A Study of the Newer Prognostic Markers in Diffuse Large B Cell Lymphomas

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Abstract

Introduction: Diffuse large B cell lymphomas (DLBCL) encompass a pathogenetically heterogeneous group of aggressive tumours that are rapidly fatal if untreated. Gene expression profiling studies have identified distinct molecular subtypes of DLBCL, one with an expression profile similar to normal germinal centre B cells (GCB subtype) and a second mimicking activated peripheral blood B cells (ABC subtype) having different prognostic significance allowing risk stratification of lymphoma patients and development of specific therapeutic strategies.

Methods: Twenty cases of DLBCL were included in the study and categorized into germinal centre and non germinal centre subtypes using the following antibody panel. CD10, Bcl-6, MUM1 and CD138. The germinal centre and non germinal centre subtypes were defined as under Germinal centre (DLBCL) CD10 + and/or Bcl-6 +, MUM1 -, CD138 - and Non germinal centre (DLBCL) CD10, Bcl-6 ±, MUM1 ±, CD138 ±.

Result: In our study of twenty cases thirteen were germinal centre DLBCL while seven of the twenty cases were non germinal centre type of DLBCL. 75% of the nodal cases and 62.5% of extra nodal cases were germinal centre B cell type. Overall survival in the GCB and non GCB groups was 91% and 14% respectively and the difference was highly significant statistically.

Conclusion: This study validates the existence of prognostic subgroups of DLBCL in the Indian population.

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Key Words : Diffuse large B cell lymphoma (DLBCL); Prognosis; Immunohistochemical subtypes; Germinal centre B subtype; Non germinal centre subtype

Introduction

Diffuse large B cell lymphomas (DLBCL) encompass a heterogeneous group of tumours that together constitute the commonest of all Non Hodgkin lymphoma and 60 to 70% of aggressive lymphoid neoplasms [1]. The pathogenetic heterogeneity has been confirmed by cytogenetic and gene expression profiling studies. As a group, DLBCL are aggressive tumours that are rapidly fatal if untreated [2]. However, with intensive combination chemotherapy, complete remission can be achieved in 60 to 80% of patients and approximately 50% remain free from disease for several years and may be considered cured.

Prognostic models based on pre-treatment characteristics, such as the International Prognostic Index (IPI), are currently used to predict outcome in DLBCL [2]. However, clinical outcome models identify neither the molecular basis of clinical heterogeneity, nor specific therapeutic targets.

The use of immunohistochemical methods has become part of the routine diagnostic procedure in several malignancies, and has revolutionized the

diagnosis and identification of lymphomas. In the last ten years, markers have been identified that influence a patient's prognosis. This has led to the proposed use of these markers for risk stratification of lymphoma patients and development of specific therapeutic strategies [3,4].

Gene expression profiling studies have identified at least 3 distinct molecular subtypes of DLBCL, one with an expression profile similar to normal germinal centre B cells (GCB subtype), a second mimicking activated peripheral blood B cells (ABC subtype) and a third, primary mediastinal large B cell lymphoma (PMBCL), typically presenting with mediastinal lymphadenopathy and displaying some molecular genetics similar to Hodgkin's lymphoma [5]. Some studies have classified these groups as germinal centre (GCB) and non germinal centre (Non GCB) subtypes. The breakthrough in the subtyping of DLBCL came with the algorithm described by Hans et al [6] and Chang et al [7].

Alacacioglu et al [8] studied 50 cases of DLBCL and categorized them into GCB and non GCB using CD10, Bcl-6 and MUM1. The overall survival (OS) and event free survival (EFS) were longer in GCB group.

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The five year OS for GCB group was 92% compared with only 44% for the non-GCB group. The OS of the GCB group was also independent of the IPI score and was longer compared to that of the non-GCB group in low IPI subgroup. Adida et al [9] have predicted that 76% patients with GCB like DLBCL will be alive at five years compared to only 16% in ABC like DLBCL even when patients with low risk disease (IPI 0 to 2) were evaluated. These observations were further confirmed in a larger study performed by the Lymphoma and leukemia molecular profiling project (LLMPP) group [4], which analysed gene expression profiles in 240 DLBCL patients treated with CHOP like regimens.

