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## Comparison of evaluation techniques, including digital image analysis, for MYC protein expression by immunohistochemical stain in aggressive B-cell lymphomas

Meghan Hupp, M.D.<sup>1</sup>, Sarah Williams, M.D.<sup>1</sup>, Brian Dunnette<sup>1</sup>, Katelyn M. Tessier, M.S.<sup>2</sup>, and Elizabeth L. Courville, M.D.<sup>1</sup>

<sup>1</sup>Department of Laboratory Medicine and Pathology, University of Minnesota, 420 Delaware St SE, MMC 609, Minneapolis, MN, 55455

<sup>2</sup>Masonic Cancer Center Biostatistics Core, University of Minnesota, 420 Delaware St SE, MMC 806, Minneapolis, MN 55455.

### Abstract

Incorporation of a MYC immunohistochemical stain in the work-up of large B-cell lymphomas has become common in hematopathology practice. Evaluation of this stain can be difficult due to staining heterogeneity and can have inter-observer variability, particularly when performed on entire tumor sections. We identified 87 cases of aggressive B cell lymphoma (34 core needle and 53 excisional biopsies) and compared the following methods of MYC immunohistochemical staining evaluation: the original pathologist's interpretation, a systematic retrospective method of evaluation by manual analysis, and a retrospective method of evaluation by digital image analysis (using scanned slides analyzed via the Aperio Nuclear algorithm). Overall, concordance among these methods was around 80% with kappa statistics showing good agreement. However, nearly one-third of our cases had a percent MYC positivity in the 30% to 50% range and, for these cases, concordance among the various methods was marginal/poor. This suggests limited utility as a prognostic or predictive marker using 40% as a cut-off value. In our series, core biopsy specimens were poor predictors of MYC gene rearrangement and there was no association between MYC immunohistochemical stain and MYC gene gain/amplification. Our retrospective digital image analysis showed strong correlation in MYC percent positivity with our retrospective manual review (correlation coefficient of 0.90) and similar concordance to pathologist interpretation as among pathologists, suggesting digital image analysis is a viable alternative to manual determination of MYC percent positivity. Digital image analysis provides further opportunities for more sophisticated and standardized scoring systems, which may be helpful in future prognostic/predictive studies.

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**Corresponding author:** Elizabeth L. Courville, MD, Department of Laboratory Medicine and Pathology, 420 Delaware St SE, MMC 609, Minneapolis, MN 55455, [ecourvil@umn.edu](mailto:ecourvil@umn.edu).

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## Keywords

MYC; diffuse large B-cell lymphoma; DLBCL; high-grade B-cell lymphoma; digital image analysis; prognosis; immunohistochemistry

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## 1. Introduction

Rearrangement of the *MYC* gene is well described in aggressive B-cell lymphomas including Burkitt lymphoma (nearly all cases) and diffuse large B-cell lymphoma (8-14% of cases)(1). A higher proportion of diffuse large B-cell lymphomas (DLBCL) show *MYC* protein expression (detected in approximately 30% of cases) than *MYC* gene rearrangement (2, 3). Translocation of the *BCL2* gene occurs in 20-30% of DLBCL cases and rearrangement of the 3q27 region involving *BCL6* is seen in up to 30% of DLBCL (1). Cases of DLBCL or cases with morphologic features of both Burkitt lymphoma and DLBCL (referred to in the 2008 World Health Organization classification as “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma”) and harboring a *MYC* (8q24) rearrangement in combination with a *BCL2* (18q21) and/or a *BCL6* (3q27) rearrangement have a relatively low complete response rate and short overall survival with R-CHOP or comparable therapies. These lymphomas, sometimes referred to as “double-hit” and “triple-hit” lymphomas, are separately classified according to the 2016 World Health Organization (WHO) classification as “high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements.” Dual positivity by immunohistochemical stains for *MYC* and *BCL2* in DLBCL (double-expression of *MYC* and *BCL2* proteins) is associated with inferior survival in most studies (2-4). Such double-expression may be a prognostic marker but does not warrant separate classification according to the 2016 WHO classification.

As *MYC* and *BCL2* protein expression may have prognostic significance and there is theoretical use for *MYC* immunohistochemical staining to triage which aggressive B-cell lymphomas require genetic studies to evaluate for double- or triple-hit, the incorporation of a *MYC* immunohistochemical stain has become common in hematopathology practice. After the standard validation process for a new immunohistochemical stain (5), our institution began offering the *MYC* immunohistochemical stain with a recommended cut-off ( 40% as positive) in early 2015. Unfortunately, immunohistochemical evaluation of this stain can have inter-observer variability, particularly when performed on entire tumor sections by a diverse group of pathologists (6). Digital image analysis is an emerging reproducible method of quantifying positivity by immunohistochemical stains (7-9).

We studied cases of aggressive B-cell lymphoma (Burkitt lymphoma, diffuse large B-cell lymphoma, and high-grade B-cell lymphoma) with the *MYC* immunohistochemical stain performed as part of routine clinical care. Our aims were (1) to compare multiple methods of *MYC* immunohistochemical staining evaluation (the original pathologist’s interpretation, a systematic retrospective method of evaluation by manual analysis, and a systematic retrospective method of evaluation by digital image analysis) and (2) to assess the ability of

MYC immunohistochemical staining to predict a *MYC* gene rearrangement or *MYC* gene gain/amplification.

