ORIGINAL ARTICLE

CD71 by flow cytometry as a diagnostic marker to differentiate between aggressive and indolent B-cell lymphomas

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ABSTRACT

Introduction: Flow cytometry is important in diagnosis and classification of mature B-cell neoplasms; however, differentiating indolent and aggressive lymphomas by flow cytometry can be challenging. The transferrin receptor CD71 mediates cellular iron uptake; an essential element of proliferation. This study looks at CD71 expression by flow cytometry in B-cell lymphomas to assess its use in differentiating between aggressive and indolent lymphomas.

Methods: From January to October 2018 results from flow cytometry, including CD71, were collected and correlated with the histopathologists' final diagnosis of 122 clonal B-cell neoplasms. The expression of CD71 by flow cytometry and the median fluorescent index (MFI) was determined. When results of Ki-67 index performed by immunohistochemistry (IHC) on tissue samples were available, these were correlated with CD71 MFI. The mean expression of CD71 for each histological diagnosis was defined. Statistical significance was assessed using the Student t-test, Pearson and Spearman coefficients, and by ROC curve analysis.

Results: The expression of CD71 correlated with a histological diagnosis of aggressive lymphomas particularly diffuse large B-cell lymphoma (DLBCL). Correlation between CD71 by flow cytometry and Ki-67 index showed a positive relationship between MFI and a high Ki-67 index.

Conclusions: CD71 by flow cytometry has the potential to contribute to the classification of lymphomas with correlations between the expression of CD71 and the diagnosis of aggressive B-cell lymphoma and between CD71 expression and the Ki-67 index.

Keywords: CD71, lymphoma, flow cytometry, Ki-67, Diffuse large B cell lymphoma.

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INTRODUCTION

Lymphomas are the 6th most common cancer in Australia with an incidence of 23 in 100,000 in 2017 (1). The majority are B-cell lymphomas, ranging from indolent lymphomas such as follicular lymphoma to aggressive lymphomas such as DLBCL and Burkitt lymphoma (2). Flow cytometry has proven to be a fundamental adjunct to morphology and molecular studies in the diagnosis and classification of NHL (3-4). However, differentiating between indolent and aggressive lymphomas by flow cytometry can be challenging (5). Analysis of the proliferation marker Ki-67 by flow cytometry is possible but due to the intranuclear location of Ki-67, additional processing of the sample is required and it remains technically challenging (6-7).

The transferrin receptor 1, CD71 is a membrane glycoprotein whose role is to mediate uptake of iron into cells through the binding of transferrin. There is internalisation of the transferrin through receptor mediated endocytosis and release of iron from the protein by decreasing endosomal pH (8). CD71 is present on all proliferating cells as it is essential for transport of iron into the cells (5,8-9). CD71 is expressed by replicating cells of all haematopoietic lineages but not by mature resting lymphocytes, monocytes, granulocytes and erythrocytes (5,9). CD71 expression by flow cytometry in both acute myeloid and lymphoid leukaemia has been described (10-11). An association between CD71 expression and Ki-67 expression by flow cytometry has previously been demonstrated in stimulated T lymphocytes, both showing upregulation after 24 hours of stimulation (12).

Previous studies have examined CD71 expression by flow cytometry in B-cell lymphomas (5, 13-15). These have found that CD71 expression is more common in DLBCL and Burkitt lymphoma compared to follicular lymphoma with higher levels of expression in Burkitt lymphoma (5,13). Increased levels of CD71 were also found in Richter transformation of chronic lymphocytic leukaemia (CLL) and has been used as an indicator of progression in CLL (14,15).

The aim of this study was to examine the use of CD71 by flow cytometry as a diagnostic marker to help differentiate between aggressive and indolent B-cell lymphomas with the hypothesis that the expression of CD71 will correlate with more aggressive lymphomas. We also examined the correlation of CD71 expression by flow cytometry and the Ki-67 index by IHC on tissue samples to see if there is an association between these two markers.

METHODS

During the study period from January 2018 to October 2018, 126 patients with a clonal B-cell population on initial flow cytometric screening panels were identified. Subsequently, a comprehensive flow cytometry panel was performed with data gathered on the type of sample: blood, bone marrow, FNA or tissue. The results were then correlated with the pathologists' histology report or haematologists blood film or bone marrow reports for the final diagnosis and where available the proliferation index from Ki-67 stain on histology samples.

