proposal was intriguingly confirmed by the results of the Non-Hodgkin's Lymphoma Classification Project, which involved the patho-morphological and immunophenotypic investigation of 1.403 lymphomas in eight different sites around the world. These cases were taken from the years 1988 – 1990, and were reviewed by experienced hematopathologists attempting at the comparison of different classification systems, the recognition of epidemiological variations in the occurrence of lymphomas, and the correlation of treatment results to histological diagnoses. The results of this study clearly established that the criteria formulated in the REAL classification and applied in the WHO classification resulted in a high inter-observer accuracy and were of significant prognostic value for the recognition of diseases with different clinical courses and behavior. It turned out that immunophenotyping was less important in some of the diseases (e.g. in follicular lymphoma), but absolutely necessary in others (mantle cell lymphoma, T cell lymphomas). Some diseases were only reliably diagnosed, if clinical data were available (e.g. mediastinal B cell lymphoma). In other cases, the differential diagnosis was at least greatly improved by the knowledge of clinical features and presentation.

For some disease entities and variants, however, diagnostic accuracy was less satisfactory. In particular, the distinction of Burkitt's lymphoma and "Burkitt's-like" non-Hodgkin's lymphoma involved a high inter-observer disagreement of more than 40 %, clearly establishing that additional and better criteria will have to be proposed for the definition of certain disease entities. Similarly, the subclassification of peripheral T cell lymphomas showed high inter-observer variability.

Prognostic factors

As was clearly shown by the International Non-Hodgkin's Lymphoma Classification Project and many other multicenter trials published within the last years, the most important prognostic factor today is the definition of the disease entity, that is the exact type of non-Hodgkin's lymphoma. Within these individual diseases, prognostic factors may influence clinical outcome. Prognostic factors and variations in grades within diseases should be distinguished from different diseases. They may be histological, biological or clinical in nature, such as stage or the International Prognostic Index. Histological grading is one method to

define types of prognostic factors. Usual approaches include the determination of cell size, nuclear features, mitotic rates, and growth pattern. In the last years, biological markers, such as genetic features, have turned out to be important prognostic factors and may even be more powerful than clinical or morphological features. Some of them may be recognized today by interphase cytogenetics (such as p53 and ATM deletions in B-CLL), or by immunohistochemistry (such as the presence of the t(2;5/ALK) rearrangement in anaplastic large cell lymphomas of T and 0 cell types, or the determination of the proliferative index in mantle cell lymphomas using the Ki67 antibody). More recently, exciting new data have become available taking into account mutated or unmutated IgVH genes in B-CLL or the subdivision of diffuse large B cell lymphomas according to their mRNA expression profiles.

These examples show how dependent a given classification is on new facts and findings that may be used for the definition of diseases in the borderline and gray zone of different diseases. It does not need great fantasy to predict that the new approaches of molecular biology will further modify our concepts on the biology of malignant lymphoma, and that in the not too distant future, our concepts of lymphoma classification will be stepwise modified and possibly reverted to a molecular definition of neoplastic processes in different lymphoma entities.

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Table 1. The WHO classification of lymphoid neoplasms

B cell neoplasms

Precursor B cell lymphoblastic leukemia/lymphoma (Precursor B cell acute lymphoblastic leukemia)

Peripheral B cell Neoplasms

- B cell lymphocytic leukemia/small lymphocytic lymphoma
- B cell prolymphocytic leukemia
- Lymphoplasmacytic lymphoma
- Mantle cell lymphoma
- Follicular lymphoma
- Cutaneous follicle center lymphoma
- Marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT type)
- Nodal marginal zone B cell lymphoma (+/- monocytoid B cells)
- Splenic marginal zone B cell lymphoma (+/- villous lymphocytes)
- Hairy cell leukemia
- Diffuse large B cell lymphoma
  - Variants: Centroblastic
    Immunoblastic
    T cell or histiocyte-rich
    Anaplastic large B cell
  - Subtypes: Mediastinal (thymic) large B cell lymphoma
    Intravascular large B cell lymphoma
    Primary effusion lymphoma
- Burkitt lymphoma
- Plasmacytoma
- Plasma cell myeloma

T cell neoplasms

Precursor T cell lymphoblastic leukemia/lymphoma (Precursor T cell acute lymphoblastic leukemia)

Peripheral T cell and NK cell neoplasms

- T cell prolymphocytic leukemia
- T cell large granular lymphocytic leukemia
- Aggressive NK cell leukemia
- NK/T cell lymphoma, nasal and nasal type
- Sezary syndrome
- Mycosis fungoides
- Angioimmunoblastic T cell lymphoma
- Peripheral T cell lymphoma (unspecified)
- Adult T cell leukemia/lymphoma (HTLV 1+)
- Anaplastic large cell lymphoma (T and null cell types)
- Primary cutaneous CD30 positive T cell lymphoproliferative disorders
  - Variants: Lymphomatoid papulosis (Type A and B)
    Primary cutaneous ALCL
    Borderline lesions
- Subcutaneous panniculitis-like T cell lymphoma
- Enteropathy-type T cell lymphoma
- Hepatosplenic g/l T cell lymphoma

Hodgkin lymphoma (Hodgkin disease)

Nodular lymphocyte predominance Hodgkin lymphoma

Classical Hodgkin lymphoma

- Hodgkin lymphoma, nodular sclerosis
- Classical Hodgkin lymphoma, lymphocyte-rich
- Hodgkin lymphoma, mixed cellularity
- Hodgkin lymphoma, lymphocyte depletion

CLINICAL IMPLICATIONS OF THE WHO CLASSIFICATION OF LYPMHOID NEOPLASMS

Hanneke C. Kluin-Nelemans

Department of Hematology, Groningen University Medical Center, Groningen, The Netherlands

Whereas clinicians in the sixties and early seventies had to be familiar with only a handful of lymphoid malignancies such as lymphosarcoma, reticulosarcoma, chronic and acute lymphoid leukaemia, they had to become familiar with more and more different names and entities since the mid-seventies. With the classifications developed by Lukes, Lennert and Rappaport, it did not became easier. The presence of more than 30-40 different lymphoid malignancies was very confusing for hematologists and oncologists. Different names were used for similar neoplasms (for example: small centrocytic, intermediate lymphocytic, type Berard, mantle cell lymphoma) creating confusion and even mistakes. In the absence of earmarked therapies for every entity apart, pathologists were urged to lump entities facilitating therapy. This resulted in an attempt to group leukaemia's and lymphomas in three major cohorts, the Working Formulation (1). This simple classification has been (and unfortunately still is) immensely popular in spite of the wrong concept by creating a mixture of all kind of different lymphomas in the same three groups: low grade, intermediate grade and high grade malignant lymphoma. T and B cell lymphomas were grouped, as were for example patients with a low grade MALT and follicular lymphoma. Patients with mantle cell lymphoma were - depending on the therapy trial group to which they were submitted - either considered as low grade (Germany), or intermediate grade (EORTC). Finally, new entities could not be classified. It is evident that clinical studies focussing on new treatment modalities ran into problems as all so-called intermediate grade malignant NHLs were lumped together, thus ignoring the large differences in outcome of patients in such a group, for example those with anaplastic large cell lymphoma, peripheral T cell lymphoma or mantle cell lymphoma (see further).

It took almost 15 years before the breakthrough with the REAL classification (2) and thereafter the WHO classification (3) was received and widely accepted, especially by the clinical specialists in the field. The remarkable product of the new WHO classification is that all unique entities (the 'real' entities) have been defined in close co-operation with a group of clinicians during the Airlie House Conference (3). This group formed a Clinical Advisory Committee, and consisted of more than 40 haematologists and oncologists from all over the world. Among the clinicians were several well known haematomorphologists. This resulted in a series of important decisions, such as split of leukaemias (which had been grouped as one disease in the REAL classification, and clearly should have been separated as B-CLL versus B-PLL) and, on the other hand, grouping of entities (Burkitt's lymphoma and ALL L3 into Burkitt's lymphoma/leukaemia; Burkitt-like lymphoma should be treated and considered as a variant of Burkitt's lymphoma; no difference anymore between lymphoblastic leukaemias and lymphoblastic lymphomas, omit the terminology L1, L2 and L3 for the acute lymphoblastic leukaemias, etc.) (3). Further details and specifications were finally published in the WHO Blue book (4).

With this new classification, new groups could be formed based upon for example lineage (B versus T), maturity (immature versus mature, pre-follicle versus post-follicle), localisation (nodal versus extranodal), or prognosis. A very good overview and validation of grouping according to prognosis is shown by Chan *et al*

(5). In this study it appeared possible to find groups of lymphomas with a 5-year survival greater than 70%, consisting of ALCL, extranodal marginal zone B cell lymphoma of MALT type and follicular lymphoma; next, a group of lymphomas with a 5-year survival between 50% and 70%, consisting of nodal marginal zone lymphoma, lymphoplasmacytoid lymphoma and small lymphocytic lymphoma; thirdly, a group with a 5-year survival of 30-50%, consisting of DLBCL, Burkitt's lymphoma, primary mediastinal B cell lymphoma; and finally the worst lymphoma group with a 5-year survival of less than 30%, consisting of precursor T lymphoblastic lymphoma, peripheral T-cell lymphoma and mantle cell lymphoma. These four groups clearly illustrate how diverse is the clinical behaviour of malignancies that were grouped in the Working Formulation as so-called lymphomas that should be treated as if having a similar prognosis. It is therefore evident that lumping is out, and that terminology like low grade lymphoma should be banned.

Although clinicians nowadays need to know much more about lymphoid malignancies, they will at the same time gain an enormous insight in these diseases. As an illustration to this, the following anecdote is important: During the Airley House conference in 1997, a voting was held whether a simplified classification should be shown representing the thirteen most occurring lymphoid malignancies (90% of all NHLs), or on the other hand the complete list including all rarities. Whereas the majority of the *pathologists* assumed that the clinicians would prefer the simplified list, this was not the case. On the contrary, almost all *clinicians* voted for the full list! And so it happened (3,4).

With a few examples it can be shown how important the new WHO classification has become for clinicians, but still will not be the last classification either:

- extranodal MALT lymphoma of the stomach should be recognised as separate entity amongst the indolent lymphomas: eradication of Helicobacter pylori can result in longstanding remissions or even cure (6);
- many trials now successfully incorporate anti-CD20 immunotherapy, necessitating a classification into B and non-B;
- patients with mantle cell lymphoma are now being treated in separate trials aiming at a better outcome;
- no different trials anymore for T-lymphoblastic NHL and T-ALL, as has been the case among paediatricians for many years.

However, it is important to be aware of the shortcomings as well; see a few other examples:

- The wastebasket DLBCL will certainly be split up in the future. The concept generated by gene expression arrays (activated B cell type versus germinal center derived) (7) need to be validated in prospective trials, but might become important. In DLBCL, prognosis is thus far much better estimated by clinical parameters (age, performance, LDH level, stage of the disease, number of extranodal sites, the so-called IPI score) (8). The pathologists (or molecular biologists?) still have a lot of work to do.
- Similar lymphomas as defined by the pathologist can have a very different prognosis depending on their localisation in the body: compare B-NHL of the skin with B-NHL of the testis or of the brain. Here, not pathology, but the localisation is a more important prognosticator. This illustrates, moreover, how important a close co-operation between the clinician and pathologist is.

In conclusion, the new WHO classification has been a major breakthrough, not only for pathologists, but also for clinicians. This classification is certainly not the last one, but an excellent beginning for a new concept, where clinicians closely co-operate with pathologists in the design of new concepts, diagnoses, and clinical trials.

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THE RECOGNITION OF MORPHOLOGICAL PATTERNS IN THE DIAGNOSIS OF MALIGNANT LYMPHOMAS

Philip M. Kluin

Department of Pathology and Laboratory Medicine,
Academic Hospital Groningen, The Netherlands

According to the modern lymphoma classifications such as the REAL and WHO classifications, malignant lymphomas are defined on basis of a combination of clinical presentation, histology and cytology, immunophenotype and molecular genetic features. This concept is different from that of previous classification schemes in which only histology (working formulation of 1982, only 20 years ago!) or only the combination of histology, cytology and immunophenotype (Kiel and Lukes classifications) were taken into account.

In spite of these achievements, in almost 90% of the cases the hematopathologist will not need the additional clinical and molecular features and instead can rely on histology, cytology and immunophenotype.

The principal differences between histology and cytology are well known, but especially inexperienced pathologists are inclined to read the histology slides with eyes of a cytologist, i.e. at a too high magnification. In consequence, they will overlook the wealth of information revealed by the architectural patterns to be recognized at low power.

In the past much more attention has been paid to pattern recognition, for instance the atlas published by Taylor et al was entirely arranged on basis of patterns. Additionally, Nathwani et al published several manuscripts on this subject. At present, this approach seems to be old-fashioned, but we have to realize that the human brain is perfectly adapted to visual pattern recognition, and can handle very fast (less than one second) with the numerous and complex data generated by a single pattern. Reading a histological slide of a lymphoma can therefore be compared with reading a map of a small city with recognition of the basic principles of the plan, the main streets, railways, parks and buildings, with knowledge of their function as a castle, church, city hall, museum, railway station or post office.

In parallel, histologic patterns can only be understood by having knowledge on the function of the structures recognized. Sometimes, the structures can be difficult to recognize in H&E stained slides and may be masked by the dominant cellular infiltrate comparable to the traffic jam that might disorientate the car driver in the city. Examples are the presence of follicular dendritic networks in angioimmunoblastic T cell lymphomas that are only unraveled by staining for CD35 or CD21 and the presence of preexistent germinal centers in (some cases of) mantle cell lymphoma which are best visualized by CD10 or BCL-2 immunostaining. Therefore immunohistochemistry is sometimes necessary to recognize a certain pattern.

In reverse, there are also conditions in which the real (malignant) nature of the condition is masked by a dominant reactive pattern. Two examples are the presence of many small granulomas in some cases of marginal zone lymphoma of the spleen, and Hodgkin's lymphoma, a disease by definition dominated by an intense relationship between reactive cells and the minority of tumor cells. In table 1 some examples are given for B cell lymphomas. This table shows that a "marginal zone" pattern (underlined in the table) can be found in many types of NHL. Because of the clinical implications, it is especially necessary to exclude mantle cell lymphoma by the application of immunohistochemistry.

Finally, in spite of their shortcomings, it is important to keep on the official terminologies for these patterns. For instance the term "follic-

ular" should be restricted to lesions in which germinal centers are present, the term "pseudofollicular" for the typical lesions in CLL that contain the larger prolymphocytes and paraimmunoblasts, and "nodular" should be used for all other lesions with a nodular appearance. Even although the (primary) follicles seen in both nodular lymphocyte predominant Hodgkin's lymphoma and mantle cell lymphoma are stuffed with follicular dendritic reticulum cells and may contain some residual follicle centers, they are called "nodules" and not "follicles".

The author will present the main and variant patterns that should be recognized by the hematopathologist as essential ingredients of lymphoma classification and the differential diagnosis of reactive lymphadenopathies.

Table 1. Some examples of histologic patterns in B cell lymphomas

Lymphoma type	Dominant pattern in the lymph node	Variant pattern in the lymph node
Precursor B & T cell lymphoblastic	diffuse	single files, interfollicular
Small lymphocytic lymphoma/ CLL	pseudofollicular	diffuse, marginal zone
Mantle cell lymphoma	diffuse, monotonous, single epithelioid histiocytes	nodular, mantle zone, marginal zone, follicular colonization
Follicular lymphoma	follicular +/- interfollicular	minimally follicular or diffuse, marginal zone, with discordant component
Nodal marginal zone	marginal zone (parafollicular)	diffuse
Splenic marginal zone	diffuse with follicular colonization, sinusoidal	-
Extranodal marginal zone	marginal zone	diffuse
Diffuse large B cell lymphoma	diffuse	cohesive, starry sky, T cell or histiocyte rich, sclerosis, compartmentalization, clear cell appearance, sinusoidal, with follicular component,
Burkitt lymphoma	diffuse, cohesive, starry sky	more loosely arranged (autolysis/poor fixation)
Plasmacytoma	interfollicular	diffuse

IMMUNOHISTOLOGICAL ANALYSYS OF MALIGNNAT LYMPHOMAS ON PARAFFIN-EMBEDDED SECTIONS WITH COMMERCIALLY AVAILABLE ANTIBODIES

Marco Paulli

Anatomic Pathology Section, Department of Human Pathology, University of Pavia, Italy

Introduction

Many purely morphological classifications for NHLs have been proposed since 1970. However, the absence of uniform diagnostic criteria for lymphoid tumors has resulted in problems for both pathologists and clinicians, hampering comparison of the various studies reported in the literature. The just published World Health Organization (WHO) Classification of tumors of Haematopoietic and Lymphoid Tissues (1) has definitely sanctioned a new approach to lymphoma classification where all available information, morphology, immunophenotype, genetic and clinical features are used to define a disease entity. As a consequence, immunophenotypic analysis currently constitutes an integral part of the work-up for lymphomas improving the accuracy of classification and dividing tumors into distinct prognostic and therapeutic groups.

Immunophenotyping of hematopoetic neoplasms

Lineage-related antigen expression is a basic principle of lymphoma immunophenotyping. The histodifferentiation of hematopoietic neoplasms roughly parallels the differentiation pathway of their normal counterparts (2): a B-cell neoplasm is expected to express antigens such as CD20 or CD79a whereas a T-cell lymphoma is expected to express CD2 and CD3 antigens. However, maturation of neoplastic lymphoid cells may arrest at any stage of differentiation; consequently, the expressional pattern of lineage specific antigens of a hematopoietic neoplasm relies on the degree of maturation. In addition antigenic aberrations (i.e. loss of lineage specific-antigens or expression of other lineage specific antigens) may occur. In addition, molecular studies have shown that most haematopoietic neoplasms may have one or more genetic abnormalities often resulting in overexpression of related proteins (i.e. bcl-1, bcl-2 and ALK). However, in spite of their biological complexity, any given haematopoietic malignancy usually expresses a constant, consistent set of antigens. Consequently a proper lymphoma subtyping has become increasingly reliant on the immunophenotypical analysis by means of antibody technique. Fortunately, numerous new antibody clones have become available that coupled with various antigen retrieval techniques have obviated the need for frozen tissues in most cases. Nowadays, pathologists may confidently investigate formalin-fixed paraffin embedded lymphoma tissues for a much wider range of antigens than was possible a few years ago thus achieving a high degree of accuracy in the lymphoma diagnosis and classification. We will briefly describe the antigenic profile exhibited on paraffin sections by each of the most relevant lymphoma subtypes which may be detectable using commercially available antibodies (3).

Major lymphoma subtypes and their antigenic profile in paraffin sections

The WHO lymphoma classification stratifies lymphoid neoplasms primarily according to cell lineage and morphology into three major categories: B-cell neoplasms, T/NK neoplasms, and Hodgkin's disease (HD). B and T-cell NHLs are furtherly subdivided in two major categories, respectively: precursor neoplasms, corresponding to the earliest stages of differentiation, and mature ("peripheral") neoplasms, corresponding to more differentiated stages.

Mature B and T/NK –cell neoplasms are also informally grouped according to their major clinical presentations: predominantly disseminated/leukemic; predominantly nodal diseases and primary extranodal forms.

Precursor B and T-cell neoplasms

B lymphoblastic lymphoma: Precursor B lymphoblastic lymphoma occurs over a wide age range from children to adults but most patients are less than 35 years of age. Lymphoma cells antigenic profile: TdT+; CD79a+, CD10/CALLA+, HLA-DR+, and CD34 -/+; CD20 is often negative. Cytoplasmic Igm can be demonstrated in some cases. Non-lineage antigen reactivities include CD43 and sporadically cytokeratin.

T lymphoblastic lymphoma: Lymphoblastic lymphoma usually affects adolescents and young adults, but no age group is exempt. Most cases present with a large mediastinal mass; others with lymphoadenopathy or primary extranodal disease. Lymphoma cells antigenic profile: TdT +; CD1a +/-, CD3 +/- (diffuse cytoplasmatic staining); CD7 + and CD4 + or CD8+ (CD4/CD8 coexpression may occur), and CD10 +/-. Non lineage reactivities include expression of myeloid markers and sporadically CD117 (c-kit).

