Indian J Hematol Blood Transfus (Jan-Mar 2019) 35(1):57–65
https://doi.org/10.1007/s12288-018-0978-1

ORIGINAL ARTICLE

MYD88 and CXCR4 Mutation Profiling in Lymphoplasmacytic Lymphoma/Waldenstrom's Macroglobulinaemia

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Received: 9 May 2018 / Accepted: 26 June 2018 / Published online: 2 July 2018

Abstract Recurrent mutations affecting MYD88 and CXCR4 gene nowadays form the basis for the diagnosis, risk stratification and use of inhibitors targeting these signalling pathways in LPL/WM which are rare B cell neoplasms. MYD88 L265P mutation analysis was performed on 33 cases of LPL/WM by AS-PCR (positivity-84.8%, n = 28/33) and by Sanger sequencing (positivity-39.3%, n = 13/33). We had only two cases with CXCR4 non-sense (NS) mutation (p.S338*) using Sanger sequencing. MYD88 (L265P) mutation detection by AS-PCR can form reliable biomarker for the diagnosis of LPL/WM in molecular labs. Although the cohort is small, still the CXCR4 mutation frequency in our study is low as compared to the published literature.

Keywords MYD88 · CXCR4 · LPL/WM · Lymphoplasmacytic lymphoma · Waldenstrom's macroglobulinaemia

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Introduction

The World Health Organization (WHO) *Classification of Tumours of Haematopoietic and Lymphoid Tissues* recognizes Lymphoplasmacytic lymphoma (LPL) as neoplasm of small B lymphocytes, plasmacytoid lymphocytes and plasma cells usually involving bone marrow (BM). LPL with BM involvement and IgM monoclonal gammopathy of any concentration is termed as Waldenstrom macroglobulinemia (WM) [1]. LPL/WM are rare B-cell neoplasm with overall incidence rate of 3 cases per one million persons [2]. LPL/WM typically has indolent clinical course with long median survival of 10 years. Risk stratification in LPL/WM is done using parameters like age, hemoglobin level, platelet count, b2 microglobulin levels, monoclonal protein concentrations and International Prognostic Scoring System for Waldenstrom Macroglobulinemia (IPSS-WM) [3]. LPL/WM lacks a disease defining immunophenotype, though it is characterized with expression of pan—B-cell markers such as CD19, CD20, CD79a, and PAX5. CD5, CD23 may be expressed in a few cases, whereas CD10 is usually negative [4, 5]. Establishing the correct diagnosis of LPL/WM is important as this disorder is associated with long-term survival and the focus is on methods to minimize the toxicity associated with therapy and to avoid late complications [6].

LPL/WM and other low-grade B-cell neoplasms showing plasmacytic differentiation and monoclonal IgM protein like SMZL, CLL cannot always be differentiated solely on the clinical, morphologic and immunophenotypic features [7]. In such conditions a biomarker is needed as a pointer to differentiate LPL/WM from other small B-cell lymphomas and plasma cell myeloma. The recent studies have shown that recurrent mutations affecting Myeloid Differentiation Primary Response 88 (MYD88) gene and

chemokine receptor (CXCR4) nowadays form the basis for the diagnosis, risk stratification and use of inhibitors targeting these signalling pathways in LPL/WM.

Treon et al. first described a recurring mutation L265P in exon 5 of the MYD88 in 91% of patients with WM by performing whole genome sequencing. The somatic mutation (T → C) at position 38,182,641 in chromosome 3p22.2 results in an amino-acid change from leucine to proline at 265 codon (L265P) in the MYD88 gene [7–9]. Subsequently various studies have shown that MYD88 L265P somatic mutation is present in 67–90% of WM/non-IgM secreting LPL patients and in a substantial proportion of activated B-cell-like subtype diffuse large B-cell (ACDLBCL) lymphoma and in a minority of cases of chronic lymphocytic leukemia (CLL) [8, 10, 11]. MYD88 is as a linker protein which controls signaling through receptors like Toll-like receptors and Interleukin-1 on surface of immune cells. Following stimulation of these receptors by pathogens or inflammatory cells, MYD88 is recruited and complexes with the proteins IRAK1 and IRAK4 which in turn stimulates the MAPK and NF-kB pathways which are important for growth and survival of Waldenstrom's cells.