## 2. Materials and Methods

Our institutional review board approved this retrospective study. We used a Sunquest CoPathPlus (version 6.1.1) database search to identify cases of large B-cell lymphoma with a MYC immunohistochemical stain performed as part of routine clinical care from the time the stain was made available in March 2015 through December 31, 2016. During this time period, the MYC immunohistochemical stain was not used as a triaging tool for cytogenetic analysis at our institution. The MYC immunohistochemical stain (clone Y69; dilution 1:229; Abcam, Cambridge, MA, USA) was performed using the standard procedures of our immunohistochemistry laboratory at the time. The original pathologist's interpretation of MYC staining (positive, negative, or equivocal) was obtained from pathology reports and is referred to as "original IHC interpretation" in the Results section. The results of cytogenetic studies (fluorescence in-situ hybridization, FISH), performed using standard methods for our laboratory at the time of biopsy, were recorded when performed on the same tissue sample as the immunohistochemical stain. A *MYC* breakapart probe was used (Vysis LSI MYC dual color break apart rearrangement probe, Abbott Molecular, Abbott Park, Illinois, USA) to determine *MYC* rearrangement and gain/amplification status. Gain/amplification of the *MYC* gene for the purposes of this study was defined as greater than 2 (>2) *MYC* fusion signals. We evaluated the hematoxylin-and-eosin (H&E) and MYC immunohistochemistry stained slides for sufficient tissue and quality of specimen/staining with any cases with insufficient tissue or missing/unavailable slides excluded. Up to ten areas on each H&E slide, measuring approximately 1 mm x 1 mm, were manually marked with a dotting pen. Areas with the best quality and most neoplasm-rich tissue were selected while areas with crush artifact, fibrosis, and necrosis were avoided. The corresponding areas on the MYC stained slides were also manually marked. Figure 1 illustrates the methods of retrospective review.

For the retrospective review by digital image analysis, an H&E and MYC immunohistochemical slide for each case were scanned at 40x using an Aperio ScanScope XT whole slide scanner (Leica Biosystems, Buffalo Grove, IL), including on-slide control tissue. These were converted to digital images and stored on a password-protected database using Aperio eSlide Manager (version 12.3, Leica Biosystems). The MYC stained slides were annotated using Aperio ImageScope viewing software (version 12.3, Leica Biosystems). The ruler tool was used to measure 1 mm x 1 mm squares in each area previously marked by dotting pen on the slide, then the free-hand pen tool or rectangle tool were used to annotate areas for analysis. The annotations on each MYC stained slide were analyzed using the Aperio Nuclear algorithm (version 9.2, Leica Biosystems), with no manipulation to the algorithm. Default settings for the nuclear algorithm were used ("Nuclear Algorithm, User's Guide" *Leica Biosystems*, MAN-0338, Revision 8; August 5, 2015). All data was saved individually by case, and the numbers of negative, 1+, 2+, and 3+ nuclei were specifically collated. We did not utilize the negative versus positive (1+ through 3+) pixel count for this study.

For the manual retrospective review, a university based hematopathologist reviewer (pathologist #1) graded the percent of cells with positive nuclear staining for each area previously marked by dotting pen on the MYC stained slide. The percentage of nuclei with brightly positive staining was recorded as well as the percentage of nuclei with any staining (that is, dim and bright alike). An average from all marked areas (up to 10 per slide) was calculated to obtain separate percent positive staining values for dim staining and any staining. When the final value for percent positivity fell between 30% and 50% by manual retrospective review, a second university based hematopathologist reviewer (pathologist #2) performed the same grading process. On-slide control tissue and the corresponding H&E stained tissue slide were evaluated as needed. Pathologists #1 and #2 were blinded to the retrospective digital image analysis, which was performed by a third pathologist.

To test the association between *MYC* gene status and variables of interest, Wilcoxon rank-sum tests and Fisher exact tests were performed, as appropriate. Correlation was evaluated using Spearman correlation coefficients and t-tests. Concordance was evaluated using the kappa statistic(10). Specificities and sensitivities were calculated. All reported p-values are two-sided. Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC) and R version 3.4.0 (<http://www.R-project.org/>).

### 3. Results

#### 3.1. General characteristics

Our cohort consisted of 87 cases: 72 (83%) DLBCL or high-grade B-cell lymphoma (as defined by 2016 WHO classification), 12 (14%) monomorphic PTLD, and 3 (3%) Burkitt lymphoma (Figure 2). Patients ranged in age from 3 to 93 years old at the time of biopsy with an average age of 58. More of the biopsies were from female than male patients (50 female: 37 male). Sampled sites included: lymph node (n=34), soft tissue and bone (26), oropharynx/sinonasal (7), gastrointestinal tract (4), liver (4), brain (4), gonadal (3), and one each from salivary gland, breast, omentum, thyroid, and lung. Thirty-four cases were core biopsies and 53 were excisional biopsies. For the manual retrospective review and retrospective review by digital image analysis, 32 samples had less than the goal of 10 areas evaluated, of these, 19 were core biopsies and 13 were excisional biopsies. For these 32 samples, an average of 5.2 areas were evaluated.

