Clinicopathologic Evaluation of MYC Expression in Primary Mediastinal (Thymic) Large B-Cell Lymphoma

K. David Li, MD,1 Rodney Miles, MD, PhD,1 Sheryl R. Tripp, MT(ASCP), QIHC(ASCP),2 Martha J. Glenn, MD,3 Sherrie L. Perkins, MD, PhD,1 and Mohamed Salama, MD1

From the 1Department of Pathology, University of Utah and ARUP Laboratories, Salt Lake City; 2ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; and 3University of Utah School of Medicine, Salt Lake City.

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ABSTRACT

Objectives: Based on previous molecular studies, a small fraction of primary mediastinal (thymic) large B-cell lymphoma (PMBL) demonstrates MYC alterations. However, no studies have evaluated MYC protein expression by immunohistochemistry (IHC) with follow-up fluorescence in situ hybridization (FISH) analysis. We aim to evaluate the clinicopathologic importance of MYC IHC expression in PMBL.

Methods: Three pathologists independently evaluated MYC IHC expression in 32 cases of PMBL for percent tumor positivity and nuclear intensity. FISH analysis for MYC rearrangement was performed on cases with high MYC IHC expression. Clinical data including treatment, follow-up, and outcome were also reviewed in a subset of cases.

Results: Variable MYC protein expression by IHC was detected in 30 (94%) of 32 cases of PMBL. One-third of the positive cases (10/30) showed high MYC IHC expression of at least 30% nuclear positivity. FISH analyses for MYC rearrangement on these 10 cases were negative. Review of clinical data on a subset of cases with high and low MYC IHC expression showed no differences in clinical outcome.

Conclusions: MYC protein expression by IHC is present in most PMBLs. Increased MYC protein expression can be seen in one-third of the cases; however, it does not correlate with genetic abnormalities by FISH. There is also no significant impact of MYC protein expression on clinical outcomes.

Primary mediastinal (thymic) large B-cell lymphoma (PMBL) is an aggressive large B-cell lymphoma that typically arises in the mediastinum from putative thymic B cells and represents approximately 2% to 4% of all non-Hodgkin lymphomas. PMBL classically occurs in young adults at a median age of 35 years, demonstrates female predominance with a male-to-female ratio of 1:2, and usually has better outcome.1,2 Initially considered a subtype of diffuse large B-cell lymphoma (DLBCL), PMBL has since been recognized as a distinct entity from DLBCL based on characteristic clinical, immunophenotypic, and molecular features, some of which appear to overlap with classic Hodgkin lymphoma (CHL).3–5 A variety of disease-driven genetic abnormalities have been identified in PMBL, including elevated expression of JAK2, PDL1, and PDL2 on chromosome 9p and amplification of REL and BCL11A on chromosome 2p.6–8 The REL gene, which encodes a protein belonging to the nuclear factor (NF)–κB family, seems to promote cell survival and inhibit apoptosis in PMBL.9 Other genetic alterations and deregulated signaling pathways involving p16, TP53, and MYC have also been recognized and are believed to contribute to the disease pathogenesis.10–13

Although MYC-induced lymphomagenesis has been studied in various other hematologic malignancies, the clinical significance of MYC alterations has not been studied in detail in PMBL. The hallmark of Burkitt lymphoma (BL), balanced translocations between MYC and immunoglobulin genes, result in deregulated MYC protein expression.1 Similar translocations of MYC also occur in 5% to 10% of DLBCLs and results in a more aggressive clinical behavior and a less favorable response to rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (R-CHOP).14 Unlike...
these other aggressive B-cell lymphomas, rearrangements of the MYC gene are absent to rare in PMBL. In the 1990s, Scarpa et al first demonstrated molecular evidence of MYC gene abnormalities in three of six cases of PMBL, with one case having major rearrangement of the MYC locus. Follow-up molecular studies with larger cohorts by Tsang et al and Scarpa et al confirmed the findings of MYC abnormalities in 19% and 25% of cases, respectively. By Southern blot, no evidence of MYC rearrangement was identified by Tsang et al, but point mutations in the MYC gene were found at the 3′ end of the first exon in three cases by polymerase chain reaction. Similarly, Scarpa et al found cases with mutations or small rearrangements at the 3′ end of the first exon and rearrangements of the second and third exons by molecular methods. These findings were similar to those observed in MYC translocations in endemic and sporadic BL. Molecular abnormalities involving the MYC gene, however, have never been studied in the form of protein expression by immunohistochemistry (IHC) in PMBL. Furthermore, in a disease in which the overall prognosis and response to treatment is generally good, whether MYC alterations have any significant clinical impact is unknown.

