Unusual histological features of Non Hodgkin's lymphoma: a clinicopathological and immunohistochemical study.

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Abstract

Morphology of tumours can vary on the haematoxylin and eosin stained sections due to artifacts, pattern of infiltration and the nature of the tissue being infiltrated. Immunohistochemistry plays a vital role in arriving at a correct diagnosis in such situations. Preliminary studies done by us showed that Non Hodgkin's Lymphoma (NHL) could mimic many other non lymphoid tumours, and that the routine use of immunostaining for Leukocyte Common Antigen (LCA) revealed the presence of a lymphoma in totally unexpected instances.

Forty cases of NHL, resembling non lymphomatous tumours and showing positivity for LCA were selected. Of these, 72.5% (29/40) were located extranodally, and 72% were of B-cell lineage. Large cell lymphoma of different sites were mimicked by carcinoma, gastrointestinal stromal sarcoma and thymoma. CD30 (Ki-1) positive lymphomas resembled melanomas. The other tumours mimicked were neuroendocrine, clear cell and germ cell tumours and squamous carcinomas. Awareness of unusual histological features especially of extranodal NHL would facilitate their correct diagnosis.

Introduction

Morphology of tumours can vary on the haematoxylin and eosin stained sections due to many reasons such as artifacts at various stages of preparation of a section, the pattern of infiltration and the nature of the tissue being infiltrated (1).

There is no fundamental unity of the morphological patterns of tumours in the body, and this fact can be appreciated only by being familiar with the diverse patterns encountered.

For example the single file infiltration pattern in lymphomas is also seen in lobular carcinoma of breast (2), infiltration of adipose tissue can simulate an adenocarcinoma pattern and a packeted arrangement or nesting pattern may result due to stromal fibrosis (2). In many laboratories abroad, problematic cases are thoroughly investigated with immunomarkers using a panel of antibodies (2).

Many Sri Lankan pathologists who work with limited resources and facilities do not have immunohistochemistry.

Preliminary studies done by us at the Department of Pathology, University of Peradeniya and others showed that Non Hodgkins lymphoma mimics many other non lymphoid neoplasms (1, 2, 3, 4, 5, 6). Routine use of LCA, a marker for lymphoid cell lineage, revealed the presence of a lymphoma in totally unexpected instances, especially when extranodal.

Therefore it would be useful to Sri Lankan pathologists to be aware of the unusual histological features of Non Hodgkin's lymphoma.

The objectives of this study was to place on record the unusual histological features of Non Hodgkin's lymphoma on haematoxylin and eosin stained sections and to document the clinicopathological profile, B and T cell lineage and pattern of mimicry in relation to the location.

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Materials and Methods

All the material were those referred to the Department of Pathology, Faculty of Medicine, University of Peradeniya.

The tumours requiring immunohistochemical demonstration of leukocyte common antigen (LCA) for confirmation of a lymphoma were selected for the study. This material had been fixed in 10% formal saline in the routine manner by the referring hospital. The fixation time and the concentration of the solution were not controlled by us. A total of 40 cases were included in this study.

All material were processed in an automated tissue processor, and were embedded in paraffin. All tumours with no apparent line of differentiation on the Erlich's haematoxylin and eosin stain were stained with a panel of antibodies and immunomarkers to detect cytokeratin (CK), epithelial membrane antigen (EMA), leucocyte common antigen (LCA) and neuron specific enolase (NSE). A 3 stage Strept avidin biotin complex (DAKO) system was used. Endogenous peroxidase activity was blocked using H₂O₂ methanol for 30 minutes. Antigen retrieval was by using a microwave oven at low heat for 10 minutes. The above monoclonal antibodies supplied by Dako was diluted to 1/50 and was applied for 1 hour. The secondary antibody applied was biotinylated rabbit antimouse immunoglobulin in 1/300 dilution.

Those tumours emerging as lymphoma on positive LCA stain (conventional CD45, clone 2B11+PD7/26) were selected for the study. When the lymphomatous nature of the tumour was confirmed, their histological appearances and patterns seen on haematoxylin and eosin stained sections were documented. The locations of those tumours were noted in relation to the pattern of mimicry. Attempts were made to identify the lymphoid cell such as centrocyte and centroblast, but this proved difficult if not impossible in most instances. Therefore lymphomas were categorized in to large, intermediate and small cell types. A Ki-1 (CD20 +ve) category was included.

Thereafter, B and T cell lineage markers were done in representative samples of both extra nodal and nodal lymphomas along with positive and negative controls.

CD 20 (clone L 26) for B-cell, CD 45RO [UCHL1] for T-cell and CD30 were applied using 1/50 dilution for one hour in room temperature, using the same technique and the results were documented.

Results

There were 29 extranodal lymphomas and 11 nodal lymphomas. The mimics of nodal lymphomas included poorly differentiated carcinoma (6), melanoma (2), paraganglioma (2) and clear cell carcinoma (1).

The extranodal lymphomas comprised 17 large cell lymphomas, 7 small cell lymphomas and 2 intermediate cell lymphomas. There were 3 Ki-1 positive lymphomas. The tumour mimics of large cell lymphoma and the location are shown in Table 1. The histology of some are shown in Figure 1 with the corresponding LCA stain. Tumour mimics and the location of small and intermediate lymphomas are shown in Table 2.

Five of the nodal lymphomas were of B-cell lineage and one was of T-cell lineage. There were two CD 30 positive nodal lymphomas. There were 13 extranodal B-cell lymphomas and two T-cell lymphomas. The 3 extranodal CD 30 positive lymphomas were in the skin and mimicked melanoma.