For each case, the original IHC interpretation was made as part of routine patient care by one of twelve different pathologists - six fellowship-trained hematopathologists practicing at a university hospital and six community-based pathologists (some with hematopathology fellowship training). The majority of cases were originally signed out by the university-based hematopathologists (72%) with the remainder signed out by community-based pathologists. We saw no pattern differences in MYC interpretation between the university hospital and community based pathologists (data not shown).

#### 3.2. Distribution and correlation of percent MYC positivity

Figure 3 illustrates the distribution of percent MYC positivity by retrospective manual review (pathologist #1) and retrospective digital image analysis for any staining and 1+ to

3+, respectively. See Supplemental Material for the distribution of bright staining only by retrospective manual review and 2+ to 3+ by digital image analysis. It can be seen there is a wide spectrum of MYC percent positivity by both methods. There was a strong positive correlation for MYC percent positivity between manual retrospective review by pathologist #1 and retrospective review by digital image analysis with a Spearman correlation coefficient of approximately 0.90 ( $p < 0.01$ ) for all comparisons (manual any staining, manual bright staining versus digital image analysis 1+ to 3+, digital image analysis 2+ to 3+). A representative scatterplot is shown in Figure 4. When we restricted analysis to the 22 cases with a percent MYC positivity between 30% and 50% (as determined by manual retrospective review by pathologist #1), the correlation to retrospective review by digital image analysis was reduced with a Spearman correlation coefficient of 0.438, and there was no correlation between pathologist #1 and pathologist #2 (Spearman correlation coefficient of 0.236,  $p = 0.291$ ).

### 3.3. Concordance among methods of MYC immunohistochemical staining evaluation

The original sign-out pathologist interpreted the MYC immunohistochemical stain as positive in 41 cases (47%), negative in 37 (43%), and equivocal in 8 (9%), with an interpretation not given in 1 (1%). Using a cut-off of 40% as positive, 51 of the 75 cases (68%) deemed sufficient material for evaluation by the manual retrospective method were positive for any nuclear staining and 30 of the 75 cases (40%) were positive for bright nuclear staining. Twelve cases were deemed insufficient material to evaluate by the manual retrospective method but were evaluated by retrospective digital image analysis. Using a cut-off of 40% as positive, forty of the 87 cases (46%) evaluated by retrospective digital image analysis were positive when 1+ through 3+ nuclear positivity was interpreted as positive and 12 of the 87 cases (14%) evaluated by retrospective digital image analysis were positive when 2+ through 3+ nuclear positivity was interpreted as positive.

Concordance values among the three methods of MYC immunohistochemical stain evaluation (comparing interpretation of “positive” or “negative”) are shown in Table 1. There was excellent concordance between the retrospective manual review and the digital image analysis on the core biopsy specimens; there was good concordance between all other comparisons. When evaluation was limited to the subset with staining in the 30-50% range (all specimens), concordance values were 50%, 60%, and 45% respectively for original IHC interpretation to manual retrospective review, original IHC interpretation to retrospective review by digital image analysis, and retrospective manual review to retrospective review by digital image analysis. Corresponding kappa scores were 0.138, 0.130, and 0.137, respectively, indicating marginal/poor agreement. There was marginal/poor agreement between pathologist #1 and pathologist #2 for the subset of cases staining in the 30-50% range.

### 3.4. MYC immunohistochemical stain versus MYC gene status

Sixty-one cases were evaluated by FISH for *MYC* gene rearrangement on the same tissue sample as the MYC immunohistochemical stain. Fourteen of 61 tested cases (23%) had a *MYC* rearrangement by FISH analysis. A summary of MYC immunohistochemical staining (as evaluated by the various methods) compared to the presence or absence of a *MYC* gene

rearrangement in excisional biopsy specimens is presented in Table 2. There was a significant association between MYC immunohistochemical staining and *MYC* gene rearrangement status for all methods when assessing excisional biopsy specimens alone and when assessing all specimens together (core and excisional biopsies,  $p < 0.01$ ). However, when evaluation was restricted to core biopsy specimens only (24 cases evaluated by FISH, 5 with *MYC* gene rearrangement detected), no significant results of association were found. A significant association was not identified between any of the variables and *MYC* gene gain/amplification. Using a cut-off of 40% as positive, Table 3 shows the sensitivity and specificity for the various methods of MYC immunohistochemical staining evaluation in predicting *MYC* gene rearrangement by FISH analysis. In Table 3, data for core biopsies and excisional specimens are presented together (“All Specimens”) and separately.

#### 4. Discussion

Original reports in the literature on interpretation of the MYC immunohistochemical stain reported high concordance rates among pathologists; however, those studies were based on tissue microarrays and/or review among few pathologists at single institutions (2, 11-13). Subsequent studies have shown less concordance among pathologists when whole tissue sections were scored by a diverse group of pathologists (6, 14). Scoring of the MYC immunohistochemical stain can be particularly difficult due to both staining heterogeneity between areas on whole tissue sections and heterogeneity of staining intensity. There is currently no guidance on how to best interpret staining heterogeneity. In our study, we used a systematic approach to retrospective review in an attempt to compensate for staining heterogeneity. In addition, we used digital image analysis as an alternative, and perhaps less biased, method of evaluating MYC immunohistochemical staining (15) (16). The percentage of MYC positivity between our manual retrospective review and retrospective review by digital image analysis showed strong correlation (Spearman correlation coefficients of approximately 0.90 for all comparisons). However, when the data was dichotomized to positive or negative for MYC based on a cut-off of 40%, concordance between the three methods of evaluation (original IHC interpretation, manual retrospective review, and retrospective review by digital image analysis) was only around 80%, with Kappa scores showing good agreement. This concordance is similar to that reported among nine different pathologists in Mahmoud, et al (6). Interestingly, concordance for the core biopsy specimens between the retrospective manual review and retrospective review by digital image analysis in our study was excellent (96%, kappa score 0.915), perhaps due to fewer areas evaluated in these specimens or better fixation of the specimen.

