The problem of classifying lymphomas: An orderly prescription for progress

C. W. Berard & R. E. Hutchison

1St. Jude Children's Research Hospital, Memphis, TN; 2SUNY-HSC at Syracuse, Syracuse, NY, USA

Summary

In the late 1960s and early 1970s, the most widely recognized 'new' classifications of the non-Hodgkin's lymphomas were those proposed by Rappaport (the 'Rappaport' classification) and by Lennert (the 'Kiel' classification). With the advent of immunologic and histochemical markers in the early 1970s, however, new concepts arose to supplement the traditional purely morphologic approach to diagnosis and classification of these tumors. Lymphomas were increasingly recognized to be neoplasms of the immune system, composed of malignant proliferations which retained many of the morphologic and functional characteristics of their normal counterparts. These advances led to a flurry of new classifications proposed in 1974-1976, leading to confusion for both clinicians and pathologists, perhaps most evident at the International Congress in Florence in 1974. To address this problem, the National Cancer Institute (USA) sponsored an international workshop of expert pathologists and clinicians on 4-5 September 1975. It became apparent at that meeting that only a well-planned retrospective study would provide data for meaningful progress and resolution of differences. From 1976 to 1980, such a massive collaborative project was accomplished and served as the basis for the Working Formulation for Clinical Usage, proposed as a vehicle for translation among the six tested schema. Since the Working Formulation was published in 1982 there have been momentous strides in scientific and clinical understanding of these cancers, fueled by contributions from immunology, cytogenetics, and molecular biology. To recognize and disseminate understanding of these newer observations, the International Lymphoma Study Group promulgated in 1994 a new proposal entitled 'A Revised European-American Classification of Lymphoid Neoplasms'. As a sequel to another international assembly of pathologists and clinicians, held at the National Cancer Institute (USA) on 21-23 March 1994, a second large-scale retrospective study has been accomplished, the results of which were presented at the Sixth International Conference on Malignant Lymphoma, 5-8 June 1996, along with data from other institutions and cooperative groups. Concurrent with these events, the World Health Organization has enlisted a committee of expert pathologists to prepare a new edition of 'Neoplastic Diseases of Hematopoietic and Lymphoid Tissues'. Composed of 10 pathology subcommittees and a clinical advisory committee, with broad international representation, this body should generate in the near future a consensus proposal with broad scientific and geographic support. These historical and ongoing efforts in lymphoma pathology are a paradigm for progress in clinicopathologic understanding of all cancers.

Key words: classification, Hodgkin's disease, lymphoma, non-Hodgkin's lymphoma, pathology

Introduction

Thomas Hodgkin's report of 1832 of seven patients with tumors of the 'absorbent glands' and spleen opened the floodgate to 140 years of subsequent observations upon which were built a succession of morphological schemes of classification for the malignant lymphomas [1]. There was general acknowledgment by the 1950s that the historical terms 'lymphosarcoma', 'reticulum cell sarcoma', and 'giant follicle lymphoma' were generic, inconsistently used, and of little clinical relevance. A plethora of alternative terms, suggested over several decades, prompted Willis in 1948 to remark that "nowhere in pathology has a chaos of names so clouded clear concept as in the subject of lymphoid tumors" [2]. Two major new classifications nevertheless gained wide recognition by the late 1960s and early 1970s. These were the schemata for the non-Hodgkin's lymphomas proposed by Rappaport (the 'Rappaport' classification) and by Lennert (the 'Kiel' classification) [3, 4]. Particularly noteworthy was the fact each appeared to have considerable clinical significance [5-7].

With the advent of immunologic and histochemical markers in the early 1970s, however, new concepts arose to supplement the traditional purely morphologic approach to diagnosis and classification of these tumors [8-11]. A breakthrough in the understanding of immunologic diseases had occurred in the 1960s with recognition that the immune system consists of two principal pathways of lymphoid differentiation, involving T (thymus-derived) and B (bursa-derived) cells [12-14]. This new insight, gleaned from studies of animals and children with an array of congenital immunodeficiencies, provided the conceptual framework for coupling morphology to function, not only for lymphoid cells but also for the companion mononuclear phagocytic system. Lymphomas were increasingly recognized to be neoplasms of the immune system, composed of malignant proliferations...
which retained many of the morphologic and functional characteristics of their normal counterparts.