Similar to MYD88 L265P mutation, whole genome sequencing showed another important somatic mutation in chemokine receptor CXCR4 in WM cases [12]. Hunter and Treon et al. [12, 13] first analysed CXCR4 gene on CD19 + ve selected lymphocytes in WM cases and showed that activating somatic mutations in this chemokine receptor C-terminal tail are seen in almost 30–40% of WM patients. These somatic mutations are primarily subclonal, and almost always associated with MYD88 L265P mutations. Very similar to the already documented germline mutations in congenital autosomal dominant WHIM (warts, hypogammaglobulinemia, infection, and myelokathexis) syndrome, somatic mutations described in LPL include both frame-shift and non-sense mutations in the C-terminal tail. WHIM like CXCR4 frameshift (CXCR4WHIM/FS) and non-sense (CXCR4WHIM/NS) mutations are gaining importance as predictors of disease presentations and resistance to targeted therapy of Ibrutinib [14, 15].

We investigated the genetic and clinicopathological profile of LPL cases with respect to MYD88 and CXCR4 gene mutation analysis in the cases referred to our molecular laboratory for MYD88 mutation analysis.

Materials and Methods

Patient Samples

This is partly retrospective and partly prospective study carried out in Department of Molecular Pathology at Tata Medical Centre, Kolkata over a period of 5 years (2011–2016). The mutation status and the clinicopathological significance of MYD88 and CXCR4 mutations was sought in 33 cases of LPL/WM (cases included had B cell lymphoproliferative disorder with raised IgM immunoglobulins and/or clinical suspicion of LPL/WM). The detailed clinic-pathological characteristics of the cases included in the study are summarized in Table 1.

All the clinical details were retrieved from the electronic medical records of the institution. Immonophenotyping in the cases where available was done using immunohistochemistry (Ventanna Systems-IHC panel) on bone marrow trephine biopsy and/or multicolour Flowcytometry (FACS Calibur, Becton–Dickinson) on bone marrow aspirate and peripheral blood. The tests done were part of standard care; Institutional Review Board approval for waiver of consent was obtained (EC/TMC/83/16).

MYD88 hotspot mutation L265P and MYD88 exon 5 were screened by conventional allele-specific polymerase chain (AS-PCR) and Sanger sequencing respectively. DNA was extracted from either fresh bone marrow aspirate/peripheral blood (n = 24) or archived bone marrow aspirate slides (n = 9) using Qiagen Blood Mini Kit as per manufacturer's recommendations. AS-PCR for MYD88 L265P mutation was performed on these cases as per protocol previously reported by Marzia Verottoni et al. [16] (Fig. 1). In addition each case was screened by bi-directional Sanger sequencing for validation using the following primers, MYD88 Ex5 forward, 5-GTTGAAGACTGGGCTTGTCC-3; MYD88 Ex5 reverse, 5-AGGAGGCAGGGCAGAAGTA-3, Big Dye Terminator v3.1 Cycle Sequencing Kit and an ABI 3500 automatic sequencer (Applied Biosystems, Foster City, USA) (Fig. 2).

As described by by Hunter et al. [12] CXCR4 gene hot spot mutations (from codon 263 to 356) were screened by Sanger Sequencing. The PCR and Sanger Sequencing for CXCR4 gene (from codon 263 to 356) was performed using the following primers, CXCR4 forward, 5-GCTGCCTTACTACATTGGGATCAGC-3; CXCR4 reverse, 5-TTGGCCACAGGTCCTGCCTAGACA-3, Big Dye Terminator v3.1 Cycle Sequencing Kit and an ABI 3500 automatic sequencer (Applied Biosystems, Foster City, USA) (Fig. 3).