In our series, core biopsy specimens were poor predictors of *MYC* gene rearrangement. When we evaluated the core biopsy specimens in isolation, no significant results of association were found between MYC immunohistochemical stain and *MYC* gene rearrangement, and sensitivities using a cut-off of 40% positivity were only 0.50 to 0.80 by the various methods used for evaluation. The sensitivity of excisional biopsy specimens was excellent by the original pathologist’s interpretation and by manual retrospective review, at the expense of specificity (sensitivities of 1.0 and specificities of 0.63 and 0.33, respectively). The drastic decrease in specificity when evaluated by manual retrospective review (performed in a more systematic fashion and not influenced by morphology and



results of additional immunohistochemical stains) suggests that, in a clinical setting, our pathologists used other clues to predict a *MYC* gene rearrangement, such as clinical behavior, Ki-67 proliferation index, and aggressive morphology. Alternatively, this could represent the individual bias of the retrospective pathologist reviewer to overestimate percent positivity in this setting. It has been recommended in the literature that *MYC* immunohistochemical staining should not be used as the sole method of *MYC* status evaluation (14). Our study findings strongly support this conclusion for core biopsy specimens. For excisional biopsy specimens, *MYC* immunohistochemical stain may be a triaging tool if used with great caution. Correlating *MYC* immunohistochemical stain results with morphologic and immunohistochemical features suggestive of Burkitt lymphoma or high grade B-cell lymphoma is invaluable. We found no association between *MYC* immunohistochemical stain and *MYC* gene gain/amplification, although we may not have had enough cases to identify an association.

In our study, digital image analysis had a similar sensitivity for *MYC* rearrangement to the original pathologist's interpretation and the retrospective manual interpretation with a similar specificity to the original pathologist's interpretation when all specimens were evaluated, although it did have a slightly lower sensitivity (0.89) when core biopsy specimens were excluded. The concordance between retrospective review by digital image analysis and the original IHC interpretation was similar to the previously reported concordance among pathologists and the correlation between our manual retrospective review and retrospective review by digital image analysis was strong(6). These findings indicate that digital image analysis performs comparably to manual analysis, and therefore is a viable alternative to manual analysis for interpretation of *MYC* immunohistochemical staining. Our method used the Aperio Nuclear algorithm without manipulation, a strategy that would need confirmation across other institutions. Advantages to digital image analysis include the possibility of a more standardized approach to interpretation of the immunohistochemical stain, the opportunity for a more sophisticated scoring system (for example, taking into account the relative distribution of nuclei with different staining intensities) and the ability of the interpretation to occur at a central location(7). Disadvantages include the processing power and time required for scanning and analyzing the slides. Our approach to digital image analysis still required manual input to identify viable areas of neoplastic tissue. Applying the nuclear algorithm to the whole slide without manual input would include non-malignant cells (endothelial cells, non-neoplastic lymphocytes, fibroblasts, etc.) in the analysis and introduce artifact from crush, overstaining, tissue folds, and degenerating neoplastic cells.

Beyond its use as a potential triaging tool, expression of *MYC* protein as determined by immunohistochemical staining is a potential prognostic and/or predictive marker. Double expression of *MYC* and *BCL2* proteins in diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) is associated with inferior survival in most studies (2, 4, 12, 17-21). Most studies, but not all, use the cut-off of 40% as indicative of *MYC* positivity or "overexpression." In our cohort, nearly one-third of our cases had a percent *MYC* positivity surrounding the cut-off of 40% and, for these cases, our concordance values among the various methods used for evaluation was marginal/poor. This suggests there is limited utility of this stain as a prognostic or predictive marker using 40% as a cut-off value without further

evaluation guidelines. Tsuyama *et al.* performed a systematic review of BCL2 immunohistochemistry using a scoring system for staining of 0 to 3+, dependent on proportion of cells staining and intensity of staining, and found that a BCL2 3+ score was a significant prognostic factor (22). A similar scoring system may prove to be valuable for MYC immunohistochemical stain interpretation. Digital image analysis may be a robust method to develop a more sophisticated scoring system, standardize the interpretation, and allow for centralized review. Our study was not designed to evaluate clinical outcome; however, we feel that our study demonstrates comparable performance of digital image analysis to manual review, therefore suggesting that future studies can incorporate digital image analysis. Clear guidelines for evaluation of MYC immunohistochemical staining interpretation is recommended for future studies regarding its prognostic or predictive value.

To summarize, our results suggest that the MYC immunohistochemical stain should not be used as a triaging tool for the *MYC* gene rearrangement in core biopsy specimens. For excisional biopsy specimens, there is potential use as a triaging tool with the caveat that the sensitivity is not 100% (as per the literature and our experience during validation of the MYC immunohistochemical stain in our laboratory) and the acknowledgement that there is a low specificity. Results of the MYC immunohistochemical stain should always be correlated with morphologic and clinical findings and results of other immunohistochemical stains. If the MYC immunohistochemical stain is to be used as a prognostic or predictive marker, clear guidelines need to be developed delineating the best method of stain interpretation for this purpose, and digital image analysis should be investigated as a potential method of stain interpretation..

