IgM monoclonal gammopathy of undetermined significance: clinicopathologic features with and without IgM-related disorders

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Abstract

A subset of patients with immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (MGUS) develop IgM-related disorders (IgM-RD) including peripheral neuropathy, cryoglobulinemia and/or cold agglutinin disease (CAD). We examined the clinical and bone marrow pathologic findings in 191 IgM MGUS patients (2016 World Health Oragnization criteria). Clonal plasma cells were identified in 41 of 171 (24%) cases by immunohistochemistry (IHC) and clonal B cells in 43 of 157 (27%). IgM-RD was identified in 82 (43%) cases, including peripheral neuropathy (n=67, 35%), cryoglobulinemia (n=21, 11%), and CAD (n=10, 5%). Cases of CAD showed distinctive features including lack of *MYD88* mutations (*P*=0.048), supporting the concept of primary CAD as a distinct clinicopathologic disorder. Following exclusion of CAD, comparison of the remaining cases with (n=72) or without (n=109) IgM-RD showed IgM-RD to be more frequent in men than women (*P*=0.02) and to be more highly associated with *MYD88* L265P (*P*=0.011). Cases with and without IgM-RD otherwise showed similar features including serum IgM concentrations, presence of lymphoid aggregates, clonal B cells by flow cytometry or clonal plasma cells by IHC. No differences were observed in overall survival between cases with and without IgM-RD. No cases in this series met criteria for plasma cell type IgM MGUS as defined in the 2022 International Consensus Classification of lymphoid neoplasms. These results show IgM-RD to be common in patients with IgM MGUS. While CAD shows distinctive features, the remaining cases of IgM-RD largely show pathologic findings similar to IgM MGUS without IgM-RD.

Introduction

In contrast to immunglobulin G (IgG) or IgA monoclonal gammopathy of undetermined significance (MGUS), which typically progress to multiple myeloma or other plasma cell neoplasms, IgM MGUS characteristically shows progression to lymphoplasmacytic lymphoma (LPL) or other B-cell lymphoproliferative disorders.[1–4] IgM multiple myeloma is rare, but also presumably develops through a precursor phase of IgM MGUS.[5–7] In the revised 4th edition of the World Health Organization (WHO) classification of hematolymphoid neoplasms, IgM MGUS is defined by the presence of an IgM paraprotein with a serum concentration of less than 3 g/dL, less than 10% clonal lymphoplasmacytic cell infiltration in the bone marrow, and lack of myeloma-defining clinical features.[8]

The criteria for diagnosis of IgM MGUS and distinction from LPL, however, has been somewhat controversial. Some cases meeting revised 4th edition WHO criteria for IgM MGUS would be classified as LPL by the criteria of the International Workshop on Waldenström's macroglobulinemia[9] or the recently published International Consensus Classification (ICC) of lymphoid neoplasms.[10] The ICC further distinguishes between IgM MGUS of plasma cell type (defined as having clonal plasma cells without clonal B cells and lacking *MYD88* mutations) *versus* IgM MGUS, not otherwise specified (all other cases) in an effort to distinguish cases that may progress to IgM myeloma *versus* those more likely to progress to lymphoproliferative disorders.[10]

It has also increasingly been recognized in recent years that patients fulfilling criteria for IgM MGUS, despite hav-

ing relatively low disease burden in the marrow, may in some cases have significant symptoms which require therapy.[11–15] The most commonly reported symptoms include neuropathies, cryoglobulinemia, or cold agglutinin disease (CAD). If these symptoms secondary to an IgM paraprotein are present, the term "IgM-related disorder" (IgM-RD) has been proposed to recognize the potential need for treatment in these patients.[9] Recent studies have shown that primary CAD represents a distinct lymphoproliferative disease with clinicopathologic findings differing from LPL or other IgM MGUS,[16–18] and the 2022 ICC recognizes primary CAD as a distinct clinicopathologic entity.[10] It is currently unclear whether other forms of IgM-RD may show distinctive clinicopathologic findings compared to asymptomatic IgM MGUS patients.

In this study, we examine the detailed clinicopathologic findings in a cohort of patients meeting 2016 WHO criteria for IgM MGUS and compare the findings in patients with and without IgM-RD. We also examine the impact of the new 2022 ICC diagnostic criteria on diagnosis of IgM *versus* the 2016 WHO system. These results provide detailed guidance for diagnosis of IgM MGUS and have implications for the most appropriate classification of these clinically heterogeneous cases.

Methods

Case selection, morphology review, and clinical annotation

After Cleveland Clinic Institutional Review Board approval, we retrospectively reviewed patients with an IgM serum paraprotein seen at our institution from 2002-2021 and identified those who underwent bone marrow biopsies. Formalin-fixed, paraffin-embedded, and hematoxylin and eosin stained tissue sections from bone marrow trephine biopsies and aspirate clot sections as well as Wright-Giemsa stained bone marrow aspirate smears were retrieved from the archives. Previously performed immunohistochemistry was reviewed, as were available results of prior flow cytometry, metaphase cytogenetics, fluorescence *in situ* hybridization (FISH) for plasma cell associated abnormalities, and *MYD88* L265P mutation testing.

Clinical data was obtained by retrospective review of the electronic medical record. The presence or absence of neuropathy not attributable to other causes per the treating physician was noted, as was previously documented cryoglobulinemia or CAD. Where previously performed, results of anti-MAG antibody testing were noted (cutoff for positive results defined as >1,000 titer units). For clinical follow up analysis, progression events were defined as the development of an overt lymphoid or plasma cell neoplasm, amyloidosis, or death.

