LYMPHOMA — HISTOPATHOLOGY IN CHANGING CLINICAL PERSPECTIVE

Peter Jacobs

On behalf of the Non-Hodgkin's Lymphoma Classification Project

Background. Lymphoma management has traditionally been dominated by nodal histopathology. Unfortunately, many different classifications coexisted and frequent revisions have often obscured clinical correlations. Some improvement in understanding histogenesis followed the introduction of immunophenotyping, while a number of new entities have been described in the last decade. In addition the whole question of lymphomagenesis is undergoing critical exploration. The use of cellular and molecular biological techniques is therefore shifting focus to the role of oncoproteins and the impact of mutation in the normally modulating suppressor genes.

To accommodate these advances the International Lymphoma Study Group has proposed the Revised European-American Lymphoma Classification. While this is an undoubted advance, it has met with persisting concerns regarding applicability to patient management.

Study setting. In determining the extent to which the latter reservation is valid, and at the same time directly testing the clinicopathological value of the new system, a group of acknowledged experts drawn from nine major academic centres worldwide analysed approximately 1 400 previously unreported cases, focusing on outcome. As part of that study 196 consecutive patients seen in Cape Town were separately examined.

Results. Findings here were similar to those of the overall experience, although distinct geographical differences emerged. Specifically in the follicular centre-cell lymphomas there was no difference in the 5-year failure-free survival rate, but these neoplasms accounted for 33% of lymphomas, which is similar to North America and London but contrasts with the 14% in the remaining six sites. Also, while mean survival for all types of peripheral T-cell lymphoma was 18% at 5 years, these accounted for 8% of lymphomas locally, as seen

Haematology Department and Bone Marrow Transplantation Unit, Constantiaberg Medi-Clinic, Plumstead, Cape Town

Peter Jacobs, MB BCh, MD, PhD, FCP (SA), FACP, FRCP (Edin), FRC Path (UK), FRSSAf

SAMJ

also in London and Hong Kong, but exceeding the 3 - 6% reported elsewhere.

Local experience, as in the other eight centres, documented good diagnostic concordance between trained haematopathologists when this classification was used by them all. Furthermore, unusual subtypes were generally well accommodated within this revised system. It should be noted that while histopathological features retain predictive value, they should not be considered the predominant factor. It was concluded that for management decisions to be appropriate, renewed and correct weighting must be assigned to other prognostic variables that include clinical features and markers of tumour biology.

Summary. This more enlightened prerequisite is the central goal that underlies optimal treatment outcome, since it determines stratification to appropriate and peer-reviewed protocols. It follows that review of histopathology needs to precede management of all newly diagnosed cases, preferably only by accredited multidisciplinary clinics. The previous anachronism of basing therapy on opinions of non-specialist pathologists, without appropriate review, is unwise. Furthermore, treatment by lone practitioners, or even single-specialty groups that lack the discipline to analyse their findings critically and regularly report their updated results, can no longer be considered standard of practice and should be discouraged.

S Afr Med J 2000; **90:** 135-141.

Evaluation of the enlarged lymph gland is an everyday problem in primary care medicine, as it is in all other disciplines. In many instances there is an underlying inflammatory lesion that resolves swiftly with appropriate treatment. Less frequently, but perhaps more importantly, the palpable node is malignant. After excluding metastatic cancer, the remaining lymphomas require investigation and management by an effectively functioning multi-specialty group, if optimal cure rates are to be achieved. In this setting three prerequisites have to be met. Firstly, diagnosis must be precise and use modern classifications that include at least immunophenotyping and preferably also cellular or molecular genetics. Secondly, prognosis needs to be defined in a way that integrates all available clinical information, including rate of progression and tumour markers such as bulk and anatomical site. Thirdly, treatment needs to be stratified according to internationally acceptable or state-of-the-art protocols, with results constantly reviewed and with all consecutively registered cases accounted for.[1] The previous practice, namely of individuals being treated outside such a broadly based group, can no longer be considered to be in the patient's best interests, and should therefore come under critical scrutiny.