These advances led to a flurry of new or updated classifications proposed in 1974-1976, leading to confusion for both pathologists and clinicians [15-22]. The latter were particularly apprehensive about the implications of novel systems of classification, of unproven clinical merit for ongoing therapeutic studies. Institutions and cooperative groups attracted to divergent schemes might have found it impossible to compare their clinical observations and outcomes. To try to resolve these difficulties a series of special meetings, with the goals of making recommendations, were held. In June of 1973, the Workshop on Classification of Non-Hodgkin's Lymphomas was conducted at the University of Chicago under the auspices of Dr. Henry Rappaport. A large number of expert hematopathologists, including all of those associated with proposed classifications, assembled, reviewed, and discussed their diagnostic impressions of 145 cases prepared in histologic slide sets with accompanying clinical histories. Many of the same pathologists, joined by even more numerous clinicians, participated on 8-12 October 1973 in London in the Symposium on Non-Hodgkin's Lymphomata, the first truly large-scale international meeting on these neoplasms. The importance of this symposium and the magnitude of the challenge were underscored by Dr. Henry S. Kaplan, who wrote as follows in the published introduction [23]: "The existence of deeply held divergent views concerning the histological identification of tumor cell types is a particularly serious obstacle, since the international adoption of a soundly based histopathological classification is a prerequisite to substantial progress at the clinical level. The difficulties experienced by the pathologists in attempting to resolve their differences by classic morphologic methods has had a salutary influence, however, by obliging them to turn their attention to new, more subtle methods for the identification and characterization of lymphoreticular cells. The Proceedings contain articles which describe several encouraging initial attempts to apply the concepts and techniques of modern immunobiology, histochemistry, and electron microscopy to this task. It may confidently be predicted that pathologists will soon acquire an exciting new armamentarium of differential tests based on surface membrane receptors and other distinctive cytochemical and immunological properties of these cells and that histopathological classifications firmly rooted in these fundamental biological attributes will emerge." Among the most notable papers from that symposium were the histopathologic contributions of Jaffe et al. [9]; Lukes and Collins [24]; and Lennert et al. [25]; coupled with major clinicopathologic reports by Rosenberg et al. [26]; Brown et al. [27]; Schein et al. [28], from the US National Cancer Institute; and van Unnik et al. [29], from Europe.

Despite Dr. Kaplan's optimism, controversy and confusion heightened at the subsequent international cancer congress in Florence in 1974. To attempt to resolve these conceptual and terminologic difficulties, Drs. Vincent DeVita and Henry S. Kaplan convened, with financial support from the US National Cancer Institute, a special closed meeting of prominent clinicians, immunologists, and hematopathologists at Airlie House in Warrenton, Virginia, on 4-5 September 1975. It was entitled 'Invitational Workshop for the Planning of Retrospective and Prospective Studies to Delineate Optimal Classifications of the Non-Hodgkin's Lymphomas'. Drs. C. W. Berard and R. F. Dorfman served as pathology cochairs for this workshop. Despite compelling presentations and often heated and exhausting debate, efforts to achieve consensus failed, mainly because inadequate clinical data were available to resolve the controversial issues [30, 31]. It became apparent at that meeting that only a well-planned retrospective study would provide data for meaningful progress and resolution of differences. From 1976 to 1980, such a massive collaborative project was accomplished, again with monetary backing by the US National Cancer Institute (for which Dr. Costan W. Berard served as NCI project officer). The six major classifications extant at that time were field tested by their expert proponents and a 'control' group of experienced hematopathologists on an aggregate of 1175 cases assembled as smaller consecutive series at four major centers in the United States and Europe. After massive data accumulation and in-depth statistical analyses, it was concluded that all six classifications were valuable and comparable in clinical correlations and reproducibility. Even as these efforts were ongoing, a series of papers perpetuated controversies regarding classification from 1977 to 1981 [32-38].