In recent years, newer monoclonal antibodies against MYC have been frequently used clinically and in studies to demonstrate overexpression of MYC protein. Studies conducted in BL and DLBCL have shown good correlation between MYC protein expression by IHC and the presence of MYC genetic translocations or other genetic abnormalities, which carry significant clinical impact in a subset of DLBCLs. Similar prognostic significance of MYC IHC expression has also been found in mantle cell lymphoma. The aims of this study are to evaluate the presence of MYC protein expression by IHC in PMBL, since this has never been formally studied. Cases with high MYC expression (in the range similar to those reported in DLBCL and BL) will be subjected to fluorescence in situ hybridization (FISH) analysis to evaluate for major rearrangements or amplification of the MYC locus to identify possible genetic lesions underlying MYC protein overexpression. Finally, impact on clinical outcome and survival with respect to MYC IHC expression will be evaluated.

Materials and Methods

Cases of PMBL, diagnosed between 1997 and 2013, were collected through the University of Utah Department of Pathology/ARUP Laboratories database. Cases include outside institution consultations as well as in-house primary diagnostic biopsy specimens. All cases were reviewed with the diagnosis confirmed according to the World Health Organization (2008) classification. Available flow cytometric and IHC results previously performed on the cases, including MIB-1 (Ki-67), CD23, CD30, BCL2, and BCL6, were also reviewed. The study was approved by the institutional review board at the University of Utah.

Immunohistochemistry

IHC stain for MYC was performed on all cases using commercially available c-MYC (rabbit monoclonal antibody; predilute): clone Y69 (catalog 790-4628, Ventana Medical Systems, Tucson, AZ). Briefly, the tissue samples were cut at 3 to 4 μm on positively charged slides and then air dried at room temperature. The slides were placed on the automated immunostainer (BenchMark Ultra, Ventana Medical Systems). The slides were deparaffinized with EZ Prep solution (Ventana Medical Systems) and pretreated with Cell Conditioner 1, pH 8.0, for 64 minutes at 95°C (Ventana Medical Systems). The primary antibody was applied for 1 hour at 36°C with the amplification kit applied to increase the antibody signal (Ventana Medical Systems). Detection of slides was performed using the IView DAB detection kit (Ventana Medical Systems), which is a goat anti-mouse/anti-rabbit biotinylated secondary, streptavidin–horseradish peroxidase system using DAB (3,3′-diaminobenzidine) as the chromogen.

IHC Analysis

IHC staining with MYC was independently reviewed by three pathologists (K.D.L., M.S., and R.M.). Two forms of IHC assessment were performed. First, the percentage of positive tumor cells was assessed for each case (0%-100%) in 10% increments. The three scores were averaged and rounded to the nearest 10%. A high MYC IHC expression was considered to have 30% or more nuclear positivity. The decision to use 30% or more as the cutoff was based on studies by Tapia et al, in which non-MYC translocated BL and DLBCL showed an average MYC IHC of 28%. Second, a nuclear intensity score (1, weak, visible only on high power; 2, moderate; 3, strong, easily visible on low power) was determined based on percent positive tumor cells, using 30% as a cutoff. For example, if a case had more than 30% positive tumor cells with moderate nuclear intensity, the case was classified as having moderate nuclear intensity and a score of 2. The three scores were averaged and rounded to the nearest 0.1. A subset of cases had treatment data as well as clinical follow-up, and they were correlated with MYC IHC expression.