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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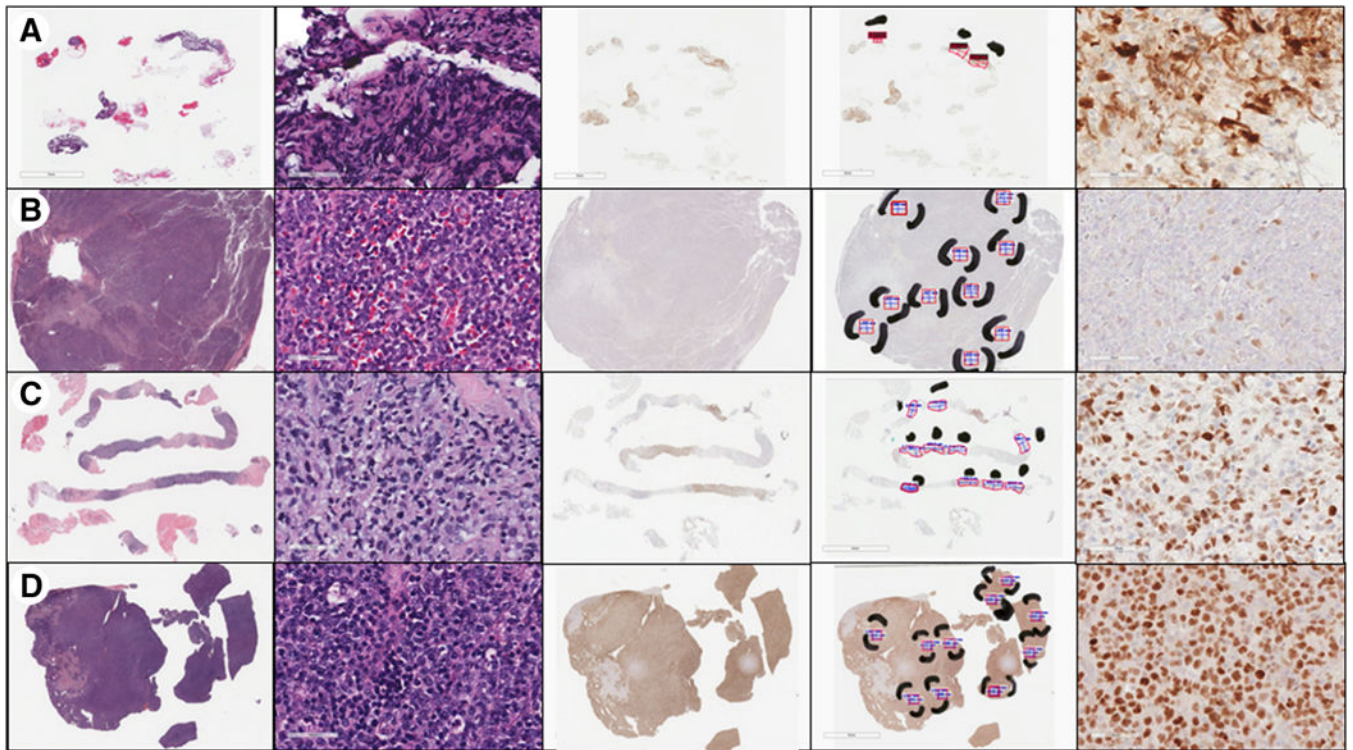
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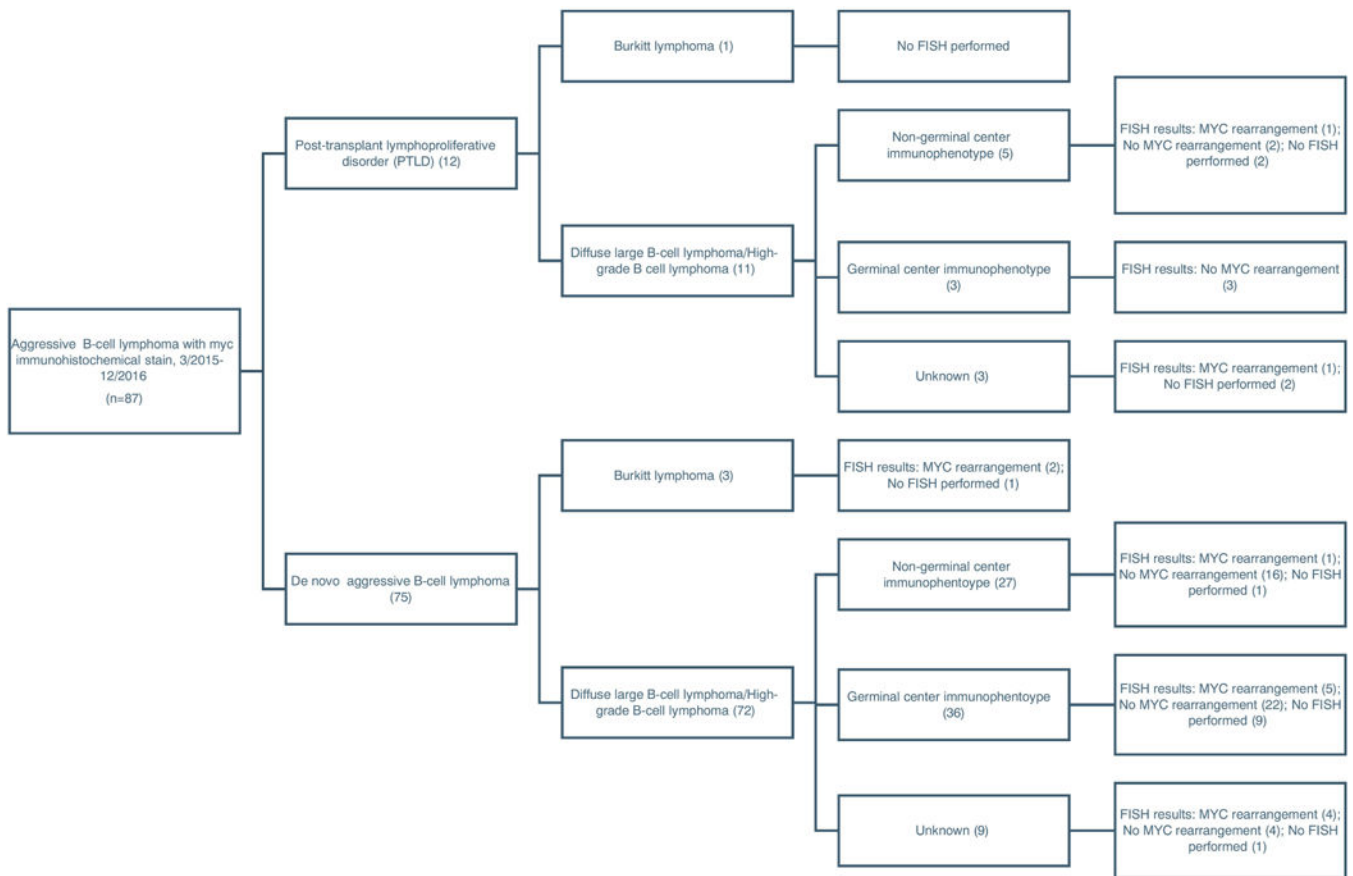
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- MYC IHC on core biopsies is a poor predictor of *MYC* rearrangement.
- The MYC IHC stain has limited prognostic utility without further guidelines.
- Digital image analysis (DIA) is a viable alternative method of MYC IHC evaluation
- DIA may be especially useful in future prognostic/predictive studies.