Historically, nodal architecture was used to divide these tumours into follicular lymphomas, lymphosarcomas, and reticulum-cell sarcomas.[2] Unfortunately, this grouping was too vague to have predictive value with regard to outcome. Subsequently cytomorphology was combined with disturbances in architecture, seeking to give clinical relevance to the growth pattern.[3] Much of that focus was changed when it was appreciated that these neoplasms originated from cells of the immune system.[4] There followed a period of enormous confusion, since as many as 20 different classifications existed concurrently, precluding uniformity of patient entry into clinical trials, or even exchange of information between centres. Some semblance of organisation was imposed on this chaos by publication of the working formulation in 1982.[5] Although intended as a means of interpreting data between systems, it became accepted as a classification in North America. In contrast, the Europeans favoured the system described by Karl Lennert.[6]

This unsatisfactory state of affairs has been reassessed recently for a number of reasons. There was a growing appreciation that new and distinct variants existed. Immunophenotyping became more readily available, so that B- or T-cell origin could be determined routinely and impact of lineage could be explored as a separate variable. Cytogenetics, as in the leukaemias, was providing relevant prediction with regard to response to therapy and outcome, but was poorly accommodated in most routine reports. Furthermore, the shortcomings of older classifications were giving way to the more clinically based prognostic index.[7]

In an attempt to redress persisting concerns, the International Lymphoma Study Group combined salient features from the working formulation with the Kiel approach to produce what is known as the Revised European-American Lymphoma (REAL) Classification.[8] Despite this undoubted advance, some reservations have persisted, particularly among clinicians. Reservations have focused mainly on the undue weight given to histopathological features, the lack of reproducibility of diagnostic criteria between centres, or indeed even by the same pathologist, and the role that immunophenotyping should play in a practical approach to classifying, understanding and treating these neoplasms.

For these reasons an international multicentre study was undertaken to examine this new proposal. The objective was to assess its acceptability and value to practising pathologists, to explore any potential geographical differences that may exist, and specifically to determine whether it had clinical relevance.[9,10] Accordingly, a collaborative effort was undertaken by clinical experts in nine areas throughout the world (Table I) working with local haematopathologists to evaluate the utility of the REAL Classification[8] in contemporary management of patients with these lymphoproliferative disorders. In addition to publication of the global results, we now separately report the Cape Town experience.

Table I. Participants in the Multicentre International Lymphoma Study Group[12]

Site	Investigator	Cases
Omaha, Nebraska, USA	Wing C Chan James O Armitage	200
Vancouver, Canada	Randy Gascoyne Joseph Connors	202
Cape Town, South Africa	Peter Jacobs Pauline Close Carol Johnson	196
London, England	Andrew Norton T Andrew Lister	120
Locarno, Switzerland	Ennio Pedrinis Franco Cavalli	80
Lyon, France	Francoise Berger Bertram Coiffier	195
Hong Kong	Faith Ho Raymond Liang	210
Würzburg/Göttingen, Germany	Alfred Schauer Wolfgang Hiddemann German Ott	200

Consultants were Saul Rosenberg at Palo Alto for study design and analysis, Nancy L Harris for advice regarding the REAL Classification, James R Anderson and Pascal Roy were statisticians, and the visiting pathologists were Jaques Diebold, Kenneth A MacLennan, H Konrad Muller-Hermelink, Bharat Nathwani and Dennis D Weisenburger.