The sequencing electropherograms were screened for nucleotide changes using SeqScape software (Applied Biosystems, Foster city, USA). The transcript for analysis of MYD88 gene Exon 5 sequence was NM_002468 (ENST00000396334.7) and for CXCR4 gene (codon 263–356) was NM_003467 (ENST00000241393.3).

Table 1 Clinical features of all the cases included in the study

No.	Age (years)	Sex	Hb (gm/dl)	TLC (×10E9/L)	Plt. Count (×10E9/L)	LDH (U/L)	IgM (mg/dl)	IgA (mg/dl)	IgG (mg/dl)	BMB	IHC/FCM	Diagnosis	AS-PCR MYD88 L265P	Direct sequencing MYD88	CXCR4 sequencing
LPL1	68	M	11.6	4.00	117	374	1754.47	Less than40	645.93	B cell LPD	FCM (PB):CD20, CD19, CD38, CD10, CD20, CD23 +ve; Kappa restriction	WM	Positive	Wild type	Wild type
LPL2	64	F	9.5	9.00	150	296	5158	61.14	1444.12	B cell LPD	IHC (BM Bx): CD20 + ve; CD5,23 −ve	WM	Positive	Positive	Wild type
LPL3	72	M	7.3	1.70	8	645	2116.9	69.7	551.2	B cell LPD	FCM (BMA):CD19, CD20, CD5, CD22 + ve; Lambda restriction	WM	Positive	Positive	Wild type
LPL4	58	M	7.8	4.40	100	545	2518	616.5	6732.5	Involved by LPL	IHC (BM BNA): CD20 + ve; CD5,23,56,138 −ve	LPL	Positive	Positive	**Wild type**
LPL5	62	M	3.9	1.39	45	1310	8410	93.6	785.7	Involved by LPL	FCM (BMA): CD19, CD38, CD20, CD22, CD23 CD5 +ve; kappa, lambda −ve	LPL/WM	Positive	Positive	Wild type
LPL6	76	M	10.5	6.10	109	268	2000.6	64.8	1159.7	LPL/WM	IHC (BM Bx): CD20+	WM	Positive	Wild type	Wild type
LPL7	69	M	7.7	5.10	204	281	6103	Less than40	761.2	B cell NHL	IHC (BM Bx): CD20+	WM	Positive	Positive	Wild type
LPL8	73	M	8.9	5.40	459	339	4308.2	58.1	936.8	Involved by LPL	IHC (BM Bx): CD20, CD23 +ve; CD5, CD38, CD56 −ve	WM	Positive	Positive	Wild type
LPL9	70	M	7.4	2.20	23	NA	1288.9	239.8	1527.3	Involved by LPL	FCM (BMA):CD19, CD20, CD22, CD23, CD25, CD38 +ve; kappa restriction	WM	Positive	Wild type	Wild type
LPL10	70	M	5.9	1.46	400	NA	1226.7	136.5	1058.7	NA	NA	WM (diagnosed outside)	Positive	Positive	Wild type
LPL11	59	F	8.1	9.50	291	NA	1974.1	108.5	1461.4	Involved by LPL	FCM (BMA): CD19, CD20, CD22, CD23, CD38 +ve; Kappa restriction	WM	Positive	Wild type	Wild type
LPL12	72	M	9	8.30	252	338	1998.2	503.7	4348	Involved by LPL	FCM (BMA): CD19, CD20, CD22, CD25, CD38 +ve; kappa restriction.	WM	Positive	Wild type	Wild type