Mature B-cell neoplasms

B-cell NHLs predominantly composed of small lymphocytes are an heterogeneous group of NHLs that have recently been defined by the WHO lymphoma classification. Seven different subtypes can be identified, respectively 1) chronic lymphocytic leukemia/ small lymphocytic lymphoma; 2) B-cell prolymphocytic leukemia 3) lymphoplasmacytic lymphoma; 4) mantle cell lymhoma; 5) extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma); 6) splenic marginal zone lymphoma and 7) hairy cell lukemia. These lymphomas can be difficult to distinguish from one another because of their morphologic similarities. However, they exhibit distinctive immunophenotyic (3), genotypic, and clinical features which may reveal useful in their accurate identification.

Small lymphocytic lymphoma/chronic lymphocytic leukemia (CLL): composed of small lymphocytes may present as either lymphomas or leukemias.(chronic lymhpocytic leukemia and the leukemia of larger cells defined as "prolymphocytic leukemia"). Lymphoma cells antigenic profile: CD20-/+, CD79A+, CD5+; CD23+; CD43+; CD10-; CD38-/+; DBA 44-; CD68-; bcl-1-; bcl-2+; bcl-6-. CD20 and CD23 expression is indeed much stronger in prolymphocytes and paraimmunoblasts than in the small cell component. Cases of CD38+ CLL have been recently suggested to originate from naive B-cells and to run a rather aggressive clinical course than negative ones. Bcl-6 is never expressed by lymphoma cells. Its presence or absence is useful to distinguish between neoplastic pseudofollicles/ proliferation centre (bcl-6 and CD10 negative but CD5 positive) and residual germinal centres (bcl-6 and CD10 positive but CD5 negative).

Lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia: such a diagnosis is often achieved by exclusion because it comprises lymphomas that do not display features that would allow their inclusion among the other subtypes of small cells lymphoma (B-CLL; MCL; MZL) or follicular lymphoma. Clinically most patients have a monoclonal IgM serum paraprotein (>3gm/dl, Waldenstrom macroglobulinemia) with consequent hyperviscosity symptoms. Lymphoplasmacytic lymphoma is composed of small lymphocytes which show tendency to differentiate towards the plasma cell stage. Lymphoma cells antigenic profile: CD20+/-; CD79a+; CD5-; CD10-; CD23-; CD43+/-; cytoplasmic IgM+; DBA-44-; CD68-; bcl-1-; bcl-2+; bcl-6-. Absence of CD5 and CD23 and strong positivity for cytoplasmic IgM are helpful in distinguishing between lymphoplasmacytic lymphoma and B-CLL.

Hairy cell leukemia: neoplastic cells which are seen in the circulation, in the bone marrow and in the red pulp of the spleen, are characterized by the presence of fine villous surface projections and bean shaped nuclei. Paraffin sections antigenic profile: CD20+; CD79a+; CD5-;

CD10-; CD23-; CD43-; DBA-44+; CD68+ (often cytoplasmatic dot reactivity); bcl-1-; bcl-2+; bcl-6-. Tumor cells also intensely express (membrane reactivity) CD25/interleukin-2 receptor.

Marginal zone B-cell lymphoma (MZL) (extranodal MALT type, nodal and splenic +/- villous lymphocytes): these lymphomas are thought to represent the neoplastic counterpart of the marginal zone cells found in the spleen and extranodal mucosa associated lymphoid tissue (MALT) but scarcely pereceptible in normal lymph node. Cytologically the lymphoma cells vary in shape from small "centrocyte-like" cells to "monocytoid B cells" to plasmacytoid cells. Lymphoma cells antigenic profile: CD20+; CD79a+; CD5-; CD10-; CD23-; CD43-/+; CD68-/+; bcl-1-; blc-2+; bcl-6-. In addition, lymphoma cells often express CD35. A positive staining for monotypic Ig at the intracytoplasmatic (perinuclear) level may be observed. To date no specific markers for marginal zone cells are commercially available but recently promising results have been obtained by Falini et al. who generated specific paraffin sections working monoclonal and polyclonal antibodies against the human IRTA1 (immune receptor translocation associated-1) protein (unpublished observation). This molecule is the product of the homologous gene involved in the myeloma-associated t(1,14) (q21;q32) translocation and it has been associated with normal and neoplastic marginal zone B-cells. The splenic marginal zone lymphoma (SMZL) may be difficult to differentiate from HCL. Clinical features and outcome (mainly) and some phenotypic differences (i.e CD25 negativity and notes of plasmacellular differentiation) allow differentiation of SMZL from HCL.

Mantle cell lymphoma (MCL): it was first identified by the Kiel group as "centrocytic lymphoma". Its pattern of antigenic expression is distinctive whereas, clinically, it usually had a high frequency of advanced stage disease with bone marrow involvement, and the lowest 5-year survival of any type of lymphoma. Lymphoma cells antigenic profile: CD20+; CD79a+; CD5+; CD43+; CD10-; CD23-; CD35-; bcl-1/cyclin-D1+; bcl-2+; bcl-6-. Bcl-1/cyclin-D1 expression but lack of CD23 are useful criteria to distinguish MCL from other small B-cell neoplasms (3).

Follicular lymphomas (FL): neoplasms of follicle centre B-cells, which exhibit at least a partially follicular pattern. Recently a FL grading (1,2,3) has been introduced based on counting of number of centroblasts in neoplastic follicles and growth pattern (follicular *vs* diffuse). Lymphoma cells antigenic profile: CD20+; CD79a+; CD10 +/-; CD5-; CD23-; CD43-; bcl-1-; bcl-2+; bcl-6+. In some cases (i.e. lymphoid follicular proliferations) a prompt distinction between a reactive versus a neoplastic follicle can be difficult to solve by light microscopy exhamination requiring immuhistochemical investigations. Positivity for Bcl-2 and MT2/CD-45RA of follicle germinal centres in conjunction with a reduced number of Ki-67 positive follicle centre cells favor a FL vs. follicular hyperplasia (4).

Diffuse large B cell lymphoma (DLBCL): This is an heterogeneous category that includes centroblastic, immunoblastic lymphomas and other variants of primary nodal and extranodal large B-cell lymphoma (i.e., anaplastic, T-cell-rich/histiocyte rich, mediastinal B-cell lymphoma, intravascular B-cell lymphoma and body cavity based). Lymphoma cells antigenic profile: CD20+; CD79a+; CD10-/+; bcl-1-; bcl-2+; bcl-6+. Sporadically CD5 and CD43 expression may occur. Tumor cells in mediastinal B-cell lymphoma intensely stain for CD23. CD30 expression is the rule in the ALCL variant (typical membrane and perinuclerar dot-like positivity) which is however ALK negative. CD30 positivity has been also noticed in a variable number of tumor cells in other subtypes, usually also providing a diffuse cytoplasmatic reactivity. The body cavity based variant usually affects HIV patients, is associated with HHV 8 infection and exhibit a peculiar antigenic profile: CD45+; HLA-DR+; CD30+; EMA+; CD38+, CD138+ in absence of both B and T-cell lineage markers.

Burkitt's lymphoma (BL): the neoplasm is monomorphic and consists of medium-sized B lymphoma cells with numerous macrophages containing cellular debris which produce a

"starry sky appearance". Lymphoma cells antigenic profile: CD20+; CD79a+; CD5-; CD10+; CD43+/-; bcl-1-; bcl-2-; bcl-6+ and Tdt-. In BL with plasmacytoid features intracytoplsmatic immunoglobulin can be demonstrated. Epstein Barr Virus (EBV) is regularly integrated in the endemic form of BL but in less than 25% of HIV negative sporadic forms and in about of 50% of HIV-related BLs. Recently the term Burkitt's-like/atypical Burkitt's lymphoma has been introduced to define cases showing greater pleomorphisms in lymphoma cells nuclear size and shape than BL but characterized by high degre of apoptosis and high mitotic index. It has been suggested that Burkitt-like lymphomas in contrast with BL are CD10 negative and bcl-2 positive. However, the Burkitt-like lymphoma issue is still open and its histogenesis remain controversial.

Plasmocytoma/Myeloma: Plasma cells neoplasm that usually lack B-cell surface antigens, but express the CD138 antigen. Cytoplasmic positivity is present for CD79a, EMA and Ig (exhibiting light chain restriction). Cytokeratin positivity in plasmocytoma/myeloma cells has been reported.

Mature T-cell and NK neoplasms

Mature T-cell lymphomas: Lymphomas derived from mature or post-thymic T-cells. Because NK-cells are closely related, and share some immunophenotypic and functional properties with T-cells, these two classes of neoplasms are considered together (5). About 90% of mature/peripheral T-cell lymphomas express CD45/45RB, T-cell lineage markers CD2 and CD3, and T-cell related markers CD45RO and MT-1/CD43. The T-cell receptor–associated protein Beta-F1 may be detected on paraffin sections in approximately 60% of T-cell lymphomas. Most cases have a T-helper phenotype (CD4+/CD8-), but T-cytotoxic suppressor (CD4-/CD8+) or aberrant (CD4-/CD8-; CD4+/CD8+) phenotype sporadically occur. However in T-cell lymphomas aberrant loss of one or more T-cell antigens (i.e. CD2, CD3, CD5 or CD7) is frequent. Loss of CD7 has been considered a common finding in mycosis fungoides. In angiocentric T-cell lymphoma, particularly in the NK/T-cell lymphoma nasal type, loss of several T-cell antigens and expression of NK-related markers (i.e. CD56, perforin, granzyme B, TIA-1 molecule) are common, suggesting their origin from NK cells or (less frequently) from cytotoxic T-lymphocytes. Expression of the CD15 antigen (usually paranuclear dot-like reactivity) has been reported in about 15% of T-cell lymphoma including MF.

CD 30+ Anaplastic Large Cell Lymphoma (ALCL) T and "null" type: Among T-cell lymphoma, ALCL deserves a special attention. Clinically, ALCLs have been divided in primary nodal/systemic and primary extranodal (cutaneous) forms, the latter characterized by a very favourable outcome in most cases. ALCL cells express, besides the CD30, other activations antigens and several cytotoxic markers (i.e. TIA-1, perforin and granzyme B). More than 50% of primary nodal/systemic ALCL express the ALK protein that is associated with a t(2;5). Highly specific anti-ALK monoclonal antibodies ALK1 and ALKc are now available and both suitable for paraffin sections immunohistochemistry. The immunohistochemical identification of ALK (6) is clinically relevant: ALK positive primary nodal ALCLs (usually affecting children and young adults) seem to have a superior prognosis than similar but ALK negative cases. Alk positivity may also be a useful marker to differentiate between primary cutaneous CD30+ ALCL (constantly ALK negative) from secondary skin dissemination of systemic CD30+ ALCL (ALK positive).

Hodgkin's lymphoma (HL)

By morphologic and immunophenotypic criteria (7), Hodgkin's lymphoma has been divided into the nodular lymphocyte predominance (NLPHL) and other subtypes termed as "classical Hodgkin's lymphoma".

NLPHL: the characteristic antigenic profile of the hallmark lympho-histiocytic (L&H) "popocorn"-cells is CD45+; CD20+; LN1/CDw75+; CD79a+; Bcl-2+. EMA and CD30 may be variably expressed, the latter if present may pro-

duce a cytoplasmic diffuse staining; CD15 is uniformly negative. Typically, in NLPHL, a subset of CD57+ small T-lymphocytes surrounds the CD20+ L&H cells.

Classical HL: the classical Reed-Sternberg cells are CD45 negative but they usually coexpress the CD15 and CD30 antigens; in some cases because of antigen masking caused by the formalin fixation the CD15 immunostaining may give up requiring additional antigen demasking procedures (i.e neuraminidase proteolytic pre-treatment). RS cells also stain for IL-2 CD25, CD71; HLA-DR, HLA-DR invariant chain/LN2/CD74; in addion, fascin and restin, two intermediate filaments–associated proteins, have been detected in tumor cells. Expression of CD20 may occur in about 20% of cases. LMP-1 antibody raised against Epstein Barr virus latent membrane protein is also often positive in RS cells.

Histiocytic disorders

Disorders originating from dendritic and histiocytic cells are rare but some of them, i.e. Langerhans cell histiocytosis (LCH) and Rosai-Dorfman disease (RDD) are well established clinicopathologic entities (8). Other histiocytic neoplasms (i.e. histiocytic, Langerhans and dendritic/follicular cell sarcoma) are exceedingly rare and poorly defined both clinically and biologically.

LCH: Three major syndromes have been recognized, respectively: a) unifocal disease (solitary eosinophilic granuloma) b) multifocal unisystem disease (Hand-Schuller-Christian disease) c) multifocal multisytem disease (Letterer Siwe disease). The LCH hallmark cell is the Langerhans cell (LC) that has nucleus containing typical linear grooves. Immunohistochemically LC are CD1a+; MT-1/CD43+, S-100+ and HLA-DR+; the CD68 is variably expressed. Recently a novel monoclonal antibody DCGM4, termed Langerin, has been developed that selectively reacts with LC on paraffin sections.

RDD: Also known as sinus histiocytosis with massive lymphoadenopathy, RDD is a non neoplastic histiocytic disorder. Lymph node sinuses are expanded by large histiocyte-like cells (Rosai Dorfman cells) which exhibit lymphocytophagocytosis. The large RD cells express the S-100 protein, CD68 and, variably, lysozyme and CD43 whereas CD1a is usually negative.

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THE DIAGNOSIS OF MALIGNANT LYMPHOMAS FROM CELL SAMPLES: COMBINED MORPHOLOGICAL AND FLOW CYTOMETRIC ANALYSIS

Veronika Kloboves-Prevodnik

Department of Cytopathology, Institute of Oncology, Ljubljana, Slovenia

At present, the diagnosis of non-Hodgkin lymphomas (NHL) is based on histopathological examination of surgically removed lymph node, supported by immunohistochemical methods (1,2). Judged from our experience and experience of some other cytopathologists abroad, fine needle aspiration biopsy (FNAB) and cytopathological examination could play an important role in pre-operative diagnostics of NHL. FNAB is a quick and patient-friendly method that allows a simple and rapid selection of the patients with lymphadenopathy in whom surgical biopsy is indicated. In patients with multiple enlarged lymph nodes, the most appropriate one for surgical biopsy can be selected. With this approach, the number of unnecessary surgical biopsies would decrease and the time needed to complete the diagnostic procedures would be considerably shorter. FNAB offers a possibility to define the cause of lymphoadenopathy in the patients in poor physical condition, so that they do not need undergo a diagnostic surgical procedure.

With standard microscopic examination of Giemsa and Papanicolaou stained lymph node aspirate, effusion and cerebrospinal fluid (CSF), it is not always possible to differentiate between reactive and neoplastic lymphocytic proliferations. Moreover, reliable subclassification of the lymphomas is hardly feasible (3). Therefore, in the late 1980s, the immunocytochemical method was introduced in the cytopathologic diagnostics of lymphomas. In this field, a pioneering work was done by Tani (Sweden) and Katz (USA) (4,5). However, due to non-standardized fixation procedures of samples, non-standardized immunocytochemical methods, and several other factors that might have influenced immunocytochemical reaction or interpretation of its results, the differentiation between the reactive and neoplastic lymphocytic proliferations in cytological samples did not reach the required diagnostic accuracy.

In a pilot study, testing different fixatives and fixation procedures, we found methanol fixation as the most appropriate one. In methanol fixed smears immunoreactive cells were brightly stained, with preserved distinct morphological features. A part of the same sample was also run by flow cytometer to verify the reliability of immunocytochemical reactions. There was a good correlation between the results of both methods; however, flow cytometric (FC) immunophenotyping provided more information on the phenotype of tumor cells and, at the same time, more data required for the identification of a prevailing clone in the tumor (7). Similar results have been reported by other authors who have also shown improved accuracy of cytopathologic diagnosis of lymphomas using FC immunophenotyping in FNAB samples (8-12).

What does flow cytometric immunophenotyping offer?

Flow cytometry is a powerful technology that allows simultaneous measurements of multiple different characteristics of the cells moving in a fluid stream. In a flow cytometer, cells pass one by one through a laser light beam. As each cell passes through the light beam, the light is scattered in different directions, depending on the physical characteristics (i.e., size and internal complexity) of the cell. At the same time, the laser light source excites fluorochrome-conjugated probes bound to the cell and endogenous fluorescent material. The light emitted from

these excited fluorochromes is simultaneously detected and measured. Depending on the flow cytometer used, fluorescence emissions of two, three, or more wavelengths can be simultaneously detected. As a result, cellular physical characteristics and binding of multiple fluorochrome-labeled probes, such as monoclonal antibodies, can be rapidly measured on thousands of cells. Usually, the cells are stained with antibodies that are directly conjugated to a fluorochrome (i.e., direct immunofluorescence). Less often, indirect immunofluorescence may be used. In this case, the cells are stained with an unlabeled antibody that is subsequently, in a second step, labeled with fluorochrome-conjugated antibody (13).

The development of flow cytometers that are compatible with clinical laboratory settings has provided an important diagnostic tool for hematopathologists. The hematopoietic cells lend themselves readily to analysis by flow cytometry because they are already in suspension (i.e., blood and bone marrow) or can be often quite easily disintegrated (i.e., lymph node, spleen). A large number of monoclonal antibodies directed toward cell surface, cytoplasmic, and nuclear antigens are available to distinguish the lineage and function of hematopoetic cells. For most clinical purposes, labeling of the cells with directly conjugated antibodies is preferable because the technique is much more rapid and easier to use than indirect immunofluorescence, particularly in multicolor FC analysis (14).

Sample preparation techniques for the detection of surface antigens by flow cytometer are highly standardized. A known number of cells (generally 0.5 to 1.0 x 10^6) is dispersed into each staining tube for direct labeling of cell surface antigens. The cells are first incubated with directly labeled antibodies according to the standard procedure. After staining, red blood cells lysis and cell fixation are recommended before running the samples on the flow cytometer (14). On the other hand, sample preparation techniques for the detection of cytoplasmic and nuclear antigens are not well established. With the exception of immunoglobulin and TdT antigens, detection of other antigens important for lymphoma diagnosis (i.e., bcl-2, bcl-6, Ki-67, cyclin D1) is still problematic.

FC immunophenotyping of hematological diseases has many advantages. It is a highly sensitive, quantitative, and high-speed technology that can be used to measure simultaneously multiple biologic properties of viable or fixed cells in suspension. One of the most powerful features of FC analysis is gating, allowing selective analysis of individual cell in a heterogeneous suspension by its physical and staining characteristics. This approach enables selective analysis of cell populations of interest even when they constitute a small fraction of heterogeneous cell mixture. The major disadvantage of FC measurements is loss of tissue architecture, which is sometimes important in diagnosis of lymphoid disorders (13,14).

The development of new monoclonal antibodies and fluorochromes with different emission spectra was followed by an improvement in acquisition and analysis techniques of FC measurements. Multiple cell surface antigens may be simultaneously assessed for and correlated to each cell under analysis. Multicolor immunophenotyping with combined light scatter and surface marker gating in the clinical laboratory has improved the diagnosis and prediction of prognosis for lymphoproliferative disorders and leukemia, and has become important in the follow-up evaluation of patients with these diseases.

Flow cytometric immunophenotyping in cytopathological diagnosis of NHL

Poor cellularity is the major problem in the analysis of cytological samples. Therefore, we modified the sample preparation technique; we started to use three- instead of two-color FC measurements, modified the acquisition technique and selected appropriate light scatter and surface marker gating. These efforts resulted in reliable immunophenotyping even when less than 0.1 to 0.2 x 10^6 cells per staining tube are analysed (0.5 to 1.0 x 10^6 cells are usually recommended). In addition, cell loss during sample preparation was minimal even in large cell lymphomas.