MATERIALS AND METHODS

Consecutive patients registered at Groote Schuur Hospital between 1 January 1988 and 1 December 1990 were identified from the records of the lymphoma clinic. The completeness of the database was confirmed by reference to the hospital central statistics department. Biopsy material, including referred slides and blocks registered in the Department of Anatomical Pathology at the University of Cape Town, was similarly scrutinised. Four hundred and twenty-six cases were identified, and after extracting pertinent clinical information, each was reviewed. Where treatment details or follow-up information was insufficient this was noted, but the case was excluded from further consideration. The lymph node and bone marrow biopsies of the remaining cases were reviewed and additional immunophenotyping was carried out where necessary. One hundred and ninety-six cases fully met the criteria for analysis (Table II). Criteria were that the tissue samples should be adequate and that all the relevant pathology materials should be available, including bone marrow aspiration and trephine biopsy samples, with immunological information being sufficient to assign the neoplasm confidently to B- or T-cell lineage. Patient characteristics, treatment data and follow-up information needed to be complete. Leukaemias were excluded.

The clinical material was compiled from review of the medical records, while histopathological preparations were reviewed by the designated site pathologist. Where necessary, additional sections were prepared and immunostains included. Other studies were performed in order that the material could be classified appropriately. Cytogenetic and molecular biological data, where available, were recorded on a standard data-capture sheet. Once compilation was complete five expert haematopathologists travelled to each of the nine centres over an 8-month period, beginning in June 1995, and spent a week reviewing all the collated information at each participating institution.

A standardised approach was used in which each of the experts first used haematoxylin and eosin- or Giemsa-stained sections to record a diagnosis when supplied only with patient age, sex, major site of disease and origin of the biopsy. The same exercise was then repeated, adding immunophenotyping together with any other cellular or molecular biological data. Finally, all available preclinical treatment information was integrated to establish a final diagnosis. At the end of each daily working session individual opinions were reviewed, with consensus defined as agreement between four of the five experts. When the site visit was complete, 20% of all the cases were randomly selected for re-review without reference to the original diagnosis.

Treatment outcomes were measured by overall and failure-free survival, with the latter defined as the time from diagnosis to first occurrence of progression, relapse after response, or

Table II. Summary of the variables collected for analysis

Clinical information	Laboratory data	Follow-up measurements
Patient identification	Serum lactic dehydrogenase	Initial treatment regimen
Sex	Absolute lymphoctye count	Therapeutic response
Ethnic origin	Presence of circulating lymphoma cells	Details of remission, progression or relapse
Date of birth	Monoclonal serum immunoglobulin	Salvage therapies
Date and site of diagnostic biopsy	History of viral infections	
Nodal areas involved	HIV status	
Maximum diameter of largest tumour mass		
Ann Arbor staging at diagnosis		
Performance status		

death from any cause. Follow-up of patients not experiencing one of these events was censored at the time of last contact. Estimates of survival were calculated using the method of Kaplan and Meier[11] and time-to-event distributions were compared using the log-rank test.

RESULTS

In the multicentre study 1.8% of cases were excluded from analysis because the diagnosis was not that of lymphoma. No such errors were recognised in the Cape Town material.

Of the consecutive locally entered patients, 33% had follicular lymphomas, 28% had diffuse large B-cell lymphomas, 8% had peripheral T-cell lymphomas, and a further 8% had small lymphocytic lymphomas. The marginal variant of the mucosal-associated lymphoid tumours was 6%, while for the mediastinal diffuse large B-cell and anaplastic large B-cell lymphomas it was 3% each, and for the mantle-cell tumours it was 1%. Twelve per cent of patients had other less frequently encountered forms of lymphoid malignancy (Table III).

As in the parent study, diagnosis based only on histology was not significantly altered by inclusion of immunophenotype when the lymphomas were follicular. These stains improved diagnostic accuracy in three other subtypes. Consequently a further 14% of diffuse large B-cell cases and an additional 39% of anaplastic large-cell cases were identified, while in peripheral T-cell variants a 45% improved recognition was possible. It should be noted that when all additional clinical data were added this was only helpful in primary mediastinal diffuse large B-cell cases. Here diagnostic accuracy improved by 37%.

It is noteworthy that when 20% of the cases were re-reviewed, the original diagnosis was verified in 80 - 90% of cases, suggesting that REAL Classification is reproducible in experienced hands. It is unlikely that such inter- or intra-observer reproducibility will apply to the individual who is not specifically committed to the in-depth study of these tumours.