Discussion

72.5% (29/40) of NHL resembling non lymphoid neoplasms were located extra nodally. The majority (72%), of them were of B-cell lineage. 11.1 % were of T-cell lineage. 16.7% were Ki-1 positive. Similar patterns of cell lineage were seen in the nodal lymphomas resembling other tumours. The report of a multicentre trial published by the Non Hodgkin's lymphoma classification project also shows a similar pattern of cell lineage, globally (7).

Table 1. Tumour mimics of large cell lymphoma and location

Pattern	Location	No
Poorly differentiated carcinoma-like pattern (figure 1)	tarsal gland, cerebellum, submandibular gland, abdominal lump, skin abscess, ethmoidal sinus, thigh, foot, liver	09
Clear cell carcinoma-like pattern (figure 2)	nose	01
Gastrointestinal stromal tumour-like pattern (figure 3)	small intestine	02
Thymoma-like pattern	mediastinum	01
Adenocarcinoma-like pattern	abdominal wall	01
Rabdoid tumour-like pattern (figure 4)	retroperitoneum	01
Haemangiopericytoma-like pattern	ankle joint	01
Germ cell tumour-like pattern (figure 5)	retroperitoneum	01

Table 2. Tumour mimics and the location of small and intermediate cell lymphomas

Type	Site	Pattern	Number
Small cell lymphoma	cervix, cerebellum, nose, ovary, heart, Parietal lobe of brain, deltoid muscle	Paraganglioma/Neuroendocrine	07
Intermediate cell Lymphoma	tonsilar lump, neck lump	Squamous carcinoma	02

The diffuse large cell lymphomas were problematic in 60% of extra nodal and 50% of nodal lymphomas. Diffuse large cell lymphoma (DLBCL) is the commonest type of lymphoid tumour worldwide (8). This category displays a significant variability in terms of cell morphology and clinical findings (8). Many cases of diffuse large cell NHL resembled a poorly differentiated

carcinoma pattern (9/17), characterized by large pleomorphic cells with prominent nucleoli and frequent mitoses. (Figure 1) Hence these cases would have been easily misdiagnosed as non lymphoid tumours if the immunomarkers, specially the leukocyte common antigen was not done.

Figure 1. Poorly differentiated carcinoma pattern

Figure 4. Rhabdoid tumour pattern

Figure 2. Clear cell carcinoma pattern

Figure 5. Germ cell tumour pattern

Figure 3. Gastrointestinal stromal tumour pattern

Figure 6. Melanoma pattern

Clearly the location of the extra nodal tumour influences the pathologist regarding the differential diagnosis, such as gastrointestinal stromal tumour (GIST) from intestinal lymphoma (Figure 3), haemangiopericytoma for a NHL arising in the ankle joint, melanoma for Ki-1 lymphoma (Figure 6) and extra renal rhabdoid tumour for retroperitoneally located large cell lymphoma (Figure 4).

Infiltration of tumour cells in to the adipose tissue gives a glandular pattern mimicking an adenocarcinoma. The large neoplastic lymphoid cells were arranged around the adipocytes creating a false glandular lumen which was clearly evident in an abdominal wall lymphoma.

A variable percentage of diffuse large cell lymphomas evoke a fibrotic reaction. This may become prominent in the retroperitoneum and in the mediastinum (8). This feature along with connective tissue reactions such as stromal fibrosis give a packeted appearance resembling a germ cell pattern (Figure 5) or a neuroendocrine pattern.

Fixation artefacts possibly contributed to the clear cell (Figure 2) and squamous carcinoma patterns of large cell and intermediate cell lymphoma respectively. In most cases it appeared to be difficult to classify the lymphoma in great detail even when the diagnosis were known. Hence classification into large, intermediate and small cell type had to be made. This again corroborates the fact that fixation artefacts and connective tissue reactions contribute to the distortion of the lymphomatous nature of the tumour, hence to the difficulties in diagnosis.

According to the presentation modalities, a high degree of suspicion is required for a correct diagnosis of Non Hodgkin's lymphoma with unusual histology, especially when dealing with extranodal tumours. The use of immunohistochemical stains such as leucocyte common antigen is mandatory in such a situation. In the last few years Non Hodgkin's lymphoma has aroused a great deal of research interests.

Awareness of prevalence of unusual histological features of nodal and extranodal NHL would indeed facilitate a correct diagnosis of Non Hodgkin's lymphoma.

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References

1. Waduge R.N, Ratnatunga NVI, Ramadasa S. Non Hodgkin's lymphoma - unusual histological features. Proceedings of the Annual Academic Sessions of the SLMA 2002 OP-39, pg 44.
2. Rosai J. ed; Ackerman's Surgical Pathology. Mosby; (USA); 1996
3. Perrone T; Frizzera G; Anaplastic large cell lymphoma; A distinct molecular pathologic entity, *American Journal of Surgical Pathology*; 1997; **21**: 1420-1432
4. Nozawa Y; Wang J; Weiss LM; et al; Extranodal lymphoma with spindle cell variant. *The Journal of Histopathology* 2001; **39**: 493-497
5. Lowe DG, Underwood JCE, eds; Recent advances in histopathology. 16; Edinburgh. Churchill Livingstone. 2001; 73- 487
6. Harris NL, Jaffe NS, Diebold J, Muller Hermerlink H.K. Classification of neoplastic diseases of the haemopoietic and lymphoid tissue; The World Health Organization report of the clinical advisory committee meeting; 1997
7. Pilleri SA, Dernhofer S, Went PH, 'Diffuse large cell lymphoma; one or more entities. *The Journal of Histopathology*; 2002; **41**: 482-509