1. Lymph nodes

The differential diagnosis of enlarged lymph nodes is a daily problem in clinical practice. Lymphadenopathy may occur due to reactive lymphocytic proliferation, metastases or lymphoma. FNAB is a well-established tool for the diagnosis of metastases, but its role in the diagnosis of lymphoma remains controversial. It is generally accepted as a method for the determination of the extent of disease at presentation, evaluation of treatment response, confirmation of the recurrent disease and obtaining tissue for ancillary studies (12). However, its use in primary diagnosis of lymphoma is limited to few institutions (5, 15).

To evaluate diagnostic sensitivity and specificity of cytomorphology in combination with flow cytometry in the diagnosis and subclassification of NHL, we analyzed 649 FNAB lymph nodes and solid tissue samples from 576 patients diagnosed at the Institute of Oncology Ljubljana from 1998 to 2001. Excluded from the study group were 69 (10.6%) cases where surgical biopsy and histological examination were not performed and 2 (0.3%) cases with missing follow-up data. Out of 578 samples, 314 (54.3%) were true positive for non-Hodgkin's lymphoma, 232 (40.1%) true negative, 17 (2.9%) false positive and 15 (2.6%) false negative. The sensitivity and specificity were 0.95 and 0.93, respectively. FC immunophenotyping was performed in three different ways, (a) two-color immunophenotyping with light scatter gating (N=141), (b) three-color immunophenotyping with combined light scatter and CD45 surface marker gating (N=176), and (c) three-color immunophenotyping with combined light scatter and CD19 surface marker gating (N=261). When only the results of 261 three-color immunophenotyping with combined light scatter and CD19 surface marker gating was considered, 0.98 sensitivity and 0.95 specificity were obtained.

Among 15 false negative cases, there were 5 T-cell lymphomas, 4 diffuse large B-cell lymphomas with abundant reactive lymphocytic background, 1 T-cell rich B-cell lymphoma, 3 marginal zone lymphomas, 1 small lymphocytic lymphoma and 1 follicular lymphoma, grade 3. These results are in concordance with the reports in the literature where most false negative cytological diagnoses have been reported in marginal zone lymphomas and large cell lymphomas with abundant reactive lymphocytic background (16). Among false positive results, there were interesting cases in which monoclonality and unusual immunophenotypic characteristics of reactive lymph nodes led to incorrect diagnosis.

In addition to differentiating between reactive proliferations and NHL, cytomorphology in combination with flow cytometry allows to determine the lineage of non-Hodgkin lymphoma and to differentiate small cell from large cell lymphoma. However, it is not certain at present whether it is accurate enough to substitute histological examination in subclassification of NHL.

2. Effusions

Involvement of body cavities with lymphoma may occur as a part of the primary presentation of lymphoma or may develop in the course of the disease. Accurate diagnosis of NHL in effusions is essential to determine the extent of disease and to evaluate treatment response, which may affect both the prognosis and therapeutic options. Cytomorphological differentiation between malignant and reactive lymphatic cell proliferation in effusions is difficult and the detection rate has been reported as low as 10% (17). There is limited published information about the use of immunocytochemical, flow cytometric and molecular genetic ancillary techniques in the cytological evaluation of effusion samples. However, several reports indicate that these techniques, in conjunction with conventional cytological evaluation, substantially enhance the detection of NHL cells in effusions (18-22).

According to our experience, the detection of minimal residual disease (i.e., less than 5% of lymphoma cells in the effusion sample) using immunocytochemical method is unsatisfactory. Due to nonspecific immunocytochemical reactions and low sensitivity of the method we rather introduced FC immunophenotyping in routine cytological diagnosis of effusion samples in the begining of 1998.

From 1998 to 2001, we performed FC immunophenotyping of 56 effusion samples in 44 patients. FC immunophenotyping was performed when abundant population of lymphoid cells was present in the effusion or in patients with previous diagnosis of NHL. Immunophenotyping was performed in three different ways, (a) two-color immunophenotyping with light scatter gating (N=13), (b) three-color immunophenotyping with combined light scatter and CD45 surface marker gating (N=13) and (c) three-color immunophenotyping with combined light scatter and CD19 surface marker gating (N=26). The final cytopathological diagnois based on morphological and immunophenotypic findings was correlated with histological findings and clinical data.

The combination of cytomorphology and FC immunophenotyping was found to be highly sensitive and specific. If only the results of three-color immunophenotyping with light scatter and surface marker gating (CD45 or CD19) were considered, the sensitivity and specificity were 1.0. When only the results of two-color immunophenotyping with light scatter gating were considered, the sensitivity decreased to 0.66 and the specificity was still 1.0. Lymphoma was misdiagnosed in one angioimmunoblastic T-cell lymphoma and one unclassified small cell non-Hodgkin lymphoma because lymphoma cells were not identified in reactive lymphocytic background either cytomorphologicaly or flow-cytometrically. With reexamination of smears, no lymphoma cells were observed; however, with the reanalysis of flow cytometric cytograms, distinct lymphoma cell populations were identified in both cases. Three- or four-color immunophenotyping with combined light scatter and surface marker gating would preclude flow cytometric misdiagnosing in these cases.

Malignant lymphocytic effusions in patients with B-cell NHL occurred in 23 cases and in those with T-cell lymphoma in 4 cases. Interestingly, in effusions caused by B-cell lymphomas, on average only 44% lymphatic cells were malignant (range: 1%-94%), the rest were reactive T-lymphocytes. In addition, less than 5% malignant B-lymphatic cells were found in 4 cases. In all malignant lymphocytic effusions, we found specific immunophenotypic characteristics of lymphoma cells that separated them from the reactive lymphocytes, i.e. monoclonal B-cell population, phenotypic features of lymphoma already present at other sites and aberrant immunophenotype in T-cell lymphomas.

3. Cerebrospinal fluid

The analysis of cerebrospinal fluid (CSF) represents the first diagnostic approach in many inflammatory neurologic diseases and the routine procedure in patients with NHL. In patients with NHL, the demonstration of central nervous system involvement is often an important determinant for clinical management. At present, the study of CSF cells is restricted to the analysis of their morphologic characteristics, which has several limitations for a specific identification of different cell types. Initially, immunocytochemical techniques have been used to improve the identification of different cell types in reactive and malignant CSF lymphocytosis (23). The presence of normal or reactive lymphocytes and especially paucity of cells in a sample can hinder the identification of lymphoma cells in CSF. To overcome these problems, FC immunophenotyping has been implemented in some laboratories.

At the beginning, low number of cells available in CSF represented a serious obstacle for FC immunophenotyping. In some studies, successful FC immunophenotyping was performed when 25 ml CSF was sent to analysis (24). However, at diagnostic lumbar puncture 5-8 ml of CSF is routinely collected (25), because larger volume causes discomfort of the patient and may provoke serious complications. With the advent of multi-color immunophenotyping with selective gating techniques FC analysis of routinely obtained, hypocellular CSF samples became possible (26-28). Reliable results were obtained in CSF samples containing at least $1x10^4$ cells/ml (27,28). In native CSF, up to 90% of cells do not survive more than 120 min after lumbal puncture. In addition, during each centrifugation step, a mean cell reduction of 16% was reported (25). To overcome technical

limitations related to cell loss, it is important to analyze CSF samples brought on ice immediately after their collection or at least within 3 hours; at the same time, centrifugation steps should be kept at minimum (27).

FC immunophenotyping in conjunction with morphological examination substantially enhanced the detection of NHL cells in CSF fluid. Finn reported 43% improvement and French 50% (26, 28). One of FC immunophenotyping strengths is sensitivity. By using selective gating procedures based on combined antigen expression and light scatter characteristics, B-cell clonal population constituting fewer than 1% of total events was detected (26, 28). This sensitivity approaches that of DNA-based PCR or Southern blot detection methods used for detection of B-cell monoclonality in lymph node samples (29). Furthermore, flow cytometry not only detects clonal B-cell populations, but can also detect additional surface antigens that may help in further characterization of the lymphoma.

Conclusions

1. Combination of cytomorphology and FC immunophenotyping is highly sensitive and specific for diagnosing NHL from FNAB samples. Three- or four-color immunophenotyping with combined light scatter and surface marker gating must be used to prevent false negative flow cytometric diagnosis. Subclassification of non-Hodgkin's lymphomas is at present not reliable enough. However, we believe that in future well-elaborated diagnostic criteria would allow accurate cytological subclassification of NHL.

2. FC immuphenotyping of effusions is indicated when abundant population of lymphoid cells is found on cytological examination or if the patient had previous diagnosis NHL. Three- or four- color immunophenotyping with combined light scatter and surface marker gating must be used to preclude false negative results because, in effusion samples, lymphoma cells are usually mixed with reactive lymphatic cells.

3. In CSF samples, sensitivity of FC immunophenotyping is high in comparison with immunocytochemical methods. Modified protocol for FC sample preparation prevents cell loss and makes successful immunophenotyping possible even in CSF samples containing 10^4 to 10^5 cells/ml.

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THE ROLE OF MOLECULAR EVALUATION IN THE DIAGNOSIS OF MALIGNANT LYMPHOMAS: TECHNIQUES AND LIMITATIONS, PROBLEMS OF INTERPRETATION

Philip M. Kluin

Department of Pathology and Laboratory Medicine, Academic Hospital Groningen, The Netherlands

Molecular (genetic) analysis has become an indispensable adjunct in lymphoma diagnosis and classification. It is typically a third step in the diagnostic workup, after histology, cytology and immunophenotyping. So far, there is no place for a routine molecular analysis as a first step in this workup.

In a standard hematopathology unit, which often receives many difficult consultation cases as well, molecular genetic analysis will be applied on no more than only 5 to 10% of all cases. This relatively low percentage is caused by the three step strategy, the continuously increasing number of antibodies that can be used as a second step in the diagnostic workup and (often based on novel insights stemming from molecular biology) the increasing knowledge how to use histology and immunohistology. For instance, monoclonal antibodies against oncogene products such as cyclin D1, ALK and BCL10 can often replace molecular tests as the detection of t(11;14), t(2;5) and its variants, and t(1;14) and t(11;18), respectively. An example of the latter is the diagnosis of mantle cell lymphoma: based on the increased knowledge fostered by the combination of clinical, histological, phenotypic and genetic data, an experienced hematopathologist can presently diagnose this entity on a single H&E stained slide in 90-95% of the cases. In contrast, before 1992, this entity was frequently not recognized on H&E stained sides and also mixed up with other entities such as lymphocytic lymphoma, marginal zone lymphoma and follicular lymphoma with a low content of centroblasts.

Molecular analysis will be performed for the following reasons:

- differentiation between reactive and neoplastic, i.e. polyclonal versus clonal;
- classification of a (neoplastic) disease, for instance the presence of t(11;14) in mantle cell lymphoma or the presence of EBV in PTLD;
- information on clonal identity of 2 or more lesions, for instance 2 synchronously or metachronously occurring lymphomas with different morphologies;
- detection of minimal residual disease.

With respect to clonality analysis the main targets are the immunglobulin (Ig) or T cell receptor (TCR) gene rearrangements that occur during the early development of B and T cells. As an alternative, the presence of any other acquired genetic alteration can be looked for, ranging from translocations or single point mutations to clonal integration of EBV. With respect to the detection of clonal Ig or TCR gene rearrangements, Southern blot analysis is still the gold standard. This method is much more laborious and time consuming than PCR analysis and can only be applied on high quality DNA from fresh or frozen material, but has a number of intrinsic advantages over PCR analysis. With an intrinsic ability to detect a clone that makes up 5-10% of the cells to be analyzed, it shares a very high sensitivity with a very high specificity of both nearly 100%.

In general this intrinsic ability of Southern blot analysis (5-10%) is good enough for lymphoma diagnosis but of course not for detection of minimal residual disease. For this purpose, PCR methods and preferably allele specific amplification techniques (ASO-PCR) are necessary. The same is true for the analysis of samples with partial involvement by tumor cells, for example in some cases of anaplastic large cell lymphoma (ALCL), T cell or histiocyte rich B cell lymphoma. However, the main dan-

ger of PCR analysis, a too high sensitivity, resulting in the detection of completely irrelevant small clones that are reactive, cannot be emphasized enough. For instance, small reactive clonal expansions of both B and T cells which can be easily picked up by PCR may be encountered in benign Epstein Barr virus infections, carcinoma patients but also in patients with CLL and other indolent lymphomas and leukemias.

The advantage of Southern blot over PCR analysis is most pronounced in Ig gene analysis. Using a single target (CDR3) for VDJ rearrangements, clonality analysis fails in approximately 40-50% of the follicular lymphomas and 20-40% of the extranodal marginal zone lymphomas, i.e. (apart from the post transplant lymphoproliferative disorders) the two single lymphoma categories for which such an analysis is most required. This low sensitivity is mainly due to the occurrence of the germinal center cell associated hypermutations that affect the annealing sites of the primers used in this PCR reaction.

To improve this unacceptable low sensitivity of the test many investigators designed other primers or primer combinations either or not in multiplex PCR tests. For instance much effort was put in primer design during workpackage 1 of the recent BIOMED program on clonality analysis coordinated by prof. dr. JJM van Dongen in Rotterdam. Indeed, using 3 (multiplex) PCR reactions a sensitivity of up to 90-95% can be achieved, however, this is accompanied by a lower specificity as well, with incidental aberrant bands being generated by some of the primer combinations.

In addition, due to the inability to generate PCR products longer than approximately 200-300 base pairs from formalin fixed and paraffin embedded tissues, only framework 2 and 3 and not framework 1 primers can be used for this analysis.

Although hypermutations are absent in TCR genes, other problems such as the limited repertoire of TCR gamma rearrangement may interfere with the PCR analysis of TCR gene rearrangements in T cell lymphomas. However, this can be overcome by using other TCR targets such as the TCR beta genes or other readout systems than fragment size analysis such as heteroduplex analysis (HDA, see below).

Clonal rearrangements are also very useful targets for identity analysis of two synchronous or metachronous lymphoma samples with different morphology. Thus, these methods have been extensively used in large B cell lymphoma, Hodgkin's lymphoma or Richter's transformation in CLL and large B cell lymphoma or Hodgkin's lymphoma development in follicular lymphoma patients. Most authors use fragment size analysis (by running the product on agarose or polyacrylamid gels or by performing gene scan analysis) or sequence analysis, but a very elegant and rapid read out system is represented by the abovementioned HDA which takes advantage of both the size and nucleotide composition of the PCR products.

Chromosomal translocations are highly characteristic for a number of B and T cell lymphomas. Some examples of these translocations occurring in mature B and T cell lymphomas are given in Table 1. Of note, at least three different mechanisms are involved in these translocations:

- juxtaposition of an intact gene to new enhancer sequences, mostly Ig or TCR enhancers,
- juxtaposition of a gene to promotor sequences of an other gene (leaving the transcription unit intact), or
- fusion of two genes, the genomic structure of both genes being disrupted and resulting in a gain of function for one or both fused genes.

Table 1. Main chromosomal translocations and involved genes in human lymphomas

Lymphoma	Translocation	Frequency	Genes	Type of translocation
Mantle cell lymphoma	t(11;14)	95%	Cyclin D1, IgH	juxtaposition of enhancer
Burkitt lymphoma	t(8;14,t(2;8), t(8;22)	95%	MYC and Ig loci	juxtaposition of enhancer
Follicular lymphoma, grades 1, 2 and 3A	t(14;18)	90%	BCL2 and IgH	juxtaposition of enhancer
Marginal zone, stomach	t(11;18)	30-40%	API2, MLT	fusion
Marginal zone, lung	t(11;18)	60%?	API1, MLT	fusion
Diffuse large B cell lymphoma	3q27	40%	BCL6	promotor substitution
	t(14;18)	20%	BCL2 and IgH	juxtaposition of enhancer
	t(8;14),t(2;8), t(8;22)	5-10%	MYC and Ig loci	juxtaposition of enhancer
Anaplastic large cell lymphoma	t(2;5) and other 2p23 translocations	40-70% dependent on age	ALK	fusion

Various methods can be applied for the detection of these translocations and include classical cytogenetics, metaphase or interphase fluorescence in situ hybridization (FISH), Southern blot analysis, regular PCR, long distance PCR or RT-PCR.

RT-PCR is only feasible for detection of fusion genes, i.e. some extranodal marginal zone lymphomas and anaplastic large cell lymphoma. Regular PCR analysis is often difficult to apply since the breakpoints are often too dispersed to be detected. For instance only 40% of the t(11;14) breakpoints in mantle cell lymphoma cluster in the major translocation cluster (mtc) and can be detected by PCR, whereas the other 60% are dispersed over a region of 300 kilobases. In follicular lymphoma and Burkitt's lymphoma this can be improved by multiplex PCR with multiple primers as devised in the BIOMED program, long distance PCR and Southern blot analysis, however the method of choice is interphase FISH, especially since this can be applied on routine formalin fixed and paraffin embedded tissues.

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ANAPLASTIC LARGE CELL LYMPHOMA (ALCL)

Marco Paulli

Anatomic Pathology Section, Department of Human Pathology, University of Pavia, Italy

Introduction

The term anaplastic large cell lymphoma (ALCL) indicates a heterogeneous group of subtypes in terms of morphology, phenotype, cytogenetic and clinical features. ALCL was originally described in 1985 by Stein et al. (1), as a peculiar lymphoma type consisting of large pleomorphic, anaplastic cells that uniformly expressed the Ki-1/CD30 antigen and that often exhibited a characteristic sinusoidal pattern of nodal involvement, mimicking metastatic carcinoma or melanoma. Subsequently, in a proportion of systemic (nodal) CD30+ ALCL mostly affecting children and young adults, the characteristic t(2;5) (p23q35) (2) and the expression of its related fusion protein NPM-ALK have been found.

Clinical classification

Four major clinical profiles (3) can be distinguished in ALCLs, respectively: A) nodal systemic CD30+ ALCL, B) primary cutaneous CD30+ lymphoproliferative disorders, C) secondary/transformed CD30+ ALCL and D) CD30+ ALCL related to various conditions of immunodeficiency.

A) Nodal/systemic CD30+ ALCLs

account for about 7% of all adult NHLs and 20-30% of large cell NHLs in children. Systemic ALCLs have a bimodal age distribution with a peak in the second decade and a smaller one in the sixth and seventh decades. Most patients present with generalised lymphoadenopathy, whereas B-symptoms occur in about 50% of cases. Extranodal localisations are frequent, preferentially involving skin and/or soft tissue, gastrointestinal tract, lung and bone but rarely bone marrow. The IPI (International Prognostic Index) is of some value in predicting outcome, but less effectively than in other lymphoma types; data accumulated indicating the ALK protein expression is the crucial parameter to predict survival in systemic CD30+ ALCL. ALK positive ALCL are characterised by typical clinical features including young age (mostly children and adults less than 35 years) and an exceptional favourable prognosis. On the other hand, systemic ALK negative ALCLs frequently have an aggressive, often poor-responsive clinical course especially if associated with secondary skin localisations. On such bases, it has been proposed that ALK positive ALCL may represent a distinct clinicopathologic entity, for which the term "ALK lymphoma" has been suggested (4).

Histology

Nodal involvement by ALCL may be sinusoidal, paracortical, mixed or diffuse. ALCL cells may exhibit a wide spectrum of cytological appearance. Since its original description (1), numerous morphologic variants (i.e. common, lymphohistiocytic, predominant small cell, Hodgkin's-like, sarcomatoid, neutrophil rich, etc.) have been described.

However, only two variants, respectively the lymphohistiocytic and the predominant small cell variants, in addition to the classic "common-type" have been included in the WHO classification of haematolymphoid neoplasms (3).

"Common type": it represents the more frequent cytologic subtype of ALCL in which a polymorphic and a monomorphic subform can be distinguished. In the polymorphic form the

lymphoma cells are large pleomorphic with chromatin-poor horseshoe-shaped nuclei containing multiple nucleoli. Cells with such cytologic features are considered hallmark cells; in addition, multinucleated cells often Sternberg-Reed-like may occur. In the monomorphic form the lymphoma cells vary in shape from large polygonal to immunoblastic.