The overall and failure-free survival rates for the Cape Town groups were 48% and 36%, respectively (Fig. 1). For patients with follicular lymphomas the corresponding figures differ by grade, both for overall survival (Fig. 2) and for those free of relapse at 7 years (Fig. 3). For the diffuse large-cell, chronic lymphocytic and peripheral T-cell lymphoma patients, overall and failure-free survival are approximately the same and can be represented by one set of graphs (Fig. 4).

DISCUSSION

Results from this local study parallel experience from other centres in supporting use of the REAL Classification on the grounds that it is currently the most appropriate approach in identifying the major types of non-Hodgkin's lymphoma.[12,13] Retrospectively it became clear that in 85% of the cases examined histopathology alone sufficed for reliable diagnosis;

Table III. Clinical characteristics and laboratory data

	Follicular lymphoma	Diffuse large B-cell lymphoma	Peripheral T-cell lymphoma	Small lymphocytic lymphoma	Marginal zone lymphoma* A	Marginal zone lymphoma* B	Mediastinal diffuse large B-cell lymphoma	Anaplastic large B-cell lymphoma	Mantle-cell lymphoma
Frequency (%)	33	28	8	8	5	1	3	3	1
Median age (yrs)	59	64	61	65	61	58	37	33	63
Male (%)	42	55	56	53	45	41	34	69	74
Stage 1 or 2 (%)	33	51	18	6	66	18	66	50	19
Positive marrow (%)	42	17	37	73	14	41	3	12	63
IPI score (%)†	39	31	14	23	38	36	44	50	19
Immunophenotype	CD20+, CD3– CD10+, CD5–	CD20+, CD3–	CD20–, CD3–	CD20+, CD3– CD10–, CD5+ CD23+	CD20+ CD3–, CD10–, CD5–, CD23–	CD20+ CD3–, CD10–, CD5–, CD23–	CD20+,CD3–	CD20–, CD3+ CD30+, CD15– EMA+,ALK+	CD20+, CD– CD10–, CD5+ CD23–, PRAD1+
Cytogenetics	t(14; 18) (q32; q21)	t(14; 18)(q32; q21) t(8; 14)(q24;q32) t(3;14)(q27;q32)	Variable	del(13q), +12	t(11; 18) (q21; q21) +3, +18	+3, +18	Variable	t(2;5)(p23; q35)	t(11;14) (q13; q32)
Oncogenes	BCL–2	BCL–2 C–MYC BCL–6	Unknown	Unknown	Un-known	Un-known	Unknown	ALK	BCL-1(Prad1)

While immunophenotyping was a requirement for entry, not all patients had cytogenetics performed; for completeness the data from the overall study are cited.[12,13] The remaining 12% were other less frequently encountered forms of lymphoid malignancy.
* Marginal zone lymphoma: A = MALT type, B = nodal type.
† IPI = International Prognostic Index.[7]

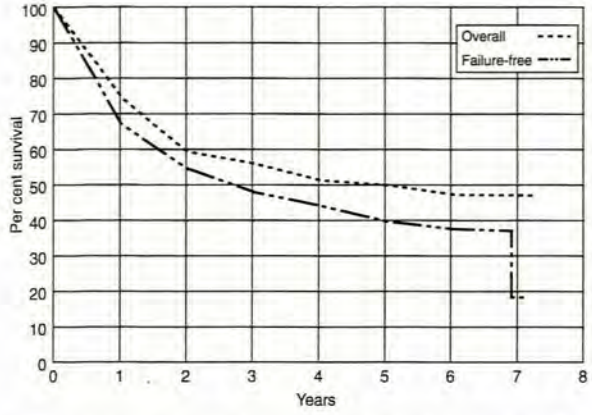

Fig. 1. Overall and failure-free survival for all patients in the Cape Town series.

