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REGULAR ARTICLE

blood advances

Quantitative MYD88 L265P and flow cytometry levels for outcome determination in IgM gammopathies: the SAL-TO study

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Key Points

- Combining MYD88 L265P and flow cytometry data enables stratification of patients with IgM gammopathies into risk groups with distinct survival outcomes.

Waldenström macroglobulinemia (WM) is a rare, indolent B-cell lymphoproliferative disorder, often preceded by a history of immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (IgM-MGUS). In this retrospective, multicenter study, we collected real-life data from 577 patients with IgM gammopathy (221 symptomatic WM [sWM], 245 asymptomatic WM [aWM], 111 IgM-MGUS) from 22 Spanish centers with a validation cohort of 166 patients (73 sWM, 71 aWM, 22 IgM-MGUS) from the University Hospital of Torino, Italy. The median overall survival (OS) was 126.7 months for the Spanish cohort and 202.8 for the Torino cohort. A multivariate analysis identified age >65 years, male gender, diagnosis of sWM, and β-2-microglobulin >3 as significant predictors of a shorter OS. In addition, age >65 years, bone marrow (BM) biopsy infiltration, hemoglobin <11.5 g/dL, and platelets <100 000/μL were associated with a shorter time to first treatment (TTFT). Pooling data from both cohorts revealed that a baseline BM quantitative *MYD88* L265P to wild-type *MYD88* ratio of >0.162 (either by droplet digital polymerase chain reaction [PCR] or quantitative PCR), together with multiparameter flow cytometry (MFC) infiltration >4.39%, had a significant impact on OS and TTFT. The combination of *MYD88* and MFC levels enabled stratification of patients into high-, intermediate-, and low-risk groups with patients with high-risk IgM gammopathy showing increased disease-related death in a competing risk analysis.

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Data are available from the corresponding author, Simone Ferrero (simone.ferrero@unito.it), on request.

The full-text version of this article contains a data supplement.

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Introduction

Waldenström macroglobulinemia (WM) is a rare, indolent B-cell lymphoproliferative disorder that is characterized by bone marrow (BM) involvement through lymphoplasmacytic cells that secrete monoclonal immunoglobulin M (IgM) protein.[1,2]

Although a familial predisposition has been demonstrated,[3] the main risk factor for WM remains a history of IgM monoclonal gammopathy of undetermined significance (IgM-MGUS)[4] with a probability of progression to WM or other lymphoproliferative disorders of 1.5% to 2% per year.[4]

Important advances in the understanding of the biology of WM have been made in the last few years. By using whole-genome sequencing, Treon et al identified *MYD88* L265P as a highly recurrent somatic mutation in 90% of patients with WM.[5] Several studies that used different techniques, such as Sanger sequencing, polymerase chain reaction (PCR), and allele-specific, quantitative PCR (ASqPCR),[6-8] confirmed the presence of the *MYD88* L265P mutation in WM. Moreover, it was also demonstrated in BM samples in 50% to 80% of patients with IgM-MGUS,[7-10] and it can also be identified in peripheral blood (PB),[11] specifically in the cell-free DNA fraction, of patients with IgM gammopathy.[12,13]

From a clinical standpoint, WM typically shows an indolent clinical course with ~25% of patients being asymptomatic at initial diagnosis, despite a heterogeneous disease behavior. Indeed, among patients with smoldering WM, there are currently no indications for the initiation of treatment until the development of significant symptoms.[14-16] However, these patients have a high risk for transformation from smoldering WM to active WM and should be monitored closely.[17]

Over the years, many attempts have been made to predict the outcomes of patients with IgM gammopathy based on clinical assessments by applying different criteria for WM or IgM-MGUS diagnosis and treatment initiation.[4,18-23] For patients with active WM who require treatment, the International Prognostic Scoring System for WM (IPSS-WM) was designed to assess survival after treatment initiation and overall survival (OS)[24] by stratifying patients into 3 risk categories with corresponding 5-year survival rates of 87%, 68%, and 36%, respectively. Later on, further revisions of the IPSS-WM refined prognostication in active WM,[25,26] whereas for patients with asymptomatic WM (aWM), Bustoros et al developed a prognostic score for disease progression that relies on BM biopsy (BMB) evaluation and basic clinical findings.[27]

Some authors have also tried to assess the impact of laboratory prognostic factors, such as multiparameter flow cytometry (MFC) features,[28] cytogenetics, and molecular biology characterization, on patient outcomes. Even in the absence of disease-defining cytogenetic abnormalities, deletions in the chromosome 6q are present in 40% to 50% of WM cases and have been shown to negatively affect patient prognosis[29,30] by being associated with a higher IPSS-WM score and a shorter time to treatment, progression-free survival (PFS), and OS.[31-34]

The impact of molecular factors in determining the prognosis for patients with WM is still not clear. Some studies have suggested that the *MYD88* L265P mutation may have an impact on OS and play a role in disease progression by promoting the transition from IgM-MGUS to WM or other lymphoproliferative diseases.[9,27,35,36] In contrast, other studies showed that the MYD88 L265P mutation had no effect on OS or time to progression[14] and that patients with MYD88WT had higher progression rates to symptomatic WM (sWM) and a more aggressive clinical course.[27,36-38] Moreover, *CXCR4*^{WHIM} mutations, the second most frequent genetic alteration in WM, have been proven to affect the response to therapy and PFS,[39,40] without affecting OS.[33,37] Finally, similar to many other neoplasms, *TP53* alterations confer worse outcome in terms of OS,[40-42] irrespectively of the IPSS-WM score; of note, such alterations were not identified in patients who had IgM-MGUS.[41,43]

Aims

This study aimed to (1) collect a large series of patients with IgM gammopathy who were treated in real-life settings; (2) determine patient outcomes in terms of OS, time to first treatment (TTFT), and PFS; (3) identify clinical factors that affect disease progression and survival; and (4) evaluate the impact of baseline molecular and flow cytometry analyses on survival and the need for WM treatment.

Materials and methods

Patient selection

Spanish cohort. The registry of monoclonal gammopathies based in Salamanca and in the region of Castilla y Leon (Spain) was investigated for IgM-secreting disorders (Figure 1); only patients with confirmed IgM-MGUS, aWM, or sWM[1] were selected for this study. All patients provided written informed consent in accordance with the Helsinki declaration.

Torino cohort. Electronic health records of patients who were observed or treated at the Centre of Torino (Division of Haematology 1, Department of Biotechnology and Health Sciences, University Hospital Città della Salute e della Scienza, University of Torino, Italy) were scanned to identify patients with IgM monoclonal gammopathy; patients with a confirmed diagnosis of IgM-MGUS, aWM, or sWM were selected by the study team using the same criteria employed for the Spanish cohort (Figure 1). All patients provided written informed consent in accordance with the Helsinki declaration.