"Lymphohistiocytic variant": first recognised by Pileri et al. (5), it is characterised by a predominant background of reactive histiocytes and plasma cells, masking the lymphoma cells, which are usually few in number and scattered or grouped in small clusters; CD30 immunostaining is very useful to highlight neoplastic elements.

"Predominant small cell variant": first recognised by Kinney et al. (6), it is characterised by a predominant population of small to medium-sized, often CD30 negative T-lymphocytes, with irregular, pleomorphic nuclei and a minor population of large CD30+ cells, which are scattered or in clusters; frequently the CD30+ lymphoma cells exhibit perivascular cuffing.

However, despite such distinctions, it is important to stress that in individual cases more than one cytological form may be observed within a single lymph node or at different biopsy sites. In addition, cytomorphologic changes (i.e. reduction in size) of the lymphoma cells have been observed following chemotherapy.

Antigenic profile

Lymphoma cells variably (about two-thirds) stain for the leucocyte common antigen (LCA/CD45) but uniformly express (membrane and paranuclear "dot-like" reactivity) the CD30 antigen (a member of the tumour necrosis receptor superfamily/TNF-R) and other activation antigens (i.e. interleukin-2 receptor/CD25, transferrin receptor/CD71, HLA-DR, HLA-DR invariant chain /CD74, CDw70 and EMA). About two-thirds of ALCLs have a mature T-cell phenotype (CD2+; CD3+; CD4 or CD8+) and contain cytotoxic molecules (i.e. TIA-1, granzyme B and perforin) (6) but only rarely they are positive for the CD56 antigen; 10-20% show a B-cell phenotype the remaining having a "null" (neither T- nor B-lineage markers) immunophenotype. Controversies still exist about the exact classification of the B-cell phenotye ALCLs which have been considered by the WHO lymphoma classification (4) a variant form of diffuse large B-cell lymphoma. Although it has been sporadically observed, the expression of CD15 RS cells related antigen is exceedingly rare in primary CD30+ ALCL. A variable percentage (ranging between about 50 to 85%) of T and null CD30+ ALCL express the NPM-ALK protein which is associated with the t(2;5) and its fusion variant forms. Specific monoclonal antibodies respectively ALK1 and ALKc, both specific for the cytoplasmic portion of ALK, are available (3) and suitable for paraffin section immunohistochemistry. ALK+ cases may show three different immunostaining patterns (respectively cytoplasmic only, cytoplasmic and nuclear, membrane) for the NPM-ALK protein and its fusion variants, depending on the subcellular distribution of the chimeric protein.

Differential diagnosis

Differential diagnoses include lymphadenopathies (i.e. necrotizing Kikuchi lymphoadenitis and infectious mononucleosis lymphadenitis) with a high content of large activated CD30+ lymphoid cells, other Hodgkin and non-Hodgkin lymphoma subtypes and non-lymphoid malignancies.

Distinction between ALCL and Hodgkin's lymphoma (i.e. syncitial variant of nodular sclerosis and lymphocyte depletion) lymphoma may reveal difficult in some cases.

ALCLs must be differentiated from large cell neoplasia (i.e. metastatic carcinoma and melanoma) which may exhibit a lymph nodal sinusoidal growth pattern or may also express (i.e. embryonal carcinoma, malignant fibrous histiocytoma, salivary glands, lung and pancreas carcinoma) the CD30 antigen. A panel of additional immune markers may usually solve most of such difficult cases; however, notably, the CD30 immunostaining pattern observed in the above-listed tumours is usually diffuse and confined to cytoplasm, thus diverging from the typical membrane and paranuclear CD30 immunoreactivity detectable in ALCLs.

Molecular biology and cytogenetic

About 90% of ALCLs clonally rearrange for the T-cell receptor (TCR) Beta and Gamma genes, including most cases with a "null" antigenic profile. A minority of cases do not show PCR-detectable TCR-monoclonality. Such negative cases probably include rare examples of true NK derived ALCLs. A non-random t(2;5) (p23;q35) is detectable in about one third of systemic ALCLs. This translocation results in a novel NPM/ALK gene and its related ALK protein overexpression, the latter involved in lymphomagenesis. Variant translocations involving fusion of the ALK-gene with another gene have also been reported resulting in a cytoplasmic-confined ALK protein immunostaining pattern. Translocations mostly occur in children and young adults in whom the ALK protein expression seems to be associated with a favourable clinical outcome (3,4).

B) Primary cutaneous CD30+ lymphoproliferative disorders

The spectrum of CD30+ primary cutaneous lymphoproliferative disorders (7) includes the lymphomatoid papulosis (LyP), anaplastic large cell lymphoma (ALCL) and a group of "borderline" lesions displaying clinicopathologic features in between LyP and ALCL. All these disorders have in common large atypical CD30+ cells some of which resemble in morphology RS and H cells, uniform negativity for the ALK protein and a relatively favourable clinical outcome (7,8).

Lymphomatoid papulosis (LyP): LyP is a rhythmic, paradoxical eruption, histologically mimicking a malignant lymphoma but clinically benign. LyP clinically presents with recurrent crops of papulo-nodular lesions (measuring in diameter from few millimetres to 1 cm), which regress spontaneously (within few weeks), leaving only a small scar or area of altered pigmentation. Histologically, the classical LyP lesions show a typical wedge-shaped pattern of dermal infiltration; the LyP cellular population is polymorphic and consists of few large atypical CD30+ cells (single or in small clusters), intermingled with a predominant reactive background of neutrophils, eosinophils, histiocytes and plasma cells. Usually, the atypical LyP cells have a T-helper cell phenotype (CD2+; CD3+; CD4+), express the CD30 and other activation antigens and contain the granule associated protein TIA-1. Limited genetic data are available but clonal rearrangement of TCR genes occurs in less than 50% of classic LyP cases. Although it has been reported an association (up to 20% in certain series) with lymphoma (mostly mycosis fungoides and Hodgkin's disease) LyP usually has a very favourable outcome, preventing patients from receiving unnecessary aggressive treatments (4,7,8).

Primary cutaneous CD30+ anaplastic large cell lymphoma (ALCL): Primary cutaneous CD30+ ALCL clinically presents as one to multiple (up to four-five) nodular or tumoral lesions. Spontaneous regression is usually not the rule, other than in cases previously classified as regressing atypical histiocytosis (RAH). Histologically, primary cutaneous CD30+ ALCL show a dense dermo-hypodermic infiltrate of large uniformly CD30+ anaplastic cells which are grouped in large cohesive sheets with few intermingled inflammatory cells. In the RAH cases the inflammatory background is abundant and often accompanied by transepidermal elimination of tumour cells. Lymphoma cells usually have a T-cell helper phenotype (CD2+; CD3+; CD4+) with expression of cytotoxic granule associated molecules. Aberrant T- (defective expression of CD2, CD5, CD7) or null phenotypes have been reported but rarely. Most primary cutaneous CD30+ ALCLs show clonally rearranged TCR genes. To date, it is widely accepted (2) that t(2;5) is not associated with the spectrum of primary cutaneous CD30+ lymphoproliferative disorders. Primary cutaneous ALCL usually affects adults and elderly patients but less frequently also children and adolescents. Although spontaneous lesional regression rarely occurs other than in RAH cases, extracutaneous disease progression occurs in less than 10% of cases and mostly confined to loco-regional lymph nodes. However, prognosis is favourable in the majority of cases (4,7,8).

Borderline lesions: These lesions have clinicopathologic features in between LyP and primary cutaneous CD30+ ALCL and are characterised by frequent divergences between the clinical and histological appearance. Although most borderline lesions clinically present with single to multiple small to large nodular lesions, they usually pursue a favourable prognostic outcome similar to LyP and often including spontaneous complete or partial lesional regression. Histologically, the cellular infiltrate involves the whole dermis and often the subcutis. The atypical CD30+ cells are grouped in small to medium-sized clusters intermingled with a variable amount of inflammatory cells, mainly granulocytes. Apoptotic features are frequent. Cases with similar clinicopathologic features have been previously classified as LyP type C, but the term borderline lesions seems to be preferable at least until more data are acquired to clarify the exact relationships of these lesions respectively with LyP and ALCL. Reports describing in details the antigenic and molecular profile of the borderline lesions are not available but from our (in part unpublished) and other experiences they closely resemble in antigenic and molecular profile the atypical LyP and ALCL cellular infiltrate. Although it has been suggested an increased risk for development of a CD30+ ALCL than patient with LyP, to date, most borderline lesions seem to pursue a favourable outcome similar to LyP (4,7,8).

C) Secondary/transformed CD30+ ALCL

Secondary CD30+ ALCLs (4) usually affect older adult, in whom they represent a progression of a pre-existing other nodal or primary extranodal lymphoma (i.e. Hodgkin's lymphoma, mycosis fungoides and peripheral T-cell lymphoma). In most of these cases the CD30+ lymphoma cells correspond in morphology to the common type previously described in the systemic form; they exhibit a T-cell or null phenotype but they are usually negative for the ALK protein. The prognosis of secondary CD30+ ALCLs is poor and is characterised by a rapid disease progression and fatal outcome.

D) CD30+ ALCL related to various settings of immunodeficiency

ALCLs have been described also in various conditions of immunodeficiency (4), including HIV infections and post-transplant lymphoproliferative disorders (PTLD). In both such conditions most of the ALCL cases exhibit a B-cell phenotype, lack the ALK protein and often harbour EBV. For such reasons most of these cases seem to be closely related to the anaplastic variant of DLBCL. However, in our series of about 780 transplants (hearth, hearth + lung, lung) recipients we have observed two cases of T-cell phenotype ALCLs, one of which confined to the skin. To date, limited data are available on the outcome of the immunodeficiency related ALCLs but it seems strictly dependent from the general status of the patient.

Conclusions

Initially, ALCL was recognised as a distinct entity on the bases of its distinctive histologic (anaplastic morphology and sinusoidal invasion) and immunophenotypic (CD30 expression) features. However, none of these features revealed to be entirely specific. A subsequent series of immuno-cytogenetic and clinical investigations better delineated the pathobiological features of ALCL thus providing a paradigm for the process applied by the WHO lymphoma classification to define new disease entities. However, to date, pathologists and clinicians must be aware that the definition of ALCL encompasses a heterogeneous group of subforms in terms of morphology, phenotype, cytogenetic and clinical features. In particular, it is crucial to distinguish between ALCL with or without anaplastic lymphoma kinase (ALK) expression. ALK positive lymphoma for which it has been proposed the term "ALK lymphoma" are characterised by peculiar clinical features including young age and very favourable prognosis. In contrast the true nature of ALK negative ALCL remains obscure calling into question whether a distinction with peripheral T-cell lymphomas not otherwise specified, is still indicated. In fact, although it

has been stressed that ALCL carry a significant better prognosis than other T-cell lymphomas, this may exclusively depend from the excellent outcome of the ALK positive subgroup.

In addition it is also very important to distinguish between primary cutaneous CD30+ lymphoproliferative disorders (including primary cutaneous CD30+ ALCL) characterised by an excellent prognosis from secondary skin localisations of systemic CD30+ neoplasms.

Despite their prognostic (favourable) similarities probably the primary cutaneous CD30+ lymphoproliferative disorders differ from systemic ALK positve lymphomas in terms of genotype, thus representing a different type of neoplasms in which an aggressive treatment is usually unnecessary.

In conclusion, the clinicopathologic spectrum of ALCL is today by one side broader and by the other narrower than originally believed. Further studies will be necessary before the ultimate definition of such intriguing lymphoma will be provided.

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PATHOLOGY AND MOLECULAR EVALUATION OF GASTROINTESTINAL LYMPHOMAS, ESPECIALLY MARGINAL ZONE AND ENTEROPATHY-ASSOCIATED LYMPHOMA

Johan H. J. M. van Krieken

Department of Pathology, University Medical Centre Nijmegen, Nijmegen, The Netherlands

Introduction

About 20% of all lymphomas present in the gastrointestinal tract and in the last few years many new data have been published concerning these lymphomas. Specific entities have been described like extranodal marginal zone lymphoma (MALT-lymphoma) and enteropathy-associated T-cell lymphoma (EATL). These lymphomas may be difficult to diagnose, partly because of the small tissue fragments that are available through endoscopic biopsies. The use of molecular techniques therefore is specifically useful is this area.

In general, the diagnosis of lymphoproliferative disorders is based on the histopathology and immunophenotype and most cases can be concluded without further techniques. However in a number of cases the differential diagnosis between a reactive process and malignant lymphoma is difficult and molecular techniques can be helpful. Especially the assessment of clonality is a useful adjunct since mature lymphocytes have rearranged immunoglobulin or T-cell receptor genes and thus almost all malignant lymphomas have at least one of these antigen receptor genes clonally rearranged. The assessment of clonality can be performed using Southern blot analysis using several restriction enzymes and many different probes. This is a reliable method but requires large amounts of high molecular weight DNA and is time consuming. Presently several different PCR-based methods have been described.

Several lymphoma types are defined by a specific translocation, which can be used in classification. One of these is a proportion of low grade gastric MALT lymphoma (the term used throughout this paper and equivalent to extranodal marginal zone lymphoma) that carry the t(11;18). Several techniques can be used to detect a translocation: classic cytogenetics, Southern-blotting, FISH and PCR or rtPCR.

The present paper deals with the use of molecular diagnostics in the diagnosis of gastrointestinal lymphomas, especially in the distinction between reactive lymphoid infiltrates and lymphoma and in the distinction of the t(11;18) positive lymphomas, which are refractory to Helicobacter pylori eradication (in contrast to low grade MALT-lymphomas that do not carry this translocation and that regress upon the eradication of Helicobacter pylori), since in these areas the results are clinically very relevant.

Clonality analysis

Funded by the European Community a group of 48 laboratories (including 32 PCR-facilities) started a collaborative study in 1997 for the development of new primers for clonality testing. The laboratories all had experiences with the molecular diagnosis of lymphoproliferative disorders and were from France, Germany, Great Britain, the Netherlands, Portugal and Spain. The first phase of the project consisted of the development of primers, which were tested in a selected series of cases that were fully analyzed by Southern blot analysis of the immunoglobulin heavy and light chain genes (IGH, IGK, IGL) as well as for the ß, ã and ä T-cell receptor genes (TCRB, TCRG, TCRD). The next phase was the testing of the selected primers in a large number of cases of all the entities of the WHO-classification for malignant lymphomas as well as a series of reactive lymphoproliferations. For all the relevant loci

specific primers were developed so that both complete and incomplete rearrangements were detectable and also that the different V-families could be recognized. This resulted in a large number of primer sets. Next a multiplex approach was developed in order to reduce the number of PCR tubes. The final number of PCR reactions for complete testing of both B- and T-cell genes could be reduced to 18 tubes.

The results of a PCR-reaction need to be analyzed. In principle there are two reliable methods and both were used: heteroduplex analysis and GeneScan analysis. For most primer sets these two methods were equally reliable, except for the IGK and IGL genes where GeneScan analysis was less suitable.

The primer sets were tested in 100 Southern blot defined lymphoproliferations and the results showed that in more than 95% of the samples not only the clone was detected by PCR but also the type of rearrangement could be defined, i.e. complete or incomplete etc. These results included cases of follicular lymphoma and multiple myeloma. In the next phase 50 or 100 cases of follicular lymphoma, marginal zone lymphoma, mantle cell lymphoma, , diffuse large B-cell lymphoma, angioimmunoblastic T-cell lymphoma, T-cell lymphoma NOS, large cell anaplastic T-cell lymphoma, and reactive lesions were investigated. The results show that in more than 90% of cases the clone could be detected. In about 10% of cases of reactive lesions also a clone was detected. These cases include EBV infections and atypical hyperplasias like Castleman's disease.

Pitfalls

During our testing several pitfalls were encountered. Occasionally the frozen tissue that was tested was not representative. This shows the importance of the frozen section H&E in case of frozen tissue being used.

We also found that when only few B- or T-lymphocytes were present in the sample we obtained "clonal" results. This is an important finding and can be explained by the fact that each mature B and T-cell will be amplified by our method and that our evaluation methods separated a dominant clone from a polyclonal background. The nature of an immune reaction is polyclonal but obviously this reflects many small clones, implying that sufficient lymphocytes are needed to obtain a polyclonal result.

Finally we found several cases of B-cell lymphomas with incomplete rearrangement of one of the T-cell receptor genes and vice versa, showing that care is needed when lineage is defined by the rearrangement of antigen receptor genes.

EATL and refractory celiac disease

Celiac disease (CD) is a chronic small bowel disorder characterised by malabsorption due to a T lymphocyte mediated gluten sensitivity with subsequent damage to the small intestinal mucosa. Treatment is straightforward with a lifelong gluten-free diet (GFD). In a subgroup of patients with CD clinical features and histological abnormalities persist or recur after initial improvement despite strict adherence to the GFD. When other causes of malabsorption with villous atrophy have been ruled out, such as bacterial overgrowth, giardiasis, eosinophilic enteritis, inflammatory bowel disease, hypogammaglobulinemic sprue, primary idiopathic collagenous colitis, tropical sprue, intestinal lymphoma and carcinoma, the syndrome is classified as "Refractory Celiac Disease" (RCD). This group probably comprises less than 5% of patients diagnosed with CD, although accurate epidemiological data on RCD are not available.

In patients with CD there is ample evidence that strict adherence to GFD results in a decrease of chronic intestinal inflammatory activity and that persistent intestinal inflammation in untreated CD and RCD has an increased risk of Enteropathy Associated T-cell Lymphoma (EATL) development. Recently several groups suggested that RCD is, in fact, a cryptic T-cell lymphoma and defined a subgroup of RCD and ulcerative jejunitis as a precursor stage of EATL, from which EATL derives.

Cellier et al described the presence of an abnormal monoclonal intra-epithelial T lymphocyte (IEL) population in nearly all patients diagnosed with RCD. It has been suggested to separate RCD into 2 categories, according to the presence or absence of an abnormal phenotype of intraepithelial T cells using a Fluorescence Activated Cell Scanner (FACS) and or the presence of a clonal T-cell population: RCD type I with a normal population of IELs and RCD type II in with an abnormal population of IELs.

RCD type I: By definition, immunophenotyping by flow cytometry shows a normal intraepithelial T-cell population in all patients. We found by PCR for TCR rearrangement polyclonal population in 8/10 patients tested.

RCD type II: The intraepithelial T lymphocytes in all patients, although histomorphologically normal, are immuno-phenotypically different from those found in RCD type I, CD and healthy controls. Characteristic is the absence of surface expression of CD3, variably the absence of surface expression of CD8, in the presence of intracytoplasmic CD3 and surface expression of CD2, CD7, CD103 (mucosa-associated), CD95 (Fas-receptor). TCR rearrangement studies showed a monoclonal population in 5 of 7 patients tested.

EATL: In all cases the immunophenotype is abnormal and the results of clonality testing showed a clonal T-cell receptor rearrangement in 8/8 cases.

Irrespective of clinical presentation of RCD, the identification of aberrant, monoclonal intraepithelial T cells determines the prognosis of patients. Although the majority of RCD patients presented with a polyclonal and normal T-cell population, weak monoclonal expansions were seen in 3 patients without any abnormality at T-cell flow cytometry. In 1/3 patients the monoclonal expansion disappeared after treatment. In contrast, 2/7 RCD type II patients had a polyclonal T-cell expansion in combination with a large population of aberrant T-cells at T-cell flow cytometry. With the currently used diagnostic approach using immunohistochemistry and TCR-rearrangement studies, these patients would not have been recognised, while both developed an EATL and died. The characteristics of the mucosal T cells play a central role in the therapeutical regimen of choice and the exact pathophysiology involved needs to be explored to come to clear treatment profiles. Pilot-studies are undertaken using chemotherapeutical treatment schedules, such as cladribine, in patients diagnosed with RCD type II with an aberrant intraepithelial T cell population.