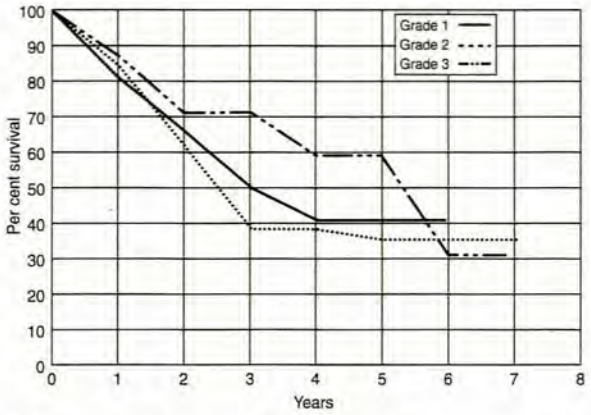

Fig. 3. Failure-free survival of patients with follicular centre cells by grade.

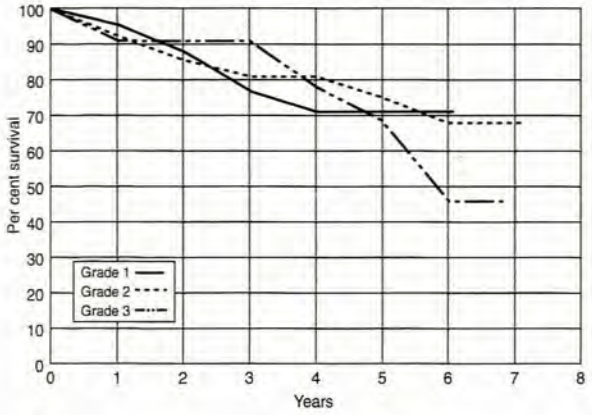

Fig. 2. Overall survival of patients with follicular centre cells by grade.

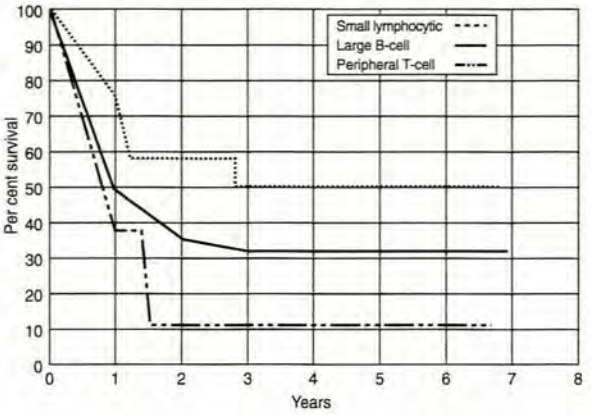

Fig. 4. Overall and failure-free survival are similar and therefore a single curve reflects data for small lymphocytic, diffuse large B-cell, and peripheral T-cell lymphomas.

this was particularly true in those cases with a follicular growth pattern. It should be emphasised that where possible an entire node together with capsule should be atraumatically removed and processed by an experienced pathologist using fixatives chosen both to maintain nuclear and cytoplasmic detail and at the same time to be appropriate for phenotyping. It is to be anticipated that many of these features will be consolidated into World Health Organisation recommendations in the immediate future.[14]

Accuracy was enhanced by adding immunostains using monoclonal antibodies. Here, consensus diagnosis improved between 2% and 14% in mucosal-associated lymphoid tumours, small lymphocytic or chronic lymphocytic leukaemia, those with lymphoplasmacytoid differentiation, high-grade or large B-cell tumours, nodal marginal zone and mantle-cell neoplasms. The striking advantage of these additional preparations in defining lineage was seen in the T-cell variants, whether precursor, anaplastic large or peripheral T-cell types. In these latter subtypes this information approximately doubled the number correctly identified over those using only sections stained with haematoxylin and eosin. Unfortunately, the need for these preparations cannot be predicted at the time of diagnosis, so that suitable planning must be made for the material to be studied using appropriate methods at a later date. It is noteworthy that flow cytometry is being increasingly employed, and that multidisciplinary groups managing these patients typically rely on this technology, using fresh tissue to avoid problems of fixation and processing.

