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## Utility of an immunoglobulin gene rearrangement assay based on multiplex PCR in detecting bone marrow involvement in B-cell non-Hodgkin lymphoma

**TO THE EDITOR:** B-cell non-Hodgkin lymphoma (B-NHL) comprises a heterogeneous group of neoplasms [1]. Traditionally, marrow involvement of NHL has been detected by examining bone marrow (BM) samples by microscopy [2]. However, even if BM samples do not show malignant lymphoid aggregates, this may not reflect the status of the entire bone marrow. Recently, diagnostic hematology has expanded with the introduction of new molecular technologies. BIOMED-2 multiplex polymerase chain reaction (PCR) (InVivoScribe Technologies, San Diego, CA, USA) has been applied to identify immunoglobulin (Ig) genes and T cell receptor (TCR) gene clonality in clinical specimens [3, 4].

The purpose of this study was to compare the utility of an Ig rearrangement assay based on BIOMED-2 multiplex PCR in detecting BM involvement in B-NHL with that of microscopic examination and positron emission tomography with computed tomography (PET-CT). A total of 28 patients (median age, 59 yrs; range, 37–95 yrs) who were diagnosed with B-NHL between January 2007 and March 2010 at Kangdong Sacred Heart Hospital, Seoul, Korea were enrolled in the study, which included 17 men and 11 women, whose histopathological characteristics are listed in Table 1. DNA was extracted from the BM aspirations from each patient according to a previously reported method [5].

We performed a BIOMED-2 multiplex PCR assay using an IdentiClone Ig (IGH: immunoglobulin heavy chain, IGK:

immunoglobulin kappa, IGL: immunoglobulin lambda) Gene Clonality Assay Kit (InVivoScribe Technologies, San Diego, CA, USA). The full study comprised the use of five kits targeting IGH (IGH<sub>A</sub>: V<sub>H</sub>FR1-J<sub>H</sub>; IGH<sub>B</sub>: V<sub>H</sub>FR2-J<sub>H</sub>; IGH<sub>C</sub>: V<sub>H</sub>FR3-J<sub>H</sub>; IGH<sub>D</sub>:D<sub>H1-6</sub>-J<sub>H</sub>; and IGH<sub>E</sub>: D<sub>H7</sub>-J<sub>H</sub>), two kits targeting IGK (IGK<sub>A</sub>: V<sub>K</sub>-J<sub>K</sub>; IGK<sub>B</sub>: V<sub>K</sub>-Kde; and J<sub>K</sub>C<sub>K</sub> intron-Kde), and one kit targeting IGL (V<sub>λ</sub>-J<sub>λ</sub>). When the first IGH test performed was negative, the IGK and IGL tests followed. Generally, PCR sensitivity is influenced by the primers used, and in the past, the most common PCR method for B-cell Ig genes amplified CDR3 regions, using FR3 and JH consensus primers [6]. Unlike other PCR methods that target only the FR3 region for IGH gene rearrangement, the BIOMED-2 multiplex PCR test uses variable target gene rearrangement of 5' primers to target FR1, 2, and 3 of the V-J and D-J regions of IGH. After PCR amplification, a heteroduplex analysis was performed. In this method, PCR products are heat-denatured and cold-renatured to form duplexes (hetero- or homo-duplexes). Then, polyacrylamide gels were used to visualize monoclonal bands upon electrophoresis at room temperature.

In our study, the overall concordance rate between the results of microscopic BM examination and IG rearrangement assay was 96.4% (27/28). Ten cases were found positive for Ig gene rearrangement (Table 1). Nine cases revealed microscopic BM infiltration; case 10 did not (Table 2). Eight of the 10 Ig rearrangements were IGH-positive, 5 were positive for IGH<sub>A</sub>, and 6 were positive for IGH<sub>C</sub> (Table 2). Similarly, a study by Abbas *et al.* [7], using a BIOMED-2 kit to assess B-cell gene rearrangements in leukemias and lymphomas, showed that 92% of cases had IGH clonality, with 74% detected by IGH<sub>A</sub>, 75.5% by IGH<sub>B</sub>, 65.1% IGH<sub>C</sub>, 26% by IGH<sub>D</sub>, and 2.1% by IGH<sub>E</sub>.