Immunohistochemistry

In cases where immunohistochemistry (IHC) had not been previously performed and paraffin blocks were available, immunohistochemical stains for CD138, κ and λ immunoglobulin light chains, and CD20 were performed. IHC staining was performed on the Ventana Benchmark automated immunostainer (Roche Diagnostics, Indianapolis, IN), using the following antibodies (concentration): CD20 mouse monoclonal L26 antibody (Dako, 1:200); CD138 mouse monoclonal B-A38 (Biocare, 1:200); κ light chains rabbit polyclonal (Dako, 1:16,000); λ light chains rabbit polyclonal (Dako, 1:32,000). Blinded scoring was performed by two pathologists (FKB, JRC); discrepancies were resolved by a third reviewer (MON).

Statistics

Statistical tests including two-tailed Student's *t*-test and Kruskal-Wallis test for continuous variables and X^2-test and Fisher exact test, when applicable, for categorical variables were performed with R (R Core Team, Vienna, Austria) and a significance threshold of $P \leq 0.05$ was used.

Results

Clinicopathologic features of immunoglobulin M monoclonal gammopathy of undetermined significance as defined by 2016 World Health Organization criteria

Out of 1,222 patients with an IgM paraprotein detected at our institution from 2002-2021, 238 patients had available bone marrow biopsies and fulfilled 2016 WHO IgM MGUS criteria. Twelve patients with concurrent or prior myeloid neoplasms, 15 patients with amyloidosis, and 20 patients with previously diagnosed and treated lymphoproliferative disorders were excluded for a final cohort of 191 patients. The clinicopathologic features of this cohort are detailed in Table 1. Overall, the average age at bone marrow biopsy was 69.6 years and there was a male predominance (male: female, 1.5:1). The median IgM serum paraprotein was 452 mg/dL and the median hemoglobin concentration was 13 g/dL.

Clonal plasma cells were identified by light chain IHC in 41 of 171 (24%) cases that could be assessed. Flow cytometric studies, performed in 157 cases, are summarized in Figure 1. Flow cytometry identified a clonal B-cell population in 43 (27%) cases including 30 cases with B cells negative for CD5 and 13 cases positive for CD5. In each of the 30 cases (100%) with CD5-negative clonal B cells, the monotypic light chain expressed on B cells matched the IgM paraprotein light chain, while the B-cell light chains and IgM paraprotein light chains were concordant in only seven of 13 (54%) CD5-positive B-cell cases (P=0.0003, Fisher exact test).

Lymphoid aggregates were present in 61 of 191 (32%)

Table 1. Clinicopathologic characteristics of all patients with immunoglobulin M monoclonal gammopathy of undetermined significance and those with and without cold agglutinin disease.

		All IgM MGUS	IgM MGUS w/o CAD	IgM MGUS with CAD	*P*
Total, N (%)		191 (100)	181 (95)	10 (5)	-
Sex, N (%)	Male	115 (60)	109 (60.2)	6 (60)	1.000
	Female	76 (40)	72 (39.8)	4 (40)	
Age at Bmbx in years, mean (median, SD)		69.6 (70.4-9.8)	69.6 (70.3-9.8)	69.6 (72.5-9.9)	0.992
IgM mg/dL mean (median, SD)		469 (452-614)	661 (444-627)	437 (497-264)	0.034
Light chain restriction by IFE, N (%)	κ	117 (61)	112 (61.9)	5 (50)	0.412
	λ	45 (24)	42 (23.2)	3 (30)	
	Multiple bands	12 (6)	12 (6.6)	0 (0)	
	PD bands	17 (9)	15 (8.3)	2 (20)	
Hgb g/dL, mean (median, SD)		12.6 (13.0-2.2)	12.7 (13.1-2.1)	10.3 (10.8-2.2)	0.007
Lymphs % in aspirate, mean (median, SD)		12.4 (11.0-7.1)	12.5 (11.0-7.0)	11.5 (8.0-9.2)	0.75
PC % in aspirate, mean (median, SD)		1.7 (1.0-2.2)	1.7 (1-1.4)	1.9 (1-1.8)	0.747
Clonal PC by IHC N=171, N (%)		41 (24)	38/163 (23.3)	3/8 (37.5)	0.400
Clonal B cells by FC N=157, N (%)		43 (27)	39/148 (26.4)	4/9 (44.4)	0.259
Lymphoid aggregates, N (%)		61 (32)	55 (30.4)	6 (60)	0.077
Abnormal karyotype N=173, N (%)		22 (13)	21/163 (12.9)	1/10 (10)	1.000
MYD88 L265P N=56, N (%)		35 (63)	35/53 (66.0)	0/3 (0)	0.048
Anti-MAG antibodies N=31, N (%)		16 (52%)	16/31 (51.6)	0/0 (0)	1.000

MGUS: monoclonal gammopathy of unknown significance; IgM: immunoglobulin M; w/o: without; RD: related disorders; CAD: cold agglutinin disease; Bmbx: bone marrow biopsy; IFE: immunofixation electrophoresis; SD: standard deviation; Hgb: hemoglobin; PD: poorly defined; Lymphs: lymphocytes; PC: plasma cells; IHC: immunohistochemistry; FC: flow cytometry; MAG: myelin-associated glycoprotein.

