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Utility of leukocyte-associated immunoglobulin-like receptor-1 (CD305) in flow cytometric detection of minimal bone marrow involvement by B-cell non-Hodgkin lymphoma

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Abstract

Multicolor flow cytometry (MFC) is crucial in detecting occult or minimal bone marrow (BM) involvement by non-Hodgkin lymphomas (NHL), which may not be detected using trephine biopsy or imaging studies. Detection of low-level BM involvement can be challenging without definite immunophenotypic aberrancies. We studied the utility of CD305 in MFC detection of minimal BM involvement by B-NHL, especially in the absence of aberrancies by commonly used markers. The study included 1084 consecutive BM samples submitted for the staging of B-NHLs (excluding CLL) over two years. Samples were studied for morphological, immunophenotypic, and histopathological assessment. MFC studies were performed using 10–13 color MFC, including CD305-antibody (clone, DX26). Minimal BM involvement was defined with a cutoff of ≤10% lymphoma cells in viable cells on MFC assessment. Of 1084, 148 samples revealed overt morphological involvement by B-NHL and were excluded from analysis. BM samples of 172/936 patients were morphologically negative but revealed involvement using MFC independently. Corresponding trephine biopsy involvement was detected in only 79/172 (45.9%) patients. On MFC, 23/172 samples showed BM involvement with >10% lymphoma cells, and 149/172 (86.6%) samples revealed minimal involvement. In 54/149 (36.24%) samples, lymphoma cells were detected only with aberrant loss of CD305 expression. In 78 of the remaining 95 samples (82.1%), it provided an immunophenotypic aberrancy addition to other markers and supported the results. CD305 is a highly useful marker in the flow cytometric assessment of minimal BM involvement by B-NHL. MFC is a superior modality to trephine biopsy in detecting low-level BM involvement.

KEYWORDS
B-cell non-Hodgkin lymphoma, bone marrow involvement, CD305, immunophenotyping, LAIR1, minimal

1 | INTRODUCTION

Bone marrow (BM) assessment is an essential part of the initial workup of lymphomas for establishing diagnosis, determining the extent of disease spread and staging of the disease (Chaganti et al., 2016). Additionally, it is the most common site of extranodal involvement in lymphoid malignancies (Adams et al., 2015a). Evidence of BM involvement on trephine biopsy definitively indicates an advanced stage of the disease. It escalates the stage of the disease to stage 4, which is known for adverse clinical outcomes (Arima et al., 2013; Campbell et al., 2006; Chung et al., 2007; El-Galaly et al., 2015; Martín-Moro et al., 2020; Min et al., 2020; Okamoto et al., 2021; Palacio et al., 2001; Stacchini et al., 2003; Soydal et al., 2016; Takahashi et al., 2012; Terziev et al., 2020; Yan et al., 1995; Yao et al., 2018). Hence, assessment of BM involvement is crucial for determining treatment strategies in lymphoma (Armitage, 2005; Cheson, 2008; Cheson, 2015; Lu, 2005; Yoo, 2022). The international prognostic index that is an integral part of the clinical assessment of patients includes age, lactate dehydrogenase (LDH), performance status, stage, and extranodal involvement. Of all these, BM status can influence both stage and extranodal involvement (The New England Journal of Medicine, 1993).

The modalities commonly used for the detection of BM involvement include BM biopsy and imaging studies such as positron emission tomography with computed tomography (PET-CT) scan or FDG-PET scan (Adams et al., 2015a; Cheson, 2008; El-Galaly et al., 2015; Soydal et al., 2016). Recent data have demonstrated that PET-CT scan is more sensitive than biopsy, indicating BM biopsy can miss the involvement of non-Hodgkin lymphomas (NHL) in a subset of cases due to the low disease or patchy involvement (Adams et al., 2015a; Barrington et al., 2014; Chen-Liang et al., 2015; Cheson et al., 2014; Goudarzi et al., 2010; Ricard et al., 2023; Teagle et al., 2017). On the other hand, PET-CT scans may not be effective in low-grade (LG) lymphomas and tend to provide false positive results (Adams et al., 2015a; Adams et al., 2015b; Adams & Kwee, 2015a; Adams & Kwee, 2015b; Adams & Kwee, 2018; Chen-Liang et al., 2017; Hao et al., 2018).

Morphologically apparent BM involvement can be easily detected on light microscopy. However, detecting low-level disease involvement is challenging using light microscopy. Although immunohistochemical (IHC) staining can help up to an extent, limitations such as single-color IHC (commonly available) and small biopsy tissue's inability to provide multiple tissue cuts for multiple markers using IHC make it difficult to detect low-level tumor cells in the background of normal hematopoietic cells and reactive/normal lymphocytes (Adams et al., 2015a; Okamoto et al., 2021; Soydal et al., 2016). Clonality staining for light chains is also difficult using IHC due to high background staining for light chains (Guo et al., 2018; Hristov et al., 2020; Rimsza et al., 2014).