In 1982, the results of the NCI-supported study were published, with proposal of the Working Formulation for Clinical Usage, to be used as a vehicle for translation among the six tested schema [39]. Even before its publication, this effort attracted worldwide attention when it was first presented to an international audience at the inaugural 1981 Lugano conference on malignant lymphoma [40, 41]. It is important to emphasize that the Working Formulation per se is a solely morphologic instrument and does not employ the designations 'B cell' or 'T cell'. Nevertheless, the Working Formulation achieved international recognition [42] and "became widely accepted, especially in North America, because it was very useful, clinically, in predicting survival and curability and was easily understood by clinicians" [43]. At the same time the Kiel classification, which codifies non-Hodgkin's lymphomas on the basis of both immunological and morphologic criteria, has gained favor with and been claimed to have clinical relevance by most European pathologists [45]. Over the ensuing years, a series of publications have attested to widespread continuing interest in classifying lymphomas [45-50]. In particular, Simon et al. [51] reported, "In 1988, a long-term follow-up of 1153 patients included in the initial 1982 NCI-sponsored study concluded that the Working Formulation for non-Hodgkin's lymphomas is a simple and useful system for selecting treatment and reporting results."

Since the Working Formulation was published in 1982, there have been momentous strides in scientific and clin-
An ever-enlarging armamentarium of such reagents has subsequently made possible the recognition and detailed delineation of numerous lineage- and/or differentiation-associated antigens on normal and neoplastic cells. Subsets of T and B cells have been characterized and tracked through normal maturation stages and complex interactions in regulation of the immune response. Initially applicable only to cellular suspensions or frozen sections, monoclonal antibodies are now available to mark many fixation-resistant epitopes in routine paraffin-embedded histologic sections. Their application to the spectrum of non-Hodgkin's lymphomas has yielded new information of both basic and clinical relevance.

Equally exciting as the advances in immunology have been the mind-boggling studies in molecular and cellular biology since the classic 1975 paper of Southern [53], paving the way to analysis of DNA rearrangements in antigen-receptor genes and a host of genomic abnormalities including translocations, deletions, and mutations [54–63]. It is important to realize that virtually this entire literature relevant to non-Hodgkin's lymphomas, for which only a few of the earliest papers are herein referenced, appeared in the years following publication of the Working Formulation in 1982 [39]. By 1986, Berard, in a summation of a monograph on lymphomas, had predicted, “In the next decade morphologic evaluation, the traditional ‘gold standard’ for diagnosis and classification, must embrace knowledge from cytochemistry, immunophenotype, precise karyotype, and gene rearrangement, alteration, and expression” [64]. It was inevitable and appropriate that these advances would lead to changes in existing classifications and the appearance of new proposals. By 1988, Stansfeld et al. [65] put forth recommendations for an updated Kiel classification. Subsequently, in April 1993, an assembly of 19 experienced hematopathologists, mainly from Europe and the United States (the International Lymphoma Study Group), convened in Berlin and reached consensus on a list of ‘lymphoma entities’, some presumably established, others provisional. They presented their conclusions to a closed meeting of other pathologists and expert clinicians at the Workshop on Lymphoma Classification held at the US National Cancer Institute in Bethesda, Maryland, on 21–23 March 1994. Their approach to lymphoma classification was to delineate a tabulation of distinct diseases which they felt could be recognized with a combination of morphological, immunological, and molecular techniques. This proposal was subsequently published in full as a ‘revised European–American classification’ [66]. Inherent in this ‘REAL classification’ is the recognition of two major shortcomings in the Working Formulation in the light of advances over the last 15 years: (1) lack of separation of lymphomas based on cell lineage, and (2) omission of several recently recognized clinicopathologic entities. In addition, the REAL classification recognizes that the lymphoid leukemias, plasma cell dyscrasias, and types of Hodgkin’s disease (HD) cannot be separated from NHL, except artificially, and are categorized in the same scheme.

The REAL classification divides lymphoid neoplasms into three major categories: B cell, T cell, and HD. This implies that the immunophenotype must be investigated or that the morphology is characteristically suggestive of a certain phenotype. Cases which appear ambiguous, anomalous, or unusual may be termed ‘unclassified’ and the reasons for this described. The specific categories of the REAL classification are shown in Table 1, and the associated terms of the Kiel classification and Working Formulation may be obtained from the proposal itself [66].