Table 1 continued

No.	Age (years)	Sex	Hb (gm/dl)	TLC (×10E9/L)	Plt. Count (×10E9/L)	LDH (U/L)	IgM (mg/dl)	IgA (mg/dl)	IgG (mg/dl)	BMB	IHC/FCM	Diagnosis	AS-PCR MYD88 L265P	Direct sequencing MYD88	CXCR4 sequencing
LPL13	45	M	7.3	5.40	150	250	7135	83	979	LPL (outside)	NA	WM	Positive	Wild type	Wild type
LPL14	57	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	WM (diagnosed outside)	Positive	Wild type	Wild type
LPL15	64	M	9	7.50	NA	NA	Igm raised	NA	NA	low grade LPD (outside)	IHC (BM Bx): CD20, CD5, CD23, +ve; Cyclin D1, TRAP, DBA44 −ve	WM	Positive	Positive	Positive
LPL16	54	F	7.3	1.50	80	NA	3301.6	259.4	2085.2	Involved by LPL	IHC (BM Bx): CD20, CD23 +ve; CD5,10 −ve	WM	Positive	Positive	Wild type
LPL17	55	F	7.5	6.00	49	799	11,444.9	Less than40	532.8	Involved by LPL	IHC (BM Bx): CD20+	WM	Positive	Positive	Wild type
LPL18	65	F	NA	NA	NA	NA	NA	NA	NA	NA	NA	WM (diagnosed outside)	Positive	Wild type	Wild type
LPL19	72	F	8.3	9.60	471	225	3334.3	158.1	3246	B cell LPD	NA	LPL/WM	Wild type	Wild type	Wild type
LPL20	45	F	3.5	2.70	29	825	561.7	6973	Less than25	B cell LPD	IHC (BM Bx): CD20+	LPL with IGA paraprotein	Wild type	Wild type	Wild type
LPL21	60	F	NA	NA	NA	NA	NA	NA	NA	NA	NA	Suspected LPL/WM (outside)	Wild type	Wild type	Wild type
LPL22	53	M	10	6.00	332	NA	7830	42.6	774.4	B cell LPD	FCM (BMA): CD19, CD20, CD22, CD23, CD38 +ve; kappa restriction	WM	Positive	Wild type	Wild type
LPL23	51	F	NA	NA	NA	NA	892.7	109.9	1012.5	NA	NA	WM	Positive	Wild type	Wild type
LPL24	60	M	8	5.4	172	NA	7264.1	40	496.1	B cell LPD	IHC (BM Bx): CD20+	WM	Positive	Wild type	Wild type
LPL25	64	M	4.7	0.9	30	NA	7737.4	148.3	1410.6	Involved by LPL	favour LPL (CD20 + ; CD5, CD10, CD23 −ve; CD138 highlights plasma cells	WM	Positive	Wild type	Wild type
LPL26	77	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	WM (diagnosed outside)	Positive	Positive	Positive

Table 1 continued

No.	Age (years)	Sex	Hb (gm/dl)	TLC (×10E9/L)	Plt. Count (×10E9/L)	LDH (U/L)	IgM (mg/dl)	IgA (mg/dl)	IgG (mg/dl)	BMB	IHC/FCM	Diagnosis	AS-PCR MYD88 L265P	Direct sequencing MYD88	CXCR4 sequencing
LPL27	69	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	WM	Positive	Positive	Wild type
LPL28	65	F	NA	NA	NA	NA	NA	NA	NA	NA	WM	WM (diagnosed outside)	Positive	Positive	Wild type
LPL29	64	M	8.5	4.6	199	NA	4628	82.2	503.3	LPL (outside)	FCM (BMA):CD19, CD20, CD22, CD23, CD38 +ve; lambda restriction	WM	Positive	Wild type	Wild type
LPL30	77	M	8.6	11.2	410	NA	4306.2	59	1161	Involved by LPL	CD20 + ve; CD10, CD5, CyclinD1−ve;138 highlights plasma cells	WM	Positive	Wild type	Wild type
LPL31	58	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	LPL (Evaluated outside)	Positive	Wild type	Wild type
LPL32	64	M	6.3	9.5	93	761	4361.8	118.5	489.5	Involved by LPL	BM BX:favor LPL, CD20 + small nodules; CD138 highlights interstitial plasma cells	WM	Wild type	ND	Wild type
LPL33	57	M	10.7	4.2	181	NA	4340.3	131	631.3	LPD	NA	LPL/CLL with raised IgM	Wild type	ND	Wild type