Sample collection and laboratory analysis

BM and PB samples were extracted from patients at first diagnosis of IgM gammopathy or at disease progression, according to locally established procedures, for both study cohorts. A BMB was performed per clinical practice, mainly when the criteria for treatment initiation or suspicion of progressive/transformed disease were met.

Genomic DNA was extracted for molecular studies from the evaluated patients, as previously described, and analyzed for the presence of the *MYD88* L265P mutation.[44] Alternatively, *MYD88* mutational status was determined by droplet digital PCR (ddPCR) on both BM and PB samples, as previously described.[12] All patient samples tested in Torino were analyzed by ddPCR. Of note, in neither of the cohorts was CD19^{+} cell selection routinely applied before the molecular study, but it was performed for selected cases in the Spanish cohort before fluorescence in situ hybridization analysis.

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Deletions of 6q were assessed in patients with IgM-MGUS and WM by either simple interphase fluorescence in situ hybridization performed on cell nuclei from whole BM samples or CD19-selected cells using a previously published technique.[31]

Immunophenotypic evaluation was done using conventional panels of monoclonal antibodies, as previously described,[45] and following the general recommendations of the EuroFlow group for the immunophenotypic evaluation of hematologic malignancies. The sensitivity of MFC assay was 0.004% for at least 500 000 events.

Statistical analyses

Statistical analyses were carried out using R (version 4.3.1). Survival curves were plotted using the Kaplan-Meier method and compared with the log-rank test. The medians between groups for continuous variables were compared using the Kruskal-Wallis test (for nonnormal variables) or the 1-way analysis of variance test (for normal variables); the χ^2 test or Fisher exact test for small study samples was employed for categorical variables. OS was measured from the date of the initial diagnosis to the date of death from any cause. For asymptomatic cases, TTFT was defined as the time between diagnosis and progression to sWM that required active treatment. PFS was defined as the time between first-line WM treatment and the date of progression or death from any cause. The Cox proportional hazards model was implemented for the univariate and multivariate survival analyses. In particular, for the multivariable model, an Akaike Information Criterion-based backward stepwise algorithm (R function stats::step) was used to perform the variable selection (restricting the data set to have no missing data) to determine the most relevant covariates and analyze potential confounding factors. Once the covariates of interest were obtained from the restricted data set, they were applied to the full data set for each endpoint.

Given the larger size of the Spanish data set, the statistical models were constructed using these data. Subsequently, the Torino data set was merged to evaluate whether there were differences between the 2 populations. An analysis to evaluate the IPSS-WM and the revised IPSS-WM scores was also performed on both cohorts (Spain, Torino). Moreover, the aWM score was evaluated in patients with aWM in the Torino series.

In addition, we worked out a cutoff for quantitative *MYD88* and MFC infiltration in BM; the cutoff points were identified using the surv_cutpoint function in R on the Spanish data set, and this was extended to the Torino cohort analysis.

Finally, a competing risk analysis was performed for OS using the cuminc function of the tidycmprsk package of R.

Institutional review board approval

Institutional review board approval was obtained from the Salamanca University Hospital Ethic Committee, Paseo de San Vicente, 58-182 37007 Salamanca, Spain; Ethical Commitee number PI91/07/2018 (date of approval: 13 August 2018) and 21/742 (date of approval: 3 November 2021). Furthermore, the ethic Committee of the Azienda Ospedaliera Universitaria Città delle Salute e della Scienza di Torino - A.O. Ordine Mauriziano di Torino - A.S.L. Città di Torino, Corso Bramante, 88/90 - 10126 Turin, Italy; Ethical Commitee number 476/2021 (date of approval 25 November 2021) also provided institutional review board approval.

Results

Patient characteristics

Data from 903 patients across 22 Spanish centers were collected between 1976 and 2019 in the considered registry; 577 cases with a local diagnosis of IgM-MGUS, aWM, or sWM were selected, whereas the remaining cases were excluded because of a diagnosis of other lymphoproliferative diseases, misregistration, or missing data (Figure 1). Among patients observed in Torino, Italy, 166 patients with IgM gammopathy with available baseline and follow-up data were selected according to the same criteria. It should be noted that these patients were diagnosed from 1988 to 2020, although the vast majority (161/166) were diagnosed after the year 2000.

The demographic and clinical data at initial diagnosis are reported in Tables 1 and 2 (Spanish and Torino cohorts).

When they were first evaluated, 111 Spanish patients were locally diagnosed with IgM-MGUS, 245 with aWM, and 221 with sWM. The IPSS-WM score at diagnosis for patients with sWM was low in 30 patients (18.2%), intermediate in 68 (41.2%), and high in 67 patients (40.6%) in the Spanish cohort, and low in 10 patients (20.0%), intermediate in 18 (36.0%), and high in 22 patients (44.0%) in the Torino cohort ($P = .804$ for the distribution in the 2 cohorts).

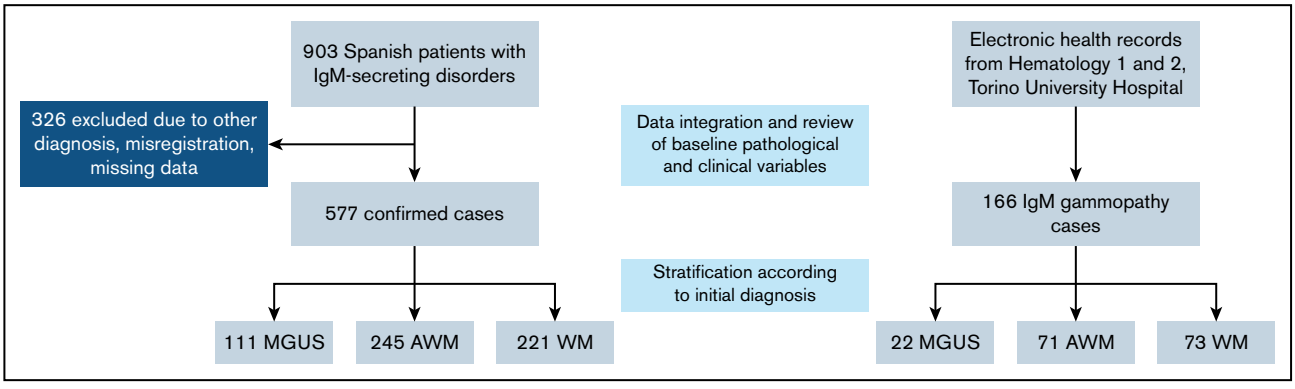

Figure 1. Consort diagram of enrolled patients in the 2 cohorts. FU, follow-up; NA, not available.

