Editorial

Malignant Lymphoma – A Changing Spectrum

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As 15 September 2009 was World Lymphoma Awareness Day, it is perhaps timely to review the subject of malignant lymphoma. There is an increasing incidence of malignant lymphomas worldwide. Correspondingly our nationwide Singapore Cancer Registry demonstrates a substantial increase in the incidence of malignant lymphomas.[1] The male and female age-standardised incidence rates (ASR) rose from 3.1 to 8.2 per 100,000 and 1.9 to 8.0 per 100,000 respectively between two 5-year periods 1968 to 1972 and 1998 to 2002. The annual rate of increase in ASR is the second highest after prostate cancer in males and second highest after breast cancer in females. However, there were only 771 males and 551 females who were diagnosed with malignant lymphoma during the period 1998 to 2002 ranking as 8th and 10th commonest cancers in males and females respectively. These figures excluded plasma cell myelomas and lymphoid leukaemias which at the time of compilation were regarded separately.

Malignant lymphoid neoplasms are traditionally categorised into 2 groups – Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Broad categorisation, however, is unsatisfactory for determining therapeutic approaches and there are now well developed chemotherapeutic and targeted immunotherapy regimens besides bone marrow or stem cell transplantation. The knowledge of disease progression, clinical outcomes and expected survival are important for physicians managing malignant lymphomas. Major advances in molecular genetics have enhanced the understanding of the pathobiology of the disease process which provide the framework for the improved classification of lymphomas. The classification of lymphomas has remained as one of the most controversial areas of tumour pathology. Most of the difficulties have been a direct consequence of the inherent biological diversity in lymphoid neoplasms; a reflection of the physiological complexity of lymphocyte development and differentiation. In the early 1980s, there were no less than 6 different lymphoma classifications. This diversity has caused confusion with regard to clinicopathological integration and interpretation of results of international trials. After several international consensus meetings among pathologists and clinicians, a classification has evolved with greater reproducibility and better correlation with clinical relevance. The World Health Organization (WHO) Classification of Tumours of the Haematopoietic and Lymphoid Tissues (2001)[2] has been revised to the 2008 WHO Classification based on new and accumulated experimental data.[3] With worldwide acceptance, it is already recognised as the gold standard in lymphoma diagnosis. It identifies individual clinicopathological entities, integrating morphology, immunophenotype, genetics, normal cell counterpart/cell of origin and clinical features and particularly molecular genetic studies.

The NHL comprises almost 85% of reported malignant lymphomas and is a heterogenous group of diseases. They are divided into 2 major groups of clonal tumours of mature and immature B lymphoid cells, and T lymphoid cells or natural killer (NK) cells at various stages of differentiation.[4] B cells and T cells are part of the adaptive immune system which have the capability for immunological defence mediated through specific receptors as the immunoglobulin (Ig) and T-cell receptor complex (TCR) respectively. Conversely, NK cells which are part of the innate immune system together with NK-like T cells and gamma-delta cells participate in mucosal and cutaneous immunity without the need to encounter antigen. Based on this, lymphomas of NK and NK-like T cells occur not infrequently in extranodal sites. The malignant lymphomas of T and B cells as well as histiocytic and dendritic cells have been considered as "non-Hodgkin lymphomas".

Hodgkin lymphoma a.k.a. Hodgkin's Disease is one of the earliest malignant lymphomas described with the recognition of the Hodgkin and Reed-Sternberg (HRS) cell, a large mononucleated or multinucleate tumour cell, as being the neoplastic cell in a background of reactive lymphocytes. Molecular techniques with the extraction of single HRS cells from tissue sections by laser-capture single cell dissection[5] have led to the detection of monoclonal Ig gene rearrangements in almost 95% of HL cases. Together with stable p53 gene mutational status the neoplastic and B-cell origin of HL has been clarified. Five per cent of HL are of a type, nodular lymphocyte predominant HL, which is a monoclonal B-cell neoplasm with histological and immunohistochemical findings strongly suggesting a relationship to follicular lymphoma.[6] The number of HL in the Singapore Cancer Registry Report[1] is small, 122 cases during the 5-year period of 1998 to 2002 compared to 1170 cases of NHL. Although HL is regarded as a tumour distinct from NHL, it is recognised that any type of NHL can occur simultaneously with HL.[7,8] Patients with HL have an increased risk of developing NHL and some cases of NHL such as B-cell chronic lymphocytic leukaemia and follicular lymphoma have been observed to progress to HL. With the recognition that classical HL is of B-cell lineage, this overlap between HL and many forms of B-cell malignancy is

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now appreciated. The term "gray zone lymphomas" has been applied to two B lineage lymphomas with features that overlap morphologically and immunophenotypically.

