EDITORIAL

CURRENT ADVANCES IN THE DIAGNOSIS OF LYMPHOMAS

Lymphoma is abnormal lymphoproliferative disorder of the lymph node which is a major anatomical component of the immune system, however about 30% arise from extra-nodal tissues. Malignant lymphoma can be divided into two major categories; Hodgkins lymphoma disease and all others which, for lack of a better term, are known as non Hodgkin's lymphoma(1-4).

Currently Hodgkins disease is further sub-classified as: lymphocyte predominant Hodgkin's lymphoma (LP-HL) and classic Hodgkin's lymphoma (C-HL).
C-HL has been further classified into four subtypes: lymphocyte rich, nodular sclerosis, with mixed cellularity and lymphocyte depleted. Non-Hodgkin's lymphoma is further classified into; B-cell neoplasm, T-cell neoplasm and natural killer cell neoplasm(1,3). There have been many rapid changes in lymphoma classification which have made Wills to comment that "Nowhere in pathology has a chaos of names so clouded clear concepts as in the subject of lymphoid tumours." These changes seem to be continuous and a new classification is always expected all the time(2,4,5).

A few recent studies on lymphoma have shown that histologic diagnosis categorisation of lymphoma has been a source of frustration for many years for both clinicians and pathologists since lymphomas may be indistinguishable from both benign lympho-proliferative disorders and malignant lesions such as poorly differentiated carcinomas and malignant melanomas, particularly in childhood tumours, such as all small round blue cell tumours, including carcinoid tumour, Alveoli rhabdomyosarcoma, Ewing's tumour of the bone (primitive neuroectodermal tumour, PNET), synovial sarcoma, small cell osteosarcoma, renal sarcoma desmoplastic small round cell tumour (DSRCT), and neuroblastoma just to mention a few. These similarities can easily lead to a wrong diagnosis without other current supporting diagnostic methods being used(6-8).

In the new World Health Organisation (WHO) classification of haematological malignancies, immuno-histochemical (IHC) analysis is important in diagnosis and sub-classification of lymphoma, which are defined by the amalgamation of five distinct parameters, namely, cell morphology, immuno-histochemistry (IHC), clinical features, molecular data and cytogenetics. This classification came into being because it was evident that a purely morphological approach to classification of lymphoma was inadequate and similar histological appearances can be shared by many different biological entities(1,2,4,8,9).

Currently lymphoma diagnosis and categorisation is based on several methods including immunohistochemistry, cytogenetics, molecular genetics, tissue micro-arrays, gene re-arrangement analysis and DNA ploidy studies by gene expression profiling. An accurate and reproducible diagnosis by pathologists is important in management of patients with lymphoma. Most of the treatment regiments are specific to a certain sub-classification of lymphoma and the diagnoses made should have clinical relevance providing information that is pertinent to the treatment and prognosis(1-3,10-13).

The common current IHC panel of antibodies used routinely in diagnosis and differentiation of lymphoid from other malignant lesion includes: lymphocyte common antigen (LCA CD45), cytokeratins, vimentin, epithelial membrane antigen (EMA), S-100/HMB-45, B-cell, T-cell, kappa, lambda light and heavy chains(14). IHC is one of the current diagnostic techniques that can help define disease entities but should not be used in isolation from morphology, cytogenetics or clinical presentation. The advantages of this technique includes remarkable sensitivity, specificity, applicability to routinely processed parameters even if stored for a long time, and the feasibility it offers of an accurate correlation with traditional morphologic parameters. It is compatible with most of the fixatives currently in use and is feasible even in decalcified materials in previously stained microscopic preparations and to electron microscopy. However like any other technique, it presents potential pitfalls that need to be acknowledged by the pathologist interpreting the reaction, in order to prevent the technique being misleading rather than helpful. These tests are also limited by the fact that they are slightly more expensive than the routine haematoxylin and eosin stain and this may limit their use in resource-poor countries; but comparing with their advantages in terms of accuracy and characterisation of lymphomas the tests are cost-effective. IHC can also be useful in making diagnosis in unusual lymphoma sites such as testis, ovary, urinary tract, central nervous system, breast, bone, thyroid gland, spleen, and nasal cavity(4, 9,12,13,15).

There are several specific monoclonal antibodies that are used in lymphoma classification and each cluster differentiation (CD) shows a different pattern of positivity in non-Hodgkin's lymphoma as follows: Pan T cell: CD 2, CD 3, CD 4, CD 7, CD 8, Pan B cell: CD 20, CD 23, CD 32, and CD 79a, Null Natural Killer cell lymphoma : CD 56, Burkitts' lymphoma: Ki-67, Histiocytic lymphoma monocytic myeloid series): CD 68 lysozymes, Neoplastic germinal centres: Bcl-2, Lymphoblastic lymphoma: TdT (Terminal Deoxynucleotidyl Transferase).