Flow cytometry method

The types of samples that were analysed included bone marrow aspirates, tissue samples, FNA samples, peripheral blood and body fluids. Samples were processed using the lyse-stain-wash method and lysed with an ammonia chloride solution followed by a washing step. The initial screening panels were used to detect evidence of a clonal B-cell disorder. These consisted of the screening B-cell panel composed of CD45 Krome Orange (KO), CD19 FITC, lambda PE, kappa APC, CD10 ECD and CD5 APC AF750 or the screening B and T-cell panel composed of CD19 FITC, lambda PE, kappa APC, CD10 ECD, CD4 PC7, CD8 PC5.5, CD56 APC AF700, CD5 APC AF750, CD3 Pac Blue and CD45 KO. If light chain restriction was demonstrated on the CD19 positive cells, the sample was further analysed with the confirmatory B-cell panel. This panel contains an antibody cocktail composed of CD71 FITC, CD200 PE, CD19 ECD, CD11c PC7, CD25 APC, CD45 KO, CD22 PC5.5, CD38 APC AF700, CD43 APC AF750 and CD20 Pac Blue (see Table 1 for antibody clones).

The samples were incubated with the antibody cocktails for 20 minutes and run on the Navios EX flow cytometer (Beckman Coulter Life Sciences, Indianapolis, IN, USA) and analysed using the Kaluza software (Beckman Coulter Life Sciences, Indianapolis, IN, USA). The MFI for CD71 on the clonal B-cell population was recorded. Samples were considered to express CD71 if >20% of the gated events were positive for CD71, depending on the position of the expression on the scatterplots (see Figure 1 for examples of scatterplots with fluorescence on the y-axis).

Correlation with histological diagnosis

The flow cytometry results were correlated with the final histology reports for tissue and FNA samples while those from blood and bone marrow were correlated with the final haematopathology report. If the histology report recorded a Ki67 index this was also used for correlation.

Statistics

Samples taken within the specified study period that were analysed with the panel containing CD71 were eligible for inclusion. Aggressive lymphomas were defined as Burkitt lymphoma, DLBCL and follicular lymphoma grade 3; other B cell NHL were counted as indolent lymphomas.

The median MFI of CD71 was assessed for the different diagnosis along with interquartile range. The expression CD71 by flow cytometry was compared with the final diagnosis and the Ki-67 index, where available. The relationship between the expression of CD71 and the Ki-67 index was measured by correlating the MFI of the samples with the Ki-67 index and analysed using the Pearson and Spearman coefficients.

A ROC curve analysis was also performed to assess the use of CD71 by flow cytometry in distinguishing between indolent and aggressive lymphomas.

RESULTS

During the study period, 126 samples with a clonal B-cell population were included in the study. Of these samples, three were excluded because on the final histological report was non-diagnostic and one was excluded as it was a non-haematological malignancy. The median MFI of CD71 expression of the different histological diagnoses is shown in Table 2 and Figure 2. Showing increased expression of CD71 in more aggressive lymphomas particularly DLBCL.

Of the 122 samples assessed, 64 had a Ki-67 index available by IHC. Of the remaining 58, the index was unavailable either because of the sample type (bone marrow, fine needle aspirate, etc) or unnecessary for routine diagnosis. The Ki-67 index was correlated with the MFI of CD71 by flow cytometry for the 64 samples (Figure 3). The Pearson coefficient for the correlation r=0.51 (P <0.001) and Spearman coefficient r=0.60 (P<0.001); showing a statistically significant correlation between the expression of CD71 and the Ki-67 index.

The ROC curve analysis for CD71 expression and the diagnosis of aggressive lymphomas is shown on (Figure 4). The AUC was 0.88 suggestive that this is a useful marker in helping differentiate between aggressive and indolent lymphomas, with increased expression of CD71 in aggressive lymphomas.

Table 1*:* Antibody clones used in the study

ANTIBODY	CLONE
CD45 Krome Orange (KO)	J33
CD19 FITC	HD37
Lambda PE	
Kappa APC	
CD10 ECD	ALB1
CD5 APC AF750	BL1a
CD19 FITC	HD37
CD4 PC7	SFCI12T4D11
CD8 PC5.5	B9.11
CD56 APC AF700	N901 (NKH-1)
CD3 Pac Blue	UCHT1
CD71 FITC	YDJ1.2.2
CD200 PE	OX-104
CD19 ECD	J3-119
CD11c PC7	BU15
CD25 APC	B1.49.9
CD22 PC5.5	SJ10.1H11
CD38 APC AF700	LS198.4.3
CD 43 APC AF750	DFT1
CD20 Pac Blue	B9E9 (HRC20)