Multicolor flow cytometry (MFC) is a powerful, highly sensitive single-cell analysis technique that simultaneously allows the evaluation of multiple surface and cytoplasmic proteins. It has been widely used for diagnosing, staging, and monitoring hematological malignancies for the last 3–4 decades (Alaggio et al., 2022; Chatterjee et al., 2017; Chatterjee et al., 2023; Falini et al., 2023; Panda et al., 2021a; Panda et al., 2021b; Sriram et al., 2022; Stetler-Stevenson & Tembhare, 2011; Tembhare et al., 2013; Tembhare et al., 2014; Tembhare et al., 2017; Tembhare et al., 2020a; Tembhare et al., 2020b; Tembhare et al., 2020c; Tembhare et al., 2022a; Tembhare et al., 2022b). MFC allows detailed immunophenotypic characterization and confirmation of clonality of suspicious cells simultaneously and detects small tumor cell populations that can be challenging to identify on BM biopsy (Boveri et al., 2009; Carulli et al., 2010; Debord et al., 2020; Martín-Moro et al., 2020; Palacio et al., 2001; Perea et al., 2004; Stacchini et al., 2003; Talaulikar et al., 2008). Such low-level involvement is usually refers to "occult or minimal involvement" (Arima et al., 2013; Talaulikar et al., 2007; Talaulikar & Dahlstrom, 2009). Furthermore, previous studies have demonstrated the clinical impact of minimal BM involvement using MFC (Duggan et al., 2000; Iancu et al., 2007; Kim et al., 2015; Sorigue et al., 2021).

B-cell NHLs (B-NHLs) with characteristic immunophenotypes, such as aberrant expression of CD10 or CD5 along with other common immunophenotypic abnormalities, can be readily identified using MFC with the inclusion of these markers (Debord et al., 2020; Perea et al., 2004; Stacchini et al., 2003; Sorigue et al., 2021) However, detecting minimal involvement in the absence of characteristic immunophenotypic aberrancies is challenging. Additionally, normal populations in BM, such as CD5 positive naïve B cells and CD10 positive transitional B-cells, can pose difficulty in identifying the true disease, especially when the tumor cell population is minimal (Chatterjee et al., 2022; Chatterjee et al., 2023; Clavarino et al., 2016; Tembhare et al., 2022a). Thus, there is a requirement to include additional markers for flow cytometric assessment of minimal BM involvement.

Previously, EuroFlow had incorporated CD305 (LAIR1) as a part of their B-NHL panel, which helped differentiate hairy cell leukemia (HCL) from other lymphomas and, along with newer markers like CD200 (van Dongen et al., 2012). CD305, also known as LAIR1 (leukocyte immunoglobulin-like receptor-1), is a transmembrane glycoprotein that acts as an inhibitor receptor and is expressed by most immune cells. The known LAIR ligands are extracellular matrix collagen and Cq1, the first component of the complement complex (Ouyang et al., 2003; Son, 2022). LAIR1 expression varies during various stages of B cell differentiation (Meyaard et al., 1997; Meyaard, 2008; van der Vuurst de Vries et al., 1999; Van Laethem et al., 2022; Zhang et al., 2014). It inhibits B-cell receptor (BCR)-mediated signaling and controls kinase pathways involved in cell

proliferation (Meyaard et al., 1997; Son, 2022). It has been studied in chronic lymphocytic leukemia (CLL) as a prognostic marker and is associated with a favorable prognosis (Perbellini et al., 2014). Thus, the role of CD305 in the immunophenotyping of B-NHL, other than CLL and HCL, remains unexplored. In this study, we determined the utility of CD305 for the assessment of minimal BM involvement by B-NHL other than CLL. We highlighted the crucial role of LAIR1 (CD305) in detecting minimal BM involvement by B-NHL, especially in the absence of common aberrancies involving CD5 or CD10 expression.

2 | PATIENT AND METHODS

We conducted a study on 1084 consecutive BM samples submitted for staging in patients recently diagnosed with B-NHLs other than CL at our hospital between January 2020 and December 2021. The clinical, laboratory, and radiological details were noted from electronic medical records. Staging BM samples from patients with solid tumors, T-NHLs, and other hematologic malignancies, including plasma cell neoplasms, were excluded. The study was approved by the Institution's Ethics Committee (IEC). The final diagnosis and subtyping of B-NHL were established based on histopathological tissue diagnosis, including morphology and immunohistochemistry evaluation.

2.1 | Cytomorphology

BM aspirate smears were stained with Wright's stain. Morphological details were studied including the adequacy of cellularity, differential count, and nuclear and cytoplasmic details of lymphocytes. BM biopsies were processed using the Hammersmith Protocol (Naresh et al., 2006). A tissue biopsy (TB) examination was conducted on hematoxylin and eosin (H&E) stained sections in conjunction with IHC studies. TB sections were performed using 5-μm thick, formalin-fixed, and paraffin-embedded tissue, and IHC was performed after heat-induced epitope retrieval as described below.