Several studies have examined the proportions of GCB and non-GCB subtypes in large series of DLBCL patients, but it remains unclear if these proportions are the same in different countries. Khera et al [10] inferred that the earlier age of onset, male dominant sex ratio and higher frequency of B symptoms sets apart DLBCL in Indian population from that in the developed countries. Assessment of data collected from other studies showed that 31% of DLBCL patients (102/330) have the GCB subtype in Asian countries, but 50% (206/416) express GCB phenotypes in Western countries. Based on these data, the occurrence of the GCB subtype of DLBCL was significantly less in Asian countries [2]. These differences in the relative proportions of NHL subtypes among developing countries and between developing countries and the rest of the world presumably arise from differences in environmental and genetic factors that influence lymphoma genesis and strongly suggest that more research in developing countries would provide valuable insights into the pathogenesis of lymphoid neoplasm.

The review of literature from around the globe emphasizes the definite existence of prognostic subgroups of DLBCL which can be easily classified using immunohistochemistry in the laboratory. The need of the hour is to standardize these markers and procedures with respect to Indian population. Therefore this study is being undertaken to assess immuno-histochemical stratification of DLBCL into prognostically significant subgroups.

Table 1

Site wise distribution of cases

Site of presentation	Total	Percent
Lymphadenopathy	12	60%
Mediastinal mass	4	20%
Stomach	3	15%
Duodenum	1	5%
Total	20	100%

Material and Methods

Twenty recently diagnosed cases of DLBCL who presented to a tertiary care hospital were included in the study after obtaining informed consent. Clinical data was obtained in these cases. The following antibody panel was selected to classify the cases into the two categories – CD10, Bcl-6, MUM1, and CD138.

The germinal centre and non germinal centre subtypes were defined as under:

Germinal centre diffuse large B cell lymphoma – CD10 + and/or Bcl-6 +, MUM1 -, CD138 - and Non germinal centre diffuse large B cell lymphoma CD10, Bcl-6 ±, MUM1 ±, CD138 ±.

Cases were categorized into germinal centre and non germinal centre according to algorithm mentioned above. This was correlated with the patient history especially with respect to survival at one year. Survival was measured as the interval between the onset of the disease and death or last follow up evaluation at one year.

Results

A total of 20 cases of DLBCL were studied over a period of one year at a tertiary care hospital. The age in the study ranged from 23 to 72 years with a mean age of 47.95 years. There were 11 (55%) male patients and nine (45%) female patients. The site wise distribution of cases is depicted in Table 1. Primary nodal involvement was the commonest presenting complaint in our study as seen in 12 of the 20 cases comprising 60% of all cases.

Eight of the 20 cases in the study were stage I by the Ann Arbor staging system. An equal number of the cases were stage II, 3 were stage III and one was stage IV (Table 2). Systemic symptoms such as malaise, weight loss, fever and anemia were present in seven of the twenty cases (35%) while thirteen cases (65%) did not show any systemic symptoms.

The cases were scored according to the IPI into four categories (Table 3).

Histological diagnosis was confirmed in all cases using standard morphological criteria (Fig. 1) and strong membrane positivity for CD20 (Fig. 2).

The cases were categorized into germinal centre and non

Table 2

Stage of disease

Stage of disease	No. of cases	Percent
Stage I	8	40%
Stage II	8	40%
Stage III	3	15%
Stage IV	1	5%

Table 3

IPI risk categorization

IPI risk category	No. of cases	Percent
Low	7	35%
Low-intermediate	8	40%
High-intermediate	3	15%
High	2	10%

Fig. 1 : Microphotograph from a case of DLBCL showing diffuse pattern with large cells and mitosis (400X, H&E).