Gastric MALT-lymphoma

MALT-type lymphoma has been recognised as a distinct entity within the category of extranodal marginal zone B-cell lymphomas listed in World Health Organisation classification. This type of lymphoma is the most frequent NHL to arise at extranodal sites, shows an indolent clinical behaviour, and may eventually disseminate to other mucosa-associated sites or sometimes to adjacent lymph nodes. It is the most frequent lymphoma to occur in the stomach, where its induction has been associated with Helicobacter pylori infection. Histologically, gastric MALT lymphomas comprises a spectrum from low- to high-grade cases. Low-grade MALT lymphoma is characterised by a diffuse infiltrate of small to medium sized lymphoid B-cells and lymphoepithelial lesions. In high-grade MALT-type lymphoma in addition large cell components and/or sheets of blasts are present. There are indications that transformation from a low-grade lymphoma into a high-grade disease can occur. In immunohistochemical studies of immunoglobulin light chain restriction and by molecular analysis of IgH rearrangements or numerical aberrations a clonal relationship between the small and large cell components has been demonstrated in gastric B-cell lymphomas. Many gastric large cell lymphomas lack features of MALT, especially in small biopsy specimens, and these are classified as diffuse large B-cell lymphoma (DLBCL) of the stomach.

Clonality assessment by PCR is a reliable tool to separate reactive from neoplastic B-cell infiltrates in the stomach. In our hands there were no false positives when the results could be

compared with immunofluoresence studies on frozen tissue specimens; furthermore 48 of 50 lymphomas did show a clonal B-cell population

Translocation detection t(11;18)

The most frequent structural abnormality associated with extranodal marginal zone lymphomas of MALT-type is t(11;18)(q21;q21) which results in fusion of the apoptosis inhibitor-2 (API2) gene on chromosome 11 and the MALT lymphoma-associated translocation (MLT or MALT1) gene on chromosome 18. This translocation can be detected by dual-color interphase fluorescence in situ hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR). Thus far t(11;18) is exclusively detected in LG MALT-type tumours of various sites, being absent in gastric HG MALT lymphoma and DLBCL, nodal and splenic marginal zone lymphoma and other NHL. Sequence analysis of the heterogeneous fusion cDNAs reveals different breakpoints both in the API2 gene and the MALT1 gene. T(11;18)(q21;q21) appears to be the sole genetic abnormality in most t(11;18)- positive lymphomas. In contrast, about half of the t(11;18)(q21;q21)-negative LG lymphomas show trisomies, especially of chromosome 3 and 18. This suggests that LG MALT lymphoma can be divided in lymphomas characterized by the t(11;18)(q21;q21) which are unlikely to transform into high-grade tumours and t(11;18)(q21;q21)-negative LG MALT-type lymphomas that may develop into HG MALT after acquisition of additional genetic aberrations.

We detected t(11;18) in about 25% of the gastric low-grade MALT. We did not find any major differences based on techniques used and the results of both FISH and RT-PCR experiments showed near identical frequencies (24% of the cases analysed by RT-PCR; 25% by FISH). Heterogeneity in the studied population might be an explanation for the variation in reported frequencies of t(11;18) in gastric low-grade lymphomas, for example selection of early versus more advanced low-grade lymphomas.

FISH can be applied both on archival frozen tissue and formalin-fixed paraffin-embedded tissue. It has been suggested that analysis of paraffin tissue might be less sensitive, because of breakdown and limited accessibility of target DNA. However, we found only minor differences in cut-off levels and frequencies of t(11;18) in frozen tissue versus paraffin embedded tissue. Because we applied probes both flanking and spanning the breakpoint at 11q21 we were able in to distinguish cases with chromosomal breakage at 11q21 and either presence or lack of a fusion to 18q21. However, in all cases breakage at 11q21 was accompanied by a translocation to chromosome 18.

The occurrence of t(11;18) is not restricted to lymphoma of the stomach: various reports have shown the occurrence of t(11;18)(q21;q21) in low-grade MALT lymphomas at sites including the lung, thyroid gland, salivary gland, orbit and lacrimal gland. These results indicate that t(11;18)(q21;q21) specifically characterizes a subgroup of low-grade MZBCL of the MALT-type that comprises approximately one third of low- grade lymphomas.

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PROGRESSION OF MALIGNANT LYMPHOMA

Hans Konrad Müller-Hermelink

Institute of Pathology, University of Würzburg, Germany

Introduction

Similar to oncogenesis of solid tumors, lymphomagenesis is considered to be a multistep process in which cumulative genetic alterations lead to autonomous growth of lymphoid cells at different stages of maturation, clinically presenting as "leukemia" and/or "lymphoma". In this process, regulatory homeostatic mechanisms controlling lymphocyte growth are overcome, resulting in the stepwise acquisition of properties referred to as "*the hallmarks of cancer*", namely, self-sufficiency in growth signals, insensitivity to antigrowth signals, escape from apoptosis, unlimited replicative potential, sustained angiogenesis and tissue invasion/metastasis.

With the advent of lymphoma classification systems integrating morphological, genetic and clinical characteristics of defined lymphoma entities, there is increasing evidence of a dynamic biological and morphological heterogeneity within one entity during the course of disease: similar to the stepwise process of oncogenesis/lymphomagenesis, lymphomas may cumulatively develop a more aggressive clinical, biological and/or morphological phenotype. This process is referred to by frequently used, but poorly defined terms, such as "transformation", "progression", "evolution", "variation", "conversion", "transmutation" or "dedifferentiation". Foulds, in his seminal work on tumor progression published in 1954, characterized progression as "*the development of a tumor by way of permanent, irreversible qualitative change in one or more of its characters*", pointing out that "*progression is different from mere extension in space and time without qualitative change, and from reversible variation due to environmental factors*…".

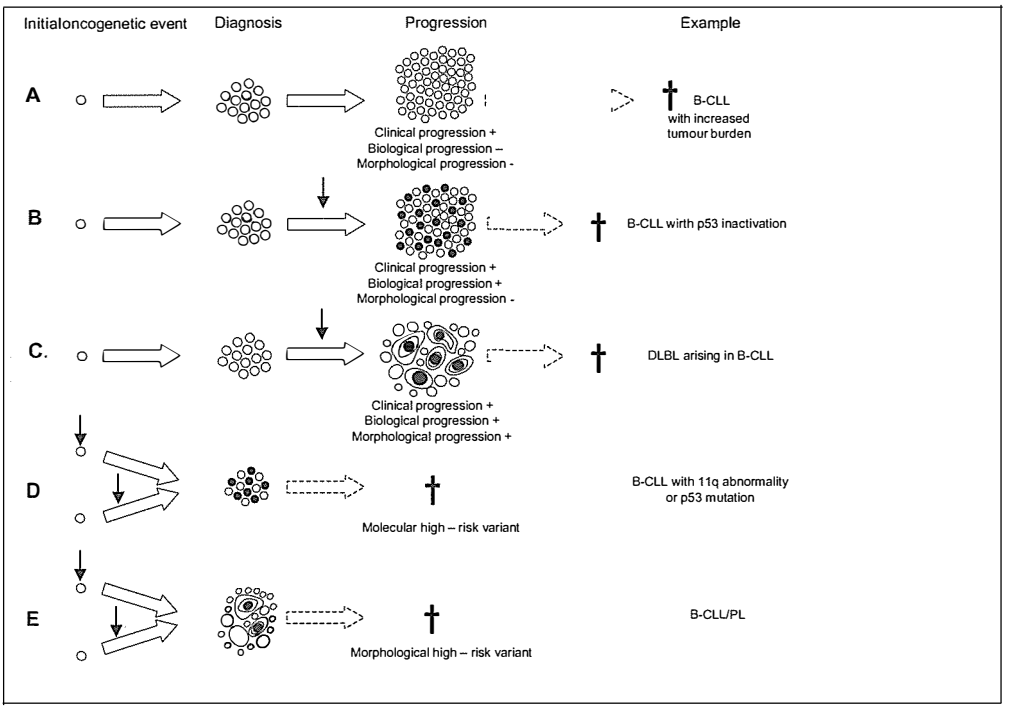
Morphological progression in lymphoma appears to be both particularly characteristic and diverse as compared to solid neoplasms. One of the earliest and most frequently cited examples of morphological lymphoma progression stems from Maurice Richter who, in 1928, reported the case of a 46 year-old male patient presenting with "*generalized reticular cell sarcoma of lymph nodes associated with lymphatic leukemia*". His purely morphological interpretation of the case was that "*the patient had chronic lymphatic leukemia...Subsequently there developed a rapidly growing, malignant tumor...* (which) *developed in, encroached upon, and destroyed tissue which was previously the seat of leukemic change*". Antedating the role of clonal identity in lymphoma progression, Richter stated that "*it is possible that the development of one of the lesions was dependent on the existence of the other...the evidence presented by the microscopic preparations... enables one only to diagnose the presence of two lesions, without giving any definite indication that they are genetically related*".

Lymphoma progression and composite lymphoma

Defining the term "lymphoma progression" in a modern sense raises several problems. The term lymphoma progression is commonly used in a descriptive sense with variable and not entirely overlapping meanings regarding morphology, clinical behavior and lymphoma biology .

Morphological lymphoma progression denotes changes of tumor cell morphology, tumor cell composition and/or pattern of growth during the course of disease or morphological heterogeneity at initial presentation as observed by

the pathologist, e.g. more mitoses, more blastoid appearance of lymphoma cells in sequential biopsies. Lymphoma *transformation* will be used as a synonym of morphological lymphoma progression in the following.



Lymphoma progression – a term with variable meanings

Various types of lymphoma progression can be distinguished. *Clinical lymphoma progression* (**A**) commonly denotes an increase in tumor burden, ultimately resulting in the death of the patient, yet no additional morphological or biological changes may have occurred in the tumor during the course of disease (e.g. B-CLL). *Biological lymphoma progression* (**B**), in contrast, reflects the biological property of tumor cells to acquire additional genetic alterations and functional capacities (symbolized by the arrow and the cells dotted black) leading to a selective growth advantage of tumor cell clones with a more malignant phenotype and increased clinical aggressiveness (e.g. B-CLL with secondary p53 inactivation). *Morphological lymphoma progression* (**C**), also referred to as *lymphoma transformation*, denotes changes of tumor cell morphology, tumor cell composition and/or pattern of growth during the course of disease, mostly associated with clinical and biological disease acceleration (e.g. DLBL arising in B-CLL). Mostly, clinical, biological and morphological lymphoma progression overlap, but they need not necessarily.

Molecular (**D**) and *morphological* (**E**) *high-risk lymphoma variants* should be distinguished from lymphoma progression. In contrast to morphological high-risk lymphoma variants (e.g. B-CLL/PL), molecular high-risk variants (e.g. B-CLL with 11q abnormality) may not be recognizable based on morphology alone. High-risk variants may either represent neoplasms with genetic alterations a priori conferring a particularly malignant phenotype, or they may represent "primarily progressed" lymphomas in which an aggressive phenotype developed at an early stage of disease before clinical lymphoma manifestation. Molecular mechanisms, however, associated with lymphoma progression or with a high-risk variant, seem to be similar.

Clinical lymphoma progression is used with regard to clinical disease presentation, commonly denoting accumulation of tumor burden (e.g. transition from localized to generalized disease with involvement of new sites) or according to treatment response (e.g. primary treatment resistance, or relapse after initial treatment response). Lymphoma dissemination or recurrent lymphoma is clinically often referred to as tumor progression.

Biological lymphoma progression denotes the biological property of tumor cells to acquire additional genetic alterations and functional capacities leading to a selective growth advantage of tumor cell clones with a more malignant phenotype.

In most instances, the different aspects of lymphoma progression will overlap: clonal acquisition of additional genetic changes in lymphoma cells ("biological lymphoma progression") will mostly be accompanied by a more aggressive morphology ("morphological lymphoma progression") and a more aggressive clinical presentation, e.g. disease dissemination and resistance to therapy ("clinical lymphoma progression").

However, these terms must not be equated in all instances. Morphological and clinical "lymphoma progression" not only include cases characterized by *clonal* development of a lymphoma into a more aggressive type (representing true biological lymphoma progression). Cases mimic "lymphoma progression" in which the "progressed" lymphoma component actually is a morphologically and clinically indistinguishable, secondary de novo, *clonally unrelated* neoplasm. Variability in lymphoma growth patterns have been referred to as morphological progression (e.g. in follicular lymphoma (FL)), but yet need not parallel biological or clinical progression. In addition, lymphoma progression may be mimicked by high-risk lymphoma variants, yet, they represent a variant of malignant lymphoma with an a priori unusually aggressive behavior.

Morphological lymphoma progression can not only lead to different morphological appearances or lymphoma types within subsequent biopsies, but even within one biopsy. In 1954, Custer introduced the term "composite lymphoma", denoting, based on morphology, the manifestation of more than one histologic pattern of malignant lymphoma occurring in different organs or within the same organ[8-10]. With the advent of immunohistochemistry and modern genetics, composite lymphoma was shown to result either from bimorphic phenotypic manifestation of one neoplastic clone or from coincident manifestation of two genetically distinct lymphoma types. Consequently, the term "composite lymphoma" should be reconsidered as a merely descriptive term for one morphologic manifestation encompassing at least two different biological processes, namely clonal transformation and coexistence of two clonally unrelated types of lymphoma which may have the same pathogenetic background (e.g. immunodeficiency-related) or may occur independently by chance.

From a biological point of view, we propose to limit the use of the term lymphoma progression only for *clonal* development of and within a lymphoma entity. The term composite lymphoma, in contrast, should be used as a merely descriptive term denoting both *clonal and non-clonal* processes. So far, the determination of a clonal relationship between two lymphoma components is hardly performed (and practicable) in daily routine and most reports on "lymphoma progression" and "composite lymphoma" use quite divergent definitions.

Patterns of morphological lymphoma progression/transformation

In general, two patterns of lymphoma progression can be observed by morphology:

- Lymphoma progression with largely maintained histomorphology of the preexisting lesion. This type of morphological progression is characterized by an increase in tumor cell/nuclear size, cellular/nuclear pleomorphism, more prominent nucleoli and increased mitotic activity. Although morphologically different from the initial malignancy, the progressed lymphoma still shows diagnostic characteristics of the initial lesion (e.g. FL

with increase in centroblasts, blastoid mantle cell lymphoma progressed from the classical variant, B-CLL/PL progressed from B-CLL). Presumably, this is the most frequent pattern of morphological lymphoma progression and likely is a clonal process in most cases; however, clonality analysis of this type of progression has only scantly been performed so far.

- Lymphoma progression with a morphological change resulting in an apparently new and distinct lymphoma type indistinguishable from an analogous de novo lymphoma, but with the same cell lineage and clonally related to the initial malignancy, such as DLBL progressed from FL or B-CLL.

In contrast, although virtually indistinguishable in daily morphology-based routine, the following patterns mimic lymphoma progression; however, they lack clonal relationship:

- Histomorphological changes in sequential biopsies indistinguishable from the latter pattern of lymphoma progression but representing a clonally unrelated process, namely the development of a secondary de novo lymphoma, such as de novo DLBL in B-CLL.
- Histomorphological changes in sequential biopsies without cellular and hence, clonal relationship to the initial malignancy, such as the occurrence of EBV-associated B-cell lymphoma in patients suffering from T-cell lymphoma or the development of a T-cell lymphoma in B-NHL patients.

Recently, single cases of coexisting, clonally related indolent lymphomas have been reported, possibly representing rare cases of a "metaplasia"-like transformation within the group of indolent lymphomas.

Frequency of lymphoma progression

Since the frequency of lymphoma progression can only be estimated based on sequential biopsies or demonstration of morphological progression within one biopsy, the exact frequencies of lymphoma progression are largely unknown for most lymphoma entities. Data on the frequency of lymphoma progression are strongly influenced by various factors such as the mere morphological definition of progression (in particular which lymphoma classification system is used), the inclusion or exclusion of patients in remission or in stable disease when calculating the frequency of progression, the number of sites biopsied at the time of diagnosis, the accessibility of a relapse site, the rebiopsy policy, the inclusion or exclusion of autopsy cases and the follow-up period/survival time of the patients.

In addition, as outlined above, in morphological lymphoma progression, a distinction between true biological progression and secondary de novo aggressive lymphoma is not routinely performed. Studies based on comparison of cytogenetics, examination of immunophenotype, immunoglobulin isotype, antiidiotype characterization or analysis of immunoglobulin gene rearrangement may not definitively demonstrate a clonal relationship between the initial and the progressed lymphoma, which can only be established by comparing the IgH/TCR CDR3 sequence. For most entities, data on frequency of lymphoma progression should be viewed with caution.

Despite the problems in determining the actual frequency of lymphoma progression, certain lymphoma types are more prone to progression than others. For example, t(11;18) positive marginal zone B-cell lymphomas (MZBL) of MALT-type have not been reported so far to progress to DLBL. Low risk variants of B-CLL with mutated IgV genes rarely progress, whereas FL implicate a high risk for progression to DLBL.

Lymphoma progression versus high-risk variants

Within (probably) all lymphoma entities, morphologically, immunohistochemically or genetically recognizable high-risk variants exist (Table 1). These high-risk variants either represent neoplasms within a defined lymphoma entity with additional genetic alterations a priori conferring a more malignant disease phenotype, or they may be "primarily progressed" lymphomas in which an aggressive phenotype developed in an indolent lymphoma "precursor" at an early stage of disease before clinical

Table 1. Mimics of lymphoma progression – morphological and molecular high-risk B-cell lymphoma variants

	Morphological high-risk variant	Molecular high-risk variant
B-CLL	CLL/PL CLL, mixed cell type Paraimmunoblastic variant Plasmacytoid differentiation ?	ATM inactivation p53 inactivation unmutated IgH CD38 expression trisomy 12 t(11;14) positivity proliferative activity (Ki-67 expression)
MCL	blastoid variant of MCL classical MCL with increased mitosis	p53 inactivation $p16^{INK4a}$ inactivation
FL	FL grade 3b ? FL with monocytoid B-cells FL with diffuse areas ? FL with sclerosis ? FL grade 2 vs. grade 1 ?	t(14;18) breakpoint p53 inactivation $p16^{INK4a}$ inactivation
MZBL of MALT	Increased number of transformed cells	t(11;18) positivity ? bcl-10 mutations
Splenic MZBL	Aggressive splenic MZBL	p53 mutation
HCL	HCL, blastic variant	

?: controversial high-risk variant

disease manifestation. In view of their rapidly progressing clinical course, these primary high-risk lymphoma variants might be misconsidered as resulting from "lymphoma progression"

Conclusion

Although morphological, clinical and biological lymphoma progression do not always overlap, readily recognizable morphological changes occurring in lymphoma along the course of disease may reflect changes in the clinical and biological lymphoma phenotype. Indolent and aggressive lymphoma in progression are either be clonally related or represent clonally unrelated neoplasms. The clonal process of lymphoma progression should be distinguished from clonally unrelated, secondary de novo or simultaneous lymphomas. "Composite lymphoma" should be used as a descriptive morphological term for different lymphoma entities in one individual either at diagnosis or occurring sequentially irrespective of clonal relationship. In contrast, we propose to use the term "lymphoma progression" in a *biological* sense denoting only clonal development of and within a lymphoma entity. All variants of aggressive B-cell lymphomas and Hodgkinžs lymphomas occur in progression of indolent B-cell lymphomas. Rarely, a "metaplasia"-like transformation within the group of indolent lymphomas is found. Within different

lymphoma entities, high-risk variants exist that mimic but are different from progressed lymphoma. Common secondary changes associated with lymphoma progression are the occurrence of secondary genetic aberrations leading to inactivation of cell cycle regulatory genes. Distinct primary genetic alterations occurring as acquired secondary lesions are associated with unusual, but well-defined lymphomas. EBV seems to play a major role in the pathogenesis of Hodgkinžs lymphoma occurring in indolent B-cell lymphomas and secondary DLBL in T-cell lymphomas.

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LYMPHOID AGGREGATES IN BONE MARROW - NEOPLASTIC, REACTIVE?