FISH

A subset of cases with high MYC IHC expression (≥30% nuclear positivity) was also subjected to FISH analysis using the LSI MYC Dual Color Break-Apart Rearrangement Probe by Abbott Molecular (LSI MYC DC BA; Abbott Molecular, Abbott Park, IL). Briefly, 3-μm-thick, formalin-fixed, paraffin-embedded sections were air-dried...
Table 1
Primary Mediastinal Large B-Cell Lymphoma With 30% or More MYC Protein Expression

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y/Sex</th>
<th>CD30</th>
<th>CD23</th>
<th>BCL2</th>
<th>BCL6</th>
<th>Mean MYC, %</th>
<th>Mean MYC Intensity Score (1-3)</th>
<th>MIB (Ki-67), %</th>
<th>sIg by FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41/F</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>30 (R: 20-40)</td>
<td>1.7</td>
<td>40</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>30/F</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>30 (R: 20-40)</td>
<td>2.3</td>
<td>70</td>
<td>Dim κ</td>
</tr>
<tr>
<td>3</td>
<td>27/M</td>
<td>+w</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>40 (R: 30-50)</td>
<td>2.3</td>
<td>90</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>31/M</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>60 (R: 40-70)</td>
<td>1.7</td>
<td>70</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>17/F</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>40 (R: 30-40)</td>
<td>2.7</td>
<td>80</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>27/M</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>30 (R: 20-40)</td>
<td>2.3</td>
<td>50</td>
<td>Dim κ</td>
</tr>
<tr>
<td>7</td>
<td>37/M</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>60 (R: 50-60)</td>
<td>2.7</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>23/M</td>
<td>+w</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>60 (R: 50-60)</td>
<td>2.7</td>
<td>80</td>
<td>λ</td>
</tr>
<tr>
<td>9</td>
<td>22/F</td>
<td>+w</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>40 (R: 30-40)</td>
<td>2.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>14/M</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>30 (R: 30-40)</td>
<td>2.3</td>
<td>60</td>
<td>κ</td>
<td></td>
</tr>
</tbody>
</table>

FC: flow cytometry; ND, not done; R, range of MYC scores; sIg: surface immunoglobulin; +, positive; +w: weakly positive; –, negative.

and oven baked for 1 hour at 55°C and then processed following the manufacturer’s recommendations on the Abbott Vysis VP2000. Slides were then denatured for 5 minutes at 80°C and hybridized overnight at 37°C on a Thermo-Brite (Vysis/Abbott Molecular, Des Plaines, IL). Following hybridization, coverslips were removed by placing the slides in a room temperature bath of 2′ SSC/0.3% NP-40 and then moved to a second bath heated to 73°C for 3 minutes and then briefly centrifuged in the SciGene Little Dipper (SciGene Corporation, Sunnyvale, CA). Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA) with DAPI was then applied to each slide, and a coverslip was applied.

Statistical Analysis
Statistical analysis was performed using SAS 9.3 software (SAS Institute, Cary, NC). Percent MYC positivity and nuclear intensity were compared between groups using the t test for the difference in means. The folded F statistic was used to determine if the variances between the two groups were unequal (P < .05). If the variances were determined to be unequal, the Satterthwaite approximation was used to calculate the P value for the t test. If the variances were assumed to be equal, a pooled method was used to calculate the P value.

Results
Thirty-two cases of PMBL were identified in our database and represented the study population. The median age was 37 (range, 14-77) years, with a male-to-female ratio of 1.3 (18 males, 14 females). Tissue samples included needle core biopsy, excisional biopsy, and resection specimens. MYC IHC expression was found in the majority of PMBLs (30/32 [94%]), with a high variability of tumor cell positivity ranging from less than 5% to 60% Image 11. There was also variability in nuclear intensity, with most cases displaying weak to moderate intensity. One-third (10/30) of the positive cases demonstrated high MYC IHC expression (≥30%) as well as higher nuclear intensity (mean score, 2.3) Image 11. The mean percent positive tumor cells in cases classified as high MYC expression was 42% (range, 30-60%), and the mean percent positive tumor cells in cases classified as low MYC expression was less than 10% (range, 0%-10%) (P < .0001). The mean nuclear intensity scores were 2.3 and 1.3 for high and low MYC expression cases, respectively (P < .0001).