Fig. 2 : Microphotograph from a case of DLBCL showing membrane positivity in immunohistochemical staining of CD20 (400X, DAB).

Fig. 3 : Microphotograph from a case of DLBCL showing membrane positivity in immunohistochemical staining of CD10 (400X, DAB).

Fig. 4 : Microphotograph from a case of DLBCL showing nuclear positivity in immunohistochemical staining of Bcl-6 (400X, DAB).

Fig. 5 : Microphotograph from a case of DLBCL showing nuclear positivity in immunohistochemical staining of MUM1 (400X, DAB).

Fig. 6 : Microphotograph from a case of DLBCL showing membrane positivity in immunohistochemical staining of CD138 (400X, DAB).

Table 4

Distribution of GCB and NGCB case in the population under study

Subtype	No. of cases	Percent
GCB	13	65%
NGCB	7	35%
Total	20	100%

germinal centre DLBCL on the basis of CD10, Bcl-6, MUM1 and CD138 (Table 4).

In our study of twenty cases thirteen were germinal centre DLBCL while seven of the twenty cases were non germinal centre type of DLBCL. 75% of the nodal cases and 62.5% of extra nodal cases were GCB cell type. IHC staining for various markers is shown in (Figs. 3-6).

It was noted in our study that during the period of follow up, eight of the twenty cases died due to the disease itself or the complications therein. Twelve of the twenty cases survived and showed response and remission of disease as confirmed clinically by PET scan and various other investigations. The overall survival (OS) amongst all the cases under study was thus found to be 60%. Of the twelve cases that survived eleven were of the GCB cell subtype and only one was non GCB cell subtype. Thus the GCB subtype accounts for 91.6% of all survivals. The correlation between outcome and subtype is shown in Table 5.

Statistical correlation between survival outcome and subtype of DLBCL showed a p value of 0.004 by Fischer Exact test and was found to be highly significant suggesting a strong correlation between subtype and survival.

Table 5

Correlation of outcome with subtype

Outcome	GCB	NGCB	Total
Survived	11	1	12
Expired	2	6	8
Total	13	7	20

Discussion

Diffuse large B-cell lymphoma is the most common lymphoma worldwide. Both morphologically and prognostically it represents a diverse spectrum of disease. Traditional morphologic sub classification often results in poor reproducibility and has not been particularly helpful in predicting outcome.

The arsenal of tools that are available in the clinical laboratory to diagnose and subclassify DLBCL is broadening. Dramatic gains have been made in our ability to predict prognosis through ancillary techniques including immunohistochemistry and cytogenetics [11]. Many of these prognostic markers have to now be reevaluated, because of differences in prognosis with regard to race, genetics, various interactions and treatment options. The hope is that therapeutic regimens will become customized and tailored to specific subtypes pertaining to their different biologic behaviours, thus achieving longer remissions and potential cures in more than just a subset of patients with DLBCL [11].

The markers useful for subclassification of DLBCL

are CD10, Bcl-6, MUM1 and CD138 [11]. Various authors have studied the significance of these markers individually as well as put together for subtyping. Though our endeavor was to subtype DLBCL, we also observed percent positivity of these markers in our study.

In the present study CD10 membrane positivity was found in 65% of the cases i.e. thirteen of the twenty cases. This is in concurrence with the study by Colomo et al [12] and Dogan et al [13]. It is also to be noted that all these are western data and no studies from India are available to the best of our knowledge. The slightly higher incidence may be because of ethnic reasons and needs to be evaluated.

In our study the expression of Bcl-6 among all the cases was found to be 20%. Hans et al [6] had found a Bcl-6 positivity of 56% and Dogan et al [13] found near 80% positivity. However, the study of Lunenburg lymphoma biomarker consortium [4] emphasizes that Bcl-6 is the most variable and difficult marker to score.