Mara Dominis[1], Sonja Džebro[1], Maruška Marušić-Vrsalović[2], Rajko Kušec[2,3]

[1] Department of Pathology and Cytology; [2] Division of Molecular Diagnostics, Institute of Clinical Chemistry; [3] Division of Hematology, Department of Medicine; University Hospital Merkur, Zagreb, Croatia

Bone marrow, an organ of lymphopoiesis, normally contains up to 20-35% lymphocytes. They are either diffusely dispersed or in form of nodular lymphocyte aggregates (LA) or in form of lymphocyte nodules i.e. nodular lymphoid hyperplasia (NLH).

Lymphocyte aggregates and nodules have been reported to occur in-between 1% to 60% of bone marrow specimens. They are more common findings in older age group (over 70yrs. of age), in female, and they are associated with certain nonhematological diseases, i.e. collagen vascular diseases, immune disorders, drug therapy, viral infections, etc).

Morphological features of benign lymphoid aggregates are well defined. They are well circumscribed, usually few in number, average 0.4 mm in size, cell population is polymorphous (lymphocytes, plasma cells, mast cells, occasional histiocytes and capillaries) within delicate reticular fibers network. They are distributed at random in the marrow, commonly non-paratrabecular.

The term nodular lymphocytic/lymphoid hyperplasia is used when more than four lymphoid nodules or aggregates are seen in low-power field, or if the nodules are greater than 0.6 mm in their greatest diameter. Lymphocytic nodules of various size and nodular lymphocytic hyperplasia are suspicious for lymphoma especially if they are located paratrabecular, around large sinuses, or if they contain fat cells. Lymphocyte aggregates are composed of B and T lymphocytes, although T lymphocytes usually predominate. Germinal centers with a mantle zone of small lymphocytes are present in a small proportion (about 5%) of lymphocytic aggregates. They are more common findings in female patients with underlying immune disorders.

The lymphocyte aggregates in patients with AIDS are distinctive. They are large, ill defined and the cells exhibit nuclear atypia with admixture of immunoblasts, and they can be misinterpreted as non-Hodgkin or Hodgkin's lymphoma.

The lymphocyte aggregates/nodules are considered as incidental findings in elderly patients. Long term studies revealed that younger patients with numerous lymphocytic aggregates may eventually develop a clonal disease, usually a low grade non-Hodgkin's lymphoma; this occurs especially in those having nodular lymphocytic hyperplasia.

Furthermore, patients with known lymphoproliferative diseases may have lymphocytic aggregates in bone marrow. They are considered to be normal or physiological findings with no association with the underlying disease if they are small and single. However, when they are larger and/or numerous, i.e. if they are in form of nodular lymphocytic hyperplasia, these findings are controversial and it is believed that they are neoplastic from the beginning.

Since there is no definitive numerical "cutoff" between lymphocytic aggregates/nodular lymphocytic hyperplasia and low grade non-Hodgkin's lymphoma, immunophenotyping should be performed in all patients in whom multiple aggregates are found as well as in those who have lymphocytic aggregates associated with already known lymphoproliferative disease.

During the period of last five years (1997-2001) the diagnosis of malignant lymphomas was made in 921 patients. For 392 patients tumor tissue was sent to us for consultation

from other institutions, while in 529 patients clinical staging was done and bone marrow biopsies were evaluated at the time of initial diagnosis (Table 1).

Myelodysplastic syndrome (MDS) is a clonal disorder that results in inhibition of normal hematopoiesis and contributes to the development of malignant hematological disease.

Table 1. Incidence of bone marrow pathology in malignant lymphomas - our experience

Type of lymphoma	No. of cases	Positive	Negative	LA/NLH
B–NHL	240	134 (55.8%)	95 (39.9%)	11 (4.6%)
T–NHL	34	11 (32.4%)	22 (64.7%)	1 (2.9%)
ALCL	49	3 (6.1%)	42 (85.7%)	4 (8.2%)
Extranodal	93	27 (29.0%)	43 (46.2%)	23 (24.7%)
Hodgkin's lymphoma	113	4 (3.5%)	105 (92.9%)	4 (3.5%)
Total	529	179 (33.8%)	307 (58.0%)	43 (8.1%)

In our patients with malignant lymphomas, the incidence of LA/NLH in bone marrow was 8.1%. Surprisingly, they were present in only 11 out of 240 patients with B-NHL, but in 23 out of 93 patients with extranodal disease presentation.

In regard to biological characteristics of B-NHL, LA/NLH were more frequently found in aggressive types than in indolent subtypes, especially in those patients with extranodal disease (Table 2).

Various immunological abnormalities are frequently observed in patients with myelodysplasia. Lymphoid aggregates can be found in bone marrow of patients with myelodysplasia. In our previous study lymphoid aggregates were found in 9% of 102 analyzed patients. There was no correlation of those aggregates and FAB morphological classification as well as no correlation with survival.

Low-grade non-Hodgkin's lymphoma associated with myelodysplasia is rather a rare event –

Table 2. Incidence of LA/NLH in B-NHL according to biological behavior - our experience

B NHL	Nodal	Extranodal	Total
Indolent	5 (45.5%)	5 (21.7%)	10
Aggressive	6 (54.5%)	18 (78.3%)	24
Total	11	23	34

The immunophenotype and molecular clonality of lymphoid aggregates in each of 43 cases were evaluated and the resultants will be presented.

estimated about 1% of cases. However, monoclonal and polyclonal expansion of lymphoid cells in patients with myelodysplasia was reported to occur in 7% to 12.5% of patients.

Concerning these data we assessed phenotypic characteristics and clonality in 29 patients out of 221 diagnosed in last five years as myelodysplastic syndrome.

The last problem to be discussed concerning lymphoid aggregates in bone marrow is the role of these aggregates in myeloproliferative syndrome (MPS). The incidence of lymphoid aggregates in our patients with myeloproliferation was 9.2% (50 out of 548 patients with myeloproliferative syndrome). Lymphoid aggregates were mostly associated with idiopathic myelofibrosis with myeloid metaplasia (MMM). It was reported that bone marrow microvessel density is an integral part of bone marrow stromal reaction and could be a useful prognostic information. The relationship of lymphoid aggregates to the network of adventitial/perisinusoidal reticular cells was assessed by low-affinity nerve growth factor receptor, and follicular as well as interstitial dendritic cells were detected by fascin expression. Furthermore, it was reported that in patients with idiopathic myelofibrosis T cells are decreased and cytotoxic cells increased in number with coincidental increase in CD5+ B-cell subpopulation. CD20 and CD5, CD3, granzyme and TIA-1 were phenotypically evaluated in the lymphoid cells comprising the aggregates in patients with idiopathic myelofibrosis. Implication of these results will be discussed concerning the relationship with fibrosis of bone marrow.

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LYMPHOMAS IN THE SPLEEN

Johan H. J. M. van Krieken

Department of Pathology, University Medical Centre Nijmegen, Nijmegen, The Netherlands

Introduction

Lymphomas in the spleen can be a really diagnostic challenge for the pathologist. There are several reasons for this. Diagnostic splenectomies are rare, so most pathologists have little experience; the morphology of lymphomas in the spleen is different from that in the lymph node; splenic marginal zone lymphoma is still ill defined. It is already known for many years that the location of lymphoma in the spleen shows the relation between the normal architecture and the specific lymphoma type. This can serve as the basis for the initial recognition. This idea then has to be confirmed by immunohistochemistry and occasionally molecular techniques. Basic knowledge of the normal spleen is therefore important so that the precise infiltration pattern can be recognised, but the main difficulties are met when one finds a primary lymphoma in the spleen.

Already many years it is clear that there is something special about lymphomas that arise in the spleen, formerly referred to as primary splenic lymphomas. These were basically clinically defined: enlarged spleens that after splenectomy appeared to contain malignant lymphoma. The morphology of these lymphomas was rather variable, from small to large cell and variable distribution in the spleen. At least some of the patients did remarkably well after splenectomy, without the need of further therapy. The scene changed when Isaacson decided to study the enigmatic splenic lymphoma with villous lymphocytes in detail. He founded the new entity splenic marginal zone lymphoma (SMZL), however regretting this nomenclature, by studying many cases from the Catovsky group that had collected many patients with villous lymphocytes in their peripheral blood. By studying the spleens that were available from these patients it became clear that there is a subgroup with rather specific morphology, but not all splenic lymphomas from patients with villous lymphocytes showed this morphology. Later studies also showed the opposite: not all patients with SMZL have villous lymphocytes in their peripheral blood. Since the publication of Isaacson new data have been presented, especially by the group of Piris.

The present overview starts with the information gathered in the WHO fascicle, which is outstanding and presently must form the basis of all lymphoma diagnosis. However, especially for such a žrecent' entity as SMZL later results are quite relevant. In fact, as in gastric MALT-lymphoma and, to a lesser extent, in mantle cell lymphoma, the recognition of this specific entity has led to a specific therapy: it has been shown recently that treatment of hepatitis C in patients with splenic lymphoma with villous lymphocytes leads to regression of the lymphoma. I have a critical note though: I am not convinced by the paper that the it deals with SMZL patients; in fact, in my opinion (without having seen the slides) these patients may have had immunocytoma. Nevertheless, the data show that it is important to recognise clinicopathological disease entities and that this recognition may result in specific therapies, even in very rare disorders.

Normal histology of the spleen

Although the normal histology of the spleen is well defined and described, it is worthwhile to repeat the specific compartments: the white

pulp, consisting of B-cell follicles and T-cell areas; the red pulp, consisting of filtering and non-filtering areas; and the perifollicular zone, the area between the red and white pulp, sometimes confused with the marginal zone. The B-cell follicles may contain germinal centres but mainly consist of IgD positive small cells forming the mantle zone, surrounded by the marginal zone consisting of IgD negative somewhat larger B-cells. There is no marginal zone around the T-cell areas (in contrast to rodent spleens). The T-cell areas often surround arteries and consist mainly of CD4 positive T-cells. In the red pulp, the non-filtering areas contain also lymphoid cells, both B- and T-cells, the latter mainly CD8 positive. The perifollicular zone between the red and white pulp consists of a cell population reflecting the peripheral blood.

The distribution of lymphomas in the spleen

Most studies and textbooks start by describing the lymphomas based on their distribution in the spleen: red or white pulp localisation. It is relevant to realise that this is a simplification. It is obvious that many B-cell lymphomas mimic white pulp. However, it is also obvious that this is *not* white pulp involvement: there are many more lymphoma nodules than there ever were follicles. If one looks carefully, one finds small nodules in the red pulp as well. It is therefore more logical to assume that lymphoma nodules start in the red pulp lymphoid tissue and grow to become nodules that mimic white pulp.

The distribution and growth pattern of lymphomas in the spleen are related to the normal counterpart of the lymphoma. Follicular lymphoma consist of žwhite pulp' nodules without pre-existent germinal centre; there are always many small nodules in the red pulp as well. Mantle cell lymphoma partly grows surrounding reactive germinal centres. Lymphocytic lymphoma and hairy cell leukaemia are mainly present in the red pulp. Most lymphomas can relatively easily be recognised in the spleen based on the growth pattern, morphology and immunophenotype, provided that one is aware of the pitfalls. The main problems arise when there is no lymph node based diagnosis and when the growth pattern, morphology and/or phenotype are not typical. In those cases often SMZL may be a potential diagnosis. Since this is a rather recent and difficult to diagnose entity, it is the main topic of this paper.

Splenic marginal zone lymphoma

Definition. The WHO defines SMZL as follows: it is a B-cell neoplasm comprising small lymphocytes which surround and replace the splenic white pulp germinal centres, efface the follicle mantle and merge with a peripheral (marginal) zone of larger cells including scattered transformed blasts; both small and larger cells infiltrate the red pulp. Splenic hilar lymph nodes and bone marrow are often involved; lymphoma cells may be found in the peripheral blood as villous lymphocytes.

As with most of the definitions of malignant lymphomas this definition is purely morphological; in contrast with other lymphoma types there is no specific phenotype and/or genotype. Together with the rarity of the disease makes it a problematic diagnosis. The most remarkable deviation in this definition of this lymphomas type is that it is defined in spleen. This obviously makes sense but makes it also not easy to separate this lymphoma from others involving the spleen. It is clear that for instance several lymphoma types (follicular lymphoma, mantle cell lymphoma) can grow in a marginal zone pattern in the spleen; and the pattern is the most defining criterion. In fact splenic lymphoma in lymph nodes is very difficult to recognise as was shown by studying splenic hilar lymph nodes. Nevertheless in general this is often very helpful in difficult cases especially since there is much more experience in recognising lymphoma entities in a lymph node compared to the spleen.

Clinical features. SMZL comprises less than 1% of lymphomas and involves the spleen, bone marrow and often the peripheral blood. Patients present with splenomegaly, may have a small monoclonal serum protein but not Waldenström's macroglobinemia. Most cases

have indolent behaviour, but response to treatment is poor. Remarkable is the good response to splenectomy, and as mentioned, to antiviral treatment in Hepatitis C positive subjects.

Morphology. The morphology of the cells is not very specific, small cells admixed with large cells. This does not separate this lesions from other marginal zone lymphomas or follicular lymphoma. It is important in the differentiation from mantle cell lymphoma, although the later lesion can best be recognised by cyclin D1 overexpression. Growth pattern is the main clue for the diagnosis: nodules of small B-lymphocytes surrounded by somewhat larger pale cells, admixed with a variable amount of B-blasts, growing in a marginal zone pattern *with disappearance of the mantel zone.* There is also red pulp involvement of both sinuses and pulp cords. This growth pattern is specific, but can be difficult to recognise. Other lymphomas, especially follicular lymphoma, can grow with a marginal growth pattern. Morphologically, blastic mantle cell lymphoma can mimic SMZL to a great extend. In the bone marrow there may be a remarkable intrasinusoidal growth pattern. It has been described that this pattern may change to more nodular after splenectomy.

Immunophenotype. Although the immunophenotype is consistent, it is not very specific. The cells have B-cell markers, not CD5, CD23, CD43 and CD10, nor cyclin D1. Often IgM, D. There is not a specific positive marker available.

Genotype. Recently several studies into the genetic profile of SMZL lymphoma were published. There is a remarkable variability in findings which strongly suggests that there are indeed severe problems with classification. SMZL cells are postfollicular with ongoing mutations, at least in some, while this was never described in SLVL. There is preferential VH1 usage. More recently a larger study showed that half of the cases are not mutated, and that this group has a worse survival, a situation similar to CLL. This needs confirmation.

There is no common specific genetic marker and larger studies indicate substantial heterogeneity. Most common is loss of 7q, which seems to be mutual exclusive with gain of 3q. Alterations of 7q21 and t(7;21) lead to overexpression of CDK6. Trisomy 3 is common, and seems to be associated with low tumour load in the peripheral blood. There is rare p53 involvement and no microsatellite instability. The t(11;18) often found in extranodal marginal zone lymphoma is not present in SMZL.

Cell of origin. The name was given to this lymphoma type because of its remarkable growth pattern with marginal zone differentiation. It is now clear that it is a misnomer: other lymphomas have also marginal zone growth pattern and the cells express IgD, in contrast to marginal zone cells. Based on the mutational data and the phenotype, a mantle cell origin is most likely. Thus the mantle zone may give rise to several lymphoma types: mantle cell lymphoma, CLL (both mutated and unmutated) and SMZL.

What defines an entity?

To answer the main question for this presentation, whether SMZL is one entity, first an entity needs to be defined. Ideally an entity is a homogenous disease with a specific clinical presentation and course, morphology, phenotype, genotype and treatment. Obviously this requirement is not met for SMZL, but this is not unique to this lymphoma type. It is more common than exception that a lymphoma type is homogenous, in fact, a spectrum including tumour progression can be seen in almost all types. Most lymphoma types can now be defined by a primary genetic event, a specific morphology and phenotype, but also this requirement is not met for SMZL. The main definition is on growth pattern and exclusion of other types and we still await a common specific feature, either genetic or phenotypic.

It is clear that in a relatively poorly defined entity a multitude of parameters can be studied, and indeed this is the case. This has led to many claims of subentities: with and without mutations, with and without plasma cell differentiation, with and without 7q, with and without increased blasts, with and without p53 mutation, with and without bcl6 mutation, with and

without hepatitis C. All these subdivisions are reported to be clinically relevant.

To me the situation is presently as follows: yes, there is an entity, which is difficult to diagnose. Within this entity there is a spectrum of pathologic and genetic findings related to tumour progression. The primary genetic event awaits discovery, after which a specific marker will be available.

Guidelines for diagnosing lymphomas in the spleen

1. Carefully recognise the infiltration pattern.
2. Define the cell type.
3. Perform immunohistochemistry, always including B- and T-cell markers, CD5, cyclin-D1, CD10, IgD, Bcl2, Bcl6.
4. SMZL can only be reliably diagnosed in the spleen. Hints to the possibility of the presence of this disease are intrasinusoidal growth of small B-cells in the bone marrow and the presence of villous lymphocytes in the peripheral blood, but both are not diagnostic. In all cases other lymphomas need to be excluded, especially mantle cell lymphoma (cyclin-D1 positive) and follicular lymphoma (IgD negative, CD10 positive) and immunocytoma (intracytoplasmic Ig, IgD negative, CD38 positive). Very helpful may be the evaluation of the splenic hilar lymph node.

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DIFFERENTIAL DIAGNOSIS BETWEEN CUTANEOUS LYMPHOMAS AND PSEUDOLYMPHOMAS

Lorenzo Cerroni

Department of Dermatology, University of Graz, Austria

Differential diagnosis of cutaneous lymphoproliferative disorders represents one of the most vexing problems in dermatology and dermatopathology. For nearly one century the diagnosis has been based only upon clinicopathologic correlation. Immunohistochemical and molecular techniques developed during the last three decades added new criteria for the differentiation of these diseases. The purpose of this presentation is to summarize the criteria for differential diagnosis of benign from malignant lymphoid infiltrates of the skin. In this context, it should be reminded that a proper classification of cutaneous lymphoproliferative disorders can be achieved only by synthesis of clinical, histopathologic, immunophenotypic, and molecular criteria, and that in some cases only follow-up data allow a precise diagnosis to be made.

Cutaneous T-cell lymphoma vs. T-cell pseudolymphoma

Mycosis fungoides has been recognized for almost two centuries as the prototype of the cutaneous T-cell lymphomas (CTCL). In the last years, several other entities of CTCL have been described, with different morphologic, immunophenotypic, and prognostic features (Table 1) (1,2). All these entities must be differentiated from benign (reactive) conditions that may simulate them either clinically, histologically, or both (cutaneous T-cell pseudolymphomas, Table 2). The recent introduction of polymerase chain reaction (PCR) analysis of the T-cell receptor (TCR) and immunoglobulin heavy chain (IgH) genes allowed to check the clonality of T- and B-cell infiltrates of the skin, respectively. Malignant lymphomas reveal a monoclonal population of lymphocytes, whereas pseudolymphomas show a polyclonal infiltrate. Although this technique is extremely powerful, allowing to detect one neoplastic cell in the background of 100 non-neoplastic lymphocytes, it is not devoid of pitfalls. In fact, in many cases of early CTCL monoclonality cannot be demonstrated by PCR; on the other hand, a monoclonal population of T-lymphocytes has been observed in more than 50% of patch-test lesions, as well as in several cases of benign dermatoses (3,4). Molecular results, although very useful for differentiation of benign from malignant lymphoproliferative disorders of the skin, should be always integrated by and interpreted together with clinical, histological, and immunophenotypic data.

The diagnosis of mycosis fungoides in early stages rests mainly on clinicopathologic correlation, and as a rule differentiation from benign reactive infiltrates cannot be achieved without correlation of the histologic features with the clinical picture. Clinical features that are helpful in the differential diagnosis of mycosis fungoides from benign inflammatory dermatoses are location on sun-protected areas such as the buttocks, presence of lesions with different morphological aspects, and history of long-standing lesions that do not tend to regress without treatment. Microscopic examination of early lesions can fail to show the typical aspects of the disease, and may be characterized only by a superficial patchy-lichenoid infiltrate located in a fibrotic, thickened papillary dermis (5-8). These features alone are suspect, but not diagnostic of mycosis fungoides.