Some categories represent previously well-recognized entities, while others describe entities only recognized since the Working Formulation was published. The previously well-recognized entities are referred to by nomenclature borrowed generally from the Working Formulation and the Kiel classification. The disorders classified by the REAL system are described in order below [66].

Precursor B-lymphoblastic leukemia/lymphoma (B-ALL) and B-cell chronic lymphocytic leukemia (CLL)/prolymphocytic leukemia (PLL)/small lymphocytic lymphoma (SLL) are established spectrums of disease. The leukemic phases are described in less detail than by the French–American–British (FAB) Cooperative Group [67, 68], which did not address the primarily tissue (lymphoma) presentations. Lymphoplasmacytoid lymphoma/immunocytoma is listed separately, as in the Kiel, and is separated from SLL by plasmacytoid features, cytoplasmic IgM, lack of CD5 and ‘lack of characteristic features of other lymphoma sub-types’. Mantle-cell (MC) lymphoma is the first of the new entities listed. This was described in 1975 as ‘lymphocytic lymphoma of intermediate differentiation’, and was felt to be morphologically intermediate between small lymphocytic lymphoma and follicular small cleaved-cell type [69]. It was included in the Working Formulation among diffuse small cleaved-cell type and in the Kiel among centrocytic lymphomas, both terms implying derivation from germinal centers. It has been subsequently determined, however, that MC lymphoma is derived from the cells of follicular mantle zones which are biologically distinct from follicle-center cells, and the neoplastic cells bear closer similarity to those of small lymphocytic lymphoma/CLL. MC lymphoma differs from B-CLL by lack of surface CD23 and by presence of the cytogenetic translocation t(11;14) with overexpression of PRAD1 and...
diffuse to vaguely nodular. A 'blastoid variant' also occurs. Lymphocytes with clumped chromatin, and the pattern is widespread disease. The cytology shows small irregular aggressively lymphoma of usually older males with subsequent production of excess cyclin D1. It is a moderately aggressive lymphoma of usually older males with widespread disease. The cytology shows small irregular lymphocytes with clumped chromatin, and the pattern is diffuse to vaguely nodular. A 'blastoid variant' also occurs. Follicular lymphomas are, of course, well recognized. The REAL classification refers to them all as 'follicle-center lymphoma, follicular', regardless of the number of large cells versus small cells. The authors suggest that pathologists rank them as provisional grades I, II, or III by the pathologist's own criteria (proportion of large cells) pending future data. Follicle-center lymphoma, diffuse, refers to diffuse lymphomas derived from small germinal center cells with or without admixed large cells.

A major group of recent entities are the marginal-zone lymphomas. These include tumors which have been referred to as low-grade lymphoma of mucosal-associated lymphoid tissue (MALT) [70], monocytoid B-cell lymphoma [71, 72], splenic marginal-zone lymphoma [73], and splenic lymphoma with circulating villous lymphocytes (SLVL) [74]. These share apparent derivation from marginal-zone B cells of the spleen, Peyer's patches, and mesenteric lymph nodes. The cytology is of cells with small variably irregular nuclei with clumped chromatin and sometimes abundant (monocytoid) cytoplasm. These cells have the capacity to mature to plasma cells as well as to monocytoid cells. They appear to distribute according to tissue-specific homing patterns and, at least in some cases, exhibit antigen-driven proliferation. Three main types are described in the REAL classification, nodal, extranodal, and splenic.

Extranodal marginal-zone lymphomas are the equivalent of low-grade lymphomas of MALT. They typically occur in glandular mucosa of patients with autoimmune diseases or helicobacter gastritis, have characteristic 'lymphoepithelial lesions', and are most often of limited extent with an indolent or potentially regressive course (in treated localized cases). They typically lack CD5 or CD10 expression as well as t(14;18) or t(11;14) translocations. (Trisomy 3 and t(11;14) have been reported.) Nodal disease usually occurs in patients with Sjögren's disease or as a manifestation of spread of extranodal disease.