In our study, two DLBCL cases (33.3%, 2/6) with negative IGH tests showed positive results in IGK tests. This result is in line with a report by van Krieken *et al.* [4], which

**Table 1.** Concordance between morphology and PCR-determined clonality of bone marrow, based on histology in cases of non-Hodgkin lymphoma.

Histology by WHO classification		BM involvement (+)		BM involvement (-)	
		PCR (+)	PCR (-)	PCR (+)	PCR (-)
DLBL	19	6	0	1	12
MZBL	1	1	0	0	0
MCL	1	1	0	0	0
FL	2	0	0	0	2
MALT	4	0	0	0	4
SLL	1	1	0	0	0
Total	28	9	0	1	18

Abbreviations: DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MALT, MALT type lymphoma; MCL, mantle cell lymphoma; NMZL, nodal marginal zone B-cell lymphoma; PCR, polymerase chain reaction; SLL, small lymphocytic lymphoma; WHO, World Health Organization.

**Table 2.** Diagnosis of review panel samples showing clonal immunoglobulin gene rearrangements.

Patient	Microscopic BM involvement	IGH tubes					IGK tubes		IGL tube	Pathological diagnosis	PET-CT results
		A FR1	B FR2	C FR3	D D-J	E D-J	A V-J	B Kde	V-J		
Case 1	+	+	-	+	-	-	NT	NT	NT	DLBL	NT
Case 2	+	+	-	-	-	-	NT	NT	NT	NMZL	+
Case 3	+	+	-	-	-	-	NT	NT	NT	DLBL	NT
Case 4	+	-	-	-	-	-	+	-	-	DLBL	NT
Case 5	+	-	-	+	+	-	NT	NT	NT	DLBL	+
Case 6	+	-	-	+	+	-	NT	NT	NT	DLBL	-
Case 7	+	+	+	+	-	-	NT	NT	NT	MCL	+
Case 8	+	+	+	+	-	-	NT	NT	NT	SLL	+
Case 9	+	-	-	-	-	-	+	-	-	DLBL	+
Case 10	-	-	+	+	-	-	NT	NT	NT	DLBL	+

Abbreviations: DLBL, diffuse large B-cell lymphoma; IGH, immunoglobulin heavy chain gene rearrangement study; IGK, immunoglobulin light kappa chain gene rearrangement study; MALT, MALT type lymphoma; MCL, mantle cell lymphoma; NMZL, nodal marginal zone B-cell lymphoma; NT, not tested; SLL, small lymphocytic lymphoma.

indicated that for DLBCL, an IGH test alone may not be sufficient to detect monoclonality. With IGH testing, the monoclonal detection rate was approximately 85%, and the use of additional IGK kits increased the detection rate to about 98%. Additionally, we detected monoclonality using only IGH tests in cases of nodal marginal zone B-cell lymphoma, mantle cell lymphoma, and small lymphocytic lymphoma, and these results were consistent with those of this report. Therefore, we suggest that in the case of DLBCL, both IGH and IGK tests be applied to detect BM involvement. The IGH<sub>D</sub> test was positive in two cases (cases 5 and 6) that were also positive for the IGHC test (Table 2). Two cases showed monoclonality in IGK tests, but not in IGH tests. This result showed that IGK tests are reliable and useful for the detection of Ig gene rearrangements in cases where they were not detected by IGH tests.

Therefore, we suggest that IGH<sub>A</sub>, IGH<sub>B</sub>, and IGH<sub>C</sub> IGH tests, together with IGK<sub>A</sub>, and IGK<sub>B</sub> IGK tests should be considered as efficient Ig clonality assays. In negative cases, the application of additional IGH<sub>D</sub> and IGH<sub>E</sub> IGH tests and IGL tests need to be considered.

Interestingly, one case (case 10) showed a discrepant result: it was microscopically normal and positive for monoclonality by IGH tests (IGH<sub>B</sub> and IGH<sub>C</sub>). The case also showed 18-F fluorodeoxyglucose (FDG) hot uptake in BM in a PET-CT analysis (Table 2). Therefore, PET-CT was confirmed to be helpful in determining BM involvement in lymphoma [8].