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Table 1. Characteristics of IgM gammopathy patients enrolled in the study: Spanish cohort

	IgM MGUS (n = 111)	Asymptomatic WM (n = 245)	Symptomatic WM (n = 221)	*P* value
Age (median [IQR])	69.5 [29.0, 87.0]	72.0 [34.0, 93.0]	71.0 [30.0, 94.0]	0.070
Sex				
Female	46 (41.4%)	88 (35.9%)	67 (30.3%)	0.120
Male	65 (58.6%)	157 (64.1%)	154 (69.7%)	
Hb (median [IQR]), g/dL	13.9 [8.1, 17.4]	13.2 [6.0, 20.0]	10.4 [3.6, 18.0]	<0.001
ECOG				
0	68 (61.3%)	136 (55.5%)	46 (20.8%)	<0.001
1	19 (17.1%)	55 (22.4%)	74 (33.5%)	
2	3 (2.7%)	12 (4.9%)	48 (21.7%)	
3	0 (0%)	3 (1.2%)	10 (4.5%)	
4	1 (0.9%)	2 (0.8%)	2 (0.9%)	
Missing	20 (18.0%)	37 (15.1%)	41 (18.6%)	
B symptoms				
No	92 (82.9%)	203 (82.9%)	117 (52.9%)	<0.001
Yes	1 (0.9%)	7 (2.9%)	73 (33.0%)	
Missing	18 (16.2%)	35 (14.3%)	31 (14.0%)	
Platelets (median [IQR]), $\times 10^9$/L	227 [73, 1970]	248 [30, 644]	210 [2, 684]	<0.001
B2M (median [IQR]), mg/dL	2.00 [0.5, 6.4]	2.46 [0.15, 18.0]	3.26 [0.4, 18.6]	<0.001
Missing	21 (18.9%)	55 (22.4%)	45 (20.4%)	
Albumin (median [IQR]), g/dL	4.10 [2.9, 5.0]	3.90 [2.3, 5.2]	3.60 [1.6, 4.9]	<0.001
Missing	20 (18.0%)	38 (15.5%)	32 (14.5%)	
IgM (median [IQR]), mg/dL	781 [87, 6130]	1460 [225, 9220]	3300 [19.0, 13 000]	<0.001
Missing	9 (8.1%)	23 (9.4%)	22 (10.0%)	
LDH ratio (median [IQR]), UI/L	0.73 [0.27, 1.8]	0.65 [0.28, 1.76]	0.65 [0.25, 4.0]	0.043
Missing	18 (16.2%)	35 (14.3%)	35 (15.8%)	
Light Ig type				
k	69 (62.2%)	160 (65.3%)	157 (71.0%)	0.039
l	37 (33.3%)	62 (25.3%)	38 (17.2%)	
Negative	0 (0%)	2 (0.8%)	1 (0.5%)	
Missing	5 (4.5%)	21 (8.6%)	25 (11.3%)	
IPSS-WM				
High	0 (0%)	13 (5.3%)	67 (30.3%)	<0.001
Int	45 (40.5%)	126 (51.4%)	68 (30.8%)	
Low	30 (27.0%)	45 (18.4%)	30 (13.6%)	
Missing	36 (32.4%)	61 (24.9%)	56 (25.3%)	
FISH				
Abnormal	4 (3.6%)	20 (8.1%)	34 (15.5%)	0.001
Normal	26 (23.4%)	93 (38.0%)	65 (29.5%)	
Not evaluable	81 (73%)	132 (53.9%)	121 (54.1%)	
***MYD88* L265P mutation**				
Negative	20 (18.0%)	25 (10.2%)	26 (11.8%)	<0.001
Positive	18 (16.2%)	111 (45.3%)	98 (44.3%)	
Not evaluable	73 (65.8%)	109 (44.5%)	97 (43.9%)	
MYD88 L265P mutational ratio (median [IQR])	0.02 [0.001, 0.0868]	0.0165 [0.00131, 1.76]	0.0362 [0.00158, 1.36]	0.004

ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridization; Ig, immunoglobulin; Int, intermediate; k, free light kappa chain; l, free light lambda chain; LDH, lactate dehydrogenase.

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Table 2. Characteristics of IgM gammopathy patients enrolled in the study: Torino cohort

	IgM MGUS (n = 22)	Asymptomatic WM (n = 71)	Symptomatic WM (n = 73)	*P* value
Age (median [IQR])	73.0 [48.0, 91.0]	67.0 [24.0, 84.0]	70.0 [43.0, 95.0]	0.091
Sex				
Female	8 (36.4%)	33 (46.5%)	24 (32.9%)	0.237
Male	14 (63.6%)	38 (53.5%)	49 (67.1%)	
Hb (median [IQR]), g/dL	10.4 [3.60, 18.0]	14.0 [8.30, 16.0]	13.0 [0, 18.0]	<0.001
ECOG				
0	16 (72.7%)	59 (83.1%)	30 (41.1%)	<0.001
1	4 (18.2%)	8 (11.3%)	15 (20.5%)	
2	1 (4.5%)	1 (1.4%)	10 (13.7%)	
3	0 (0%)	0 (0%)	7 (9.6%)	
4	0 (0%)	0 (0%)	1 (1.4%)	
Missing	1 (4.5%)	3 (4.2%)	10 (13.7%)	
BMB% (median [IQR])	0 [0, 0]	30.0 [10.0, 90.0]	70.0 [10.0, 95.0]	<0.001
Missing	15 (68.2%)	8 (11.3%)	10 (13.7%)	
B symptoms				
No	20 (90.9%)	69 (97.2%)	53 (72.6%)	0.002
Yes	1 (4.5%)	0 (0%)	10 (13.7%)	
Missing	1 (4.5%)	2 (2.8%)	10 (13.7%)	
Platelets (median [IQR]), $\times 10^9$/L	209 [114, 315]	260 [71.0, 632]	203 [29.0, 543]	0.013
B2M (median [IQR]), mg/dL	2.52 [1.4, 6.6]	1.95 [1.1, 13.2]	3.10 [1.5, 12.6]	0.002
Missing	10 (45.5%)	23 (32.4%)	42 (57.5%)	
Albumin (median [IQR]), g/dL	3.80 [3.0, 5.1]	4.00 [3.0, 5.3]	3.75 [2.5, 4.8]	0.012
Missing	11 (50.0%)	34 (47.9%)	35 (47.9%)	
IgM (median [IQR]), mg/dL	781 [195, 5360]	1370 [210, 5480]	2960 [205, 9620]	<0.001
Missing	0 (0%)	5 (7.0%)	11 (15.1%)	
LDH (median [IQR]), UI/L	0.8 [0.3, 1.8]	0.8 [0.3, 1.4]	0.7 [0.4, 2.4]	0.554
Missing	8 (36.4%)	25 (35.2%)	22 (30.1%)	
Light Ig type				
k	15 (68.2%)	50 (70.4%)	52 (71.2%)	0.982
l	4 (18.2%)	15 (21.1%)	15 (20.5%)	
Missing	3 (13.6%)	6 (8.5%)	6 (8.2%)	
IPSS WM				
High	0 (0%)	4 (5.6%)	22 (30.1%)	<0.001
Int	15 (68.2%)	30 (42.3%)	18 (24.7%)	
Low	6 (27.3%)	23 (32.4%)	10 (13.7%)	
Missing	1 (4.5%)	14 (19.7%)	23 (31.5%)	
FISH				
Abnormal	0 (0%)	2 (2.8%)	2 (2.9%)	0.193
Normal	0 (0%)	2 (2.8%)	0 (0%)	
Not evaluable	22 (100%)	67 (94.4%)	68 (97.1%)	
***MYD88* L265P mutation**				
Negative	3 (13.6%)	5 (7.0%)	10 (13.7%)	0.376
Positive	11 (50.0%)	50 (70.4%)	53 (72.6%)	
Missing	8 (36.4%)	16 (22.5%)	10 (13.7%)	
MYD88 L265P mutational ratio (median [IQR])	0.00497 [0.000551, 0.0371]	0.0216 [0.000520, 0.283]	0.0278 [0.000400, 0.713]	0.003

ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridization; Ig, immunoglobulin; Int, intermediate; k, free light kappa chain; l, free light lambda chain; LDH, lactate dehydrogenase.

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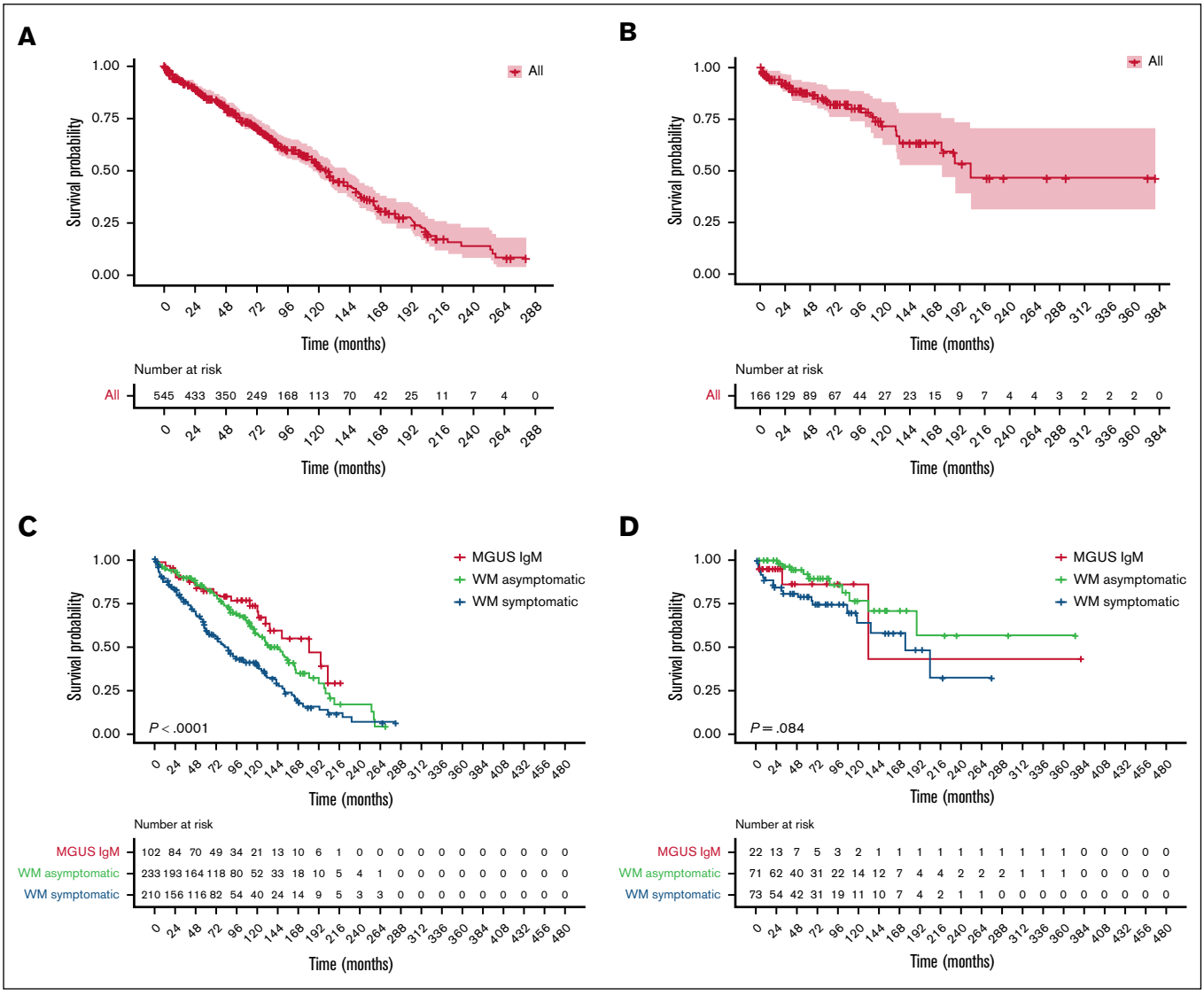

Figure 2. Overall survival by cohort and diagnosis. (A) Spanish cohort. (B) Torino cohort. (C-D) Overall survival stratified by initial diagnosis of MGUS, aWM, or sWM.

Treatments

In the Spanish retrospective series that comprised patients observed from 1976 to 2019, 78 of 577 (13.5%) patients had missing first-line treatment details and were excluded from the analysis of treatments received. Among 499 patients with available details of treatments received, 321 (64.3%) (diagnosed with IgM-MGUS or aWM) were observed in a watch and wait (W&W) approach, whereas 178 of 499 (35.7%) patients with WM received initial therapy (supplemental Table 1). At last follow-up, 208 of 321 (64.8%) patients remained asymptomatic, whereas 41 of 321 (12.8%) had progressed to WM and required active treatment; follow-up data were missing for 72 patients.

Most employed first-line treatments included chlorambucil-based regimens (n = 98/178, 55%), dexamethasone-rituximab-cyclophosphamide scheme (n = 19/178; 10.7%), and fludarabine-containing regimens (n = 6/178; 3.4%). Overall, 51 of 178 patients (28.6%) received rituximab as first-line treatment (single agent or any combination).

In the Torino cohort, 5 of 166 patients were excluded from the analysis of treatment received for missing information; 60 of 161 patients (37.3%) remained asymptomatic and did not receive therapy during follow-up. First-line treatments used at any time included dexamethasone-rituximab-cyclophosphamide (n = 27/73; 37%), rituximab-bendamustine (n = 8/73; 11%), and chlorambucil-based schemes (n = 6/73; 8.2%). Rituximab was administered as first-line treatment to 69.8% of treated patients.