The 2008 WHO Classification has introduced 2 new categories of high-grade B-cell lymphomas: entities in which features of diffuse large B-cell lymphoma (DLBCL) overlap with Burkitt lymphoma (BL) or with classical Hodgkin lymphoma (CHL). The DLBCL/HL category encompasses lymphomas that exhibit the morphology of classical HL but immunophenotype of DLBCL or vice versa, leading to the term "B-cell lymphoma unclassifiable with features intermediate between DLBCL and classical Hodgkin lymphoma". An example is that of primary mediastinal large B-cell lymphoma (PMBL) and classical HL as HL can occur in the mediastinum and with the latest molecular studies they share similar molecular signature features.[9-11] These gray zone lymphomas generally have a more aggressive clinical course and poorer outcome than classical HL or PMBL and there is no consensus on the optimum treatment although some groups recommend treating these cases as aggressive B-cell lymphomas. The DLBCL/BL category encompasses cases that resemble BL morphologically but have one or more immunophenotypic or molecular genetic deviations that would exclude it from the BL category: conversely some cases have immunophenotypic and/or genetic features of BL but display cytologic variability, unacceptable for BL.[3] The designation of these 2 new "gray zone" case groups as formal diagnostic entities has taken some pressure off pathologists who previously felt compelled to classify the unclassifiable. The diagnostic specifications of BL, DLBCL, and CHL are well defined so that only truly intermediate cases are included within the new entities. We should not consider them as representing single diseases. Further study in due time will allow assignment to discrete groups with predictable clinical behaviour and optimal therapy.

The first-line approach to the diagnosis of malignant lymphomas is morphological assessment with immunophenotyping. Molecular studies are helpful research tools but in routine practice they are applied only when morphologic and immunohistochemical studies yield inconclusive findings. DNA analysis can provide information on the clonality of the lymphoid population and nucleic acid amplification testing also known as the polymerase chain reaction (PCR) can help in distinguishing lymphoma from reactive lymphoid hyperplasia and define the lineage of the lymphoma.[12] Practically all B-cell lymphomas show rearrangements of the immunoglobulin (Ig) heavy chain and light chain genes, whereas T-cell lymphomas show rearrangements of the T-cell receptor (TCR) beta and gamma chain gene. NK cell lymphomas and histiocytic neoplasms lack both Ig and TCR gene rearrangements. These gene rearrangements are sensitive and specific indicators of the lineage of the lymphoma and neoplastic clones and can be detected in small fractions of the tissue. However, there can be a significant false negative result due to the failure of the "universal" primer to hybridise the rearranged Ig or TCR gene due to partial rearrangement or somatic hypermutation. Recent developments with BIOMED-2 multiple PCR strategy are shown to reduce this.[13,14] While lineage is the defining feature of most lymphoid malignancies, in recent years there has been a greater appreciation of lineage plasticity within the haemopoietic system.[15] Lineage switch or demonstration of multiple lineages is however less frequent in mature lymphomas than immature haematopoietic neoplasms, in particular acute leukaemias. The detection of genetic features continue to play an important role in the defining of lymphomas and fluorescence-in-situ hybridisation (FISH) and comparative genomic hybridisation techniques are used for detecting specific chromosomal translocation e.g. t(14:18) in follicular lymphoma and t(8:14) in BL.

The commonest lymphomas worldwide are the mature B-cell lymphomas of which DLBCL and follicular lymphomas (FL) account for more than 60% of all lymphomas.[16] In a review of the pathology reports and standardisation according to the WHO Classification, there were 1410 cases of NHL reported to the Singapore Cancer Registry during 1998 to 2002 of which 78% or 1095 cases were mature B-cell lymphomas and 17% were mature T-cell and NK-cell lymphomas.[17] About half of the B-cell lymphomas (507 cases) were DLBCL. The mature (post-thymic) T-cell and NK-cell lymphomas collectively referred to as peripheral T-cell lymphomas (PTCL) account for less than 15% of all NHL worldwide. There is an overall higher frequency of PTCL in Asia and Central/South America than in Western Countries,[18] especially of those induced by Epstein-Barr Virus (EBV) and Human T-lymphotropic Virus-1 (HTLV-1), the latter particularly in Japan. The entity Nasal NK/T-cell lymphoma with its association with EBV is not uncommon in Singapore and extranasal sites have been reported but adult T-cell lymphoma leukaemia due to HTLV-1 infection is a rarity.[19,20] Irrespective of their pathobiological heterogeneity, PTCL are, with few exceptions, aggressive diseases with poor outcome with standard chemotherapy.[21]