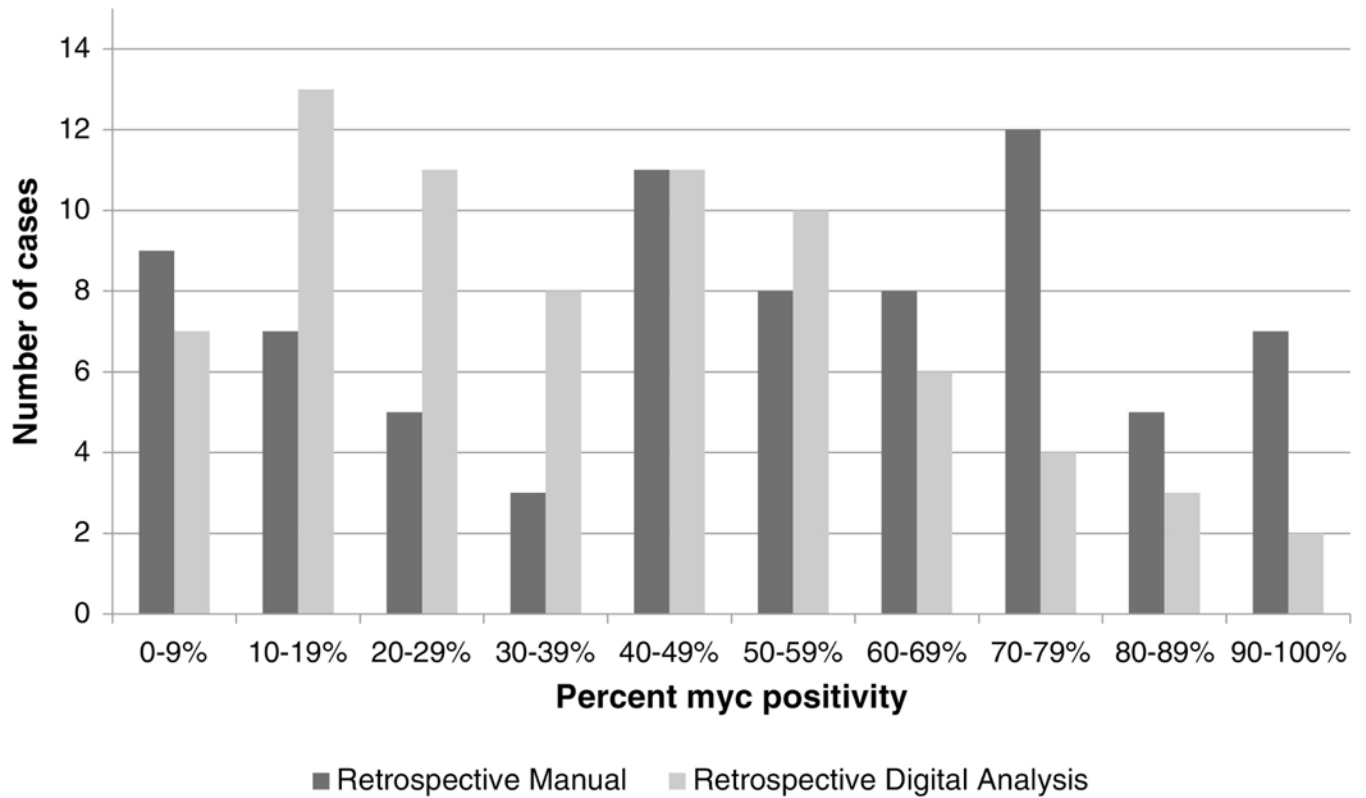


**Figure 1:**

Images from representative cases showing (row A) insufficient material for review, (row B) negative MYC immunohistochemical (IHC) staining, (row C) equivocal MYC IHC staining (percent positivity in 30-50% range), and (row D) positive MYC IHC staining. The first column shows whole-slide images of the hematoxylin and eosin (H&E) stained slide. The second column shows a portion of the H&E slide at 400x magnification, in one of the selected areas of best tissue. The third column demonstrates whole-slide images of the MYC IHC slide. The fourth column shows the Aperio ImageScope annotations on the MYC IHC slide, with black marking pen indicating the areas of best quality tissue selected as the areas to evaluate. The red boxes select tissue to analyze, while the text and ruler icons in blue measure the area selected, with the goal of 1 mm<sup>2</sup> in each area. The fifth column shows the MYC IHC slide at 400x magnification, within one of the evaluation areas.