Survival analyses

OS. In the Spanish cohort, at last follow-up, 305 (52.9%) patients were alive at a median follow-up of 100.6 months (interquartile range [IQR], 55.5-166.6). The median OS was 126.7 months (IQR, 58.7-193.6). The OS at 5 years was 74.0% (95%

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confidence interval [CI], 70.1-78.2), and at 10 years, the OS was 52.5% (95% CI, 47.3%-58.2%; Figure 2A). The median OS for patients with IgM-MGUS vs aWM vs sWM was 180.6 (IQR, 111.3-not reached) vs 143.6 (80.9-199.7) vs 85.0 (38.8-151.6) months ($P < .001$, hazard ratio [HR] for death in sWM group, 2.481) in the univariate analysis (Figure 2C).

In the Torino cohort, at last follow-up, 132 (79.5%) patients were alive at a median follow-up of 71.2 (IQR, 39.6-115.4) months. The median OS was 202.8 months (IQR, 109.0-not reached); the OS at 5 years was 85.7% (95% CI, 80.0-91.8) and 71.6% (61.7-83.1) at 10 years from initial diagnosis (Figure 2B). The median OS stratified for initial diagnosis was 131.0 (131.0-not reached) months for IgM-MGUS, not-reached (131.4-not reached) for aWM, 174.4 (65.9-not reached) months for sWM ($P = .084$; Figure 2).

TTFT. The TTFT was evaluated for all asymptomatic patients at baseline, including those with IgM-MGUS and aWM. For the Spanish cohort, the median TTFT was 228.5 months (IQR, 146.9-not reached) with 89.1% of patients not requiring treatment after 5 years of follow-up (95% CI, 85.5-92.9). For patients observed in Torino, the median TTFT was 165.6 months (IQR, 49.4-207.9), and the probability to remain asymptomatic was 71.4% at 5 years (95% CI, 60.9-83.7) (Figure 3).

PFS. PFS was evaluated for patients who received treatment for sWM at any time. Among patients with available data, the median PFS was 42.4 months (IQR, 13.6-91.0) for the Spanish cohort and 38.0 months (IQR, 13.9-75.6) for the Torino cohort (Figure 3, bottom panels). At 2 years, the PFS probability was 64.6% (95% CI, 58.8-70.9) and 63.3% (54.2-73.8) for the Spanish and Torino cohorts, respectively. Importantly, there was no difference in the PFS outcome between patients who received active treatment at diagnosis and patients who initially underwent the W&W approach and then progressed to WM ($P = .34$ and $P = .24$ in the 2 cohorts).

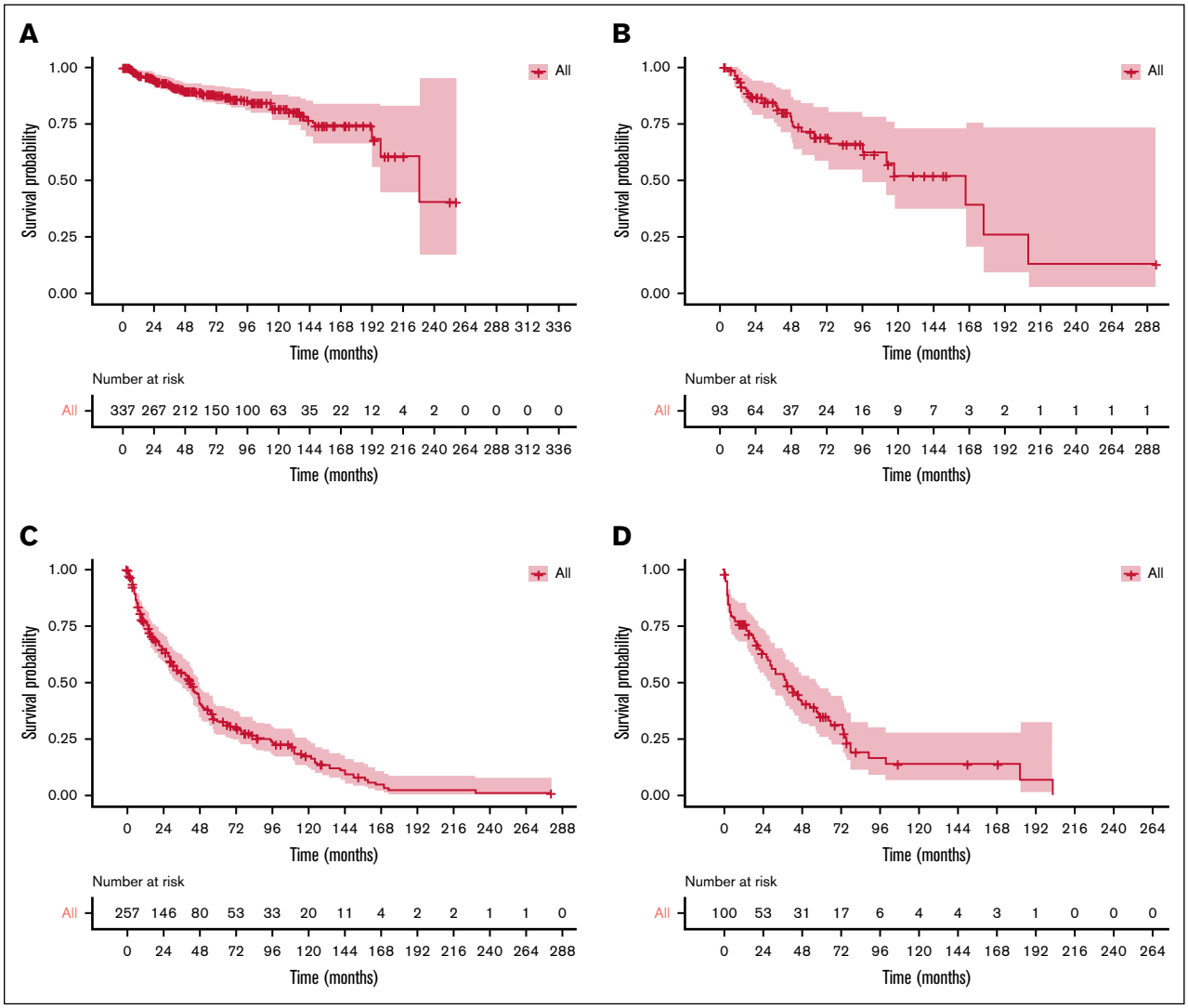

Figure 3. TTFT in asymptomatic patients and progression-free survival in treated patients by cohort. (A) Asymptomatic patients belonging to the Spanish cohort. (B) Torino cohort. PFS among patients who required treatment at any time in the Spanish cohort (C) and Torino cohort (D) (bottom panels).