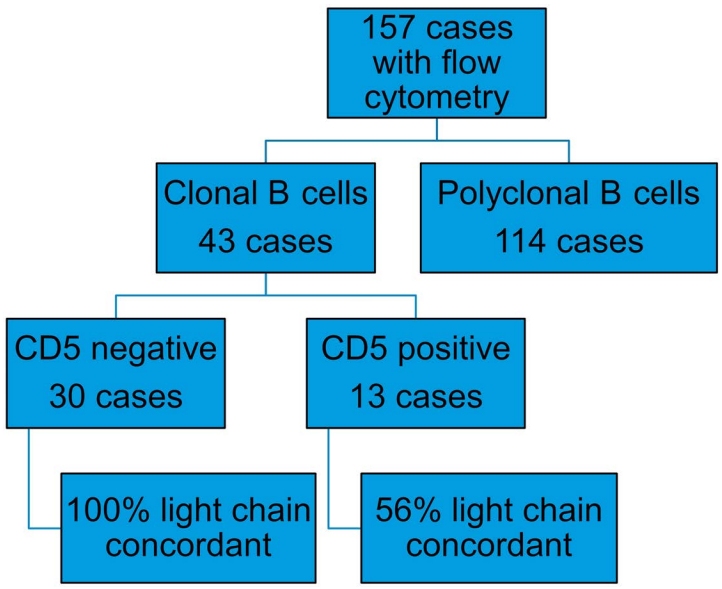

Figure 1. Summary of flow cytometric findings in 157 cases of immunglobulin M monoclonal gammopathy of unknown significance.

samples, but only six cases contained lymphoid aggregates in the core biopsy as well as clonal plasma cells by IHC and clonal B cells by flow cytometry. Of these six cases, one case displayed T-cell-rich aggregates interpreted as being reactive, two case showed clonal plasma cells plus a CD5-positive B-cell population with light chains that did not match the IgM paraprotein, consistent with synchronous IgM MGUS and monoclonal B lymphocytosis, and three cases contained B-cell-rich aggregates representing <10% of the core biopsy as well as CD5-negative B-cell populations with light chains that match clonal plasma cells by IHC and the IgM paraprotein light chain. These latter three cases (3/191, 1.6%) are compatible with a diagnosis of LPL using 2022 ICC criteria or Waldenstrom Workshop criteria (Figure 2).[9,10] Additional cases with abnormal, interstitial lymphoplasmacytic infiltrates but without aggregates, which would meet the Waldenstrom Workshop definition of LPL but not the 2022 ICC definition, were not identified in this cohort.

Metaphase karyotyping was performed in 173 cases (90.6%); 22 (13%) cases had an abnormal karyotype (*Online Supplementary Table S1*). FISH testing for myeloma related abnormalities was performed in 86 of 191 (45%)

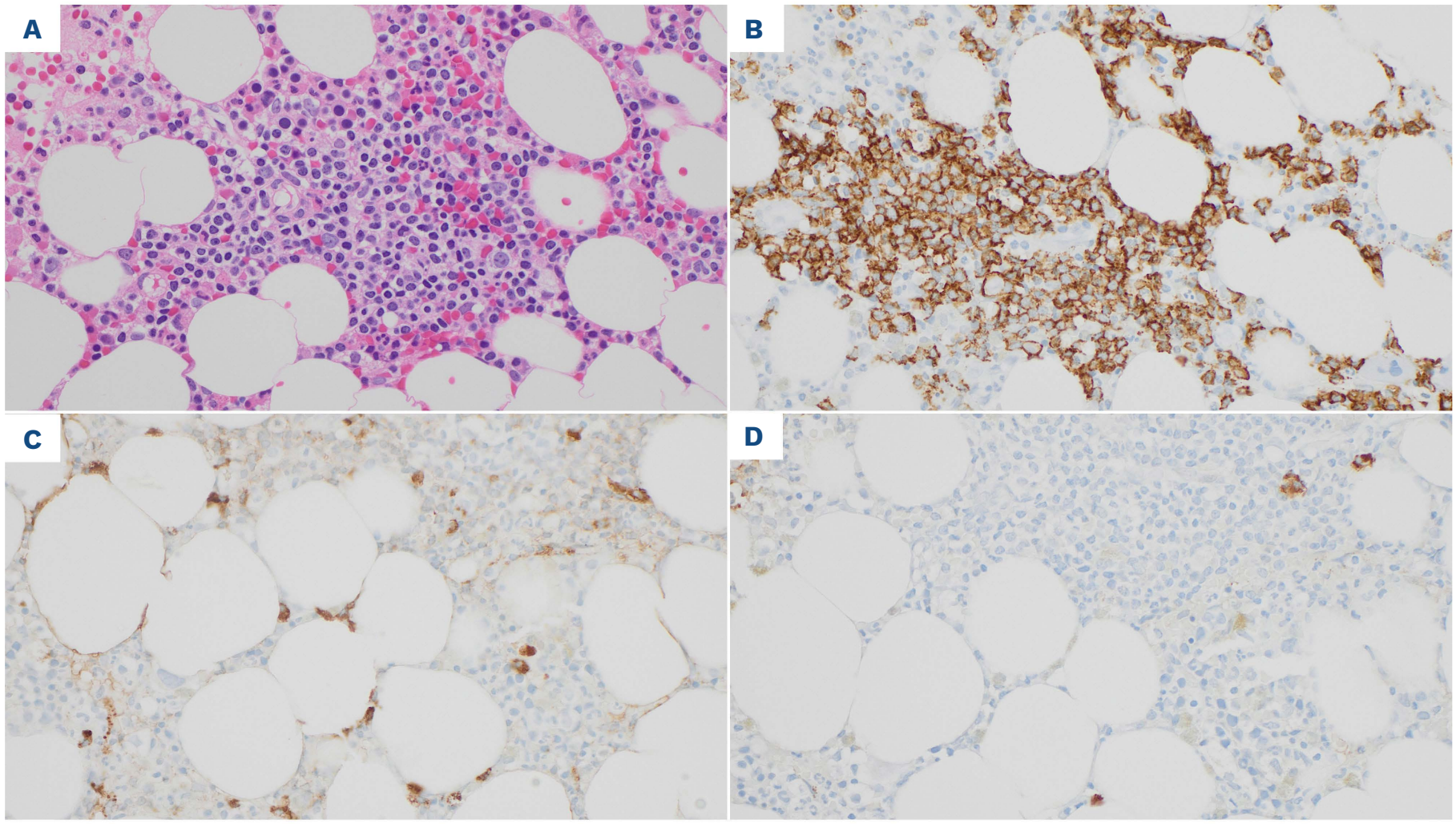

Figure 2. Histologic features in a case meeting 2016 World Health Organization criteria for immunglobulin M monoclonal gammopathy of unknown significance but diagnostic of lymphoplasmacytic lymphoma using 2022 International Consensus Classification criteria. The aggregate in this case (A) (hematoxylin and eosin staining, 40X magnification) contains numerous CD20-positive small cells (B) (40x magnification) representing <5% of bone marrow cellularity admixed with numerous κ-positive (C) (40x magnification) and only rare λ-positive (D) (40x magnification) plasma cells. This case also displayed κ monotypic B cells by flow cytometry.