With regard to minimal residual disease (MRD), Brisco *et al.* [9] reported that the IGH gene rearrangement assay would be useful in monitoring of MRD in B-cell lymphoblastic leukemia, and if a case did not show BM involvement morphologically but showed monoclonality in IGH tests, it would be associated with poor prognosis and a high-risk relapse rate. We also followed one case with microscopic involvement and monoclonality detected in IGK tests but

not in IGH tests (case 9). Complete hematological remission was achieved, based on BM microscopic examination, after chemotherapy. The case exhibited negative result on Ig gene rearrangement assays (IGH, IGK and IGL). Therefore, discrepancy cases need to be followed closely.

Recently, there was another study using BIOMED-2 kits and the heteroduplex method on formalin-fixed paraffin-embedded tissue from Hodgkin's lymphoma (HL) cases. IGK and IGL gene rearrangement assays were found to be reliable methods that increased the clonality detection rate. Of the HL cases tested, 94% were found positive for clonality [10].

On the whole, this study showed a high concordance rate (96.4%, 27/28) between the results of microscopic BM examination and Ig gene rearrangement assay. These results are in line with those of Shin *et al.* [11], which indicated a relatively high concordance rate (70.6%, 84/119) in B-cell neoplasias. We recommend simultaneous application of both BM microscopy and Ig gene rearrangement assays to more accurately diagnose and determine the prognosis of cases. Henceforth, more data on the discrepancy between BM exam and clonality assay should be accumulated; it will be helpful to make a therapeutic decision or develop a protocol. Additionally, we found a concordance rate of 96% (24/25) between results of the Ig gene rearrangement assay and PET-CT, although three cases were not examined by PET-CT (data not shown). A limitation in our research was that we could not compare our data with that of the capillary EP method by nucleotide sequencing to detect monoclonality in malignant lymphomas. We believe that application of this method might have improved the sensitivity and specificity of diagnosis of B-NHL.

In this study, we evaluated the utility of multiplex PCR-based Ig gene rearrangement assay in detecting BM involvement in malignant lymphoma. When it is difficult to detect BM involvement in malignant lymphoma based

on morphology, the Ig gene rearrangement assay can provide more accurate information regarding BM involvement in malignant lymphomas.

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#### Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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## The imbalance of procoagulant and anticoagulant factors in patients with chronic liver diseases in North India

**TO THE EDITOR:** Patients with chronic liver diseases (CLD) tend to experience severe hemostatic anomalies because of reduced levels of most of the coagulant proteins and anticoagulant factors such as protein C, protein S, and antithrombin. In contrast, it has been observed that levels of certain procoagulant factors such as factor VIII and von Willebrand factor (vWF) levels may be increased [1]. Various mechanisms such as increased levels of vWF antigen and reduced synthesis of ADAMTS 13 cleavage protease have been described to explain elevated factor VIII levels in these patients [2]. Additionally, the fact that factor VIII is an acute-phase reactant could elucidate partly these findings [3]. Not only increased procoagulant factor levels but a reduction in these factors may also lead to prothrombotic tendency in these patients. Concurrent reduction in protein C and factor VIII may also result in the procoagulant imbalance. It is important to distinguish the mechanism for increased factor VIII level in CLD patients since its sustained elevations may provoke the thrombosis. This study was aimed to compare the levels of factor VIII and protein C in CLD patients with a superimposed acute insult [acute-on-chronic liver failure (ACLF)] and in patients with compensated cirrhosis (CC), and to detect the correlations between the these factors and the disease activity using Model for End-Stage Liver Disease (MELD) scores in these respective groups. Furthermore, the ratio of factor VIII and protein C levels was evaluated as an indicator of the severity of liver disease in both groups.

This prospective study comprising 2 groups of patients with underlying CLD in a tertiary care center in North India was approved by the institutional Review board, with written informed consent obtained from all participants. Group 1 included 58 patients with ACLF (Asian Pacific Association for the Study of the Liver criteria [4]), and group 2 included 58 patients with biopsy-proven CC. The blood samples for coagulation study were collected from