One of the striking features of this study is that it confirms previous concerns that arose shortly after publication of the REAL Classification and were strongly reflected in the Cape Town experience, namely the importance of integrating all available information within a prognostic index, with particular emphasis given to patient data. This point is most remarkably demonstrated in primary mediastinal large B-cell tumours. However, the value of careful attention to clinical features, many of which reflect disease biology, cannot be underestimated. Logically it must, therefore, be acknowledged that previous preoccupation with purely microscopic features is

no longer acceptable. Correct stratification of patients to appropriate treatment programmes needs to integrate biochemical markers that include lactic dehydrogenase and additional imaging in order to document tumour distribution and bulk.

Assuming that optimum treatment protocols are used, the Cape Town experience again mimics that of the multicentre analysis in recognising four survival patterns.[12] Consequently, overall 5-year survival exceeded 70% in patients with anaplastic large-cell lymphoma, marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue, and the follicular lymphomas. A second group of these tumours occupied an intermediate position and comprised the nodal variant of marginal zone B-cell lymphomas, those with lymphoplasmacytoid differentiation and small lymphocytic lymphoma or its chronic leukaemic equivalent. In the third category, where overall survival is approximately 50%, the majority of cases involved diffuse large B-cell neoplasms. In the remainder of cases this figure dropped to 30% and largely involved T-cell and mantle-cell lymphomas.

Although these results have improved over the last 5 years, they leave much to be desired. One area where new technology can be expected to improve outcome significantly is selection of treatment regimen based on inclusion of cellular and molecular biological data. In this context karyotyping or fluorescent *in situ* hybridisation that reveals the presence of chromosomal rearrangement or deletions is already proving valuable, both in diagnosis and in predicting results from conventional therapy. For example, the integration of such information into selection criteria for any particular treatment programme means that high-risk patients can rationally be assigned to intensive chemotherapy, often coupled with irradiation, and myeloprotection with haematopoietic stem-cell transplantation as first-line protocol management. A specific example will involve cases that at presentation have intermediate or high-risk features defined by the International Prognostic Index.[7]

A number of observations that emerge from the local study echo findings in other centres. The REAL Classification is therefore reproducible provided that the initial material is interpreted by an experienced pathologist, preferably one who is a regular participant in ongoing review workshops at international level. Secondly, cytomorphological and architectural features are no longer acceptable as the only criterion for diagnosis, with accuracy often being improved by inclusion of immunophenotyping and karyotypic analysis. Thirdly, there is growing acceptance that all relevant clinical information, including imaging and biochemical measurements, needs to be used in allocating patients to a risk category in the International Prognostic Index. Fourthly, and perhaps of particular relevance in developing countries, is the appreciation that work-up and management must be centred in multidisciplinary clinics that are at least nationally, and preferably internationally, accredited. This necessitates audit of every patient referred and peer-review of outcome in consecutively enrolled patients. Data accumulated in this way can be further monitored when it forms part of multicentre or collaborative studies.

In contrast, the previous practice of management by an occasional therapist, or even within a single-discipline practice, has shortcomings. These include over-treatment on the one hand, and failure to disclose the availability of innovative or investigational options on the other, which may lead to the equally unacceptable under-treatment. The clinical trials group of the South African Society of Medical Oncologists has taken a leadership role in this direction.[15] Here ongoing local studies are being catalogued and efforts are being made to co-ordinate relevant approaches at national level with guidelines for conventional management, while concurrently advancing scientific standards through collaborative programmes. Issues that are suitable for such investigation include evaluation of topoisomerase inhibitors, high-dose chemoradiotherapy with myeloprotection using haematopoietic stem and progenitor cell transplants or anti-B-cell monoclonal antibodies. There are compelling advantages, particularly in the Third World and as managed health care programmes permeate all levels of practice, for the small number of centres that have earned a reputation for excellence to be identified and designated as such. This step is needed to maintain standards, to disseminate information about what expectations exist with regard to treatment outcomes, and to provide a reliable base both for referral of new patients and to ensure that the guiding principles of interdisciplinary consultation are observed. Failure to act in this direction will fuel the downward spiral in understanding and therefore the correct contemporary management of patients with lymphoma.