cases, of which 27 (31.4%) yielded insufficient plasma cells. Among 59 cases that were assessed, one case of IgM MGUS with a normal karyotype showed loss of chromosome 15 in 70 of 100 plasma cells. No myeloma associated *IGH* translocations were identified. The *MYD88* L265P mutation was detected by allele specific polymerase chain recation (PCR) in 35 of 56 (63%) cases analyzed.

Clinically, IgM-RD was identified in 82 of 191 (43%) patients, including neuropathy in 67 of 191 (35%) cases, followed by cryoglobulinemia in 21 of 191 (11%) and CAD in ten of 191 (5%) (Figure 3). Patients with cryoglobulinemia presented with concurrent neuropathy in 14 of 21 cases (67%), and rarely with concurrent cryoglobulinemia and CAD (2 cases, 1%). Neuropathy and CAD were not observed in the same patients in our cohort.

Findings in primary cold agglutinin disease

The ten cases with clinical and laboratory evidence of CAD were considered separately (Table 1): compared to cases without CAD, the IgM serum concentration and hemoglobin level were significantly lower (P=0.034 and P=0.007, respectively). Clonal plasma cells were found by IHC in three of eight (38%) of cases. Flow cytometry showed a clonal B-cell population in four of nine cases (44%) including two CD5-positive and two CD5-negative B-cell clones. Cases with CAD showed lymphoid aggregates more frequently than cases without CAD (60% *vs.* 30.4%), although the difference did not reach statistical significance (P=0.077). None of the three CAD patients tested showed the *MYD88* mutation (P=0.048, Fisher exact test).

Findings in immunoglobulin M monoclonal gammopathy of undetermined significance with and without immunoglobulin M-related disease

After exclusion of primary CAD cases, patients with IgM-RD represented 40% (72/181) of the cohort *versus* 60% (109/181) without IgM-RD (Table 2). Patients with IgM-RD showed a greater male predominance (P=0.020) and had higher hemoglobin levels compared to those without IgM-RD (13.4 g/dL *vs.* 12.3 g/dL; P<0.001). Anti-MAG antibodies were identified in 52% (16/31) of tested patients, all of whom presented with IgM-RD due to neuropathy (16/27, 59%) and three of whom also had cryoglobulinemia. Four patients without neuropathy or cryoglobulinemia tested negative for MAG antibodies (P=0.043). *MYD88* L265P mutations were detected in 84% (21/25) of IgM-RD cases