Splenic marginal-zone lymphoma is listed separately and is stated to be distinct from the other two lesions, overlapping with 'splenic lymphoma with villous lymphocytes', a chronic B-cell leukemia with features intermediate between B-cell and hairy-cell leukemia. Splenic mantle- and marginal-zone involvement is typical, red pulp involvement is often also prominent, and there is frequent bone marrow and peripheral blood disease. Its relationship to and separation from other chronic leukemias is not detailed.

Plasmacytoma/plasma-cell myeloma is listed and briefly described. This is a previously well-characterized disease. The immunophenotype differs from B-cell lymphomas by presence of cytoplasmic immunoglobulin and surface CD38 in the absence of most lymphoid markers.

The REAL classification diverges significantly from the Working Formulation in regard to diffuse large-cell and immunoblastic lymphoma. The REAL divides them into B cell and T cell. B-cell cases are lumped under diffuse large B-cell lymphoma, with a single subgroup of primary mediastinal large B-cell lymphoma, which is considered a distinct clinicopathologic entity. The others,
whether of follicle-center cells, immunoblasts, or anaplastic large B cells, and including T-cell-rich and histiocytoid-rich cases, are considered as one category. The authors state that subclassification is impractical is the same, but acknowledge that several diseases are likely represented. This category also overlaps with small non-cleaved-cell (Burkitt's) lymphoma. T-cell large-cell lymphomas are extensively subdivided, on the other hand.

Burkitt's lymphoma is well known. The term high-grade B-cell lymphoma, Burkitt-like, replaces small non-cleaved-cell, non-Burkitt, and is utilized for cases histologically intermediate between diffuse large B-cell and Burkitt's lymphoma. This includes cases which are biologically related to either.

T-cell and putative natural killer cell (NK) neoplasms comprise the second major group of tumors. Of these, precursor T-lymphoblastic lymphoma/leukemia and T-cell chronic lymphocytic leukemia/T-prolymphocytic leukemia are well described, though only lymphoblastic lymphoma is included in the Working Formulation.

Large granular lymphocyte (LGL) leukemia, T-cell and NK-cell types, are more recently described diseases which would have previously been regarded as small lymphocytic lymphoma, CLL, or chronic lymphocytoses. These share the morphology of large granular lymphocytes and often show splenic red pulp involvement. All express CD2 and many CD8. T-cell cases express CD3, usually CD16, and sometimes CD57. NK-cell cases lack CD3 but variably express CD16, CD56, and/or CD57. The spectrums of phenotypes and clinical behavior are still being elucidated, and while aggressive variants occur, most cases are indolent.

Mycosis fungoides/Sézary syndrome is dealt with in the REAL classification as a familiar clinicopathologic entity. Cases of T-cell lymphoma previously listed in the Working Formulation as diffuse large-cell, immunoblastic, or diffuse mixed type are listed either generically as peripheral T-cell lymphomas, unspecified (with provisional categories based on cell make-up), or as a series of specific new clinicopathologic entities.

The unspecified cases show diffuse or interfollicular infiltrates of small and large atypical cells in variable proportions and frequently with admixed eosinophils and histiocytes. The majority of cells present are clonal T cells (with clonal T-cell receptor genes.) Reed-Sternberg-like cells, but not true RS cells, are sometimes present. The authors recommend stratifying the cases by the number of large cells as medium-sized, mixed medium- and large-cell, and large-cell types. Hepatosplenic gamma-delta T-cell lymphoma and subcutaneous panniculitic T-cell lymphoma (associated with hemophagocytic syndrome) are listed as provisional entities under the unspecified category.

Specific categories of peripheral T-cell lymphoma are also listed. Angioimmunoblastic T-cell lymphoma, previously referred to as angioimmunoblastic lymphadenopathy or immunoblastic lymphadenopathy, is now considered to be a lymphoma rather than an abnormal immune reaction. This disease shows lymph nodes with diffuse but sparse infiltrates of clonal T cells with prominent PAS+ hyalized high endothelial venules, often clusters of clear cells, and frequently admixed eosinophils, plasma cells, and histiocytes. Angiocentric lymphoma is characterized by an angiocentric and angioinvasive mixed infiltrate with associated necrosis and has previously been referred to by terms including lethal midline granuloma, polymorphic reticulosis, and nasal T-cell lymphoma. Some cases of lymphomatoid granulomatosis may fall in this category and associated hemophagocytic syndromes may occur. The cell of origin is of NK-cell or undefined T-cell lineage.