In addition to MYC IHC expression, the mean MIB-1 results also appeared to be higher in cases with high MYC compared with those that were low MYC (65% vs 52%). However, this finding did not reach statistical significance (P = .09). CD30, CD23, BCL-2, and BCL-6 by IHC were performed on a subset of cases. CD30 was positive in 19 (91%) of 21 cases. CD23 was positive in 20 (83%) of 24 cases, including all cases with high MYC expression, which is similar to previous reports.21,22 BCL-2 was positive in five (56%) of nine cases. BCL-6 was positive in 14 (93%) of 15 cases, including all cases with high MYC expression. Of the 32 cases, 19 had available flow cytometry results. Five (26%) cases had negative flow cytometry results by report, 11 (58%) cases had surface immunoglobulin light chain restriction by report, and three (16%) cases had no detectable surface immunoglobulin light chains by report. Of the 11 surface immunoglobulin light chain–positive cases, four were outside institution consultation cases; therefore, flow cytometry histograms were not available for review. The remaining seven cases had flow cytometry performed within our institution and showed either dim or normal-intensity surface immunoglobulin light chain restriction Image 21.

The 10 cases with high MYC IHC expression were subjected to follow-up FISH analysis, including three cases with 60% expression. However, no MYC abnormalities in the form of translocation or amplification were identified in all cases using this approach.

Clinical data, including treatment modalities, follow-up, and outcome, were available in most of the high MYC expression cases (8/10) and a subset of low MYC expression cases (6/22) Table 21. The mean clinical follow-up duration was 45.8 months for cases with high MYC expression and 36.8 months for cases with low MYC expression. Patients with
H&E and MYC immunohistochemistry (IHC) of a case with a mean MYC expression of 60% and a mean nuclear intensity score of 2.7. MIB-1 for this case is 80% (not shown). C, D, H&E and MYC IHC of a case with a mean MYC expression of 30% and a mean nuclear intensity score of 2.7. MIB-1 for this case is 80% (not shown). E, F, H&E and MYC IHC of a case with a mean MYC expression of less than 10% and a mean nuclear intensity score of 1.7. MIB-1 for this case is 30% (not shown). (All images are at ×400.)
high MYC IHC expression were younger than those with low MYC expression (26.9 vs 43.6 years; \( P = .0013 \)). There was no significant difference in sex distribution. The treatment modalities were comparable for the most part with R-CHOP and localized radiation as the most commonly used regimen. Two patients, one with high MYC expression and the other with low MYC expression, were both treated with dose-adjusted etoposide, doxorubicin, cyclophosphamide, prednisone, and rituximab without radiotherapy, and both patients achieved comparable clinical remission. All patients with available clinical data, regardless of MYC IHC expression status, achieved clinical remission at the end of treatment and continued to be in clinical remission at the last follow-up visit.