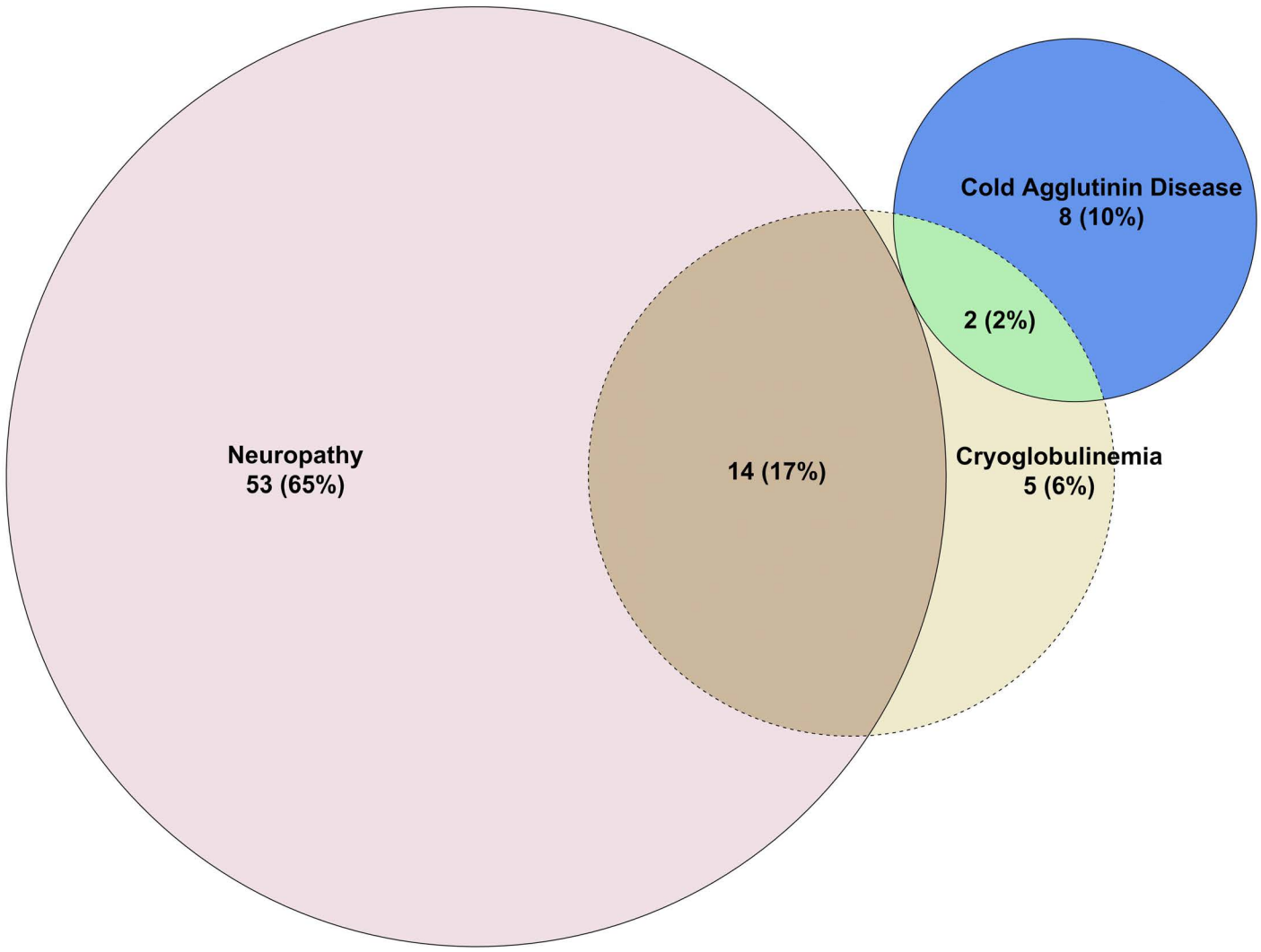

Figure 3. Venn diagram of the symptoms of patients with immunglobulin M monoclonal gammopathy of unknown significance with and without immunglobulin M-related disorders and patients with cold agglutinin disease.

compared to 50% (14/28) in those without IgM-RD (*P*=0.011).

Comparison of immunoglobulin M monoclonal gammopathy of undetermined significance with and without clonal B cells and/or plasma cells

We next examined the clinicopathologic features of IgM MGUS (excluding CAD) based on the presence or absence of clonal B cells and/or clonal plasma cell populations. This analysis was restricted to the 148 patients where results of flow cytometry and immunohistochemistry was available for review (Table 3). Cases with clonal plasma cells, with or without clonal B cells, were associated with higher levels of IgM serum concentration (*P*<0.001) and were more likely to contain lymphoid aggregates in the bone marrow (*P*=0.086). Cases with monotypic plasma cells, regardless of the presence of clonal B cells, showed the *MYD88* L265P mutation in 100% of cases analyzed. The subset with both clonal B cells and monotypic plasma cells showed the highest IgM serum concentration, highest incidence of IgM-RD (62%), and all patients were male. Cases lacking both detectable clonal plasma cells and clonal B-cells showed the lowest IgM serum paraprotein concentrations, infrequently harbored lymphoid aggregates, showed the lowest incidence of an abnormal karyotype, yet showed the *MYD88* L265P mutation in 57% (12/21) of cases. Abnormal karyotypes were present at similar levels in all subsets of cases.

Clinical outcome

The mean follow-up time post bone marrow biopsy for the cohort including cases with IgM MGUS with and without IgM-RD (excluding CAD) was 4.17 years (median 2.78 years; range, 0.03-19.48 years). Only two patients, both IgM MGUS without RD, showed progression to an overt disease on available follow-up. One patient (with polyclonal B cells and polyclonal plasma cells in the bone marrow) was diagnosed with diffuse large B-cell lymphoma and a second patient (with polytypic plasma cells and no flow cytometry data) developed renal amyloidosis with a different immunoglobulin light chain. Overall survival (OS) was similar between IgM MGUS and IgM-RD (*P*=0.43, Figure 4). *MYD88* mutation status, available in 56 patients across the entire cohort, was not significantly associated with survival (*P*=0.27). Within CAD patients, no progression events occurred with a mean follow-up of 3.16 years (median 3.16 years).

Discussion

MGUS has long been recognized as a precursor condition that may progress to multiple myeloma, other plasma cell neoplasms, or lymphoproliferative disorders, but the approach to diagnosis and classification of MGUS has changed over recent years.[1–4] Recognizing the biologic differences between IgM MGUS, which typically progresses

Table 2. Clinicopathologic characteristics of patients with immunoglobulin M (IgM) monoclonal gammopathy of unknown significance and with and without IgM-related disorders (after exclusion of cold agglutinin disease).

		IgM MGUS w/o IgM-RD	IgM MGUS with IgM-RD	*P*
Total, N (%)		109 (60)	72 (40)	-
Sex, N (%)	Male	58 (53.2)	51 (70.8)	0.020
	Female	51 (46.8)	21 (29.2)	
Age at Bmbx in years, mean (median, SD)		70.6 (71.7-9.9)	68.2 (68.5-9.5)	0.103
IgM mg/dL, mean (median, SD)		714 (517-671)	581 (363-549)	0.152
Light chain restriction by IFE, N (%)	κ	66 (60.6)	46 (63.9)	0.390
	λ	25 (22.9)	17 (23.6)	
	Multiple bands	6 (5.5)	6 (8.3)	
	PD bands	12 (11.0)	3 (4.2)	
Hgb g/dL, mean (median, SD)		12.3 (12.7-2.1)	13.4 (13.7-1.9)	<0.001
Lymphs % in aspirate, mean (median, SD)		11.8 (11.0-6.0)	13.4 (11.0-8.2)	0.168
PC % in aspirate, mean (median, SD)		1.9 (2-1.5)	1.5 (1-1.3)	0.054
Clonal PC by IHC N=171, N/N (%)		24/96 (25)	14/77 (18.2)	0.577
Clonal B cells by FC N=157, N/N (%)		22/90 (24.4)	17/58 (29.3)	0.568
Lymphoid aggregates, N (%)		32 (29.4)	23 (31.9)	0.743
Abnormal karyotype N=173, N/N (%)		13/100 (13)	8/63 (12.7)	1.000
MYD88 L265P N=56, N/N (%)		14/28 (50)	21/25 (84)	0.011
Anti-MAG antibodies N=31, N/N (%)		0/4 (0)	16/27 (59.3)	0.043