About 50% or more of non GCB cell subtypes are known to express MUM1 [14]. Hans et al [6] and Chang et al [7] had introduced it as a marker of non GCB cell subtype of DLBCL. Falini et al [14] suggested that DLBCL related to GCB cell subtype do not express the MUM1 protein. In our study also 43% of the NGCB were seen to express MUM1. CD138 is known to be expressed by plasma cells and is seen in plasmablastic variant. In our study only one case showed positivity for CD138 and on morphology showed plasmablastic differentiation in accordance with WHO 2008.

Considering the expression of CD10, Bcl-6, MUM1, CD138, Bcl2, CD44 and other biomarkers, different algorithms to identify GC and Non GC DLBCL have been proposed [6,7,12] but confirming the relevance of most of them is hampered by failures in reproducibility and validity [11]. The algorithm of Hans et al [6] still remains the most valid and used till date which is what was used in this study as well.

Our study showed 65% of the cases to be of the GCB cell type and 35% to be of the non germinal centre subtype. According to western studies the incidence of non GCB cell lymphoma is higher than that of GCB cell lymphoma [4,6]. However Dogan et al [13] found 58% of his cases to be GCB in contrast with 42% of NGCB in a study of 64 patients in Netherlands. Moreover, Shia et al [15] in their study have suggested that Asians may have an increased incidence of GCB subtype. No data was available in literature on the subtypes in Indian population. Therefore, there is need to study the prevalence of the subtypes in an Indian setting and the local population.

The IPI is the commonest prognostic marker used

currently but it is purely clinical and fraught with errors. It does not take into account the cell of origin, or presence of bulky disease. The rapidly expanding knowledge about gene expression patterns and protein expression has put forth a number of prognostic markers which on IHC have been found to be equally reproducible. Alizadeh et al [5] found that even in low risk IPI scores two subsets of patients were identified, one with worse outcome. Thus the molecular dissection of DLBCL and the IPI apparently identify different features of the patient that influence their survival. Numerous studies [4,6,8] world over have suggested a better overall survival for the GCB-cell subtype of DLBCL which is determined by the immuno-histochemical markers designated above. The findings in our study are similar, with an overall survival in the GCB and non GCB groups being 91 and 14% respectively and were highly significant statistically.

We must remember that pathogenesis of DLBCL is not isolated but an intricate interaction between various markers and factors. The best and the most consistent of these have to be identified and authenticated with respect to different sub populations so that tailor made, individualized and effective therapy can be instituted.

Conflicts of Interest

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Intellectual Contribution of Authors

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References

1. Lossos I S. Molecular pathogenesis of B cell lymphoma. *J Clin Oncol* 2005; 23: 6351-7.
2. Hans CP, Dennis D, Timothy CG. Confirmation of the molecular classification of diffuse large B cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; 103: 275- 82.
3. Gustaaf WIV, Evert Jan GB, Holt BVD. Prognostic impact of germinal centre associated proteins and chromosomal breakpoints in poor risk diffuse large B-cell lymphoma. *J Clin Oncol* 2006; 24: 4135-42.
4. Daphne de J, Rosenwald A, Chhanabhai M et al. Immuno-histochemical prognostic markers in diffuse large B-Cell lymphoma: Validation of tissue microarray as a prerequisite for broad clinical applications. A study from the Lunenburg Lymphoma Biomarker Consortium. *Journal of Clinical Oncology* 2007; 25: 805-12.
5. Alizadeh AA, Eisen MB, Davis RE et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; 403: 503-11.
6. Hans CP, Weisenburger DD, Greiner TC et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma

- by immunohistochemistry using a tissue microarray. *Blood* 2004; 103: 275-82.
7. Chang CC, McClintock S, Cleveland RP et al. Immunohistochemical Expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. *Am J Surg Pathol* 2004; 28: 464-70.
 8. Alacacioglu I, Ozcan M, Ozkal et al. Prognostic significance of immunohistochemical classification of diffuse large B-cell lymphoma. *Hematology* 2009; 14: 84-9.
 9. Adida C, Haioun C, Gaulard P et al. Prognostic significance of surviving expression in diffuse large B cell lymphoma. *Blood* 2000; 96: 1921-5.
 10. Khera R, Jain S, Kumar L, Thulkar S, Vijayraghawan M, Dawar R. Diffuse large B cell lymphoma: experience from a tertiary care centre in north India. *Medical Oncology* 2009; 27: 211-2.
 11. Kristin E, Kaaren KR. Diffuse large B cell lymphoma. *Arch of Pathol, Lab Med* 2008; 132: 118-24.
 12. Colomo L, Lopez-Guillermo A, Perales M et al. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. *Blood* 2003; 101: 78-84.
 13. Dogan A, Munson P, Isaacson PG. CD10 and BCL6 expression in paraffin sections of normal lymphoid tissue and B cell lymphomas. *Am J Surg Pathol* 2000; 24: 846-52.
 14. Falini B, Fizzotti M, Pucciarini A et al. A monoclonal antibody (MUM1) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. *Blood* 2000; 95: 2084-92.
 15. Shia AKH, Jairaman S, Peh SC. High frequency of germinal centre derivation in diffuse large B cell lymphoma from Asian patients. *J Clin Pathol* 2005; 58: 962-67.

Journal Scan

Strumitzen S, Hofmann TB, Gradi B, Grnenbeck M, Hofmann BM, Mittlboeck M. Mesh graft infection following abdominal hernia repair: risk factor evaluation and strategies of mesh graft preservation. A retrospective analysis of 476 operations. *World Journal of Surgery* 2010; 34: 1702-9.

Mesh infections are the bugbear of prosthetic mesh hernioplasties. Lack of comprehensive studies on conservative management tilts opinion towards safety of mesh removal on its recognition.

This study by Stremitzer S et al from Medical University of Vienna, Austria, is path breaking one which analyzes factors associated with mesh infections and outcome of conservative management. Outcome of 476 patients, with mesh hernioplasty for incisional hernias, over five years period (2000 to 2005), has been retrospectively audited. Gender, age, body mass index (BMI), hernia type, hernia size, diabetes mellitus, renal function, liver function, steroids use, smoking habit, duration of operation, surgeons expertise, surgical technique, class of wound, additional intraoperative procedures, type/ size of mesh/ sutures, peri-operative antimicrobial therapy, intraoperative saline wound irrigation before wound closure and drainage were the factors evaluated. Of the 476, 31 patients (6.5%) developed mesh infection. Skin commensals coagulase negative/ positive *Staphylococci*, *Streptococcus epidermidis*, enteric commensals- *Enterococcus faecalis*, *Corynebacteriae* and *Pseudomonas* were the predominant organisms isolated. Univariate analysis singled out BMI among demographic factors, mesh size and duration of surgery, among

operative factors, as those influencing mesh infections. Multivariate analysis, however, singled out only duration of surgery as the vital factor. Laparoscopic mesh repairs were found associated with lesser infections compared to open surgery, although, the difference was statistically insignificant. Conservative management could salvage 55% of meshes but the duration of treatment and cost of treatment was prolonged and high (antibiotics for two weeks and open/ vacuum dressing for mean of 81 days, cost - 70 Euros/ day). 100% of absorbable meshes, with large pore sizes, such as, polygalactin could be salvaged whilst only about 20% of non absorbable meshes, with small pore sizes, such as, polypropylene and PTFE could be salvaged. Use of prophylactic postoperative antibiotics, surgery in recurrent hernias and presence of coagulase negative staphylococcus and corynebacterium were factors adversely affecting mesh preservation.

This study dispels numerous myths surrounding mesh infection and its management, such as, inevitability of mesh removal and safety on use of prolonged prophylactic antibiotics, postoperatively. It clearly differentiates meshes to be preserved from ones to be expeditiously removed. However, the cost and duration of conservative management may prove forbiddingly high and long for wide adoption, forcing more meshes to be removed than necessary, in an economically challenged country like India.

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