DIFFERENTIATING PARAMETERS BETWEEN HODGKIN'S DISEASE AND ANAPLASTIC LARGE CELL LYMPHOMA

By
Mohamed Fawzy M

From
Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

ABSTRACT
The differentiation between anaplastic large cell lymphoma and Hodgkin's disease sometimes represents a problem. Both diseases are dissimilar in prognosis and management therefore the aim of this work was to study the clinicopathological and immunohistochemical features that can differentiate between both diseases.

This work was carried out on 50 cases (30 HD and 20 ALCL), selected from Mansoura University Hospitals and from private cases in the period from 1995-2000. Excision biopsies were done and studied by routine histopathological techniques as well as by immunohistochemical stains including CD45RB, CD30, CD45RO, CD20, CD15 and EMA.

In this study we noticed that ALCL showed predilection for older age group, while younger age group was affected by HD. Males were more affected by ALCL while females were more affected by HD. Nodal involvement took the upper hand in both HD and ALCL vs extranodal involvement.

It was found that the accurate diagnostic criteria regarding pattern of growth, number and shape of blast cells and Reed Sternberg cells and detailed cellular morphology were the most differentiating features in 90% of cases. The most valuable immunohistochemical markers were CD30, CD15 and EMA that could differentiate between the problematic cases.

INTRODUCTION
Malignant lymphomas constitute...
one of the most diagnostic difficulties for pathologists as it has many variable differential diagnoses among both benign and malignant lesions. In this respect, anaplastic large cell lymphoma (ALCL) and some types of Hodgkin's diseases (HD) constitute a special diagnostic problem (1).

Anaplastic large cell lymphoma represents a generally recognized group of large cell lymphomas characterized by proliferation of predominantly large lymphoid cells with strong expression of cytokine receptor CD30 (2). It represents 2-8% of all lymphomas (3).

Morphologically, ALCL consists of large blast cells having pleomorphic and bizarre shaped nuclei and sometimes multiple nuclei. Some of these cells have Reed Sternberg like nuclei reminiscent to typical Reed Sternberg cells of Hodgkin's disease. Variable admixture of granulocytes and macrophages are present among tumor cells (4,5).

The morphological diagnosis of ALCL may face some overlap with HD especially syncytial variant of nodular sclerosing and lymphocytic depletion subtypes that contain atypical and pleomorphic Reed Sternberg cells similar to blast cells of ALCL (6,7). Even with the advanced application of immunohistochemistry there were persistent expression of CD 30 in both Hodgkin's RS cells and blast cells of ALCL, a fact which not only failed to detect differentiation between both cell types, but also underlines some overlap in determination of precise cell of origin and may suggest a common pathogenetic mechanism (6,8,9,10). This overlap leads to confusion in the variable terms used to name ALCL in the classification of lymphomas such as the inaccurate term(ALCL-Hodgkin's like) in REAL classification which was revised latter and deleted (11,12).

Perfect diagnosis is highly valuable because prognosis as well as clinical management are completely dissimilar in both diseases.

Therefore the aim of the present work was to study the clinical, morphological and possible immunohistochemical features of Hodgkin's disease and ALCL, expressing focus on the most significant immunohistochemical features for the easier differential diagnosis between both diseases.
PATIENTS AND METHODS
This study was carried out on 50 patients (i-HD 30 and ALCL 20) admitted at Mansoura university hospitals (forty cases), and private cases (ten cases) for whom excision biopsy was done in the period from 1995 to 2000.

Clinical data were collected including age, sex, anatomical site (nodal vs extranodal), and distribution (organs affected).

Paraffin blocks of formalin-fixed biopsies were sectioned at 4-5 micron and examined using:

(1) Hematoxylin and Eosin staining for assessment of morphologic criteria only including: architecture (partial or complete loss), pattern of growth (sinusoidal, paracortical, nodular), size of blast cells (in relation to RBCs and endothelial cells), shape and number of blast cells and RS cells per high power field.

(2) Immunohistochemistry: using panel of monoclonal antibodies including CD45RB (leukocyte common antigen), CD20 (a pan B cell marker), CD45RO (a pan T cell marker), epithelial membrane antigen (EMA), CD15(Leu M-1) and Ki-1 (CD30) with full comment on pattern and distribution of the staining in both blast cells and Reed Sternberg cells (13,14,15).