With the sequencing of the human genome, we enter the period with hope that personalised genomic medicine may contribute to better survival outcome of malignant lymphomas. Through gene expression profiling, Alizadeh et al in 2000 have identified 2 major groups of DLBCL. One termed germinal centre B-cell like (GCB) with the gene expression profile of germinal centre B cell, accounted for 45% to 50% of cases studied.[22] The other termed activated B-cell like (ABC) has the profile of activated peripheral B cells. The third molecular subtype, primary mediastinal DLBCL (PMBCL), was subsequently identified and shares similarities with classical HL.[11] The genetic and molecular differences between these subtypes of DLBCL strongly suggest that they correspond to different clinical entities. Rosenwald et al[23] found that patients with these tumours differ in clinical outcome with 5-year survival rates of 59%, 39% and 64% in GCB, ABC and PMBCL patients respectively. The peculiar clinical phenomenon of late relapses seems to occur mainly in lymphomas with GCB profile.[24] Prognostic models based on pre-treatment characteristics such as the International Prognostic Index are currently used to predict outcome in DLBCL. Can we translate the results of gene expression profiling of DLBCL to prognosticate results in

clinical practice? Unfortunately the correlation between gene expression subtypes of DLBCL with those defined as GC and non-GC large B-cell lymphoma by morphology and immunohistochemistry is variable.[25] Combination chemotherapy with R-CHOP has significantly improved the survival of patients with DLBCL. Whether gene expression signature correlates with survival after treatment of DLBCL has been the subject of further study. The Lymphoma/Leukaemia Molecular Profiling Project embodying major research institutes created a multivariate model from gene expression signatures termed B-cell "stromal-1" and "stromal-2" which predicted survival both in patients receiving CHOP and R-CHOP chemotherapy.[26] The prognostically favourable signature stromal-1 reflected extracellular matrix deposition and histiocyte infiltration. By contrast, the prognostically unfavourable stromal-2 signature reflected tumour blood-vessel density. The conclusion is that survival after treatment of DLBCL is influenced by differences in immune cells, fibrosis and angiogenesis in the microenvironment of tumour. Targeted therapy with angiogenesis inhibitor may form a basis for clinical trials in DLBCL and combined treatment that targets oncogenic mechanism as well as tumour microenvironment may prove synergistic.

Gene expression profiling has now been applied to several small series of peripheral T-cell lymphomas.[27] The largest series to date has been performed on 151 lymphomas as part of the International PTCL Project[21] where 1314 cases of PTCL and NKTCL were studied with a participating consortium of 22 institutions which included Singapore General Hospital. Preliminary results have shown that there are distinctive molecular classifiers for subgroups as angioimmunoblastic T-cell lymphomas, ALK positive anaplastic large cell lymphoma and adult T-cell leukaemia/lymphomas. The peripheral T-cell lymphoma – unspecified was molecularly heterogenous although there is a molecular subgroup with worse survival.[28] If these gene classifiers reflect the biology of tumour cells as well as their microenvironment, we can look forward to the more precise identification of oncogenic pathways and tumour-host interactions to lead to better therapies and outcomes in future.