**Discussion**

PMBL is a distinct type of large B-cell lymphoma that occurs in young adults and carries a relatively good prognosis. Molecular evidence suggests similar disease pathogenesis with CHL rather than DLBCL, which partly explains the better outcome with multiagent chemotherapy compared with DLBCL. In addition to JAK-STAT and NF-\( \kappa \)B as the principle signaling pathways, other genetic alterations such as MYC are also believed to contribute to the disease process. Since the discovery of MYC gene alterations in PMBL in the 1990s, detailed protein expression by IHC and its clinical impact has not been studied. Our results showed that in most PMBLs, MYC protein expression is low and comparable to the expression levels seen in normal mature B cells in benign lymphoid tissue.\(^{23}\) One-third (33%) of the cases demonstrated increased MYC protein expression similar to those seen in DLBCL and BL; however, no evidence of MYC rearrangements or amplification was identified by FISH. Based on earlier molecular studies by Scarpa et al\(^ {10,12} \) and Tsang et al,\(^ {11} \) in which most MYC alterations were point mutations, the negative FISH results were not surprising, but we were unable to confirm any genetic alteration by molecular methods.
Prior to our study, research conducted by Tapia et al\textsuperscript{18} demonstrated MYC protein expression correlated with gene status in BL and DLBCL. Overall, MYC translocated lymphomas showed MYC nuclear positivity in 70% of neoplastic cells, whereas MYC nontranslocated lymphomas were positive in only 28% of the cells\textsuperscript{18}. When reviewed independently, BL had a mean positivity in 81% of the cells (range, 50%-100%), and DLBCL had a mean positivity in 61% of the cells (range, 5%-95%). Given these findings, the rationale for using 30% or more tumor positivity as a cutoff for high MYC expression in our study seemed most logical. Even though the mean percent of high MYC IHC expression in our study was 43\%, there were three cases with MYC protein expression of 60\% with moderate nuclear staining intensity. In comparison to MYC IHC expression in BL and DLBCL with MYC rearrangement, the observed nuclear staining intensity in PMBL was considerably less. Since most cases of BL and DLBCL that harbor MYC rearrangement show strong MYC IHC, we would hypothesize that the possibility of finding a major rearrangement by FISH in PMBL is low. As expected, we did not identify any genetic abnormality by FISH analysis, either translocation or amplification, in all cases with 30\% or more MYC protein expression. One possible explanation for the absence of MYC abnormalities by FISH could be alternative gene activation or posttranscriptional upregulation through other signaling pathways such as JAK-STAT and NF-kB. Our findings support the hypothesis that the increased MYC protein expression is not secondary to major gene rearrangement and suggest that MYC is not the principle driver in lymphomagenesis of PMBL. We did, however, observe a trend of slightly increased MIB-I expression in cases with high MYC expression, which can be evidence for a minor or an indirect effect of MYC abnormalities on tumor proliferation.

Another interesting finding was the detection of surface immunoglobulin light chain expression by flow cytometry. In contrast to the published criteria that PMBL does not typically express surface immunoglobulin light chain,\textsuperscript{1,24} we found surface immunoglobulin light chain restriction in approximately half of the cases, and less than 20\% of the cases showed no detectable surface immunoglobulin light chain expression. However, we were only able to confirm the expression of surface immunoglobulin light chains in seven (36\%) cases through review of the histograms. The significance of this observation is unknown and could be skewed by the small number of cases. Nevertheless, we believe that cases of PMBL with surface immunoglobulin light chain expression do exist, but additional large standardized studies using present-day high-sensitivity methods are required to determine the exact frequency.

Our study also did not reveal any differences in clinical outcome in the patient cohort with respect to the status of MYC IHC expression. Patients with both high and low MYC expression treated with similar regimens did equally well with no disease relapse. An interesting finding is the demographics of our patients who had high MYC protein expression. These patients were significantly younger than those with low MYC expression (26.9 vs 43.6 years; $P = .0013$). The significance of this finding is unknown but does not appear to affect overall survival.

In contrast to BL and DLBCL, the utility of MYC IHC expression as a screening tool for MYC gene translocation does not appear to be clinically or prognostically applicable in PMBL. Since the finding of DLBCL with MYC translocation has high prognostic impact and benefits from more aggressive chemotherapy than conventional DLBCL, the use of MYC IHC is warranted given the relative good
correlation with gene status. In PMBL, however, MYC IHC expression is variable and usually low, and high MYC expression does not appear to equate to actionable MYC genetic abnormalities by FISH, and there is no significant impact on clinical outcomes.

Address reprint requests to Dr Salama: ARUP Laboratories, 500 Chipeta Way, MS115-G04, Salt Lake City, UT 84108; mohamed.salama@path.utah.edu.

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