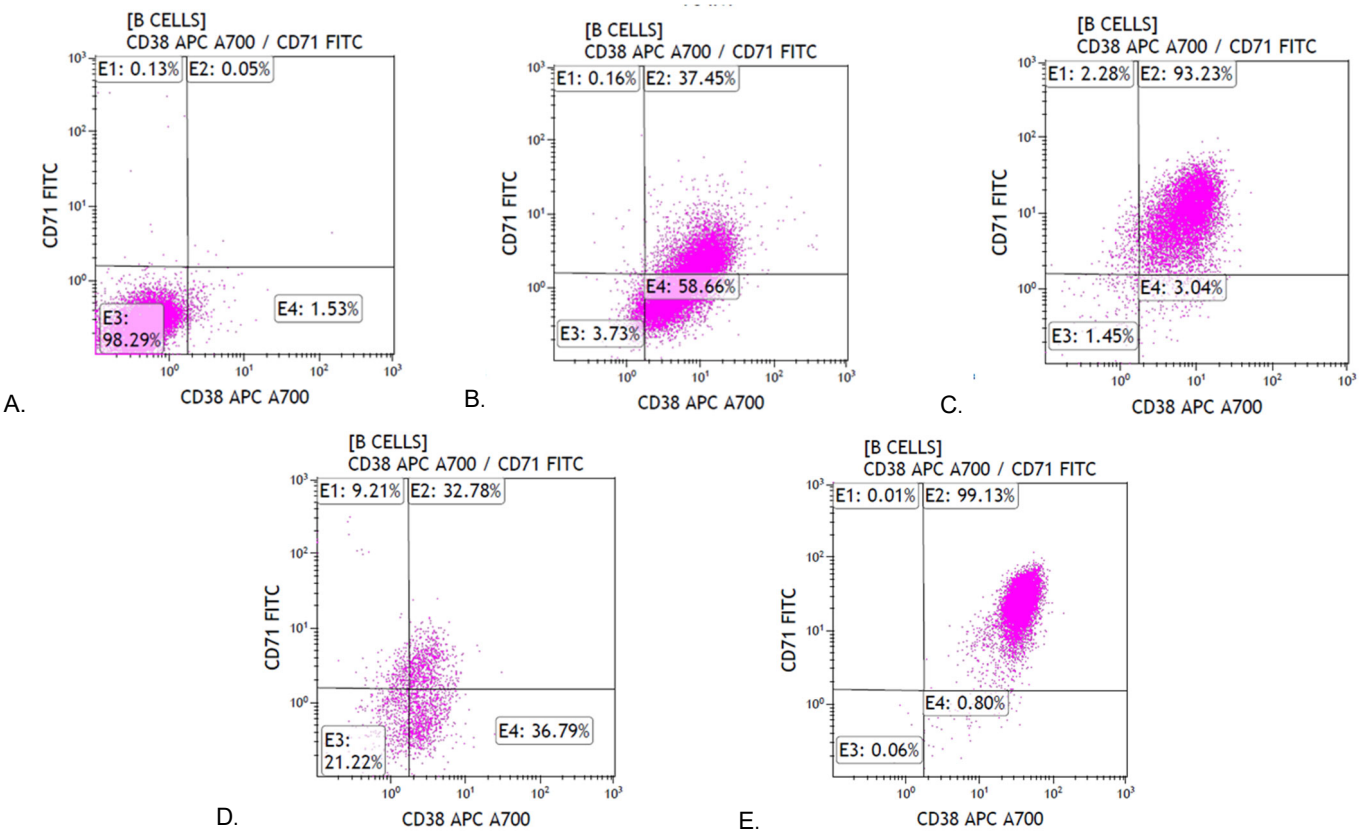

Figure 1. Examples of flow cytometry scatter plots used in the study showing various expressions of CD71.
A*. Negative CD71 expression. (SLL)* ***B****. Partial CD71 expression. (Follicular lymphoma grade 2)* ***C.*** *Positive CD71 expression. (DLBCL)* ***D.*** *Heterogeneous CD71 expression. (DLBCL)* ***E.*** *Bright CD71 expression. (Burkitt lymphoma)*

Table 2: Median MFI of CD71 expression by flow cytometry for the different histological diagnoses.