2.2 | IHC Studies

IHC was performed using avidin-biotin complex method on formalin-fixed paraffin sections using automated immunostainers (Ventana Benchmark XT) as described previously (Tembhare et al., 2022b). The panel of antibodies for IHC studies included- LCA, CD3, CD5, CD4, CD8, CD10, CD15, CD20, CD23, CD30, CD34, CD43, CD117, CD138, CD163, AE1/AE3, ALK-1, BCL2, BCL6, BOB1, C-myc, CK7, CK20, EMA, Granzyme-B, MIB1, myeloperoxidase (MPO), MUM-1, OCT2, PAX-5, terminal deoxynucleotidyl transferase (TdT), and EBVLMP1. The selection of IHC panels was primarily based on the tissue adequacy, availability of reagents at the time of evaluation, and judgment of the reporting pathologist.

2.3 | MFC immunophenotyping

BM samples were processed for MFC immunophenotyping using the bulk lyse and stain method described elsewhere (Tembhare et al., 2022b). In brief, the cell suspension was prepared by bulk erythrocyte lysing with ammonium chloride-based lysing reagent (0.15 M NH4Cl, 1.0 g KHCO3, 37 mg EDTA, and 1 L distilled water). After the lysis and washing step, the cells were resuspended in phosphate-buffered saline with 5% bovine serum albumin. The cell count was adjusted to get a final concentration of 2×10^6 cells in 80 μL and stained for immunophenotyping using 11–13 color antibody panels. The panel included an anti-CD305 antibody (DX26, BD Biosciences). The details of clones and fluorochrome combinations are mentioned in Table 1. The cells were either acquired within 4 h of staining or fixed with 0.5% paraformaldehyde and acquired within 24 h of fixation. Samples were acquired on a three-laser 13-color Cytoflex instrument (Beckman Coulter, BC), and a minimum 500,000 events per tube were collected. The instrument calibration, quality control, and voltage and compensation were performed as per the manufacturer's instructions. For cases in which BM involvement was morphologically evident, we proceeded directly with a dedicated 10–13 color antibody panel (Table S1), whereas in morphologically uninvolved cases, we used the 13-color comprehensive lymphoma screening tube (LST) including CD305 (Table 1). The normal range for lymphocyte percentage in BM aspirate is approximately 11%–20% (Sovani, 2021), and in our experience, BM involvement with more than 10% of lymphoma cells is not easily missed on morphology and commonly used immunophenotypic markers. Hence, we defined minimal BM involvement with a cutoff of ≤10% lymphoma cells in viable cells on MFC. The expression pattern of all markers, including CD305, was categorized as negative, dim or weak, moderate or intermediate, bright or strong, partial, and variable based on its expression on internal negative and positive control population (shown in DATA S1) as described earlier (Sriram et al., 2022). Normal mature B-cells showed moderate to variable expression of CD305 and were taken as an internal positive control, and precursor erythroid cells (nucleated red cells) were taken as an internal negative control.

3 | RESULTS

We studied BM samples from 1084 patients submitted for B-NHL staging. As shown in Figure 1, 148 out of 1084 samples revealed overt involvement by lymphoma cells upon morphological evaluation, which was further confirmed on immunophenotypic evaluation. Since this study focused on minimal BM involvement evaluation, we excluded these patients from further analysis. Of the remaining 936 patients, 172 did not show microscopic features of morphological involvement but revealed BM involvement by B-NHL cells on MFC independently, with a median tumor burden of 1.05% (0.005%–24%). The distribution of these cases is shown in Figure 2. CD5 and CD10 are commonly expressed aberrant markers in B-cell lymphoma and are routinely used to identify clonal abnormal B-cells in the background of

TABLE 1 A lymphoma screening antibody panel for NHL.

	BV786	BV650	BV605	BV510	BV421	FITC	PE	ECD	PC5.5	PC7	AF647	APC AF_700	APC AF_750
Antibody	CD16	CD14	CD5	CD4	sCD3	CD8	CD7	CD34	CD19	CD10	CD305	CD45	CD38
Clone	3G8	M5E2	L17F12	RPA-T4	UCHT1	B9.11	8H8.1	581	J3-119	ALB1	DX26	J.33	LS198-4-3
Company	BD	BD	BL	BL	BD	BC	BC	BC	BC	BC	BD	BC	BC
	and	X	X	and	X	and	and	X	and	X	X	X	X
Antibody	CD56			CD20		Kappa	Lambda		TCR γδ				
Clone	NCAM16.2			2H7		Polyclonal	Polyclonal		IMMU510				
Company	BD			BL		Dako	Dako		BC				

normal cells. To assess the value of CD305 as an independent marker, we divided these 172 cases into three groups based on CD5 expression and CD10 expression such as CD5-positive B-NHL (35/172, 20.3%), CD10-positive B-NHL (77/172, 44.7%), and CD5 and CD10 double-negative B-NHL (60/172, 34.8%). Of the 172 cases, 23 cases showed BM involvement with >10% tumor cells on MFC, and 149 (86.6%) cases revealed minimal BM involvement.