Positive and negative controls were used for each marker.

RESULTS
Clinical data of patients diagnosed as HD versus those diagnosed as ALCL:

• Age distribution. Table (1-A)
This table illustrates that there are marked predominance of Hodgkin’s disease among younger age group, mostly in the third decade of life. On the other hand, ALCL shows preponderance of older age group, mostly in the fifth and sixth decades of life.

• Sex distribution. Table (1-B)
There is prevalence of Hodgkin’s disease more among females with male to female ratio 1:1.5

ALCL shows male predominance with male to female ratio 1.5:1

• Different anatomic sites involved by both HD and ALCL. Table (2)
In both diseases nodal distribution is by far the most common ana-
tomical site. In Hodgkin's disease, no extranodal involvement occurred among all 30 cases, while in ALCL there were two cases out of 20 cases, were diagnosed in gastric biopsies.

Details of morphologic analysis of both diseases

(1) Pattern of growth: Table (3)

ALCL cells usually grow in cohesive pattern (mimicking carcinoma) and preferentially run in sinusoidal distribution usually with partial effacement of the nodal architecture, and extranodally usually infiltrates the tissue in a pattern similar to that of carcinoma. In Hodgkin's disease the tumor tissue not uncommonly leads to complete or interfollicular effacement, with no cohesive pattern and sinusoidal distribution.

(2) Blast cells and RS cells:

The proportion of blast cells to the rest of tumor cells was > 20% in ALCL and <10% in HD. Mitotic figures and apoptosis were typically more prominent in ALCL than in HD which may indicate a higher proliferation in ALCL rather than HD.

The typical wreath like nuclear arrangement of nuclei in giant cells and horse shoe nuclei are characteristic for ALCL (Fig 1), that are absent in Hodgkin's disease.

(3) Reactive cells:

The number of lymphocytes and prolymphocytes (slightly larger than small lymphocytes with prominent central nucleolus) was smaller in ALCL than in HD. Admixture of eosinophils and histiocytes together with blood vessels are invariably present in both diseases that cannot be a point of differentiation.

Details of immunohistochemical analysis

Panel of antibodies that recognize antigens that survive fixation and conventional processing have been applied to 30 cases of Hodgkin's disease and 20 cases of anaplastic large cell lymphoma.

(1) Immunoreactivity in both diseases for antibody panel: Table (4)

Reed-Sternberg cells in HD were positive for CD30 in all cases, positive with CD15 in 90%, positive with CD20 in 30%, positive for CD45RO in 30% and positive for CD45RB (LCA) in
10% of cases. Reed-Sternberg cells were negative for epithelial membrane antigen.

The distribution of immunoreactivity of ALCL were similar to that in HD, except that none were positive for B-cell marker (CD20) and 40% were positive for T-cell marker (CD45RO). All cases were positive for CD30, all were negative for CD15. 80% of ALCL were positive for CD45RB (LCA) (Fig 2) while 50% were positive for EMA.

(2) Immunologic reaction pattern with CD30 in both diseases:
CD 30 reactivity in ALCL, as compared to HD, was stronger and present in a larger number of cells (Fig 3,4).

The correlation between histopathology and immunohistochemistry. Table (5)

Precise morphologic characterization of both diseases was the main source for diagnosis, based on hematoxylin and Eosin-stained slides without the use of any auxiliary technique.

Morphology alone was diagnostic in 29 Hodgkin’s cases, with exception of one case showing some cellular cohesion and necessitate immunohistochmecal panel to differentiate it from ALCL.

Regarding ALCL, 17 cases out of 20 were perfectly diagnosed using morphologic criteria only, while three cases showed diagnostic difficulty that needed immunohistochemical study (Fig 5,6).

Finally diagnosed cases in relation to other cases which need further molecular genetic determination. Table (6)

In 30 cases of Hodgkin’s disease used in this study, the use of both morphology and panel of monoclonal antibodies were sufficient to reach final diagnosis. On the other hand, one case of ALCL was improperly characterized after the use of both morphology and immunohistochemistry. That is to say that after the use of both morphologic and immunohistochmical studies to diagnose both diseases, there still need to use further investigation on molecular pathologic bases.
Table (1) showing clinical data of patients diagnosed as HD versus those diagnosed as ALCL

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<td>5</td>
<td>3</td>
<td>9</td>
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Table (2) Shows different anatomic sites involved by both HD and ALCL