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Table 3. Multivariable analysis for OS, TTFT, and PFS in the Spanish and Torino cohorts

	OS			PFS			TTFT		
Variables	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Spanish cohort									
Age									
≤65	–	–		–	–		–	–	
65-75	2.221	1.514-3.260	**<.001**	1.389	0.931-2.072	.107	1.002	0.483-2.083	.996
≥76	4.136	2.812-6.085	**<.001**	2.561	1.675-3.918	**<.001**	0.238	0.075-0.757	**.015**
Sex									
Female	–	–					–	–	
Male	1.651	1.192-2.287	**.003**				2.115	0.971-4.606	.059
Hb									
≤11.5							-	-	
>11.5							0.353	0.136-0.918	**.033**
MGUS	–	–							
aWM	1.172	0.722-1.902	.521						
sWM	2.086	1.277-3.407	**.003**						
BMB									
Not evaluable				–	–		–	–	
Negative				0.937	0.423-2.074	.873	1.498	0.523-4.291	.452
Positive				1.332	0.950-1.869	.096	2.902	1.337-6.298	**.007**
Platelets									
≤100				–	–	–	–	–	
>100				1.567	0.950-1.869	.091	0.096	0.027-0.345	**<.001**
B2M									
≤3	–	–		–	–				
>3	1.617	1.200-2.180	**.002**	1.262	0.905-1.761	.171			
Albumin									
<3.5							–	–	–
≥3.5							0.449	0,200-1.010	.053
LDH									
≤1				–	–				
>1				0.533	0.326-0.872	**.012**			
Hyperviscosity									
No	–	–							
Yes	1.407	0.943-2.098	.094						
Torino cohort									
Age									
≤65	–	–		–	–		–	–	
65-75	2.221	1.514-3.260	**<.001**	1.389	0.931-2.072	.107	1.002	0.483-2.083	.996
≥76	4.136	2.812-6.085	**<.001**	2.561	1.675-3.918	**<.001**	0.238	0.075-0.757	**.015**
Sex									
Female	–	–					–	–	
Male	1.651	1.192-2.287	**.003**				2.115	0.971-4.606	.059
Hb									
≤11.5							–	–	
>11.5							0.353	0.136-0.918	**.033**

Variables with a statistically significant effect in the multivariable models are indicated in bold.

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Table 3 (continued)

Variables	OS			PFS			TTFT		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
MGUS	–	–							
aWM	1.172	0.722-1.902	.521						
sWM	2.086	1.277-3.407	**.003**						
BMB									
Not evaluable				–	–		–	–	
Negative				0.937	0.423-2.074	.873	1.498	0.523-4.291	.452
Positive				1.332	0.950-1.869	.096	2.902	1.337-6.298	**.007**
Platelets									
≤100				–	–	–	–	–	
>100				1.567	0.950-1.869	.091	0.096	0.027-0.345	**<.001**
B2M									
≤3	–	–		–	–				
>3	1.617	1.200-2.180	**.002**	1.262	0.905-1.761	.171			
Albumin									
<3.5							–	–	–
≥3.5							0.449	0.200-1.010	.053
LDH									
≤1				–	–				
>1				0.533	0.326-0.872	**.012**			
Hyperviscosity									
No	–	–							
Yes	1.407	0.943-2.098	.094						

Variables with a statistically significant effect in the multivariable models are indicated in bold.

Finally, 22 of 577 (4.8%) and 5 of 166 (3.1%) patients in the 2 examined cohorts experienced transformation from IgM gammopathy to aggressive lymphoma during follow-up and showed very poor survival (supplemental Figure 1). No significant difference in the incidence of transformation in the 2 groups was found ($P = .51$).

Clinical prognostic factors

The clinical data at baseline, including age, sex, IgM levels, hemoglobin (Hb), platelets, Eastern Cooperative Oncology Group Performance Status, the presence of hyperviscosity, BMB infiltration, albumin, β-2-microglobulin (B2M), lactate dehydrogenase, *MYD88* L265P mutational ratio, and MFC infiltration in BM, were evaluated for prognostic significance in terms of OS, TTFT (for asymptomatic patients), and PFS (for patients treated at any time). The significant variables for each cohort are summarized in Table 4.

A multivariable model for OS, PFS, and TTFT (summarized in Table 3) was built, as previously described, for the Spanish cohort. This model was then used to evaluate a merged population (Spain + Torino) to identify differences between the 2 cohorts. Because a *P* value of > .05 was found, we may conclude that there was no evidence of differences between these groups.

For the OS prediction, the algorithm identified age >65 years, male sex, initial diagnosis of sWM, and B2M >3 as statistically significant variables. In contrast, the multivariable analysis for TTFT was built for asymptomatic patients (IgM-MGUS, aWM) and produced a model with age, BMB infiltration, Hb <11.5 g/dL, and platelets <100 000/μL as significant variables. Finally, a multivariable analysis for PFS in patients with sWM (sWM at diagnosis + patients progressing from W&W approach to sWM) was performed, and age and lactate dehydrogenase ratio were identified as statistically significant.

In addition, we evaluated the IPSS-WM and revised IPSS-WM scores in Spanish patients with WM and found that they both retained statistical significance ($P < .001$ for both scores; supplemental Figure 2), also confirmed in the multivariable analysis. In contrast, the aWM score could not be evaluated in patients with aWM from the Torino series because of the low number of patients with available complete data (n = 29).

Prognostic significance of molecular and flow cytometry factors

For patients with available BM *MYD88* L265P mutation evaluation data at baseline (n = 298; 51.6% for the Spanish cohort; and n = 142; 85.5% for Torino cohort), we analyzed the outcome in terms of OS and TTFT using both quantitative PCR methods results.

Patients from the Spanish cohort and Torino cohorts were pooled and divided into high vs low *MYD88* groups (cutoff point set at 0.162, defined as previously described).

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Table 4. Univariate analysis for OS, TTFT, and PFS with molecular and flow cytometry variables in the Spanish and Torino cohorts

	OS			PFS			TTFT		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Spanish cohort									
***MYD88* ratio: cutoff >0.1624318**									
Neg	–		–	–		–	–		–
Low	0.995		.982	0.789		0.333	0.864		.754
High	1.823		.061	1.013		0.969	3.478		**.049**
Flow ratio: cutoff >4.39%									
Neg	–		–	–		–	–		–
Low	0.912		.792	**<0.001**		0.995	3.215		.268
High	1.421		.279	**<0.001**		0.995	7.196		.056
Torino cohort									
***MYD88* ratio: cutoff >0.624318**									
Neg	–		–	–		–	–		–
Low	1.153		.822	0.811		0.575	0.724		.670
High	3.063		.16	1.430		0.434	2.874		.395
Flow ratio: cutoff >4.39%									
Neg	–		–	–		–	–		–
Low	**<0.001**		.998	**<0.001**		0.997	0.35		.396
High	**<0.001**		.998	**<0.001**		0.997	1.48		.621

Variables with a statistically significant effect in the multivariable models are indicated in bold.

In the univariate analysis, the MYD88^{low} group showed better OS ($P = .005$; HR, 0.44) and TTFT ($P = .024$; HR, 0.33) than the MYD88^{high} group (Table 4). In the multivariable analysis, statistical significance was not confirmed ($P = .622$) for OS, although there was a trend toward significance for TTFT ($P = .06$).