3.1 | Correlation between BM biopsy and MFC findings

Among 172 patients without morphological findings of involvement in BM aspirate smears, BM biopsy revealed involvement in 79 out of 172 (45.9%) patients, while no involvement was found in the remaining 93 (54.1%). IHC was performed in 51 of 79 BM biopsies with B-NHL involvement. Among the remaining 93 BM biopsies, IHC was performed in 29 samples where suspicion of BM involvement arose during H&E evaluation, and these were finally reported as uninvolved. In the remaining 64 samples, IHC was not performed due to the absence of suspicion of BM involvement in H&E. Thus, IHC was not performed in the majority of biopsy samples in the absence of suspicion of involvement in H&E section evaluation. Upon comparison of MFC results, the median level of tumor burden in these 93 patients (median, 0.4%; range, 0.005%–6.4%) was significantly lower compared with that in 79 patients with trephine biopsy involvement (median, 3.3%; range, 0.01%–24%) ($p < 0.0001$) indicating low-level involvement can be easily missed on trephine biopsy evaluation. This highlights the impact of MFC in BM involvement analysis for B-NHL staging.

3.2 | CD305 expression

In 95/149 cases (63.75%) with minimal disease, lymphoma cells were identified using the aberrant or altered expression pattern of CD5, CD10, CD11c, CD20, CD38, CD45, and forward scatter in the background of normal B-cells. In the remaining 54/149 cases (36.2%), the tumor population was identified using only CD305 negative expression (see Figure 3). Thus, CD305 was indispensable in detecting BM involvement in these cases, which lacked immunophenotypic abnormalities based on commonly used markers such as CD5, CD10, CD11c, CD20, CD38, and so on.

Moreover, out of 149 samples, CD305 aided in detecting minimal BM involvement in 78 out of the remaining 95 (82.1%) cases, along with one or more additional markers from the panel used. The median (range) of tumor burden in minimal BM involvement cases was 0.59% (0.016%–9.7%). These 95 cases include 43 cases of diffuse large B-cell lymphoma (DLBCL), which exhibited other immunophenotypic aberrancies such as CD10 expression and high forward scatter characteristics (FSC) in tumor cells; 24 cases of follicular lymphoma (FL), which showed weak CD10 and negative CD38 expression; 13 cases of mantle cell lymphoma (MCL) which showed weak-variable CD5

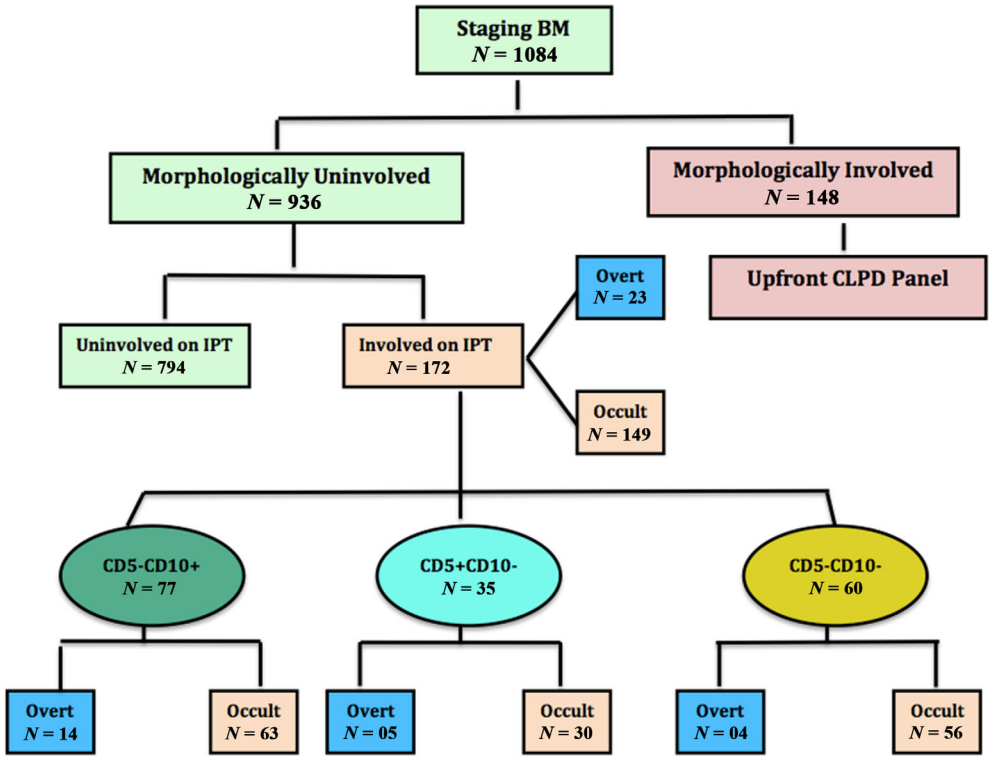


FIGURE 1 Flowchart representing the distribution of B-NHL patients studied. "Overt" BM involvement includes patients with no morphological involvement but tumor cells >10% of all viable cells on flow cytometric immunophenotyping. "Occult" BM involvement includes patients with no morphological involvement but tumor cells ≤10% of all viable cells on flow cytometric immunophenotyping. BM, bone marrow; FCI, Flow cytometric immunophenotyping; CLPD, chronic lymphoproliferative disorders; IPT, immunophenotyping. [Color figure can be viewed at wileyonlinelibrary.com]