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Table (3): pattern of growth in both diseases:

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- Table (4). Immunoreactivity in both diseases for antibody panel:

<table>
<thead>
<tr>
<th></th>
<th>CD45RB (LCA)</th>
<th>(EMA)</th>
<th>CD20</th>
<th>CD45RO</th>
<th>CD30</th>
<th>CD15</th>
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<td>0%</td>
<td>30%</td>
<td>30%</td>
<td>100%</td>
<td>90%</td>
<td>100%</td>
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<tr>
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Table (5) shows the correlation between histopathology and immunohistochemistry:

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Table (6) shows number of finally diagnosed cases in relation to other cases which need further molecular genetic determination:

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<th>Further investigations needed</th>
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<td>0</td>
<td>0%</td>
<td>30</td>
</tr>
<tr>
<td>ALCL</td>
<td>19</td>
<td>95%</td>
<td>1</td>
<td>5%</td>
<td>20</td>
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</table>
Fig (1) : A case of ALCL showing atypical nuclear features with variable giant cells including Reed Sternberg- like cells and Giant cells with Wreath-like nuclei (arrow) (H & E, X 400)

Fig (2) : A case of ALCL stained with CD45RB (LCA) shows positive membranous staining of tumor cells (Immunohistochemistry , AEC chromagen , Hematoxylin counterstain, X400)

Fig (3) : shows Pattern of CD30 staining in a case of Hodgkin's disease shows selective cytoplasmic strong staining of RS cells, which are scattered and few (DAB chromagen, hematoxylin counter staining, X 400).

Fig (4) : shows pattern of CD30 staining of blast cells in a case of ALCL. Increased number of blast cells and RS-like cells (binucleated immunoblast with basophilic nucleoli) (DAB chromogen, hematoxylin counter staining, X 400)
Fig (5): A case of ALCL shows Reed Sternberg-like cells and bizarre blast cells with diffuse pattern of proliferation that constitute a problematic mimicry to HD. (H&E, X 250)

Fig (6): The previous case stained with EMA showed positive staining of tumor cells. The use of EMA was of value in differentiating ALCL from HD (Immunohistochemistry, DAB chromogen, Hematoxylin counterstain, X 250)

DISCUSSION
Precise diagnosis for any lymphoma subtype necessitates correlation of histopathological, Immunophenotypic characters as well as molecular features with clinical data, even including the response to chemotherapy. In this respect the perfect diagnosis of either ALCL or the histologically similar types of HD is highly essential as prognosis and management are completely dissimilar in both diseases(11).

Pathological understanding of large cell lymphoid neoplasms was significantly advanced in 1985 by Stein and coworkers (9) with the discovery of the Ki – antigen (later clustered as CD 30 lymphoid activation antigen). Almost all nodal and extranodal large cell tumors expressing the CD 30 antigen were referred to as Ki-1 lymphoma " with ill-defined morphologic criteria, and irrespective of B or T or null cell phenotype. However, since the classifications (Kiel , REAL and WHO) of malignant lymphomas were based on morphology and not on antigen expression, the term Ki-1
lymphoma was replaced by anaplastic large cell lymphoma (ALCL) (9, 11).

Many authors have paid attention to the possible overlap between ALCL and HD. They noted a continuous spectrum of nuclear profile, immunophenotypes and immunogenotypes between both types of lymphomas. Thus some cases may be difficult for final diagnosis as either ALCL or HD (7, 8, 16, 17).

While typical cases of ALCL were clearly distinct (by pathologic, cytogenetic, and clinical criteria) from Hodgkin's disease, there is a variety of histologic and immunophenotypic patterns that overlap those of ALCL and HD; most of these would be classified as HD lymphocyte depletion (LD) or syncytial type of nodular sclerosis (NS) (18).

Blast cells in Anaplastic large cell lymphoma (ALCL) may cytologically resemble Hodgkin's cells and may be distributed among reactive cells in a pattern similar to Hodgkin's disease (1).

For a perfect diagnosis, clinical history including age, sex, anatomical distribution (skin rarely affected by HD but could be affected in ALCL) (4, 19) and the presence of systemic symptoms (more with HD) may be helpful (18).