In Hodgkin's lymphoma the cluster differentiation (CD) positivity pattern is different depending on the variant of Reed Sternberg cells. They are best characterised as classical Reed Sternberg cells -(cells

with a bi-lobed nucleus, one lobe resembling the mirror–image of the other): CD15, CD30, Pop corn cells -(cells with irregular outline with infoldings): CD20, CD45, mummified cells; (cells with shrunken nuclei corresponding to apoptotic tumour cells), lacunae cells; (cells with clear halo around the nuclei and large in size), undifferentiated giant multinucleated cells; (cells with multiple nuclei and large in size) (3,9,18).

Electron microscopy which has been used to characterise lymphomas can be used for specific lymphoid diseases such as Langerhans cell histiocytosis and various tumours although its role on lymphoma diagnosis has been overtaken by other cheap and readily available methods such as IHC and genetic molecular techniques.

Flow cytometry has many important research and clinical applications. It is used to examine DNA ploidy from fluids or material from fine needle aspirations or from tissue sections and has shown a good correlation with microscopic grades of malignant lymphoma. The technique consists of the measurement of various parameters while a suspension of cells flows through a beam of light past stationary detectors. Cellular features that can be evaluated with flow cytometry include cell size, cytoplasmic granularity cell viability, cell cycle time (S-phase fraction DNA content (DNA ploidy) surface maker phenotype and enzyme content. This method of analysis has become a routine procedure in leukaemia and lymphoma in many institutions, although the prognostic information above and beyond that obtainable from conventional morphology and immunohistochemistry remains controversial(19).

Proliferative indices are also important in lymphoma diagnosis such as Cyclin Dependent Kinases (CDKs), p53, bcl-2, bcl-1, bcl-6, p27, p16 $^{INK\ 4a}$ c-myc, and cell proliferative maker Ki-67 (MIB-1) are useful prognostic indicators and provide information independent of other histological and clinical variables(13,16,17). The detection of specific translocations by using cytogenetics and molecular genetics involving the c-myc, bcl-2, or cyclic D1 by molecular analysis is required in making a definite diagnosis of Burkitt's like follicular and Mantle cell lymphoma respectively(16,17). The molecular genetic methods currently available to study lymphoid diseases have evolved in a world of their own, which is quite remote from the modest aims of this editorial.

Gene re-arrangement analysis can be detected by southern blot or polymerase chain reaction (PCR). Southern blot hybridisation procedure is used to assess the size of re-arranged fragments using radio-labelled DNA hybridisation probe specific for DNA sequences in or around the constant region of immunoglobulin of lymphocytes. This procedure results in an autoradiogram in which a re-arranged fragment can be identified as a dark band. Gene re-arrangement technique is applied in diagnosis of lymphoid neoplasms for differential diagnosis between benign and malignant lesions, as a marker for B or T cell derivation, and as a marker for the presence of multiple lymphocytic clones in a single patient. The technique can be adapted to fine needle aspiration material and to formalin fixed, paraffin embedded material.

Tissue micro-array (TMA) is a method of creating a single paraffin block containing hundreds of well ordered tissue samples each roughly a diameter of a propelling pencil lead. This method has proven particularly powerful for correlating gene and protein expression pattern in intact tissues with clinical outcomes in large patient populations though more prosaic application including inter-laboratory quality assurance and intra-laboratory control standards has made TMAs common in research and routine anatomical pathology practice. The high density of tissue samples as a single TMA slide permits high volume, well controlled parallel histological assays to be performed with minimum of reagent and effort. The entire range of routine tissue based analysis including IHC, mRNA *in situ* hybridisation (ISH) and chromosomal fluorescence *in situ* hybridisation (FISH) have been done successfully on TMAs. Despite the small size of the sampled tissue in TMA cores, with adequate core replications (often less than four) TMA data usually confirm clinical-pathological correlations both in protein expression, IHC and FISH analysis of gene application obtained with larger normal tissue analysis(7,18,20,21).

To my knowledge, it has been rather difficult to do IHC leave alone other current lymphoma diagnostic methods due to limited resources in the developing countries. This compromises the recommended WHO classification for lymphomas and the progress in the understanding and treatment. The use of other methods is far much more costly and beyond the reach of most of developing countries. In Kenya, like most developing countries, the sole methods of diagnosis are usually using the morphology only, using the Haematoxylin and Eosin stains but no special stains or immunologic data.

In conclusion, immunohistochemistry is important for lymphoma diagnosis, treatment and follow up, though current lymphoma diagnosis is in its infancy in Kenya and most of African countries. By not using the current advanced diagnostic techniques, proper therapeutic approach will be based on limited knowledge of the kinetic and molecular characteristics of individual lymphoma and the value of treatment will be much affected. Current trends in developed countries show that there are plans in the near future for genetic treatment being devised by introducing DNA fragment into the genome of neoplastic cells so that apoptosis can be induced and functions that have been lost could be re-introduced in the transformed cells. In order to keep up march with these current advances and rapidly changing lymphoma diagnosis and categorisation, there is need to develop current diagnostic parameters within

the developing countries and commit resources in few viable institutes to initiate these important techniques for better understanding, management, and follow up of patients to improve survival and maintain standard of care of our patients.