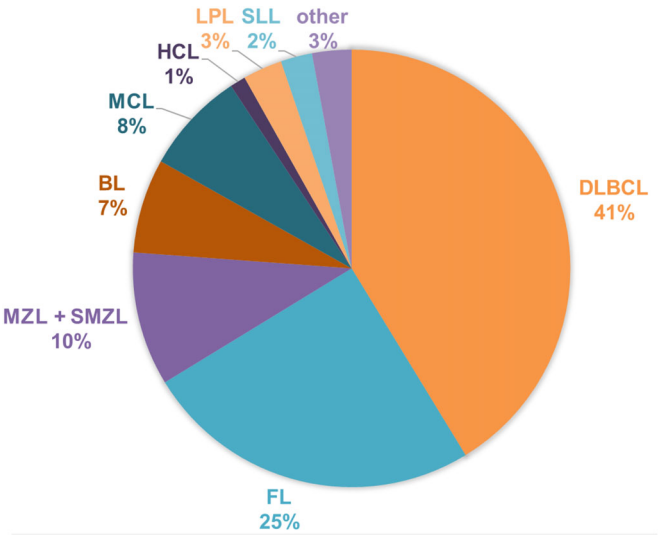


FIGURE 2 Distribution of patients with minimal bone marrow involvement ($n = 172$). BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HCL, hairy cell leukemia; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; SLL, small cell lymphoma; SMZL, splenic marginal zone lymphoma. [Color figure can be viewed at wileyonlinelibrary.com]

expression; 9 cases of Burkitt lymphoma (BL) which showed co-expression of CD10 and CD38; 3 cases of small cell lymphoma (SLL) which showed weak-variable CD5 and CD20 expression; 2 cases of HCL which showed strong CD19, CD20, CD11c, and CD305 expression and one case of marginal zone lymphoma (MZL) with weak CD11c expression.

The expression pattern of CD305 in these patients is shown in Figure 4 and DATA S2. Overall, CD305 was downregulated in 152 out of a total of 172 cases (88.4%; negative, 145 and weak, 7). Eleven patients (6.39%) showed moderate CD305 expression, and two patients (1.1%) showed bright CD305 expression, both diagnosed with HCL. The eleven patients with moderate expression of CD305 included six patients with DLBCL and one each with splenic marginal zone lymphoma (SMZL), BL, MCL, LG B-NHL not-classified, and high-grade (HG) B-NHL not-classified.

Twenty-three patients showing overt (>10% lymphoma cells) BM involvement included 14 patients with CD10 positive FL, 5 patients with CD5 positive B-NHL and 4 patients with CD5 negative, CD10 negative B-NHL, which were detected using abnormal downregulation of CD305.

4 | DISCUSSION

Multiparameter flow cytometry (MFC) is essential for accurately staging B-NHLs (Debord et al., 2020; Stacchini et al., 2003; Stetler-Stevenson & Tembhare, 2011; Tembhare et al., 2022b). A carefully selected panel of antibodies and their appropriate combinations along with knowledge of immunophenotypic expression patterns of various hematopoietic cells in BM, allows prompt detection of BM involvement with high sensitivity (Ramalingam et al., 2022; Tembhare et al., 2014; Tembhare et al., 2020a; Tembhare et al., 2020c; van Dongen et al., 2012). A comprehensive antibody panel that includes markers such as CD5, CD10, CD11c, CD38, CD45, CD200, and Kappa/Lambda light chains helps detect BM involvement by B-NHL with high sensitivity (Alaggio et al., 2022; Debord et al., 2020; van