Regarding the prognostic impact of MYD88 on OS specifically in patients with IgM-MGUS, data were available for 37 patients classified as MYD88 low (n = 17) or MYD88^{WT} (n = 20), whereas no patient was classified as MYD88 high; no difference in OS was observed in the univariate analysis between these 2 subgroups ($P = .2$; supplemental Figure 6).

In addition, the molecular burden was compared with the percentage of marrow infiltration in the BMB (available only for the Torino cohort), which showed a moderate positive Pearson correlation (r = 0.653; $P < .0001$). The infiltration reported by BM MFC also showed a strong positive linear correlation (r = 0.73; $P < .0001$). Patients in the MYD88^{high} group generally showed higher BMB infiltration (~70%-90%), although rare outliers with low marrow infiltration (as low as 20%) were also observed, indicating possible sampling errors and biologic heterogeneity.

The pooled population was also analyzed for the prognostic significance of baseline clonal B-cell infiltration by MFC in available BM samples with a cutoff point set at 4.39%. In the univariable analysis, MFC^{low} patients had increased OS ($P = .033$; HR, 0.65) and TTFT ($P = .008$; HR, 0.37) when compared with the MFC^{high} group (Table 4). In the multivariable analysis, MFC lost statistical significance for OS ($P = .51$); however, it retained significance for TTFT ($P = .025$; HR, 0.37). Similarly, for patients with IgM-MGUS, the MFC burden affected the TTFT ($P = .006$; supplemental Figure 7) but did not show an impact on OS ($P = .5$).

In addition, we combined both the MFC and *MYD88* baseline levels and stratified patients into low-, intermediate-, and high-risk groups (low: MFC^{low}/*MYD88*^{low}; intermediate: either MFC^{low}/*MYD88*^{high} or MFC^{high}/*MYD88*^{low}; high: MFC^{high}/*MYD88*^{high}). Based on MFC and the molecular results, no patients with IgM-MGUS was assigned to the HR group; 16 HR patients had sWM, whereas 8 had aWM. Moreover, among the patients with sWM with high IPSS-WM risk, 7 patients were in the low-risk group, 18 patients were in the intermediate group, and 6 were in the high-risk group.

In the univariate analysis, the high-risk group differed significantly from the others in terms of OS (n = 156; $P = .005$; HR, 3.28; Figure 4) and TTFT (n = 92; $P = .003$; HR, 9.61). For TTFT, the intermediate-risk group ($P = .015$; HR, 4.76) was also significantly different from the reference group (low; supplemental Figure 3).

A multivariable analysis confirmed these results, highlighting the strong prognostic significance of the combination of molecular and MFC evaluations (for OS: $P = .038$; HR, 2.81; for TTFT: $P = .026$; HR, 7.91 for intermediate vs low risk and $P = .003$; HR, 24.68 for high vs low risk).

Finally, a competing risk analysis was performed. Among the 545 evaluable cases, at 5 years from diagnosis, 12.0% (95% CI, 9.6%-16%) of deaths were not related to WM, whereas 7.1% (95% CI, 5.0%-9.6%) were considered related (supplemental Figure 4). Among patients in the *MYD88*/MFC high-risk group, there was a statistically significant increase in disease-related, but not unrelated, deaths at 5 years ($P = .002$; 2.2%; 95% CI, 0.17%-10% vs 19%; 95% CI, 5.6%-38% for LR vs HR; supplemental Figure 5).

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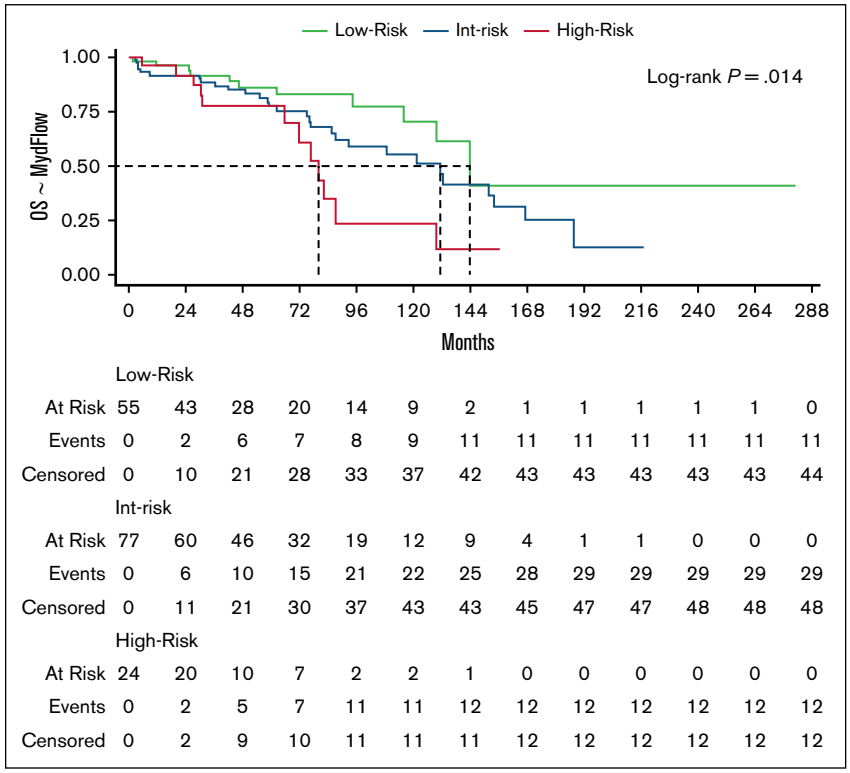
Figure 4. Outcome in terms of OS according to the BM MYD88^{L65P} and BM MFC levels.



Low-Risk													
At Risk	55	43	28	20	14	9	2	1	1	1	1	1	0
Events	0	2	6	7	8	9	11	11	11	11	11	11	11
Censored	0	10	21	28	33	37	42	43	43	43	43	43	44
Int-risk													
At Risk	77	60	46	32	19	12	9	4	1	1	0	0	0
Events	0	6	10	15	21	22	25	28	29	29	29	29	29
Censored	0	11	21	30	37	43	43	45	47	47	48	48	48
High-Risk													
At Risk	24	20	10	7	2	2	1	0	0	0	0	0	0
Events	0	2	5	7	11	11	12	12	12	12	12	12	12
Censored	0	2	9	10	11	11	11	12	12	12	12	12	12

Discussion

Previous real-life studies that reported on the survival outcomes of patients with WM, for instance, the Rory Morrison Registry in the United Kingdom and the Swedish Lymphoma Registry, showed a 5-year OS that ranged from 60% to 90%, both with a median follow-up of ~6 years.[20,46] In our series that also included aWM and IgM-MGUS, the 5-years OS was comparable (85% for the Torino cohort, 74% for the Spanish cohort), after a median follow-up of 71 and 100 months, respectively. It should be noted that the slightly superior OS outcome of the Torino cohort might be explained by the more recent IgM gammopathy diagnoses and the more extensive adoption of rituximab-containing regimens in first and subsequent lines of treatment.