Diagnosis	MFI	IQR
Diffuse large B cell lymphoma	2.77	1.55-5.07
Follicular lymphoma (Grade 1-2)	0.68	0.54-0.99
Follicular lymphoma (Grade 3)	0.91	0.80-1.26
Chronic lymphocytic leukaemia/Small lymphocytic lymphoma	0.41	0.32-0.50
Marginal zone lymphoma	0.60	0.32-0.74
Lymphoplasmacytic lymphoma	0.36	0.31-0.41
Mantle cell lymphoma	0.71	0.64-0.78
Hairy cell leukaemia	0.81	0.74-0.87
Burkitt lymphoma	24.29	
Reactive	0.54	0.37-0.71

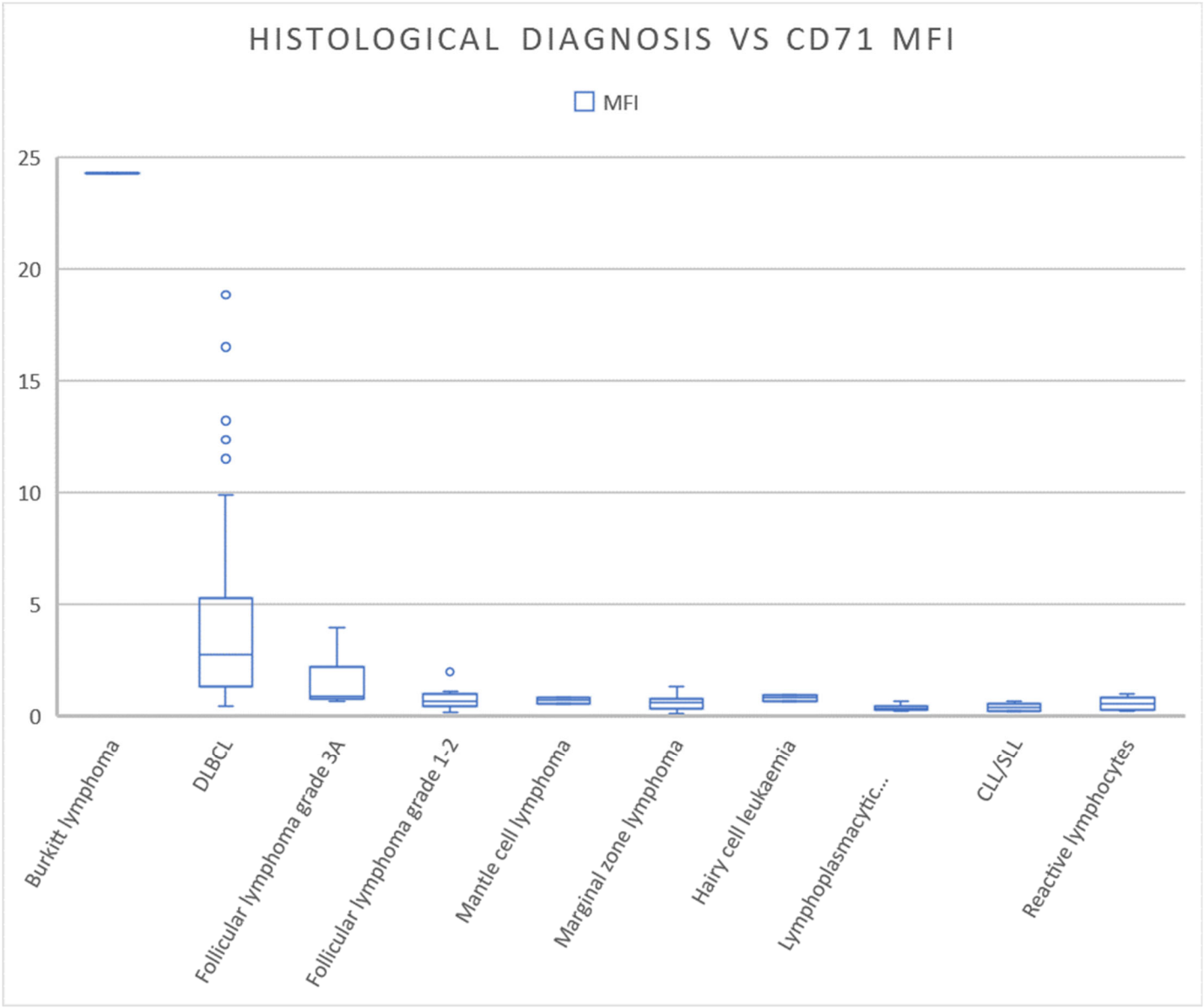

Figure 2: Box and whisker plot of the MFI of the different diagnoses

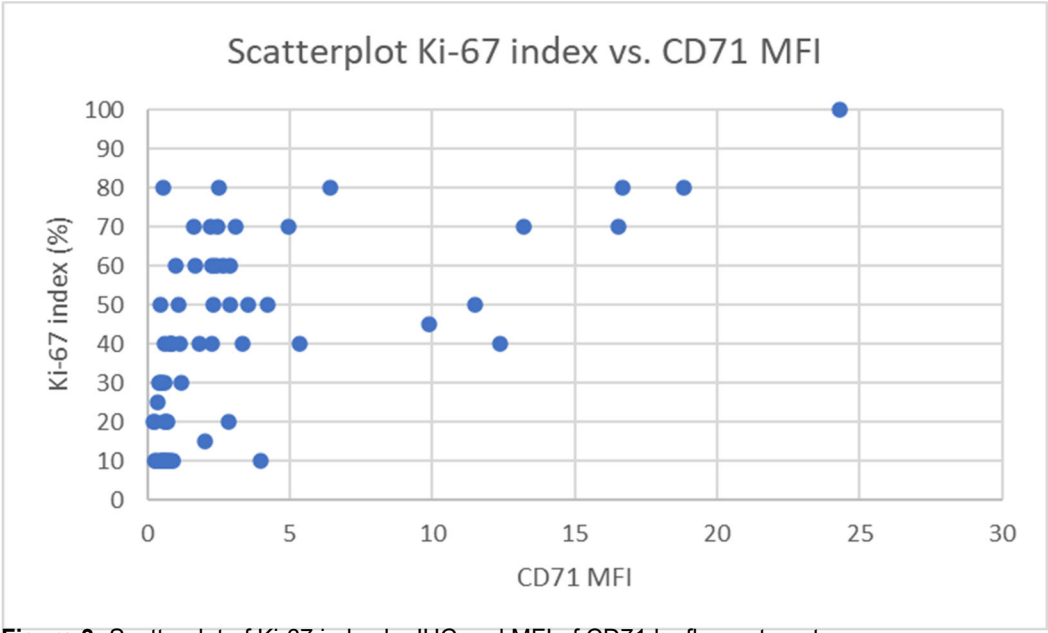

Figure 3: Scatterplot of Ki-67 index by IHC and MFI of CD71 by flow cytometry

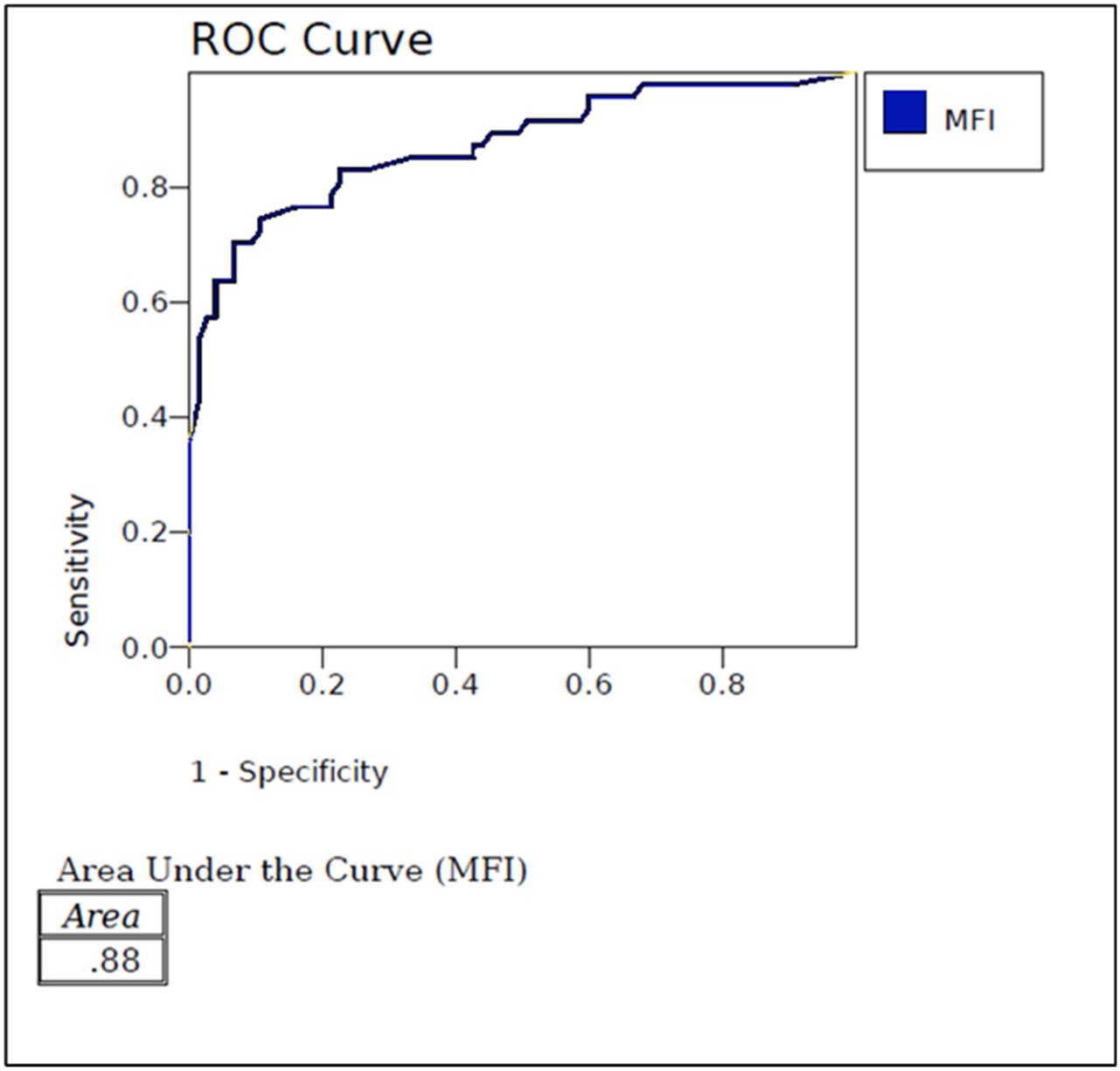

Figure 4: ROC curve analysis – showing the expression of CD71 by flow cytometry and the diagnosis of high-grade B-cell lymphomas

DISCUSSION

This study suggests that CD71 by flow cytometry is helpful in distinguishing indolent from aggressive B cell lymphomas. This is shown by the high concordance between the increased MFI of CD71 by flow cytometry with a diagnosis of an aggressive lymphoma and also, by the ROC curve analysis showing an AUC of 0.88. These results are consistent with the findings from other studies using CD71 as a flow cytometry marker in the diagnosis of lymphoma (5,13).