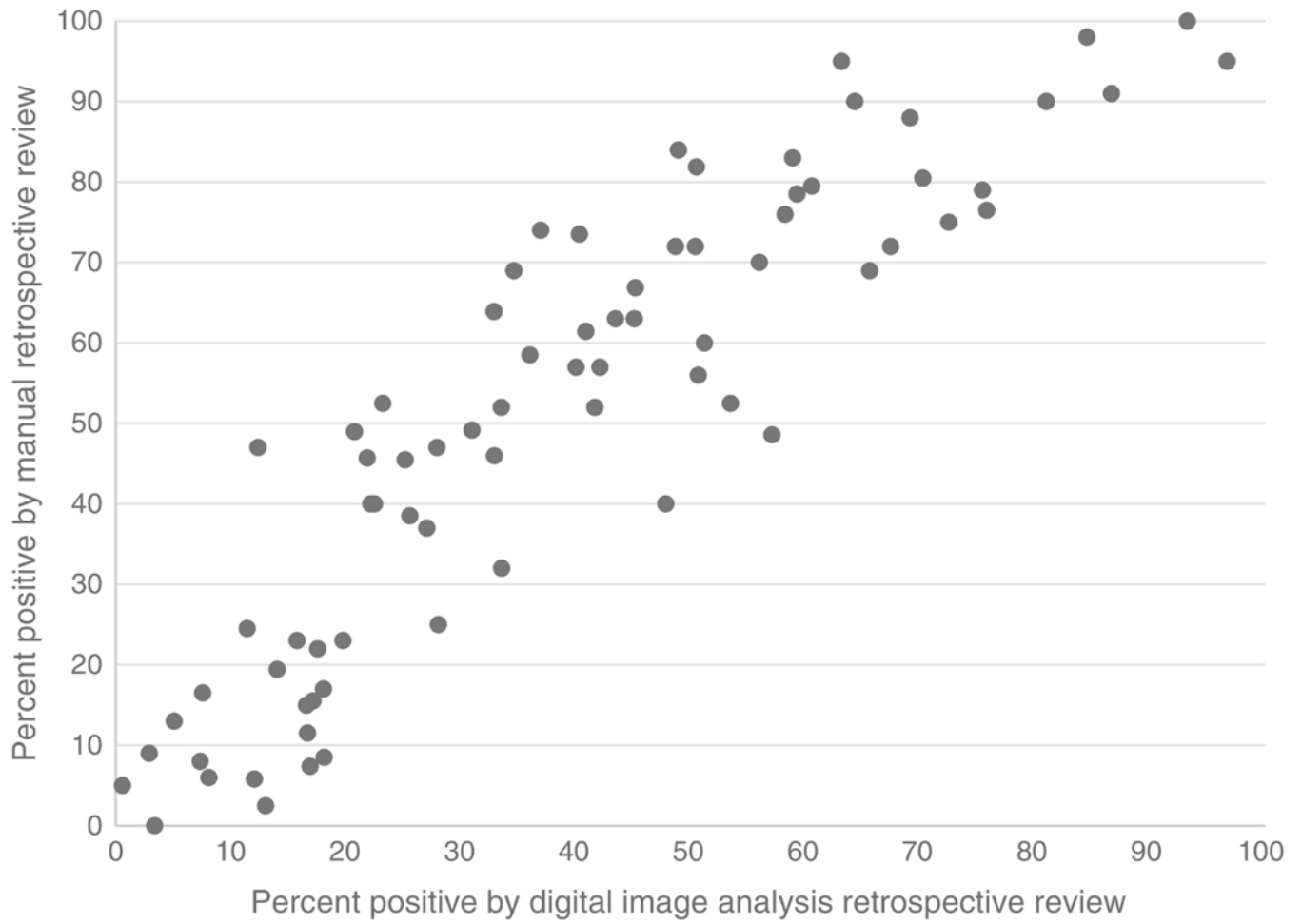


**Figure 2:** Flow chart outlining the classification of aggressive B-cell lymphomas included in our study.



**Figure 3:** Distribution of percent MYC positivity by retrospective manual review (pathologist #1) and retrospective digital image analysis. Represented in this figure are the percentages using any nuclear staining (retrospective manual) and 1+ to 3+ nuclear positivity (retrospective digital analysis). Both the manual and digital analysis methods show wide variability in percent MYC positivity, including many cases surrounding the suggested cut-off of 40%. The manual method showed a bias toward higher estimation of MYC percent positivity, while the digital analysis method showed a bias toward lower estimation of MYC percent positivity.