Histologically, many cases of anaplastic large cell lymphoma demonstrate at least focal involvement of the lymph node sinuses, in which tumor cells grow in a cohesive pattern. In addition, cells with multiple atypical nuclei arranged in a wreath-like pattern (doughnut-cells) in which multiple prominent comma shaped or rounded nucleoli are often found (20). Variable admixture of reactive cells is usually present. While in Hodgkin's disease, the diagnostic Reed Sternberg cells rarely show cohesiveness pattern and sinusoidal distribution (6). Their nuclei contain single eosinophilic nucleolus and seldom exhibit wreath like pattern. Reactive cells are more prominent than ALCL (1).

Immunohistochemically, although the neoplastic cells of both anaplastic large cell lymphoma and Hodgkin's disease express CD30, the use of CD45RB, CD45RO, and CD15 allows diagnosis in most cases (the first three antigens often found in anaplastic large cell lymphoma and the last antigen in Hodgkin's disease. In addi-
tion, the neoplastic cells of anaplastic large cell lymphoma are often positive for epithelial membrane antigen, an antigen infrequently expressed in classic Hodgkin's disease (21, 22).

In the present study, Clinical data showed marked predominance of Hodgkin's disease among younger age group, mostly in the third decade of life. This data is in concordance with that worldwide (23). On the other hand, ALCL shows preponderance of older age group, mostly in the fifth and sixth decades of life, this is controversial to that reported by previous authors showing prevalence among younger age in first decade of life (19). The preferential occurrence of ALCL in older age group in this study may be in agreement with Nakamura S et al., 1997 (24) who reported bimodal age distribution with one peak in second decade and the other in the fifth one. It may be attributed to the absence of some environmental factors, especially viruses in Egypt.

There was a prevalence of Hodgkin's disease among females with male to female ratio 1: 1.5, while ALCL shows male predominance with male to female ratio 1.5:1. In this respect, the worldwide predominance of males is the rule in both disease (19, 23). The controversy in the female predominance in HD in this study may be explained as these cases were randomly chosen, and they constitute a small proportion from all lymphoma cases.

Nodal distribution was the most prevalent among our patients in both diseases. This is consistent with Jaffe et al., 1994 (18). It was reported that HD very lately affects extranodal sites. This fact may denote either diagnosis of HD in early stages or the biopsies were taken from superficial lymph nodes, easily accessible to our surgeons. ALCL showed two cases of extranodal involvement, both of which were gastric. Gastric affection is experienced to be highly symptomatic, that urges the patient to seek medical advice and hence gastric affection usually presents early.

The morphologic analysis of both diseases in this study showed that Mixed cellularity HD can be diagnosed easily and could be excluded from the start depending on the definite criteria of typical RS cells. This also was applied for lymphocyte predominant HD. This is agreed with Chittal et al., 1988 (25).
Lymphocyte depletion HD, and nodular sclerosing Hodgkin's disease were an important challenge for differentiation based on morphologic criteria. So, in some instances confirmation was needed using a panel of monoclonal antibodies as mentioned by Stein et al., 1985 (9) and Gatter et al., 1987 (26).

Specific cohesive sinusoidal pattern of growth in ALCL was rarely noticed in HD. Proportion of Blast (large cells) was higher in ALCL rather than HD. Presence of wreath like nuclei in giant cells of ALCL were rarely seen in HD. The number of lymphocytes and prolymphocytes was smaller in ALCL than in HD. These criteria are consistent with that reported by Greer et al., 1991 (19) and Bizjak Schwarzbarti, 1997 (1).

The immunohistochemical results found in this study revealed specific immune reactivity for both diseases, in which HD was positive for CD30 in all cases, positive with CD15 in 90%, positive with CD20 in 30%, positive for CD45RO in 30% and positive for CD45RB (LCA) in 10% of cases and negative for EMA. While in ALCL, none were positive for B-cell marker (CD20) and 40% were positive for T-cell marker (CD 45 R0). All cases were positive for CD30, all were negative for CD15. 80% of ALCL were positive for CD45RB (LCA) while 50% were positive for EMA. All these immunohistochemical data were consistent with that reported by Thomas and Said, 1985 (14) and Sarker et al, 1992 (15) for Hodgkin's disease and with that reported by greer et al., 1991 (19) and Pileri et al., 1990 (4) for ALCL.

Correlation between histopathology and immunohistochemistry

Precise morphologic characterization of both diseases was the main source for diagnosis, based on hematoxylin and Eosin-stained slides without the use of any auxiliary technique. This indicates the persistent value of consensus between pathologists regarding the fixed data that differentiates between both diseases (18,27).