ACKNOWLEDGEMENTS

To the Director, KEMRI for permission for this editorial to be published. All my colleagues for constructive criticism and encouragement.

G. Z. Mutuma, MBChB, MMed (Path), Dip Forens Med, DMJ (Path), Principal Research Officer and Head, Pathology and Oncology Research Unit, Centre for Clinical Research - Kenya Medical Research Institute, P.O. Box, 20778-00200, Nairobi, Kenya.

REFERENCES

1. Chan, J.K.C., Banks, P.M, Cleary, M.L., Delson, G., Wolf-Peeters, C.D.E., and Gatter, K.C. A proposal for classification of lymphoid neoplasm by the International Lymphoma Study Group. *Histopathology.* 1994; **25:**517-536
2. Harris, N.L., Jaffe, E.S., and Stein, N. H. A Revised European – American classification of lymphoid neoplasms: A proposal from the International Group. *Blood.* 1994; **84:**1361-1392,
3. Diebold, J., Jungman, P., Molina, T. and Audouin, J. Recent Advances in Hodgkin's disease: an overview and review of the literature current diagnostic pathology. *Histopathology.* 1995; **2:**153-165
4. Lennert, K. The proposal for a new revised European American Lymphoma Classification- a new start of a trans-Atlantic discussion. *Histopathology.* 1995; **26:**481-483.
5. O'Connor, N. New classification of Lymphomas. *Lancet.* 1995; **345:**1521-1522.
6. Leoncini, L., Lazzi, S. Bellan, C. and Tosi, P. Cell kinetics and cell cycle regulation in lymphomas. *J. Clin. Path.* 2002; **55:**645-655.
7. Classification and diagnosis of cutaneous lymphoproliferative diseases. *Recent advances in Histopathology;* No 20. By David Lowe and James Underwood. T*he Royal Society of Medicine Press Limited.* 2004.
8. The diagnostic Challenge of paediatric small round cell tumours. *Progress in Pathology;* Vol 6. Edited by Nigel Kirkham and Neil Shepherd. *Greenwich Medical Media Limited.* 2003.
9. Mutuma, G.Z., Musibi, A. and Shiramba, T. The role of immunohistochemistry in lymphoma diagnosis. *Health Line.* March. 2004.
10. Peleri, S.A., Dimhofer, S., Ascani, S., *et al* Present controversies and possible tools for its sub-classification. *Histopathology.* 2002; **41:**462-502.
11. Meas, B. and De Wolf-Peeters. Marginal zone cell lymphoma - an update on recent advances. *Histopathology.* 2002; **40:**117-123.
12. Cool, C.D. and Bitter. M. The malignant Lymphomas in Kenya. Morphology, immunophenotype and frequency of Epstein –Barr Virus in 73 cases. *Human Pathology.* 1997; **28:**1026-1032.
13. Leonicini, L., Lazzi, Bellan, C. and Tossi P. Cell kinetics and cell cycle regulation in lymphomas. *J. Clin. Path.* 2002; **55:**648-655.
14. Lee, F.D. Unusual sites and types of lymphomas. *Recent Advances in Histopathology.* No **16:**73-93.
15. Oudejans, J.J. and Van der Valk, Immunohistochemical classification of B cell neoplasms. *J. Clin. Path.* 2003; **56:**193-194.
16. Bellen, C, Lazzi S, De Falco G Nyongo A, Giordano A, and Leoncini L. Burkitt's lymphoma: New insights into molecular Pathogenesis. *J. Clin. Path.* 2003; **56:**188-192.
17. Harris, L.N., Jaffer, E.S., Diebold, J., Flandrin, G., Muller-Hermelink, H.K., Vardiman, J., Lister, T.A., and Bloomfield, C.D. The world health organization classification of neoplastic diseases of the haematopoietic and lymphoid tissues. Report of the clinical Advisory Committee meeting Airline House, Virginia, 1997. *Histopathology.* 2000; **36:**69-87.
18. Pileri, S.A., Ascani, S.A., Leoncine, L., Sabattini, E., Zinzani, P.L., Piccaluga, P.P., Peleri, A Jr, Giunti, M., Falini, B., Bolis, G. B., and Stein, H. Hodgkin's lymphoma: the pathologist viewpoint. *J. Clin. Path.* 2002; **55:**162-176.
19. Recent advances in Histopathology No 18. Clinical Application of Flow cytometry. Edited by David Lowe and James Underwood. Church *Hill Living stone.* 1999.
20. Margret A shipp. Gene expression profiling in diffuse large B cell lymphoma: new insights into molecular heterogeneity and rational treatment targets. M*od. Pathol.* 2003; **16:**183-184.
21. Camp, R.L., Chung, G.G., De Marzo, A.M., and Rubin, D. L. Automated sub-cellular localization and quantification of protein expression in tissue microarrays. *Nat. Med.* 2002; **8:**1323-1327.