This study also shows a positive correlation between the increased expression of CD71 by flow cytometry and the Ki-67 index by IHC. Both markers showed significantly higher expression in aggressive lymphomas. Methods are available to assess Ki-67 by flow cytometry (7) however, to the authors knowledge there does not appear to be any studies in the literature of a direct comparison of CD71 and Ki-67 expression by flow cytometry.

Limitations of the study include only looking at the use of CD71 as a flow cytometry marker to aid in the diagnosis of lymphoma, it did not assess the clinical progress of the patients or other prognostic markers. Some of the lymphomas that we have classified as indolent lymphomas, such as mantle cell lymphoma, can have a more aggressive clinical course particularly if they have a poor prognostic score at diagnosis (2). The MFI values are dependent on machine settings, so we are unable to compare with other laboratories however, we are currently working on addressing this by comparing MFI to normal bone marrow to allow interlaboratory studies in the future.

Further research into the use of CD71 by flow cytometry in lymphoproliferative disorders could look at the expression levels in different lymphomas to aid diagnosis, classification and assess whether it has value as a prognostic marker by assessing correlation between expression and prognosis. Comparative studies of both CD71 and Ki-67 expression both by IHC and flow cytometry could also be performed to add further insights into the usefulness and application of this marker.

CONCLUSION

This study suggests that CD71 expression by flow cytometry is a useful diagnostic marker in differentiating between indolent and aggressive B-cell lymphomas. There was a correlation between the expression of CD71 by flow cytometry and the histological diagnosis of more aggressive lymphomas particularly DLBCL. There is also a clear positive correlation between the expression of CD71 by flow cytometry and the Ki-67 index by IHC. In the authors laboratory, CD71 expression is becoming a useful adjunct tool along with clinical, morphological, and other flow cytometric features for the assessment and reporting of flow cytometry in suspected lymphoma cases.

CONFLICT OF INTEREST

The authors have no competing interests.

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