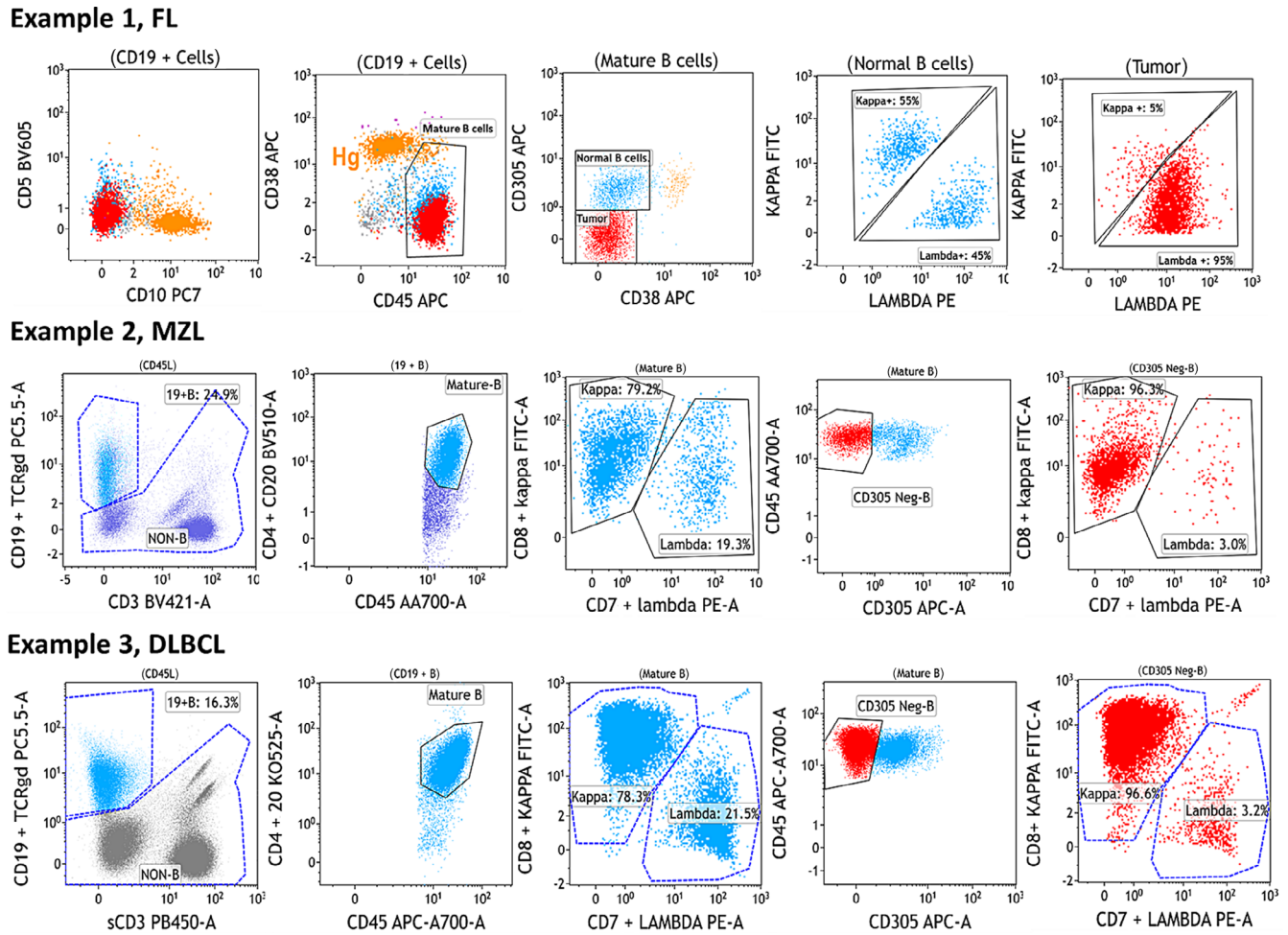
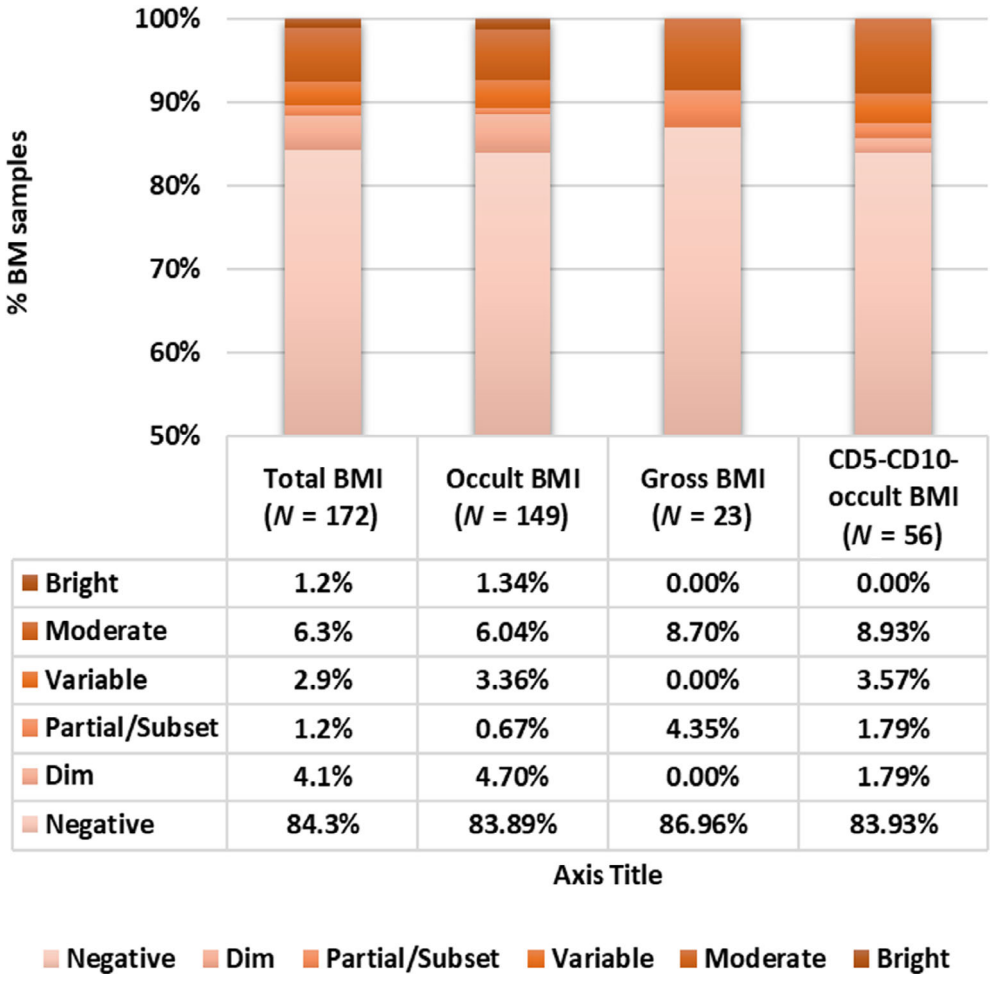