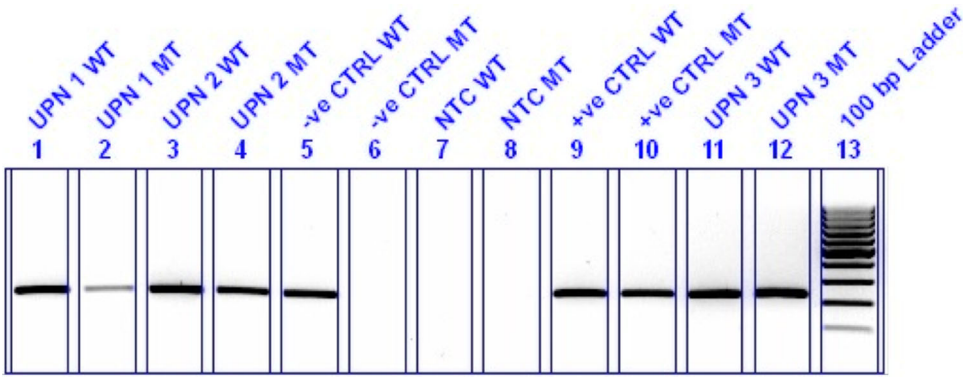

Fig. 1 Gel electrophoresis image of MYD88 L265P AS-PCR. [UPN1,2,3 are MYD88 L265P mutation positive cases showing band in both wild type lane (1,3,11) and mutant lane (2,4,12) respectively. Lanes 5,6 show wild type control whereas lanes 9,10 show MYD88 L265P positive control. Lane 13 shows 100 bp ladder]

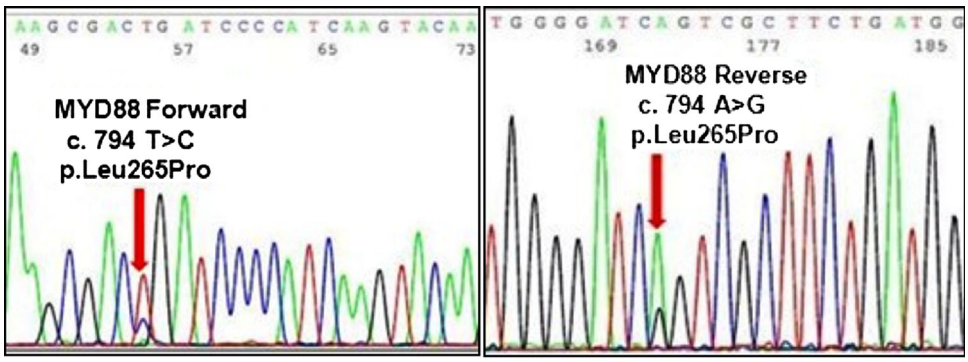

Fig. 2 Sanger sequencing electropherogram of exon 5 of MYD88 gene (representative picture of forward sequence—c.794T > C change and reverse sequence—c.794A > G change)

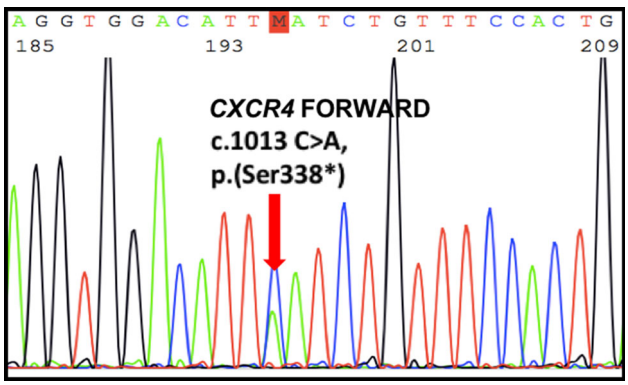

Fig. 3 Sanger sequencing electropherogram of CXCR4 gene (representative picture of forward sequence—c.1013C > A; p.Ser338* change)

Statistical Analysis

The data was analyzed using SPSS version 21 and student *t* test for equality of means was used to study the correlation of the clinicopathological features within the MYD88 L265P mutated and wild type cohort. All the *p* values were two-tailed and the statistical significance was set at $p < 0.05$.