**Figure 4:** Scatterplot demonstrating correlation between retrospective manual review of MYC immunohistochemistry positivity and digital review by Aperio ImageScope with nuclear algorithm (Spearman correlation coefficient of 0.92).

**Table 1:**Concordance Among Methods of MYC Immunohistochemical Stain Evaluation<sup>1</sup>

	Concordance	Cohen Kappa Statistic	Agreement <sup>2</sup>
Original IHC interpretation to manual retrospective review (n=69)	80%	0.588	Good
Original IHC interpretation to retrospective review by digital image analysis (n=78)	81%	0.616	Good
Retrospective manual review to retrospective review by digital image analysis (n=75)	80%	0.606	Good
<b>Core Biopsy Only</b>			
Original IHC interpretation to manual retrospective review (n=23)	87%	0.738	Good
Original IHC interpretation to retrospective review by digital image analysis (n=30)	80%	0.598	Good
Retrospective manual review to retrospective review by digital image analysis (n=24)	96%	0.915	Excellent
<b>Excisional Biopsy Only</b>			
Original IHC interpretation to manual retrospective review (n=46)	76%	0.513	Good
Original IHC interpretation to retrospective review by digital image analysis (n=48)	81%	0.626	Good
Retrospective manual review to retrospective review by digital image analysis (n=51)	73%	0.474	Good

<sup>1</sup>Concordance evaluated using any nuclear staining by manual retrospective review and 1+ through 3+ nuclear positivity by digital image analysis to determine percent myc positivity, and using a cut-off of 40% as positive.

<sup>2</sup>As defined by Le (10).

**Table 2.**

MYC immunohistochemical staining versus *MYC* gene rearrangement status (Excisional biopsy specimens only)

	<i>MYC</i> gene rearrangement (N=9) <sup>I</sup>	No <i>MYC</i> gene rearrangement (N=28) <sup>I</sup>	P- value
Myc immunostain percent positivity by:	Median (25%, 75%)	Median (25%, 75%)	
Digital image analysis (1++-3+)	65.6 (63.1, 80.9)	29.5 (14.7, 43.4)	0.001
Digital image analysis (2++-3+)	30.7 (19.3, 51.3)	14.0 (5.3, 23.8)	0.02
Manual retrospective (any staining)	90.0(81.9, 91.0)	48.6 (23.0, 63.9)	0.0005
Manual retrospective (bright staining)	67.5 (38.1, 85.0)	25.0 (9.0, 42.8)	0.009
Original pathologist's interpretation of myc staining:	Number (percentage)	Number (percentage)	0.002
Positive	9 (100)	9 (32.1)	
Negative	0 (0.0)	15 (53.6)	
Equivocal	0 (0.0)	4 (14.3)	

<sup>I</sup>Overall N may vary by variable.

**Table 3:**

Sensitivity and specificity of the various methods of MYC immunohistochemical stain interpretation for *MYC* rearrangement determination

<b>Original IHC Interpretation</b>				
	Sensitivity		Specificity	
All Specimens (n=54)	0.93		0.65	
Germinal Center Subtype (n=28)	0.80		0.70	
Non-germinal center subtype (n=16)	1.00		0.57	
Core (n=21)	0.80		0.69	
Excision (n=33)	1.00		0.63	
<b>Manual Retrospective Review<sup>1</sup></b>				
	Sensitivity		Specificity	
	Any	Bright	Any	Bright
All Specimens (n=55)	0.92	0.62	0.40	0.69
Core (n=19)	0.75	0.50	0.53	0.67
Excision (n=36)	1.00	0.67	0.33	0.70
<b>Retrospective Review by Digital Image Analysis<sup>2</sup></b>				
	Sensitivity		Specificity	
	Any	Bright	Any	Bright
All Specimens (n=61)	0.86	0.50	0.66	0.98
Core (n=24)	0.80	0.60	0.63	1.00
Excision (n=37)	0.89	0.44	0.68	0.96

Note: Of the 61 cases with FISH analysis, 6 were deemed insufficient to evaluate by retrospective manual review and 7 were deemed equivocal by original pathologist's interpretation, accounting for varying "n".

<sup>1</sup>For the manual retrospective review, "Any" refers to MYC ihc percent positivity coming from nuclear staining of any intensity (bright or dim) and "Bright" refers to MYC ihc percent positivity coming from nuclear staining of only bright intensity.

<sup>2</sup>For the digital image analysis, "Any" refers to MYC ihc percent positivity coming from 1+ through 3+ nuclear positivity and "Bright" refers to MYC ihc percent positivity coming from 2+ through 3+ positivity.