FIGURE 3 The figure shows representative dotplots with only CD19-positive events (from three representative examples) that included hematogones (Hg, orange dots), normal polyclonal B-cells (blue dots), and tumor B-cells (red dots). Tumor B-cells (red dots) are negative for CD5, CD10, and CD38 expression and show bright CD45 expression. Tumor cells are separated with negative CD305 expression and their clonality is confirmed with lambda or kappa light chain restriction. "Example 1" is a case of follicular lymphoma (FL), "Example 2" is a case of marginal zone lymphoma (MZL), and "Example 3" is a case of diffuse large B-cell lymphoma (DLBCL with discordant BM involvement). In Example 3, tumor B-cells (red dots) were small and hence, it was labelled as discordant BM involvement. [Color figure can be viewed at wileyonlinelibrary.com]

Dongen et al., 2012). However, the lack of aberrant expression of these markers may fail to isolate lymphoma cells in the background of normal B-cells, leading to false negative results (Wang et al., 2021). Additionally, adding an extensive panel including antibodies specific to each subtype of B-NHL is expensive, consumes more sample quantity, and may not be feasible. We studied the utility of CD305 (LAIR-1) for detecting BM involvement by B-NHL involvement using a lymphoma screening panel, especially in minimal BM involvement. Our study was focused on evaluating its role in the absence of commonly known immunophenotypic abnormalities such as CD5- and CD10-negative lymphomas.

Previous studies have reported the frequency of BM involvement by B-NHL using flow cytometry between 18% and 31% (Sorigue et al., 2021). However, many of these reports were limited by less than 8-color immunophenotyping and restricted to one or two B-NHL subtypes such as DLBCL and follicular cell lymphoma or MZL (Boveri et al., 2009; Iancu et al., 2007; Martín-Moro et al., 2020; Palacio et al., 2001; Sorigue et al., 2021; Talaulikar et al., 2008; Wang et al., 2021). A few studies that include all B-NHL subtypes, including CLL or lymphoplasmacytic lymphoma, have reported high BM involvement frequency (Carulli et al., 2010; Kim et al., 2015; Merli et al., 2010; Statuto et al., 2020). Since CLL has a distinct immunophenotypic signature and is always associated with BM involvement, we excluded these patients from our study. Thus, our data included patients with non-CLL B-NHL and revealed BM involvement in 320 of 1084 samples (29.5%) submitted for staging. The prevalence of BM involvement in our data may be biased as our cohort also included a few patients who might have received a few cycles of chemotherapy or steroids before reaching our hospital. Nevertheless, it represents a real-world practice in tertiary reference centers.

BM involvement was overtly detectable in 148/320 cases by morphology and immunophenotyping, whereas 172/320 cases were detected based on immunophenotyping alone. In 23 cases, tumor burden was greater than 10%, and involvement was readily identified



	Total BMI (*N* = 172)	Occult BMI (*N* = 149)	Gross BMI (*N* = 23)	CD5-CD10-occult BMI (*N* = 56)
Bright	1.2%	1.34%	0.00%	0.00%
Moderate	6.3%	6.04%	8.70%	8.93%
Variable	2.9%	3.36%	0.00%	3.57%
Partial/Subset	1.2%	0.67%	4.35%	1.79%
Dim	4.1%	4.70%	0.00%	1.79%
Negative	84.3%	83.89%	86.96%	83.93%

FIGURE 4 Expression patterns of CD305 in tumor cells from samples with bone marrow involvement (BMI). The attached table shows the distribution of BM samples with different levels of CD305 expression (also refer to DATA S2). [Color figure can be viewed at wileyonlinelibrary.com]

based on immunophenotypic abnormalities of commonly used markers and clonality testing by light chain restriction. Usually, lymphoma cells are identified using a panel of surface markers, including CD5, CD10, CD11c, CD19, CD20, CD38, CD45, and forward scatter, in the background of normal hematopoietic precursors in BM (Debord et al., 2020; van Dongen et al., 2012). However, a small population of mature CD5-positive naive B-cells and CD10-positive transitional B-cells can be challenging to distinguish from tumor cells in cases of MCL, FL, and CD5 or CD10-positive DLBCLs (Clavarino et al., 2016; Debord et al., 2020; Ramalingam et al., 2022; van Dongen et al., 2012). Our data showed that, in such cases, the abnormal downregulation of CD305 with kappa or lambda light chain restriction helps identify the lymphoma cells and confirm the BM involvement.