MGUS: monoclonal gammopathy of unknown significance; w/o: without; RD: related disorders; CAD: cold agglutinin disease; Bmbx: bone marrow biopsy; IFE: immunofixation electrophoresis; SD: standard deviation; Hgb: hemoglobin; PD: poorly defined; Lymphs: lymphocytes; PC: plasma cells; IHC: immunohistochemistry; FC: flow cytometry; MAG: myelin associated glycoprotein.

to LPL or other lymphoproliferative disorder, *versus* non-IgM (i.e., IgA or IgG) MGUS which generally progress to multiple myeloma, these two entities were separately defined in the 2014 update to the International Myeloma Working Group diagnostic criteria,[19] and these changes were incorporated into the 2016 WHO classification of plasma cell neoplasms.[20] In addition, there has been increasing interest in recognizing the subset of patients who meet criteria for diagnosis as MGUS but who may nevertheless have clinically significant symptoms that may require treatment.[11–15] In patients with IgM MGUS, the most common symptoms include neuropathy, cryoglobulinemia and CAD, which have collectively been described under the term IgM-RD.[21,22] Prior studies of patients with IgM-RD have primarily focused on clinical manifestations, and it has been unclear to what extent the bone marrow pathologic features may differ in patients with and without IgM-RD.

In the current study, we have examined the detailed clinicopathologic findings in a large series of patients with IgM MGUS. Overall, IgM-RD (including CAD) was identified in 82 of 191 (43%) of cases. This finding is similar to that reported in the literature, where peripheral neuropathy alone has been reported in approximately 30-50% of IgM MGUS patients, emphasizing that symptomatic disease is not rare in IgM MGUS.[23,24] Indeed, this study likely represents an underestimate of symptomatic disease as we limited our definition of IgM-RD to neuropathy, cryoglobulinemia and CAD. We elected to focus on these manifestations of IgM-RD as these conditions are reported to be relatively common, and because cryoglobulinemia and CAD may be readily documented by routine laboratory testing. This analysis did not include other conditions considered part of the spectrum of IgM-RD by some investigators such as autoimmune thrombocytopenia or IgM-related nephropathy.[1,15,25] Similarly, amyloidosis was excluded from this analysis as it is recognized as a distinct clinicopathologic entity in the 2016 WHO classification[8] and the 2022 ICC system.[10] Prospective studies of IgM MGUS patients with systematic assessment for all po-

Table 3. Clinicopathologic characteristics associated with the presence of clonal B-cells and/or monotypic plasma cells in patients with immunoglobulin M (IgM) monoclonal gammopathy of unknown significance with and without IgM-related disorders.

		Clonal B Polyclonal PC	Polyclonal B Clonal PC	Clonal B Clonal PC	Polyclonal B Polyclonal PC	*P*
Total N=148, N (%)		27 (18.0)	25 (17.0)	12 (8.0)	84 (57.0)	-
Sex, N(%)	Male	19 (70.4)	14 (56.0)	12 (100.0)	46 (54.8)	0.016
	Female	8 (29.6)	11 (44.0)	0 (0.0)	38 (45.2)	
Age in years, mean (median, SD)		72.4 (72.7-9.0)	69.9 (70.6-10.0)	71.4 (73.8-13.1)	68.3 (68.9-9.6)	0.263
IgM mg/dL, mean (median, SD)		629.0 (432.5-462.2)	1,096.0 (1,030.0-777.9)	1449.0 (768.0-1,239.0)	480.0 (357.0-371.0)	<0.001
Light chain restriction by IFE, N (%)	κ restricted	19 (70.4)	19 (76.0)	7 (58.3)	51 (60.7)	0.523
	λrestricted	5 (18.5)	3 (12.0)	3 (25.0)	21 (25.0)	
	Multiple bands	0 (0.0)	2 (8.0)	2 (16.7)	6 (7.1)	
	PD bands	3 (11.1)	1 (4.0)	0 (0.0)	6 (7.1)	
Hgb g/dL, mean (median, SD)		12.4 (13.0-2.5)	12.9 (13.1-1.8)	13.8 (13.6-2.3)	12.9 (13.2-2.0)	0.269
Lymphs % in aspirate, mean (median, SD)		13.5 (13.0-5.2)	11.5 (10.0-5.4)	18.6 (16.0-14.1)	11.7 (10.0-6.5)	0.090
PC % in aspirate, mean (median, SD)		1.6 (2.0-0.9)	2.5 (2.0-2.4)	1.2 (1.0-1.1)	1.8 (2.0-1.3)	0.208
IgM-Related disorders, N (%)		10 (37.0)	8 (32.0)	7 (58.3)	34 (40.5)	0.483
Lymphoid aggregates, N (%)		9 (33.3)	12 (48.0)	6 (50.0)	21 (25.0)	0.086
Abnormal karyotype N=132, N/N (%)		10/26 (38.5)	7/24 (29.2)	2/10 (20.0)	14/72 (19.4)	0.256
MYD88 L265P N=56, N/N (%)		3/10 (30.0)	8/8 (100.0)	9/9 (100.0)	12/21 (57.1)	<0.001
Anti-MAG antibodies N=29, N/N (%)		1/6 (16.7)	2/3 (66.7)	3/3 (100.0)	7/15 (46.7)	0.110

B: B cells; PC: plasma cells; IFE: immunofixation electrophoresis; SD: standard deviation; Hgb: hemoglobin; PD: poorly defined; Lymphs: lymphocytes; PC: plasma cells; IHC: immunohistochemistry; FC: flow cytometry; MAG: myelin-associated glycoprotein.