However, in published registry studies, molecular evaluations and MFC data were limited or not available, and thus, prognostic evaluations relied solely on clinical factors. Indeed, prognostication for sWM was historically established based on the IPSS-WM score and its revised version[24,25] that stratified patients based on age and baseline characteristics.

In this study, both the IPSS-WM and revised IPSS-WM were confirmed to correctly identify the risk of patients with sWM, whereas for those with aWM, the score proposed by Bustoros et al was not reproducible, probably because of the low number of patients with data available on the percentage of invasion in BMBs.[27] Moreover, analyzing the whole IgM gammopathy series (IgM-MGUS, aWM, and sWM taken together), clinical factors were confirmed to be important for both OS (age, sex, B2M, and diagnosis) and TTFT (age, Hb, platelet, and BMB involvement). Indeed, the IPSS-WM was initially developed at a time (before 2002) when nucleoside analogs represented around one-third of the first-line therapies, whereas rituximab was only administered to 4% of patients. Nevertheless, it was still confirmed to be a feasible and powerful tool. However, in the rapidly changing field of WM in which both an understanding of its biology and its treatment were revolutionized by the discovery of the *MYD88* L265P mutation, the need to incorporate translational knowledge into prognostic assessment is still unmet. Indeed, although *MYD88* L265P mutation testing is already considered part of standard initial evaluation in suspected WM and is particularly helpful in the differential diagnosis of other lymphoproliferative neoplasms, its role in prognostication is still debated.

Indeed, previous findings have highlighted that patients with *MYD88*^{WT} WM have inferior survival outcomes[36] and show slower and less profound responses with Bruton tyrosine kinase inhibitors, especially with ibrutinib.[40] However, a large cohort study by Abeykoon et al did not show inferior OS or time to next treatment in patients with *MYD88*^{WT} vs those with *MYD88* L265P WM, whereas there was an increased occurrence of transformation to diffuse large B-cell lymphoma.[14]

For patients with IgM-MGUS, a study by Varettoni et al showed lower progression rates to overt WM in *MYD88*^{WT} cases than in cases with *MYD88* L265P.[9] In the same study, the *MYD88* L265P allele burden, as determined by ASqPCR on BM CD19^{+} selected mononuclear cells, showed a significant correlation with progression from MGUS to WM, albeit with a mild effect. These findings were later confirmed in a recent study by Moreno et al using ddPCR in IgM-MGUS and aWM cases.[47] Of note, among patients with

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IgM-MGUS, the reproducibility of the prognostic effect of molecular factors might be affected by low disease burden; the use of highly sensitive techniques is therefore key to correctly estimate prognosis. Indeed, past studies have shown heterogeneous results because of the different PCR techniques, the variable application of $CD19^+$ sorting, and the adoption of different diagnostic criteria.[48]

To date, however, there were no published data regarding the effect of high vs low *MYD88* L265P baseline levels on OS and TTFT in patients with WM. In this study, highly sensitive techniques for *MYD88* quantitative evaluation, either by ddPCR or ASqPCR, together with MFC analysis, allowed for a precise definition of disease burden at the time of IgM gammopathy diagnosis. Interestingly, the identified MFC cutoff points (4.39% clonal B-cell infiltration in BM) that separated high vs low disease burden was actually quite low, close to the median results in the aWM subgroup (3.55%). In contrast, the *MYD88* L265P cutoff point (ratio mutated/WT, 0.162; allele frequency, 13.4%) was higher than the median *MYD88* L265P identified in the sWM group (ratio mutated/WT, 0.04; allele frequency, 3.85%), thus identifying patients with very high molecular disease burden. We also observed a strong correlation between the *MYD88* L265P results and BM MFC infiltration, higher than the one between *MYD88* L265P and the percentage of infiltration in BMB by conventional histopathology observation.

From a technical standpoint, the molecular analysis was not homogeneous between the 2 cohorts. All Torino samples were analyzed using ddPCR, which offers higher sensitivity, whereas the vast majority (>95%) of the Spanish samples were assessed by ASqPCR, reflecting clinical practice at the time of initial diagnosis. Although both techniques correctly identified high molecular risk patients, this discrepancy represents a limitation of this retrospective, real-world study and highlights the absence of standardized molecular approaches for this disease. This lack of harmonization underscores the need for future efforts aimed at method standardization to ensure comparability and consistency across studies and clinical settings.

In the multivariable analysis, the combination of MFC and molecular variables identified a high-risk group that consisted of ~15% of the evaluable patients. Interestingly, a minority of aWM cases (~9%), but no patients with IgM-MGUS, were classified as high risk in terms of the *MYD88*/MFC score; thus, it is conceivable that this kind of evaluation could help to determine the risk for progression and disease-related death, even in asymptomatic patients. Indeed, a significant fraction (~22%) of the patients who scored high in the IPSS-WM was reclassified as low risk by the molecular/MFC score.

Conclusion

To our knowledge, this is the first time that quantitative *MYD88* L265P evaluation and MFC degree of infiltration in BM were proven to be prognostic of patients' survival and need for treatment in a large IgM gammopathy series. Moreover, molecular and flow cytometry results have never been combined before to generate translational data. Validation of these results in prospective studies might add greatly to prognostic evaluation of IgM gammopathy, particularly to improve the definition of patients with high-risk WM.

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Authorship

Contribution: I.D., C.J., D.D., R.G.-S., and S.F. conceptualized the study; I.D., V.P., M.F., D.M., S.M., S.R., and G.M.Z. contributed to data curation; I.D., C.J., V.P., and E.A. wrote the original draft; D.M., S.M., G.M.Z., and M.G. developed the methodology; I.D., V.P., G.B., E.M.O., A.R., I.M., F.E., C.A., M.A.G., A.G.d.C., R.H., J.D., F.C., N.P., V.G.d.l.C., S.R., M.C., C.C., E.A., M.d.C.C., A.M., N.C.G., S.F., and R.G.-S. performed investigation; S.F., B.B., M.G.D., and R.G.-S. provided supervision; and all authors reviewed and edited the manuscript.

Conflict-of-interest disclosure: S.F. is a consultant for Janssen, EUSA Pharma, AbbVie, and Sandoz; is on the advisory boards of Janssen, EUSA Pharma, Recordati, Incyte, Roche, AstraZeneca, Italfarmaco, and Behring; reports speaker's honoraria from Janssen, EUSA Pharma, Recordati, Lilly, BeiGene, Gilead, and Gentili; and research funding from Gilead and MorphoSys. G.B. reports serving on the speaker's bureau and advisory boards of Janssen, Bristol Myers Squibb, GSK, and Novartis. The remaining authors declare no competing financial interests.

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