Results

The 33 cases of LPL/WM had median age of 64 years (range 45–77 years) with 69.7% males (n = 23) and 30.3% females (n = 10). Almost all cases had cytopenia of one or more lineage with median IgM levels of 4306.2 mg/dl (range 561.7–11,444.9 mg/dl, n = 25), median lymphocyte count of 35% (range 11–70%, n = 27) and median IPSS-WM of 3. Of the total LPL/WM (n = 33) cases screened by AS-PCR technique, twenty-eight (84.8%, n = 28) harbored MYD88 L265P (Fig. 4). This MYD88 L265P mutant cohort had mean age = 63.9 years, M:F = 3:1, median IPSS-WM 2.57 (n = 21) and raised IgM (mean = 4447.4 mg/dl, range 561.7–11,444.9 mg/dl, n = 21) ($p = 0.280$).

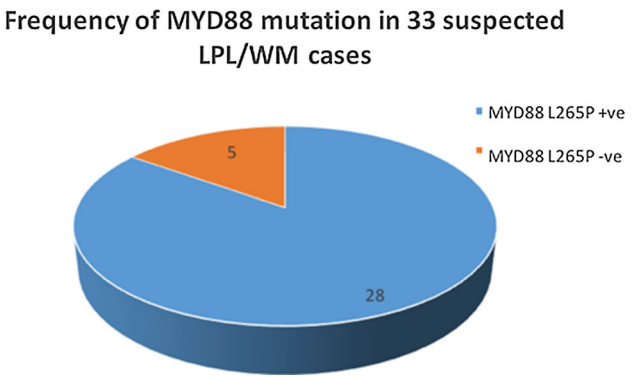

Fig. 4 Frequency of MYD88 L265P mutation

All cases had anaemia (Hb: < 11.5 gm/dl) and BM involvement by lymphoplasmacytic cells. Table 2 shows the other laboratory variables of both AS-PCR MYD88 L265P positive and wild type LPL/WM cases.

Of the five AS-PCR MYD88 mutation negative cases, one case [LPL20] was of LPL with IgA paraproteinemia (6973 mg/dl), the second (LPL33) had a differential diagnosis of LPL/CLL with IgM paraproteinemia (4340.3 mg/dl) and the third (LPL32) was a case of LPL with raised IgM (4361.8 mg/dl). In two of the MYD88 L265P mutation negative LPL cases (LPL19 and LPL21) no specific clinical details were available.

MYD88 L265P was detected by Sanger Sequencing only in 13/28 (46.4%) AS-PCR positive cases. All these cases had heterozygous misense mutation and not a single case had homozygous mutation.

Amongst the MYD88 L265P mutated cohort screened (n = 28), CXCR4 gene mutation was noted in only two cases. Both the cases had a MYD88 L265P mutation positivity by AS-PCR as well as Sanger Sequencing and had heterozygous CXCR4 non-sense (NS) mutation (c.1013 C > A, p.Ser338*) which resulted in premature truncation of the protein at codon 338 (Fig. 3). All the five AS-PCR MYD88 L265P mutation negative cases were wild type for CXCR4 gene mutation on Sanger Sequencing.