tential IgM-related conditions may be helpful to further refine the incidence of symptomatic disease

The prior literature contains limited information regarding the flow cytometric findings in IgM MGUS, primarily focusing on the number of total B cells and plasma cells identified rather than the characteristics of clonal B-cell or plasma cell populations.[26,27] Using a complex gating strategy involving 17 different antigens, Pavia *et al.* reported clonal B-cell populations detectable in 15 of 20 (75%) MGUS patients.[28] In this series, clonal B-cells were identified in 43 of 157 (27%) patients using a standard clinical flow cytometry approach, including 13 cases positive for CD5 and 30 cases negative for CD5. Importantly, in all cases of CD5-negative clonal B-cell populations, the light chain identified by flow cytometry matched the monoclonal protein's light chain. In contrast, for cases with CD5-positive B-cell clones by flow cytometry, the light chain on B cells matched the monoclonal protein in only seven of 13 (54%) cases. This observation suggests that at least many CD5 positive clonal B-cell proliferations detected in IgM MGUS are unrelated to the monoclonal protein. In such cases, a diagnosis of concurrent IgM MGUS and monoclonal B lymphocytosis may be appropriate. Definitively evaluating the relationship between a CD5-positive B-cell population and clonal plasma cells expressing the same light chain would require molecular studies on sorted B-cell and plasma cell populations and such studies are rarely if ever available in routine clinical practice. In this situation, the possibility that the CD5-positive B-cell population is unrelated to the paraprotein should be noted and staging studies are appropriate to rule out an overt CD5-positive B-cell lymphoproliferative disorder elsewhere.

CAD has been recently reported to show characteristic findings that distinguish these cases from LPL/IgM MGUS including lack of *MYD88* L265P mutations and the presence of recurrent chromosomal trisomies of chromosomes 3, 12 and 18.[16–18] CAD was identified in ten of 191 (5%) cases in this cohort. These patients showed a lower hemoglobin level (P=0.007) and a trend towards more frequent lymphoid aggregates (P=0.077) compared to other cases of IgM MGUS and *MYD88* L265P was absent in all

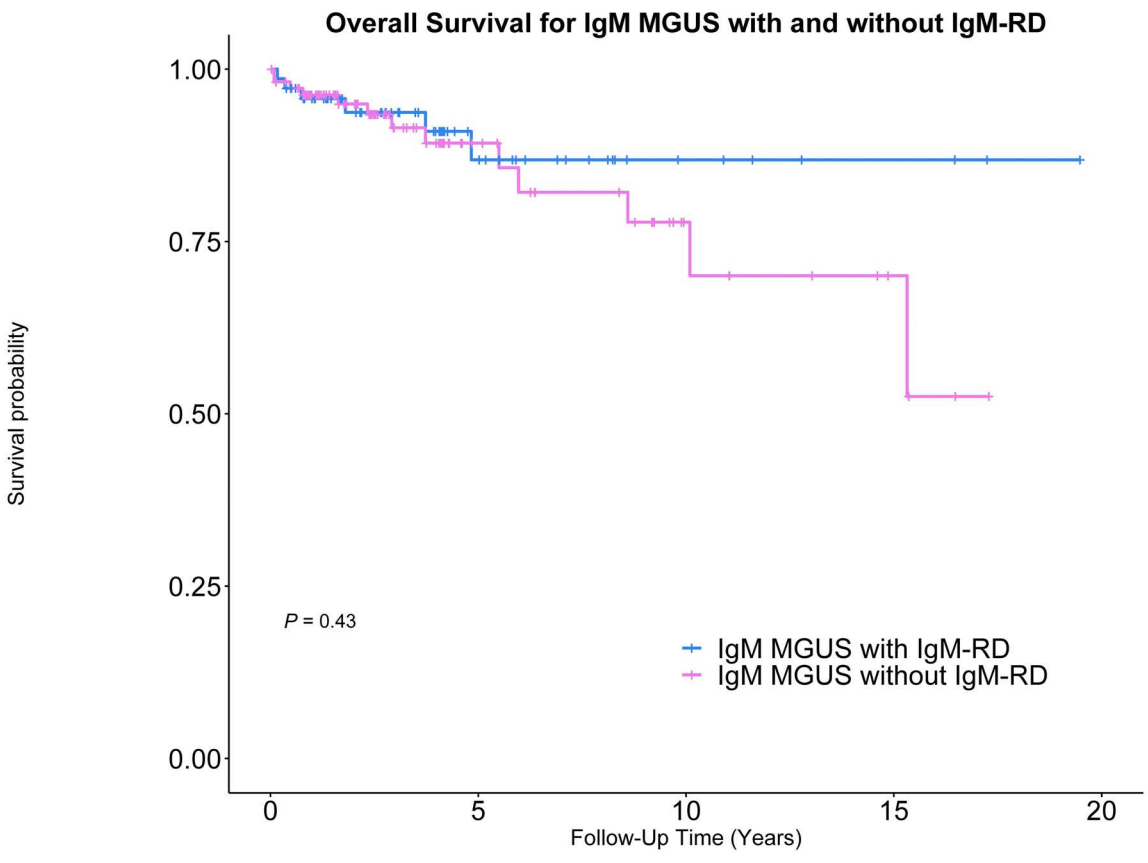

Figure 4. Overall survival of immunglobulin M monoclonal gammopathy of unknown significance patients with or without immunglobulin M-related disorders. MGUS: monoclonal gammopathy of unknown significance; IgM: immunoglobulin M; RD: related disorders.

tested CAD cases. Our results confirm the distinctive nature of CAD compared to IgM MGUS and support the approach of the 2022 ICC system which separately recognizes primary CAD as a distinct lymphoproliferative disorder.[10] FISH studies for chromosomal trisomies were not performed as part of this study, and additional studies are warranted to determine whether cases of CAD may be more similar to cases of marginal zone lymphoma than they are to cases of LPL/IgM MGUS.