On the other hand, in cases with CD5- and CD10-negative lymphomas, identifying low-level disease can be challenging in the background of normal polyclonal B-cells. Even kappa or lambda light chain restriction may not help as no prominent immunophenotypic aberrancies are present for isolating the small tumor population. Our data included 54/149 (36.2%) CD5- and CD10-negative lymphoma cases where BM involvement was confirmed using only negative/weak CD305 expression combined with light chain restriction. In these samples, no other immunophenotypic aberrancy was detected.

Euroflow has reported the expression pattern of CD305 in chronic lymphoproliferative neoplasms (CLNs) with strong expression in HCL, moderate to weak expression in most of CLL and MCL, and occasionally in DLBCL tumor cells (van Dongen et al., 2012). In our data with minimal BM involvement, CD305 was downregulated in 152/172 (88.4%) cases. Of the remaining patients, it was strongly positive in HCL ($n = 2$), moderate in DLBCL ($n = 6$), followed by MZL, BL, MCL, LG-BNHL not-classified, and HG-BNHL not-classified each. In the remaining seven cases, it was variable or partially positive. CD305 downregulation helped confirm 78/95 (82.1%) cases with minimal BM involvement by B-NHL and was indispensable in 54/149 (36.2%) cases, which could have been missed without its inclusion in the antibody panel. CD305 was found prominently useful in the assessment of BM involvement in patients with MZL/SMZL, DLBCL, FL, and LPL. This is because no other markers were found useful in separating abnormal lymphoid populations from background normal polyclonal B lymphocytes. BM involvement in patients with BL, MCL, HCL, and CLL could be identified using common markers, such as CD5, CD10, CD38, and so forth, along with kappa or lambda light chain restriction.

Our data demonstrated the utility of the addition of CD305 in the B-NHL antibody panel to evaluate the low-level BM involvement. One should be aware of a few concerns while interpreting low-level

minimal BM involvement. One such concern is the clinical significance of minimal BM involvement. The studies evaluating the clinical significance of minimal BM involvement are limited. Studies by Arima et al. (2013), Martín-Moro et al. (2020), and Talaulikar et al., (2007) have shown that DLBCL patients with minimal BM involvement (confirmed by flow cytometry) had poor clinical outcomes compared to uninvolved BM.

Similarly, using clonal immunoglobulin gene rearrangements studies, Berget et al. (2014) have also shown the prognostic significance of minimal BM involvement in FL. Such data in other LG B-NHL is scarce, and there is a need to perform the prospective clinical trial to evaluate it systematically.

Further, with a low-level BM involvement in MFC, it is difficult to distinguish between true BM involvement versus the presence of circulating tumor cells due to BM hemodilution. The minimal BM involvement due to hemodilution can be excluded if peripheral blood is negative for tumor population performing the same assay. Rarely, in elderly patients, one also needs to be aware of non-CLL type monoclonal B-cell lymphocytosis (MBL) which may coexist with well-established B-NHL. It can be confirmed if B-NHL's immunophenotype significantly differs from that of clonal B-cells detected in BM sample. MBL can be divided into two types CLL-type and non-CLL type. CLL-type of MBL demonstrates immunophenotype features overlapping with CLL, however, non-CLL MBL immunophenotypically and genetically significantly differ from CLL (Nieto et al., 2010; Oliva-Ariza et al., 2023; Rawstron et al., 2017). Non-CLL type MBL has been described in a smaller proportion with reported prevalence up to 2% in elderly adults (Nieto et al., 2010; Oliva-Ariza et al., 2023). Its prevalence has been found to be higher in a hospital-based registry from Uganda. Non-CLL MBL can be divided into two groups: CD5+ and CD5− MBL cases and may raise the suspicion of BM involvement by B-NHL (Rawstron et al., 2017). Hence, one should be aware of such condition while interpreting the minimal BM involvement by B-NHL.

In summary, CD305 (LAIR-1) is a highly useful marker in the flow cytometric evaluation of BM involvement in B-NHL. Hence, compared with normal B-cells, its downregulation is a common abnormality and substantially aids in identifying low-level disease. Its inclusion in the antibody panel of MFC, along with kappa and lambda light chains, improves the sensitivity of MFC-based BM staging for B-NHLs, especially in cases with minimal BM involvement and no definitive immunophenotypic abnormalities.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethical Committee, Tata Memorial Centre.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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