The two MYD88L265P/CXCR4 WHIM-NS mutation positive cases (LPL15 and LPL26, Table 2) were referred to our center with diagnosis of B-cell lymphoproliferative disorder. The patient (LPL15) had complaints of weakness and easy fatigability. The CBC findings were: Hb-9.4gm/dl, WBC-7500 with predominant neutrophils and normal Platelet count. The serum electrophoresis and immunofixation electrophoresis (IFE) showed M-band and IgM Kappa band respectively. The LDH and B2 microglobulin levels were elevated. The BM aspirate was found to have 73% atypical lymphoid cells which on immunohistochemistry done on BM biopsy had dim positive expression of CD20, CD5, CD23 and negative for Cyclin D1, TRAP and DBA44. As MYD88 L265P mutation was positive, the patient was diagnosed as WM (taking into consideration the raised serum IgM levels, immunophenotypic findings of atypical lymphoid cells in bone marrow). This patient after completing 5 cycles of BR (Bendamustine, Rituximab) chemotherapy regimen had repeated fever and weakness. Subsequent BM biopsy showed 2–3 focal granulomas with central necrosis and multinucleated giant cells. The patient was also found to have warts on face which were suspected to be due to Herpes infection. However, further the patient was lost to follow-up.

The second MYD88L265P/CXCR4-NS mutation positive case [LPL26] presented with anaemia and neutropenic fever and was diagnosed at outside center as WM on bone marrow biopsy. The patient was subsequently managed by Bortezomib and Rituximab based regimen.

Discussion

MYD88 L265P mutation is now emerging as the single most important biomarker for LPLs. It not only increases the diagnostic accuracy of LPL but it has been shown to have prognostic impact with MYD88 L265P mutation having a protective effect, mutants having longer overall survival as compared to wild type (WT) cases. Somatic mutations in CXCR4 are the second most common mutations described in LPL. In WM cases, the CXCR4 mutations have been described to affect both clinical presentation and disease outcome. In this study we therefore analyzed the incidence and utility of both MYD88 L265P mutations and CXCR4 in LPL/WM cases.

The positivity rate of MYD88 L265P in this study was 84.8% by conventional AS-PCR technique. Ondrejka et al. [4] and Marzia et al. [16] had reported a frequency of 100% positivity for MYD88 L265P mutation in LPL/WM cases they studied using AS-PCR. Overall the MYD88 L265P somatic mutation has been described in 67–90% of WM/non-IgM secreting LPL patients. There is only a single study by Patkar et al. [17] in Indian population which

Table 2 Clinical Features of MYD88 L265P positive and Wild Type cases

Clinical features	MYD88 L265P positive	MYD88 wild type	*p* value
Mean age (years)	63.9 (n = 28)	59.6 (n = 5)	0.395
Mean Hb (gm/dl)	8.0 (n = 21)	7.2 (n = 4)	0.633
Total WBC count (×10E9/l)	5.09 (n = 21)	6.5 (n = 4)	0.503
Plt count (×10E9/l)	179 (n = 20)	193.5 (n = 4)	0.895
Mean IgM conc (mg/dl)	4447.4 (n = 21)	3149.5 (n = 4)	0.280
Median Lymphocte % age	35.96% (n = 23)	36% (n = 4)	0.996
Median International Prognostic Index (IPSS-WM)	2.57 (n = 21)	2.75 (n = 4)	0.587
LDH (U/L)	495 (n = 11)	603.6 (n = 3)	0.645

shows frequency of MYD88 L265P mutation to be 84.3% (using AS-PCR) in 32 WM cases which is almost similar to this study. It has been shown in various studies that MYD88 wild type LPL cases are clinically and biologically different than MYD88 mutant cases—which have high tumour burden and poor therapeutic response [18]. As the number of MYD88 wild type cases are less in this study we were not able to establish statistically significant differences between different clinical findings (Table 2) in both the cohorts.