Most cases of mycosis fungoides and Sézary syndrome show in the early phases a T-helper phenotype (CD3+, CD4+, CD5+, CD8-), indistinguishable from that seen in benign chronic

inflammatory dermatoses (9,10). Only a minority of cases exhibit a T-suppressor lineage (CD3+, CD4-, CD5+, CD8+) (11). It has been suggested that in early stages of mycosis fungoides, in contrast to benign (inflammatory) cutaneous infiltrates of T lymphocytes, there is a loss of expression of the T-cell-associated antigen CD7 (12,13). However, this finding has not been confirmed by other studies showing normal CD7+ populations in early mycosis fungoides (10,14). In addition, T-lymphocytes in some cases of benign inflammatory dermatosis can also show partial loss of CD7 (14,15). At present, the value of CD7 staining in the differential diagnosis of cutaneous T-cell infiltrates is still unclear (16).

Immunohistochemical analysis of the TCRs has been advocated for differentiation of early mycosis fungoides from chronic, benign inflammatory conditions. The TCR consists of a constant and a variable region. Two types of TCR may be distinguished with respect to the constant regions, namely, a/ß and g/d heterodimers. Analysis of these receptors shows in most cases of early mycosis fungoides an a/ß phenotype (ßF1+/TCRd1-), similar to that seen in benign cutaneous T-cell infiltrates (17). More interesting results have been obtained with the analysis of the variable regions of TCR. In benign T-cell infiltrates these differ from one cell to another, whereas malignant proliferations usually exhibit a monoclonal expression of these determinants. Jack et al. (18) could show a monoclonal population in 10 out of 16 cases of plaque or tumor stage mycosis fungoides using antibodies specific for the Vß8 and the Vß5 determinants. However, monoclonality could not be demonstrated in patch stage mycosis fungoides. The frequent expression of the same variable region in different cases of mycosis fungoides could reflect similarities in the etiology and/or pathogenesis (i.e., a distinct population of virus-infected cells) of this condition (18).

Molecular analysis of TCR gene rearrangement is a further criterion helpful in the differentiation of mycosis fungoides from benign skin conditions. However, it must be underlined that early lesions of mycosis fungoides reveal a monoclonal rearrangement only in about 50% of the cases, and that several benign dermatoses have been shown to harbour a monoclonal population of T lymphocytes (i.e., lichen planus, lichen sclerosus) (19-21). The reasons for the low sensitivity of gene rearrangement analysis in mycosis fungoides may reside in the very low number of neoplastic lymphocytes in early phases of the disease, and some studies showed that the sensitivity can be increased upon microdissection of the specimen (22). However, specificity of this technique, when applied to the diagnosis of early mycosis fungoides, is low as well, and the presence or absence of a monoclonal pattern of TCR gene rearrangement cannot be considered as a crucial criterion in the early diagnosis of mycosis fungoides.

Recently, a new group of CTCL with cytotoxic phenotype distinct from mycosis fungoides / Sézary syndrome has been described. These lymphomas may either show a natural killer (NK)-cell phenotype, or may derive from the cytotoxic T-lymphocytes. Cytotoxic cells typically express markers such as TIA-1, granzyme-B, and perforin. Of particular importance in this group is the so-called subcutaneous panniculitis-like T-cell lymphoma (SPTCL), which may mimic lupus profundus (lupus panniculitis) both clinically and histologically. SPTCL presents histologically with prominent involvement of the fat lobules, often simulating a lobular panniculitis (23). Neoplastic cells usually show a CD3+, CD4-, CD8+, CD56-, a/b phenotype. A useful feature for differentiation of SPTCL from a benign panniculitis is the "rimming" of fat cells by pleomorphic, neoplastic T-lymphocytes that are positive for proliferation markers (16). It must be reminded that rimming of fat lobuli by lymphocytes is not a diagnostic feature *per se*, as it can be observed in several benign and malignant lymphoid infiltrates showing a lobular panniculitis. In contrast to lupus panniculitis, B-cells and germinal centers are not a prominent feature in SPTCL.

An important group of CTCL is characterized by the expression of the CD30 antigen by neoplastic cells. In the skin, CD30+ cells are a characteristic feature of anaplastic large cell

lymphoma (ALCL) and lymphomatoid papulosis (LYP). It is important to remember that there is no clear-cut boundary between cases of LYP and cutaneous ALCL. Histopathologic and immunohistologic criteria alone are not sufficient to distinguish between these two entities, and usually differentiation can be achieved only upon correlation with clinical features (self-healing lesions, number of lesions, size, etc.). (24). In skin lesions, moreover, CD30-positivity alone does not imply a diagnosis of LYP or cutaneous ALCL. In fact, CD30+ large blasts have been observed in several reactive conditions including, among others, various viral infections, arthropod reactions, and drug eruptions (25,26). However, CD30+ lymphocytes in reactive lesions are present in small numbers scattered throughout the infiltrate, and are usually not arranged in clusters or sheets as observed in LYP or ALCL.

Cutaneous B-cell lymphoma vs. B-cell pseudolymphoma

Most of the B-cell malignancies arising primary in the skin belong to the groups of follicle center cell lymphoma (FCCL) and marginal zone lymphoma (MZL) / immunocytoma (IC) (Table 1). It may be difficult to differentiate them from benign infiltrates of B-lymphocytes (B-cell pseudolymphomas - Table 2) (2,27). Many of the cases classified in the past among the B-cell pseudolymphomas are considered today to be examples of low-grade malignant cutaneous B-cell lymphoma (CBCL) (28).

Clinical features that favour a diagnosis of CBCL are the presence of clusters of irregularly shaped papules and nodules surrounded by erythematous patches and plaques. The typical locations are the head and neck region and the trunk, especially the back (so-called reticulohistiocytoma of the back). B-cell pseudolymphomas, in contrast, usually present with solitary lesions, but exceptions to this rule are well known (i.e., drug-induced B-cell pseudolymphoma).

In lesions showing a follicular pattern, histopathologic features that suggest a diagnosis of CBCL are the monomorphism of the follicles, the lack of a well-formed mantle zone, and the absence of tingible body macrophages (29,30). It must be underlined that many CBCLs, especially of the type of MZL, reveal a mixed cell infiltrate with presence of eosinophils and small granulomas, and that differentiation from reactive conditions can be very difficult on histopathologic grounds alone (28).

Immunohistochemical criteria for differentiation of benign from malignant infiltrates rely upon the analysis of the immunoglobulin light chains (k and l), which can be studied on routinely fixed, paraffin-embedded biopsy specimens (16,31). Malignant cell populations of B-lymphocytes usually show a monoclonal restriction to either k or l light chain, whereas benign infiltrates exhibit a polyclonal pattern with expression of both light chains. Unfortunately, however, there are several cases of B-cell lymphoproliferative disorders, both benign and malignant, in which the cells do not express immunoglobulins. The frequency of immunoglobulin negative B-cell proliferations appears to be higher in the skin than in the lymph nodes (32).

A useful immunohistochemical criterion in cases with follicular pattern is the presence of CD10+ and/or Bcl-6+ clusters of cells outside the follicles (29,30). In contrast, B-cell pseudolymphomas with follicular pattern reveal a population of CD10+/Bcl-6+ cells that is confined to reactive germinal centers. Another useful clue for the diagnosis of FCCL with follicular growth pattern is the diminished proliferation activity of malignant germinal centers as outlined by the Ki67/MIB-1 antibody (16). Reactive germinal centers show a high proliferation, whereas malignant ones often are characterized by a much lesser degree of positivity.

Molecular analysis of CBCLs often shows a monoclonal rearrangement of immunoglobulin heavy and light chain genes. However, it is not unusual that cases of FCCL do not show a monoclonal rearrangement. One possible explanation resides in the high number of somatic hypermutations found in these cases that may render the annealing of DNA primers impossible. Monoclonality of B lymphocytes in B-cell

pseudolymphomas is infrequent, but rare cases of otherwise typical *Borrelia burgdorferi*-associated lymphocytoma cutis with monoclonal rearrangement of the IgH gene have been observed.

About 80-85% of follicular lymphomas and 15-30% of high-grade malignant non-Hodgkin's lymphomas in the lymph nodes are characterized by the t(14;18), which is associated with an overexpression of the *bcl-2* oncogene (33). The oncogene maintains a normal structure, resulting in the production of higher amounts of a normal *bcl-2* protein. Using the anti-*bcl-2* protein antibody 85% to 100% of the follicular lymphomas in the lymph nodes stain positive, and negativity is considered as a diagnostic criterion of follicular hyperplasia rather than follicular lymphoma in the lymph nodes. Investigation of cutaneous cases demonstrated that the t(14;18) is exceedingly rare in primary CBCLs, and that the majority of cases of cutaneous FCCL do not show expression of *bcl-2* protein by neoplastic cells (34). Thus, in the skin *bcl-2* protein expression cannot be used as a criterion for differentiation of FCCL from B-pseudolymphoma with follicular pattern.

Low-grade malignant lymphomas of B-cell lineage may show aberrant expression of some T-cell-associated markers. CD5 is a pan-T-cell marker that reacts with the cells of most cases of B-cell chronic lymphocytic leukemia (B-CLL). Normal B lymphocytes are CD43-, but several low-grade B-cell lymphomas are CD43+ (35). The detection of an aberrant phenotype of the B-lymphocytes (CD20+, CD5+, CD43+) is considered as a sign of malignancy. The majority of plasmacytic tumors express epithelial membrane antigen (EMA) and may also be positive for cytokeratins, thus representing a pitfall in the immunohistochemical diagnosis. These lesions are usually negative for common pan-B-cell markers, but may be positive for CD79a and are stained by anti-CD138 antibodies. Demonstration of immunoglobulin light chain restriction is the main clue for differentiation of malignant from reactive cutaneous plasma cell infiltrates. This is especially important for differentiation of cutaneous plasmacytoma from cutaneous lesions of so-called plasma cell granuloma (inflammatory pseudotumor, plasma cell type).

Table 1. Main types of primary and secondary cutaneous lymphomas

Cutaneous T-cell lymphomas
Mycosis fungoides and variants
Pagetoid reticulosis, solitary type (Woringer-Kolopp)
Sézary syndrome
Lymphomatoid papulosis
Anaplastic large T-cell lymphoma (CD30+)
Large T-cell lymphoma, CD30-
Granulomatous slack skin
Subcutaneous panniculitis-like T-cell lymphoma
Small/medium-sized pleomorphic T-cell lymphoma
Aggressive epidermotropic CD8+ cutaneous T-cell lymphoma
Nasal-type NK/T-cell lymphoma
Blastic NK-cell lymphoma
Intravascular large T-cell lymphoma
T-lymphoblastic lymphoma
Cutaneous B-cell lymphomas
Follicle center cell lymphoma (follicular or diffuse)
Marginal zone lymphoma
Immunocytoma
Large B-cell lymphoma of the leg
Cutaneous plasmacytoma
Intravascular large B-cell lymphoma
B-lymphoblastic lymphoma
B-chronic lymphocytic leukemia

Table 2. Main clinicopathologic entities of cutaneous pseudolymphomas

T-cell pseudolymphomas

- Actinic reticuloid
- Lymphomatoid contact dermatitis
- Lymphomatoid reactions to arthropod bites
- Lymphomatoid drug eruption, T-cell type
- Solitary T-cell pseudolymphoma
- Lichenoid keratosis
- Lichen aureus
- Lichen sclerosus
- Orf / Milker's nodule
- Herpes simplex / zoster
- Pseudolymphomas in tattoo

B-cell pseudolymphomas

- Lymphocytoma cutis (lymphadenosis benigna cutis)
- Lymphomatoid drug eruption, B-cell type
- Inflammatory pseudotumor (plasma cell granuloma)
- Morphea, inflammatory stage
- Syphilis II
- Pseudolymphoma after vaccination
- Lupus panniculitis

Cutaneous B-cell lymphomas, especially of low-grade malignancy, may show a distinct T-cell population, which occasionaly can be numerically predominant. Immunohistochemical criteria for diagnosis of B-cell lymphoma in cases where T lymphocytes are the majority include normal phenotype of the T lymphocytes, monoclonal pattern of immunoglobulin light chain expression by the B cells, and cytomorphologic-phenotypic correlation. Cases with only a few B-cell blasts interspersed among a prominent population of small T-lymphocytes have been classified as cutaneous T-cell rich B-cell lymphomas. This type of lymphoma is exceedingly rare in the skin. A helpful technique for determination of the lineage of neoplastic cells in such cases is represented by molecular genetic analysis of single cells isolated by laser-based microdissection technique (36). This method allows to isolate single cells or a group of cells for PCR analysis of TCR and/or IgH genes, avoiding artifacts due to contamination caused by other lymphocytes within the infiltrate.

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Pomožne snovi: kalcijev hidroksid ali klorovodikova kislina (za uravnavanje pH), voda za injekcije.

TERAPEVTSKE INDIKACIJE: • Preprečevanje pojava tromboembolijskih bolezni: posebej tistih, ki so povezane s splošno in ortopedsko kirurgijo; pri bolnikih na oddelku za intenzivno nego, pri katerih je tveganje zelo veliko (odpoved dihanja in/ali okužbe dihal in/ali srčne insuficience); pri drugih zelo ali zmerno ogroženih bolnikih. • Zdravljenje tromboembolijskih bolezni. • Preprečevanje nastanka strdkov med hemodializo. • Zdravljenje nestabilne angine pektoris in akutnega miokardnega infarkta brez zobca Q.

ODMERJANJE IN NAČIN UPORABE: Zdravilo ni namenjeno za intramuskularno uporabo.

Preprečevanje pojava tromboembolijskih bolezni
Splošna kirurgija: Zdravilo dajemo subkutano v enkratnem dnevnem odmerku po 0,3 ml, in to vsaj, dokler bolnika ni možno začeti zdraviti ambulatno, najmanj pa 7 dni. Prvi odmerek naj dobi 2 do 4 ure pred operacijo.

Ortopedska kirurgija: Začetna odmerka je treba dati 12 ur pred operativnim posegom in 12 ur po njem. Dnevne odmerke je treba prilagoditi bolnikovi telesni masi:

ortopedska kirurgija	količina Fraxiparina, ki se jo subkutano vbrizga ENKRAT na dan	
telesna masa (kg)	pred operacijo in 3 dni po njej	od 4. dne dalje
< 50	0,2 ml (1900 I.E. AXa)	0,3 ml (2850 I.E. AXa)
50–69	0,3 ml (2850 I.E. AXa)	0,4 ml (3800 I.E. AXa)
≥ 70	0,4 ml (3800 I.E. AXa)	0,6 ml (5700 I.E. AXa)

Zdravljenje je treba nadaljevati še najmanj 10 dni. Profilaksa traja ves čas bolnikove velike ogroženosti oz. vsaj, dokler ga ni možno začeti zdraviti ambulatno.

Bolniki na oddelku za intezivno nego: Dnevne odmerke je treba prilagoditi bolnikovi telesni masi:

telesna masa (kg)	količina Fraxiparina, ki se jo subkutano vbrizga ENKRAT na dan
≤ 70	0,4 ml (3800 I.E. AXa)
> 70	0,6 ml (5700 I.E. AXa)

Drugi zmerno ali zelo ogroženi bolniki: Odmerek je treba prilagoditi bolnikovi telesni masi in stopnji ogroženosti:

ogroženost	telesna masa (kg)	količina Fraxiparina, ki se jo subkutano vbrizga ENKRAT na dan
zmerna		0,3 ml (2850 I.E. AXa)
velika	51–70	0,4 ml (3800 I.E. AXa)
	> 70	0,6 ml (5700 I.E. AXa)

Trajanje zdravljenja sovpada s časom ogroženosti bolnika.

Zdravljenje tromboembolijskih bolezni
Zdravilo dajemo subkutano dvakrat na dan (na vsakih 12 ur), ponavadi 10 dni. Odmerek prilagodimo bolnikovi telesni masi:

zdravljenje tromboembolijskih bolezni	
telesna masa (kg)	količina Fraxiparina, ki se jo subkutano vbrizga DVAKRAT na dan
< 50	0,4 ml (3800 I.E. AXa)
50–59	0,5 ml (4750 I.E. AXa)
60–69	0,6 ml (5700 I.E. AXa)
70–79	0,7 ml (6650 I.E. AXa)
80–89	0,8 ml (7600 I.E. AXa)
≥ 90	0,9 ml (8550 I.E. AXa)

Peroralno zdravljenje z antikoagulanti začnemo takoj, ko je to mogoče, razen če obstaja kakršnakoli kontraindikacija. Zdravljenja se ne sme končati, dokler ne dosežemo želenega INR.

Preprečevanje strjevanja krvi med hemodializo
Običajno najprimernejšega odmerka za bolnika je individualno glede na tveganje za krvavitve in tehnične pogoje dializiranja. Ponavadi se da pred začetkom hemodialize v arterijsko linijo en odmerek. Pri bolnikih, pri katerih ni zvečane nevarnosti krvavenja, glede na njihovo telesno maso dajemo:

preprečevanje strjevanja krvi med hemodializo	
telesna masa (kg)	količina Fraxiparina, ki se vbrizga pred dializo
< 50	0,3 ml (2850 I.E. AXa)
50–69	0,4 ml (3800 I.E. AXa)
≥ 70	0,6 ml (5700 I.E. AXa)

Bolniki, pri katerih obstaja nevarnost krvavitve, dobijo polovični odmerek. Kadar traja dializa več kot štiri ure, lahko bolniki dobijo še dodatne majhne odmerke. Po potrebi se pri naslednji dializi njegov odmerek prilagodi glede na začetni učinek.

Zdravljenje nestabilne angine pektoris in akutnega srčnega infarkta brez zobca Q
Zdravilo dobijo bolniki skupaj z acetilsalicilno kislino v odmerku do 325 mg na dan. Začetni odmerek je 86. I. E. anti-Xa/kg v obliki intravenskega bolusa, nato pa dajemo 86. I. E. anti-Xa/kg v obliki subkutane injekcije na vsakih 12 ur. Običajno traja zdravljenje 6 dni. Odmerki, prilagojeni bolnikovi telesni masi, so:

zdravljenje nestabilne angine pektoris in akutnega srčnega infarkta brez zobca Q			
telesna masa (kg)	vbrizgana količina Fraxiparina		
	začetni bolus i.v.	subkutano vbrizganje (na vsakih 12 ur)	odgovarjajoče I.E. anti-Xa
< 50	0,4 ml	0,4 ml	3800
50–59	0,5 ml	0,5 ml	4750
60–69	0,6 ml	0,6 ml	5700
70–79	0,7 ml	0,7 ml	6650
80–89	0,8 ml	0,8 ml	7600
90–99	0,9 ml	0,9 ml	8550
≥ 100	1,0 ml	1,0 ml	9500

Kontraindikacije: • preobčutljivost za nadroparin • trombocitopenija zaradi zdravljenja z nadroparinom v anamnezi • aktivna krvavitev ali zvečano tveganje za njen pojav, povezano s hemostaznimi motnjami, razen kadar diseminirana intravaskularna koagulacija ni posledica uporabe heparina • poškodbe organov, povezane s krvavitvijo (npr. aktivna peptična razjeda) • hemoragični cerebrovaskularni dogodek • akutni infekcijski endokarditis

Previdnostni ukrepi in opozorila:
Opozorila: Heparin lahko povzroči trombocitopenijo, zato je treba med zdravljenjem s Fraxiparinom redno preverjati število trombocitov. Redna kontrola trombocitov je potrebna pred zdravljenjem in med njim. Pri bolnikih, pri katerih se je v preteklosti med zdravljenjem s heparinom trombocitopenija že pojavila (tako s standardnim kot z nizkomolekularnim) in je zdravljenje s heparinom nujno, lahko uporabimo Fraxiparine. V tem primeru je nujoo natančno klinično opazovanje; vsaj enkrat na dan je treba določiti število trombocitov. Če se pojavijo znaki trombocitopenije, je treba zdravljenje takoj ustaviti.