Conclusion

In South Africa, as elsewhere in the world, the goal should be one of treating lymphoid malignancies with the intention of improving disease-free survival, or more directly, achieving the highest possible cure rate. The extent to which successful outcome is achieved depends on exacting diagnosis and stratification of patients to the most effective treatment option designed to capitalise on assignment-by-risk category, with appropriate weight being given to inclusion of clinical characteristics such as tumour progression and biology. The local experience, part of a much larger international study project, cogently argues for referring any patient with suspected lymphoma to an established multidisciplinary group without delay. In this way evaluation will be comprehensive and recommendations can be made about inclusion in peer-reviewed protocols.

Equally important is the obligation to monitor the response in each case objectively, so that appropriate changes to treatment can be made immediately there is any deviation from

anticipated outcome. Acceptance of these principles excludes the older practice of dabbling by occasional therapists, or even therapy given by single-discipline practices. These are largely curable neoplasms, and however unpalatable, there is no substitute for constant guidance — even supervision if appropriate — from the properly constituted and impartially functioning combined clinic.

I thank Fred Ullrich and James Anderson, from the University of Omaha in Nebraska, for the separate statistical analyses of the cases from Cape Town; Patricia Marais and Gillian Ganz for help with the manuscript typing; and Christine Dölling for bibliographic assistance. Pauline Close, from the Department of Anatomical Pathology at the University of Cape Town, was the site pathologist. Carol Johnson, Radiation Oncologist, collated the clinical material. James O Armitage, Professor of Medicine at the University of Nebraska Medical Centre, kindly reviewed the manuscript. These studies were supported by the Medical Research Council and the Cancer Association of South Africa.

References

1. Jacobs P, Wood L. The lymphomas: contemporary management. *Specialist Medicine* 1998; **20**(3): 16-26.
2. Gall EA, Mallory TB. Malignant lymphoma: a clinico-pathologic survey of 618 cases. *Am J Pathol* 1942; **18:** 381-415.
3. Rappaport H, Winter WJ, Hicks EB. Follicular lymphoma: a re-evaluation of its position in the scheme of malignant lymphoma, based on a survey of 253 cases. *Cancer* 1954; **9:** 792-821.
4. Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. *Cancer* 1974; **34:** 1488-1503.
5. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a Working Formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. *Cancer* 1982; **49:** 2112-2135.
6. Lennert K, Mohri N, Stein H, Kaiserling E. The histopathology of malignant lymphoma. *Br J Haematol* 1975; **31:** Suppl, 193-203.
7. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med* 1993; **329:** 987-994.
8. Harris NL, Jaffe ES, Stein H, *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; **84:** 1361-1392.
9. Weiss NS. Geographical variation in the incidence of the leukemias and lymphomas. *Natl Cancer Inst Monogr* 1979; **53:** 139-142.
10. Correa P, O'Conor GT. Geographic pathology of lymphoreticular tumors: summary of survey from the Geographic Pathology Committee on the International Union Against Cancer. *J Natl Cancer Inst* 1973; **50:** 1609-1617.
11. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association* 1958; **53:** 457-481.
12. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood* 1997; **87:** 3909-3918.
13. Anderson JR, Weisenburger DD, Armitage JO, for the Non-Hodgkin's Lymphoma Classification Project. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. *Ann Oncol* 1998; **9:** 717-720.
14. Jaffe ES, Harris NL, Diebold J, Muller-Hermelink HK. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: a progress report. *Am J Clin Pathol* 1999; **111:** suppl 1, S8-S12.
15. Jacobs P. SASMO Clinical Trials Group: The haematologic malignancies. *The Cape Doctor* 1998; **38:** 16.

Accepted 20 May 1999.