Only 46.4% of the AS-PCR MYD88 L265P mutant cases were positive using Sanger sequencing technique highlighting the limitation of using Sanger sequencing for detection of this mutation. The discrepancy between AS-PCR and Sanger Sequencing technique might be due to the low tumour (lymphocyte) percentage or because of the sensitivity of the technique to detect mutant allele. Studies have shown that limit of detection of mutant allele by Sanger Sequencing technique is low (15–20%) [19] whereas AS-PCR is a highly sensitive assay (limit of mutant allele detection up to 1.5%), thus the lower frequency of MYD88 L265P mutation detection by Sanger Sequencing in our cohort indicates that the samples which were wild type by Sanger techniques might have had a low mutant allele. No significant co-relation was noted between the median lymphocyte percentage in the MYD88 L265P Sanger positive (median-41%, n = 10, range 11–70%) and wild type cases (median-32%, n = 15, range 12–66%) ($p = 0.270$).

MYD88 L265P mutation is usually absent or rarely described in multiple myeloma, marginal zone lymphoma (MZL) or IgM-monoclonal gammopathy of undetermined significance (IgM-MGUS) patients. Thus, detection of this mutation as adjunct to the clinical features is valuable for the differential diagnosis of WM from these low-grade B-cell neoplasms [20].

Hunter and Treon et al. [12, 13] performed CXCR4 mutation analysis on CD19 + ve selected lymphocytes in WM cases and have shown that activating somatic mutations in CXCR4 chemokine receptor C-terminal tail are seen in almost 30–40% of WM patients. These mutations are primarily subclonal, and almost always associated with MYD88 L265P mutations. CXCR4 is a chemokine receptor with frame-shift and non-sense mutations in C-terminal tail similar to those documented in autosomal dominant congenital disorder WHIM (Warts, Hypogammaglobulinemia, Infection, and Myelokathexis) syndrome. WHIM like CXCR4 frameshift (CXCR4WHIM-FS) and non-sense (CXCR4WHIM-NS) mutations are gaining importance as predictors of disease presentations and resistance to targeted therapy of Ibrutinib [14, 15].

We had a very low positivity rate of CXCR4 mutations (only two cases) among the MYD88 L265P mutant cases as compared to the literature. This could be because various studies in literature have utilized CD19 + ve selected lymphocytes for performing CXCR4 mutation analysis where as we have used unselected whole bone marrow/peripheral blood for this CXCR4 mutation analysis. Another reason for this lower frequency could also be due the sensitivity of the Sanger sequencing technique.

Patients with MYD88 L265P and CXCR4WHIM-NS have significantly higher serum IgM levels, bone marrow involvement as compared to MYD88L265P/CXCR4WHIM-FS, MYD88L265P/CXCR4WT, and MYD88WTpatients [13, 14], though this was not evident in our study (Table 2).

WHIM syndrome is autosomal dominant inherited disorder with multigenerational pattern of the disease in families. Several point mutations leading to a premature stop codon (R334*, S338*, G336*, and E343*) as well as missense mutation (E343K) involving last 10–19 amino acids of the C-tail of CXCR4 have been reported from the genomic DNA of patients with WHIM syndrome and their family members [21–23]. Both of the MYD88L265P/CXCR4 WHIM-NS mutation positive cases in our study had a non-sense mutation at codon 338 which is in the highly conserved region of the receptor C-terminal region. The C-terminal tail conserved region is generally associated with germline mutations in the inherited WHIMs syndrome cases. The germline status of the CXCR4 gene could not be analyzed as both the cases were lost to follow. Since the CXCR4 mutations described in WM cases also involve the C-terminal tail conserved region, a cautious approach is needed while interpreting the CXCR4 mutation analysis done from whole blood DNA and it would be prudent to rule out germline mutations.

Conclusion

AS-PCR is a highly sensitive method for MYD88 L265P mutation analysis and could be of great help for the recognition of LPL/WM in molecular labs. This is the first study from India documenting the CXCR4 mutations in LPL/WM cases. Although the number of cases are only few, still the positivity rate for CXCR4 mutations in the study is low as compared to the literature.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval This study was approved by the Ethics Committee and Institutional Review Board (EC/TMC/83/16) of the Tata Medical Center, Kolkata, India.

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