One case of HD and 3 cases of ALCL needed auxiliary study (immunohistochemical and molecular). The use of these techniques increased the accuracy of diagnosis, however for economic reasons and because that most cases were diagnosed by morphology alone, we sug-
gest that the use of these techniques should be reserved for problematic cases that are impossible to be diagnosed by morphology alone.

**Finally diagnosed cases in relation to other cases which need further molecular genetic determination**

In 30 cases of Hodgkin's disease used in this study, the use of both morphology and monoclonal antibodies panel was sufficient to reach final diagnosis. On the other hand, one case of ALCL was improperly characterized after the use of both morphology and immunohistochemistry. The presence of residual problem after the use of both morphology and immunohistochemistry, opened a new field for molecular study in order to search for genetic structural criteria responsible for specific antigen expressions.

**SUMMARY AND CONCLUSION**

- In this study we noticed that the Egyptian ALCL shows preponderance of older age group which is controversial to that reported all over the world.

- Nodal involvement took the upper hand in both HD and ALCL vs extranodal involvement.

- Males are more affected by ALCL while females are more affected by HD.

- The use of morphology alone in differentiation between Hodgkin's lymphoma and Anaplastic large cell lymphoma was accurate in more than 90% of cases, but still there was some problematic cases in which the use of immunohistochemistry was mandatory.

- The panel method used in this study offers an important tool in the characterization and diagnosis of both HD and ALCL. The most valuable of these were CD15, CD30 and EMA.

- We can conclude from this study that accurate diagnostic characters regarding pattern of growth, number and shape of blast and RS cells and the detailed cellular morphology are the most important tools to differentiate between most cases of HD and ALCL.

- Some problematic cases still in need for the use of immunohistochemical and molecular techniques. The use of such techniques will increase the accuracy of diagnosis and can be an engine for more research to clarify the origin of both diseases.

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26. Gatter KC, Ralfidaer E et al. (1987): Immunophenotyp-

المعايير المميزة بين مرض هودجكين والورم الليمفاوي ذو الخلايا الليمفاوية الكبيرة عالية الخصائص

د. محمد فوزى
قسم الباثولوجى - كلية طب المنصورة

إن التفرقة بين الورم الليمفاوي ذو الخلايا الليمفاوية الكبيرة عالية الخصائص ومرض هودجكين أحيانا مايمثل مشكلة. ولأن كل المرضيين مهتمين في النهاية المتوقع للمعية وطريقة العلاج فإن الغرض من هذا البحث هو دراسة الخصائص الإكلينيكية والباثولوجية والهستوكيميائية المناعية التي تستطيع التفرقة بين كلا المرضين.

أجرى هذا البحث على عدد 50 حالة (30 حالة هودجكين و 20 حالة ورم ليمفاوي للخلايا الليمفاوية الكبيرة عالية الخصائص) مأخوذة من مستشفى جامعة المنصورة وبعض الحالات الخاصة في الفترة من سنة 1995 إلى 2000. وقد درست هذه الحالات بالطريقة الهستوكيميائية العادية وكذلك بطريقة الهستوكيميائية المناعية باستخدام س. د. 45 - س. د. 30 - س. د. 20 - أ. د. 15 - 10 

وقد لوحظ في هذه الدراسة أن الورم الليمفاوي للخلايا الليمفاوية الكبيرة عالية الخصائص يحدث في الذكور ذوى العمر الكبير بينما مرض هودجكين يحدث في الإناث ذوات العمر الصغير وتحدث كلا المرضيين في الغدد الليمفاوية أكثر من حدوثه في الأعضاء الأخرى.

وقد وجد أن التشخيص بطريقة دقيقة قد حدث في 90/9 من الحالات بالطريقة الهستوكيميائية العادية اعتماداً على معرفة طريقة نمو الخلايا وعدد وشكل الخلايا الليمفاوية (البلست) وخلايا ريدستيرنبرج وكذلك الشكل النسيجي للخلايا.

وقد وجد أن الدلالات الهستوكيميائية المناعية ذات القيمة في التفرقة بين الحالات التي تمثل مشكلة في التشخيص هي س. د. 45 - س. د. 30 - أ. د. 15 و إى أم أهى.

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