Intestinal T-cell lymphoma (with or without enteropathy) is a jejunal tumor frequently associated with gluten-sensitive enteropathy which contains variable proportions of anaplastic large cells and also adjacent mucosal infiltrates of intraepithelial T cells from which it is postulated to arise. The course is aggressive.

Adult T-cell lymphoma/leukemia is a well-defined entity that is the aggressive form of T-cell lymphoproliferative disorder associated with infection by human T-lymphotropic virus type I (HTLV-1). Endemic areas occur in southern Japan and, to a lesser extent, in the Caribbean, and sporadic cases occur. The typical acute form shows cutaneous and leukemic involvement including cells with cloverleaf nuclei, hypercalcemia, and a rapid course. The lymph node histology is variable, but frequently shows a mixed infiltrate including pleomorphic large cells. The immunophenotype is CD3+/CD4+/CD25+/CD7- and both clonal T-cell-receptor genes and clonally integrated HTLV-1 are present.

In addition to these T-cell lymphomas, anaplastic large-cell (CD30+) lymphoma (T- and null-cell types) is listed in the REAL classification as a distinct entity. Anaplastic large-cell lymphoma (ALCL) is a tumor consisting of variable proportions of large cells with abundant cytoplasm which infiltrate, among other areas, lymph node sinuses or skin. It mimics epithelial tumors and usually expresses CD30 and epithelial membrane antigen (EMA). Cases may or may not have T-cell receptor gene rearrangement (T cell or null cell) but only rarely show B-cell lineage. The (t,2,5) cytogenetic translocation has been found in some cases, particularly in young patients. A provisional entity of ALCL, Hodgkin's-like, is also listed, which has morphologic similarities to nodular sclerosis Hodgkin's disease but does not respond well to Hodgkin's therapy.

Hodgkin' disease (HD) itself is also listed in the REAL classification. It was not discussed in the Working Formulation, and the REAL classification essentially conforms to the previous Rye classification [75]. Lymphocyte predominance (paragranuloma), nodular sclerosis, mixed cellularity, and lymphocyte depletion types are listed, as well as a provisional entity of lymphocyte-rich classical HD (with small numbers of classical RS cells).

What lies ahead and how do we proceed? There is always some consternation expressed in reaction to a new classification, especially one seemingly as complex as the REAL proposal on initial review [76]. Pathologists fa-
miliar with the updated Kiel classification will readily recognize most of the listed entities, but a recent survey of American pathologists revealed that 93.7% use the Working Formulation and only 5% are conversant with the Kiel classification [77]. As was true 20 years ago when the NCI-supported study was launched, what we must generate now are convincing data regarding the new proposal. There is general agreement with the oft-repeated concept that secondary lymphomas are a paradigm for progress in clinico-pathologic understanding of all cancers.

The Working Formulation project demonstrated that pathologists, clinicians, and statisticians can together successfully achieve now are convincing data regarding the new proposal [66]. Over the past several months, a large-scale retrospective study has been conducted by five pathologists at eight sites. Their findings and the accompanying clinical data were presented at the Sixth International Conference on Malignant Lymphoma, along with data from other institutions and cooperative groups. Concurrent with these events the World Health Organization has enlisted a committee of expert pathologists to prepare a new edition of 'Neoplastic Diseases of Hematopoietic and Lymphoid Tissues'. Composed of 10 pathology subcommittees and a clinical advisory committee, with broad international representation, this body should generate in the near future a consensus statement with broad scientific and geographic support. The Working Formulation project demonstrated that pathologists, clinicians, and statisticians can together successfully accomplish large-scale studies incorporating new concepts and terms. These historical and ongoing efforts in lymphoma pathology are a paradigm for progress in clinico-pathologic understanding of all cancers.

References