Previdnostni ukrepi: Previdnost je potrebna pri: • jetrni in ledvični odpovedi • hudi arterijski hipertenziji • peptični razjedi ali drugih organskih poškodbah, ki krvavijo, v anamnezi • okvarah ožilja v žilnici in mrežnici • v obdobju po kirurškem posegu na možganih, hrbtenjači ali očeh. Možnost nastanka spinalnih/epiduralnih hematomov je večja pri bolnikih, ki imajo vstavljen epiduralni kateter, nevarnost je večja tudi po travmatski ali ponavljajoči se epiduralni ali spinalni punkciji, zato je pri teh bolnikih potrebna nevrološka kontrola.

Medsebojna učinkovanja zdravil: Hkratno zdravljenje z acetilsalicilno kislino ali drugimi salicilati, nesteroidnimi protivnetnimi sredstvi in protitrombocitnimi sredstvi ni priporočeno zaradi večje nevarnosti pojava krvavitve. Previdnost je potrebna pri bolnikih, ki dobivajo perioralno antikoagulante, sistemske (gluko-) kortikosteroide in dekstrane.

Nosečnost: Uporaba se ne priporoča, razen kadar je pričakovana korist nost večja od morebitnega tveganja.

Stranski učinki: • krvavitve • trombocitopenija, včasih trombogena • redki primeri kožne nekroze • majhne podplutbe na mestu vbrizganja • kožne reakcije • zvečane vrednosti transaminaz

Preveliko odmerjanje: V hujših primerih je treba razmisliti o uporabi protaminovega sulfata.

Inkompatibilnost: Zdravila se ne sme mešati z drugimi preparati.
Rok uporabe: 3 leta
Pogoji shranjevanja zdravila: Hranite pri temperaturi do 30 °C.
Oprema: škatle z **10 napolnjenimi injekcijskimi brizgami** (brez količinskih oznak) **z varnostnim mehanizmom po 0,3 ml** (2850 I.E. AXa), škatle z **10 napolnjenimi injekcijskimi brizgami** (brez količinskih oznak) **z varnostnim mehanizmom po 0,4 ml** (3800 I.E. AXa), škatle z **10 napolnjenimi injekcijskimi brizgami** (s količinskimi oznakami) **z varnostnim mehanizmom po 0,6 ml** (5700 I.E. AXa), škatle z **10 napolnjenimi injekcijskimi brizgami** (s količinskimi oznakami) **z varnostnim mehanizmom po 0,8 ml** (7600 I.E. AXa), škatle z **10 napolnjenimi injekcijskimi brizgami** (s količinskimi oznakami) **z varnostnim mehanizmom po 1,0 ml** (9500 I.E. AXa).

NAČIN IZDAJANJA ZDRAVILA: Uporaba samo v bolnišnicah, izjemoma se izdaja na zdravniški recept pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju.

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Dovoljenje za promet z zdravilom izdano: 2. 6. 2000 oz. 22. 2. 2001

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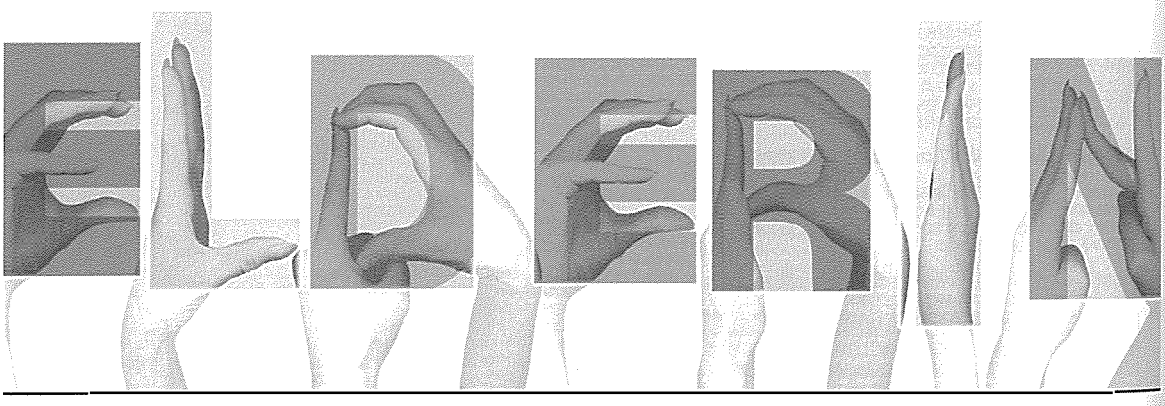

selektiven za COX-2 • varen • dober analgetik • Elderin SR® - enkrat na dan

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ELDERIN • **etodolak** • **Sestava:** V tabletah po 300 mg je 300 mg etodolaka, v SR tabletah po 600 mg je 600 mg etodolaka. • **Indikacije:** vnetne revmatične bolezni, artritisi pri vezivnotkivnih boleznih, s kristali povzročeni artritisi, osteoartroza, zunajsklepni revmatizem, blage in srednje hude bolečine. • **Odmerjanje in način uporabe:** Elderin, tablete po 300 mg: Bolnikom navadno predpišemo po 1 tableto zjutraj in zvečer. Lahko vzamejo tudi 1 do 2 tableti zjutraj in 1 do 2 tableti zvečer. Elderin SR, tablete po 600 mg: Navadno predpišemo1 tableto na dan, lahko tudi 1 tableto zjutraj in 1 tableto zvečer. Tablete je treba vzeti med jedjo. • **Neželeni škodljivi učinki:** dispepsija (10 %), slabost, driska, vetrovi, bolečina v žlički, gastritis, melena, bruhanje, vrtoglavica, glavobol. • **Posebna opozorila in previdnostni ukrepi:** krvavitve iz prebavil v anamnezi, Chronova bolezen, ulcerozni kolitis, hujša jetrna ali ledvična okvara, visoka starost, oslabelost, astma, hudo srčno popuščanje, bolniki na antikoagulacijski ali kortikosteroidni terapiji, alkoholiki; lahko prikrije znake infekcijskih bolezni. • **Kontraindikacije:** preobčutljivost za etodolak ali pomožne sestavine zdravila, salicilate in druge nesteroidne antirevmatike, razjeda želodca ali dvanajstnika, krvavitev iz prebavil, hude motnje v delovanju jeter ali ledvic, nagnjenost h krvavitvam, nosečnost ali dojenje, uporaba pri otrocih. • **Izdajanje zdravila:** samo na zdravniški recept. • **Imetnik dovoljenja za za promet:** Lek, tovarna farmacevtskih in kemičnih izdelkov, d.d., Verovškova 57, 1526 Ljubljana, www.lek.si • Informacija pripravljena: januarja 2002.

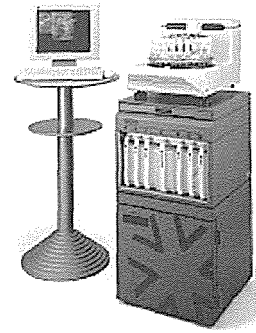
lek

A - Ž Consulting
Ljubljana, Miklošičeva 38
Slovenija

tel.: +386 (0) 1 433 62 22, 230 8 84
fax.: +386 (0) 1 230 19 85
E-mail: az.consulting@siol.net

Instrumenti in reagenti za: **IMUNOHISTOKEMIJO (ICH),**
IN SITU HIBRIDIZACIJO (IHC),
OBDELAVO TKIVNIH VZORCEV
ANATOMSKO PATOLOGIJO

VENTANA MEDICAL SYSTEMS, INC.



NOVO CASTRA

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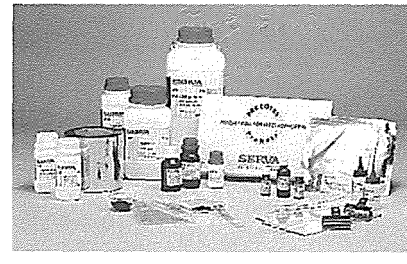

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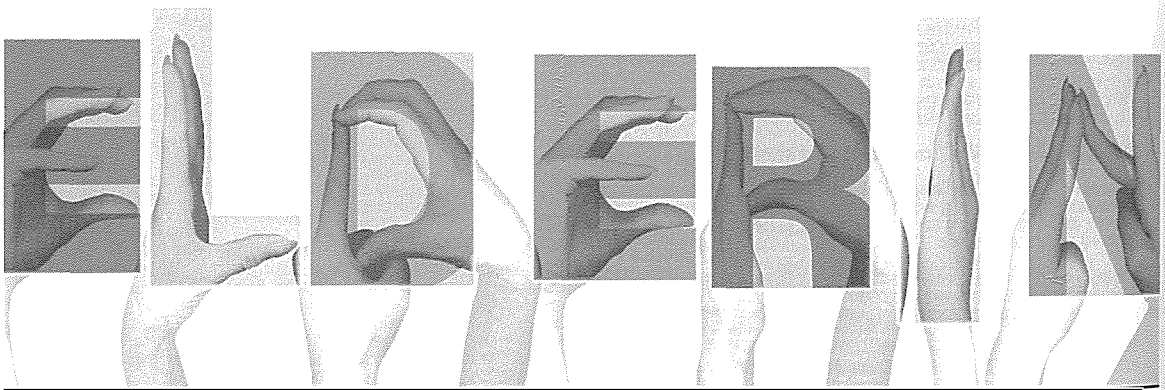

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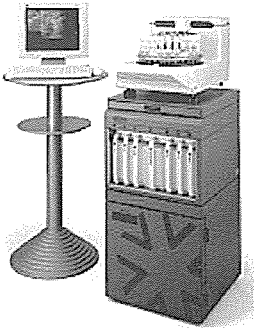
ANATOMSKO PATOLOGIJO

VENTANA MEDICAL SYSTEMS, INC.



NOVOCASTRA LABORATORIES

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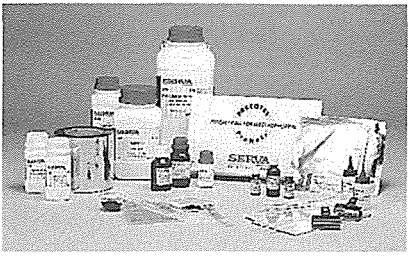

Lindron®

tablete po 10 mg *natrijev alendronat*

Kakor skala kost.

- vodilno mesto v kliničnih smernicah za zdravljenje osteoporoze po vsem svetu
- z raziskavami dokazana zmanjšana pogostost vseh osteoporotičnih zlomov
- veliko povečanje mineralne gostote kosti

VSAKODNEVNA SKRB ZA TRDNOST KOSTI

Indikacije: Lindron je namenjen za zdravljenje osteoporoze pri ženskah po menopavzi. **Odmerjanje in način uporabe:** Odmerek za zdravljenje osteoporoze je 1 tableta (10 mg) na dan. Učinek Lindrona bo največji, če bolnik vzame tableto zjutraj, najmanj pol ure pred prvim obrokom hrane, pijače ali zdravil. Ob tem mora popiti poln kozarec (2 do 3 dl) navadne vode (ne mineralne vode, kave, čaja ali soka) in se nato ne sme uleči vsaj 30 minut. Bolnik tablete ne sme žvečiti ali jo raztopiti v ustih, ker obstaja možnost nastanka razjede v ustih in žrelu. Če bolnik s hrano ne zaužije dovolj kalcija in vitamina D, naj ju jemlje v obliki tablet poleg Lindrona. **Kontraindikacije:** Preobčutljivost za katero koli sestavino zdravila; hipokalcemija, nenormalnosti na požiralniku, zaradi katerih je praznjenje požiralnika upočasnjeno (npr. striktura požiralnika ali ahalazija), nezmožnost stati ali sedeti vzravnano vsaj 30 minut. **Previdnostni ukrepi in opozorila:** Lindron lahko tako kot drugi bisfosfonati povzroča lokalno draženje sluznice zgornjega dela prebavnega trakta. Posebna pozornost je potrebna pri bolnikih, ki že imajo težave v zgornjem delu prebavnega trakta, kot so disfagija, bolezni požiralnika, gastritis, duodenitis ali razjede, ker se lahko osnovna bolezen poslabša. Lečeči zdravnik mora biti zato posebej pozoren na znake in simptome, ki nakazujejo možne reakcije v požiralniku. Če se pri bolniku pojavijo oteženo požiranje, bolečine pri požiranju ali bolečina za prsnico, mora takoj prenehati jemati Lindron in poiskati zdravniško pomoč. Tveganje za resne neželene učinke, povezane s požiralnikom, je večje pri bolnikih, ki ne upoštevajo navodil o jemanju Lindrona oziroma ga ne prenehajo jemati, ko se pojavijo znaki ali simptomi, ki kažejo na reakcijo požiralnika. Zato je zelo pomembno, da lečeči zdravnik bolnika natančno pouči, kako naj jemlje Lindron, in se prepriča, da je bolnik navodila tudi razumel. Lindrona ne priporočamo bolnikom z ledvično okvaro, ki imajo kreatininski očistek manjši od 35 ml/minuto. Hipokalcemijo je treba pozdraviti pred začetkom zdravljenja z Lindronom. Tudi preostale motnje pri presnovi mineralov (npr. pomanjkanje vitamina D) je treba učinkovito zdraviti. **Medsebojno delovanje z drugimi zdravili:** Sočasno jemanje mineralnih dodatkov (kalcija, železa), antacidov in nekaterih peroralnih zdravil vpliva na absorpcijo Lindrona. Bolniki, ki jemljejo Lindron, smejo vzeti antacid ali druga zdravila najmanj po preteku 30 minut. **Neželeni učinki:** Najpogostejši stranski učinki v prebavilih so: bolečine v trebuhu, slabost, dispepsija, zaprtje, flatulenca, regurgitacija kisline, vnetje požiralnika (lahko z razjedami na požiralniku, motnjami pri požiranju, bolečinami pri požiranju, bolečino za prsnico ali v prsih), bruhanje, motnje pri požiranju in napenjanje. Redko lahko pride do strikture požiralnika, razjed na želodcu ali dvanajstniku. Drugi neželeni učinki so lahko še glavobol in mišično-skeletne bolečine (artralgije, mialgije). Redko se pojavijo alergijske reakcije, vključno z angioedemom, izpuščajem (občasno s preobčutljivostjo za svetlobo) in srbenjem. **Oprema in način izdajanja:** 28 tablet po 10 mg; samo na zdravniški recept

Datum priprave besedila: maj 2002

Podrobnejše informacije so na voljo pri proizvajalcu.

KRKA

Krka, d. d., Novo mesto
Šmarješka cesta 6
8501 Novo mesto
www.krka.si

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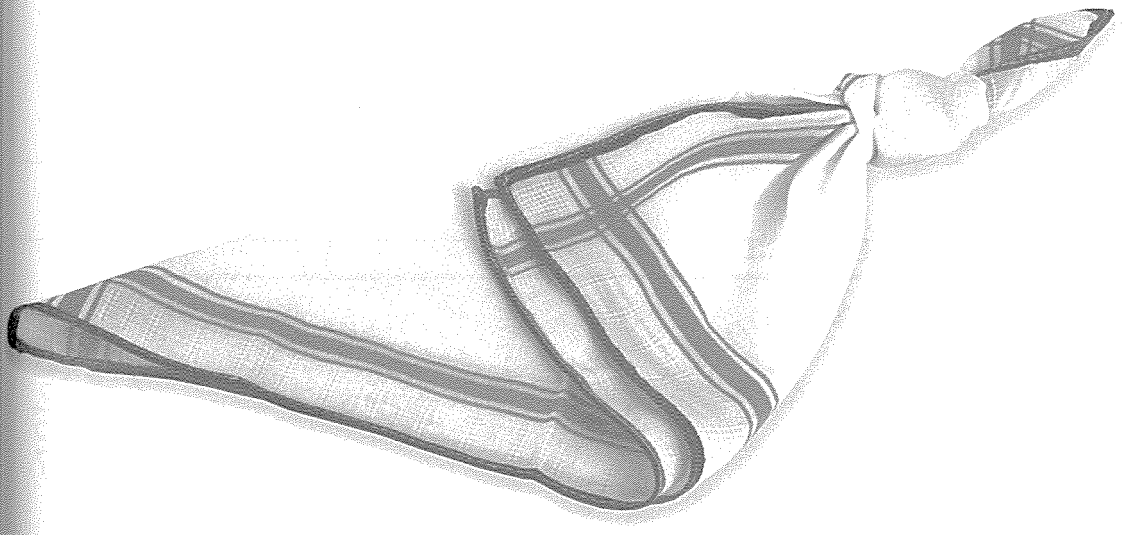

Če ste pozabljivi, naredite vozel na robcu! Naj vas spominja, da je treba srečo iskati. Sodelujte v eni od iger na srečo, ki jih prireja Loterija Slovenije. Pa še to. Če pride, naj ne ostane samo vaša. Delite jo z drugimi.
Sicer pa boste že z nakupom ene same srečke pripomogli k uresničitvi tisoč plemenitih želja. Loterija Slovenije ocenjuje, da bo letos namenila 3, 6 milijarde tolarjev Fundaciji za financiranje invalidskih in humanitarnih organizacij ter Fundaciji za financiranje športnih organizacij.

Torej SREČA ZA SREČO! Ne pozabite na to!

LOTERIJA SLOVENIJE, d.d.
Gerbičeva ulica 99, Ljubljana

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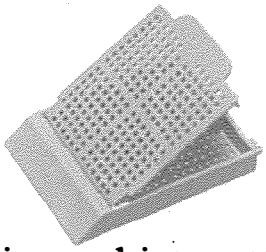
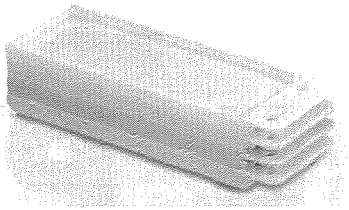
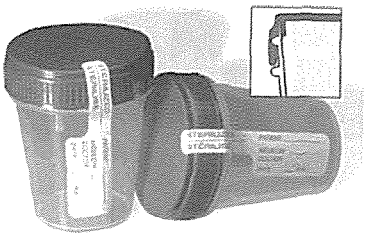


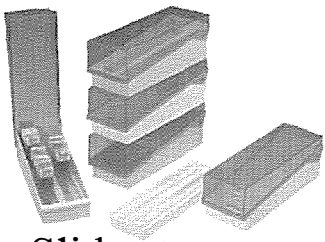
Biopsy histosette



Slide mailer



Specimen containers



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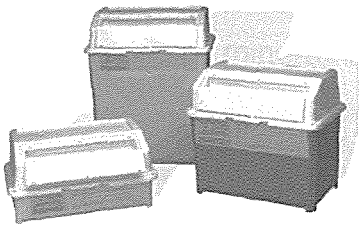
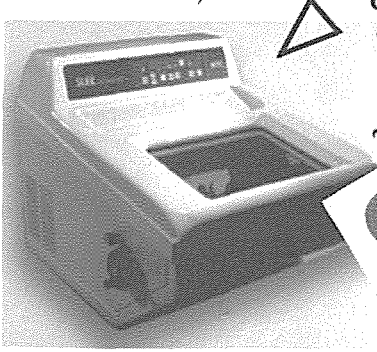


Safest containers

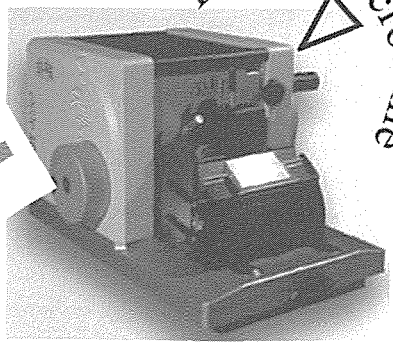
Simport LTD PLASTICS



Benchtop cryostat

SLEE MAINZ

Rotary microtome



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Savska cesta 3, 1000 Ljubljana

Telefon: 01 437 77 45
Telefaks: 01 437 77 47
E_mail: mediconsult@siol.net
www.mediconsult.si