REFERENCES

1. Seow A, Koh WP, Chia KS, Shi LM, Lee HP and Shanmugaratnam K. Trends in Cancer Incidence in Singapore, 1968-2002. Singapore: Singapore Cancer Registry, 2004. Report No. 6.
2. Jaffe ES, Harris NL, Stein H and Vardiman JW, editors. WHO Classification of Tumours. Pathology and Genetics of Tumours of the Haematopoietic and Lymphoid Tissue. Lyon: IARC Press, 2001.
3. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. editors. WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues. Lyon: IARC Press, 2008.
4. Jaffe ES. Pathobiology of peripheral T-cell lymphomas. Hematol Am Soc Hematol Educ Program 2006;126:317-22.
5. Stein HS and Hummel M. Hodgkin's disease: biology and origins of Hodgkin's and Reed-Sternberg cells. Cancer Treat Res 1999;25:161-8.
6. Marafioti, Hummel M, Anagnastopoulos I, Foss HD, Falini B, Delsol G, et al. Origin of nodular lymphocyte predominant Hodgkin's disease from a clonal expansion of highly mutated germinal-centre B cells. N Engl J Med 1997;337:453-8.
7. Jaffe ES, Muller-Hermelink HK. Relationship between Hodgkin's disease and non-Hodgkin's lymphoma. In: Mauch PM, Armitage JO, Diehl V, et al, editors. Hodgkin's Disease. Philadelphia: Lippincott, Williams and Wilkins, 1999:181-93.
8. Muller-Hermelink HK, Zettle A, Pfeifer W, Ott G. Pathology of lymphoma progression. Histopathology 2001;38:285-306.
9. Savage KJ, Monti S, Kutok JL, Cattoretti G, Neuberg D, de Laval, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphomas. Blood 2003;102:3871-9.
10. Traverse-Glehen A, Pittaluga S, Gaulard P, Sorbara L, Alonso MA, Raffeld M, et al. Mediastinal gray zone lymphoma, a missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. Am J Surg Pathol 2005;29:1411-21.
11. Rosenwald A, Wright G, Leroy K, Yu X, Gaulard P, Gascoyne RD, et al. Molecular diagnosis of primary mediastinal B-cell lymphoma identifies a clinically favourable subgroup of diffuse large B-cell lymphoma related to Hodgkin lymphoma. J Exp Med 2003;198:851-62.
12. Sklar AL. The molecular diagnosis of lymphomas and related disorders. In: Canellos GP, Lister TA, Sklar AL, editors. The Lymphomas. Philadelphia: WB Saunders, 1998:129-50.
13. Segal GH. Assessment of B-cell clonality by the polymerase chain reaction: a pragmatic overview. Adv Anat Path 1996;3:195-203.
14. van Dongen JJ, Langerak AW, Bruggeman M, Evans PA, Hummel M, Lavender FL, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of BIOMED-2 concerted action. BMH4-CT98-3936. Leukaemia 2003;17:2257-317.
15. Cobaleda C, Brussinger M. Developmental plasticity of lymphocytes. Curr Opin Immunol 2008;20:139-48.
16. Anon. A clinical evaluation of the International Lymphoma Study Group Classification of non-Hodgkin's Lymphoma. The non-Hodgkin's Lymphoma Classification Project. Blood 1997;89:3909-18.
17. Sng I. Trends in cancer incidence in Singapore 1968-2002 with distribution of major histological types of non-Hodgkin Lymphoma 1998-2002. Presented at the 96th Annual Meeting of the Japanese Society of Pathology, Osaka, Japan, 14 March 2007.
18. Rudiger T, Weisenburger DD, Anderson JR, Armitage JO, Diebold J, MacLennan KA, et al. Peripheral T-cell lymphoma (excluding ALCL): results from non-Hodgkin's Lymphoma Classification Project. Ann Oncol 2002;13:140-9.
19. Ng SB, Lai KW, Murugaya KM, Lee KM, Loong LE, Chong SF, et al. Nasal-type extranodal natural killer/T-cell lymphomas: a clinico pathologic and genotypic study of 42 cases in Singapore. Mod Pathol 2004;17:1097-107.
20. Sng I, Levin A, Jaffe ES, Ng HW, Sim CS, Blattner WB. T-cell lymphoma in Singapore: Pathology, clinical findings and association with HTLV-1 antibodies. Histopathology 1992;21:101-13.
21. International Peripheral T-cell and NK/T-cell Lymphoma Study: Pathology findings and clinical outcomes. International Peripheral T-cell Lymphoma Project. J Clin Oncol 2008;26:4124-30.
22. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503-11.
23. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large B-cell lymphoma. N Engl J Med 2002;346:1937-47.
24. de Jong, Glass A, Boerrigter L, Hermus MC, Dalesio O, Willemse E, et al. Very late relapse in diffuse large B-cell lymphoma represents clonally related disease and is marked by germinal centre features. Blood 2003;102:324-7.
25. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of molecular classification of DBCL by immunohistochemistry using a tissue microarray. Blood 2004;103: 275-82.
26. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large B-cell lymphomas. N Engl J Med 2008;359:2313-23.
27. Piccaluga PP, Agnostinelli C, Califano A, Rossi M, Basso K, Zupo S, et al. Gene expression analysis of peripheral T-cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. J Clin Invest 2007;117:823-34.
28. Igbal J, Weisenburger DD, Greiner TC, Vose JM, McKeithan T, Kucuk C, et al. Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. Blood 2009. In press.