After exclusion of CAD, we compared the findings in the 181 patients with IgM MGUS with (n=72) or without (n=109) IgM-RD. These cases were similar in terms of the serum IgM concentrations, presence of lymphoid aggregates, clonal B-cell populations by flow cytometry, and clonal plasma cells by IHC. We also saw no differences in overall survival in patients with or without IgM-RD, although the follow-up data was limited for this cohort. These findings suggest the symptomatology in patients with IgM-RD is a function of the specificity of the IgM antibody produced rather than a result of distinctive underlying lymphoproliferative disorders in the bone marrow. This data supports the approach of the 2022 ICC system which does not recognize IgM-RD as a distinct disease, but instead encourages the use of terms such as "monoclonal gammopathy of clinical significance" as descriptors which may be appended to a pathologic diagnosis.[10] Intriguingly, however, the *MYD88* L265P mutation was detected in a higher proportion of patients with IgM-RD compared to those without IgM-RD (P=0.011). It is unclear whether this result may reflect a quantitative distinction, i.e., a lower burden of clonal cells in IgM MGUS without IgM-RD such that the *MYD88* mutations may not be detectable within the limits of the allele specific assay employed, or whether this may reflect a qualitative distinction with *MYD88* wild-type MGUS cases being less likely to develop these specific forms of IgM-RD. Additional studies on sorted populations of B cells and plasma cells may be necessary to further examine this phenomenon.

In the 2022 ICC system, two subtypes of IgM MGUS were recognized.[10] IgM MGUS of plasma cell type, the presumed precursor of IgM myeloma, is defined as cases with myeloma-associated cytogenetic abnormalities or as cases with clonal plasma cells but lacking clonal B cells by flow cytometry and lacking *MYD88* mutations. IgM MGUS, NOS, on the other hand, encompasses all other cases of IgM MGUS which are presumed to progress to LPL or other lymphoproliferative disorder rather than myeloma. In an effort to understand the frequency and significance of these subsets, we compared cases with and without clonal B cells and/or plasma cells. Among cases with clonal plasma cells but no clonal B cells, *MYD88* L265P was detected in all cases tested (8/8, 100%). Similarly, none of the 59 cases of IgM MGUS tested by FISH showed an *IGH::CCND1* rearrangement or other myeloma-associated *IGH* translocation. While our data is limited due to *MYD88* and FISH testing being performed in only a subset of patients, the available data suggests that IgM MGUS of plasma cell type is very uncommon and no cases of this subtype were identified in this cohort.

The diagnostic criteria for distinguishing IgM MGUS from LPL have been controversial. In the 2016 WHO system, a diagnosis of LPL requires a clonal lymphoplasmacytic infiltrate representing ≥10% of bone marrow cellularity, while cases showing less than 10% of an infiltrate may be classified as an IgM MGUS if other remaining criteria are met.[8] In contrast, the International Workshop on Walden-

ström's macroglobulinemia criteria for diagnosis of LPL includes cases with clonal lymphoplasmacytic infiltrates of any extent,[9] and the 2022 ICC system defines LPL based on the presence of neoplastic lymphoplasmacytic aggregates at any percentage of bone marrow infiltration.[10] The cohort of cases in this study was defined using 2016 WHO criteria because the 10% threshold is relatively easy to objectively apply, and because cases with ≥10% infiltration would fulfill criteria for LPL in 2016 WHO, 2022 ICC, and Waldenström Workshop criteria. Of the 191 cases, only three of these showed abnormal aggregates of clonal B cells and clonal plasma cells meeting criteria for LPL by 2022 ICC criteria or Waldenström Workshop criteria. This observation provides reassurance that most cases historically classified as IgM MGUS in recent years would still remain best classified as IgM MGUS in the recently published criteria of the 2022 ICC system. Nevertheless, lymphoid aggregates were identified on hematoxylin and eosin sections in 61 of 191 (32%) of the entire cohort, and a multiparameter approach including flow cytometry and immunohistochemistry is required to distinguish these relatively common reactive aggregates from cases diagnostic of LPL by 2022 ICC criteria.

In conclusion, this study has provided detailed clinicopathologic findings in a large series of IgM MGUS as defined by 2016 WHO criteria. The precise incidence of IgM-RD is difficult to determine as we did not assess for all conditions that may be secondary to an IgM paraprotein, leading to a likely underestimate of IgM-RD, while at the same time, as our institution represents a tertiary referral center, there may be referral bias leading to overrepresentation of symptomatic patients. Nevertheless, this study shows that IgM-RD, defined here as neuropathy, cryoglobulinemia or CAD, is common. While cases of CAD show distinct features warranting their recognition as a separate clinicopathologic entity, other cases of IgM MGUS with and without IgM-RD are not clearly pathologically distinguishable. The presence of IgM-RD is therefore best designated as a clinical descriptor attached to the underlying pathologic diagnosis of IgM MGUS, rather than being recognized as a distinctive entity. Finally, IgM MGUS of plasma cell type, defined by the recent 2022 ICC system as the putative precursor of IgM myeloma, is rare with no cases of that subtype identified in this cohort.

Disclosures

No conflicts of interest to disclose.

Contributions

JC and MN designed the study. FB, PM, EB and MN collected data. FB, PM, MN, and JC analyzed and interpreted the data. FB wrote the draft. All authors provided important scientific insights, critically revised and edited the manuscript. All authors approved the final version of the manuscript.

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Data-sharing statement

Data are available from the corresponding author upon reasonable request.

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