Inflammation in Waldenström macroglobulinemia is associated with 6q deletion and need for treatment initiation

Waldenström's macroglobulinemia (WM) is a rare mature B-cell lymphoproliferative disorder, characterized by bone marrow (BM) infiltration of lymphoplasmacytic cells and the presence of a monoclonal IgM. *MYD88*^{L265P} mutation has been identified as the main driver event, with more than 90% of patients harboring this mutation.[1] MYD88 is an adaptor protein of the Toll-like receptors (TLR) and IL-1 receptors signaling cascades, and is consequently key in the activation of the NF-kB and JAK/STAT pathways.[1] The second most commonly mutated gene is *CXCR4* (30-40%),[2] including around 50% of mutations located in the S338 codon (referred to as *CXCR4*^{WHIM}). The most frequent cytogenetic abnormality is 6q deletion (del6q), found in 30% to 55% of cases.[3] Other chromosomal abnormalities are, in decreasing order of frequency: trisomy 18 (tri18), del13q, del17p, tri4 and del11q.[4] Thirty percent of patients are asymptomatic at diagnosis. Of those, 68% will require treatment at 10 years.[5] Main reasons for therapy initiation are anemia (67%), increasing IgM levels, peripheral neuropathy (20%), hyperviscosity (15%), and organomegaly (10%).[6]

A significant association between WM and dysimmune conditions has been reported,[7] supporting the hypothesis that chronic immune stimulation could contribute to lymphomagenesis. Clinical practitioners caring for WM patients also notice that a fraction presents with chronic inflammatory syndrome, for which there is no obvious cause besides WM itself. Chronic inflammation may be the cause of and/or contribute to the development of anemia, the leading cause of WM treatment initiation. However, very few data exist on the association between WM and inflammatory syndrome. One prospective clinical trial published in 2001 including 231 WM patients showed that 51% of the cohort had a C-reactive protein (CRP) value of at least 1 mg/L at enrollment, which was associated with a higher risk of treatment initiation and lower progression-free (PFS) and overall survival (OS) rates.[8] Authors have postulated that the association between WM and inflammation could be mediated by interleukin 6 (IL-6), an inductor of CRP synthesis,[9] with higher levels in WM patients compared to healthy controls, and significant reduction after treatment.[10] IL-6 production is mediated through NF-kB activation, itself the consequence of *MYD88*^{L265P} mutation. However, not all patients present with inflammation, suggesting that other mechanisms are at work. Main objectives of our study were to estimate the prevalence of inflammatory WM and to compare clinical and biological characteristics between inflammatory and non-inflammatory patients.

We conducted a retrospective analysis including all WM patients who consulted in the Clinical Hematology Unit of Pitié-Salpêtrière hospital (Paris, France) between 1988 and 2020[4] with available information regarding CRP, at diagnosis and/or before treatment initiation. We defined "inflammatory" status as a CRP value equal or superior to 5 mg/L (according to local normal values) and confirmed once at a minimum interval of 1 month, with no obvious cause besides WM itself. Written consent for clinical, biological and bone marrow (BM) analyses were obtained in accordance with the Declaration of Helsinki and with ethical approval from national (CNIL 2212382) and local (CPP Ile-De-France 05/21/2014) ethics committees.

Main characteristics of the whole WM cohort are detailed in the *Online Supplementary Table S1*. No significant difference was observed between WM patients with available CRP or not. The study population comprised 222 patients, of which 66% were male. Median age at WM diagnosis was 64.5 years old (range, 28.4-88.2). During follow-up, 167 of 222 (75%) WM patients required first-line (1L) therapy. Those therapies consisted of chemoimmunotherapy, chemotherapy, anti-CD20 monoclonal antibody alone or in combination with proteasome inhibitor in 117 (70%), 33 (20%), 13 (8%) and four (2%) of cases, respectively. Median follow-up for the whole WM cohort was 7.2 years. Median treatment-free survival (TFS) was 3.5 years. Five-year PFS for patients receiving 1L therapy was 57%, and 5-year OS for the whole cohort was 92% (data not shown). Median CRP level was 16.7 mg/L (range, 0-263), including 103 (46%) patients with CRP values strictly below 5 mg/L, and 119 (54%) patients with CRP values ≥5 mg/L (thereafter referred to as "inflammatory"). Among the 119 patients with inflammatory WM, patients harboring CRP levels between 5-19, 20-49 and ≥50 mg/L were 66 (55%), 31 (26%) and 22 (18%) respectively (Figure 1A). The mean albumin value was significantly lower in the inflammatory group, as expected. Inflammatory WM patients more frequently presented with lymphadenopathies and anemia (Table 1; *Online Supplementary Table S2*). All other routine clinical and biological parameters were similar between inflammatory and non-inflammatory WM groups (Table 1; *Online Supplementary Table S2*, whether CRP was studied as a negative/positive value or a continuous value

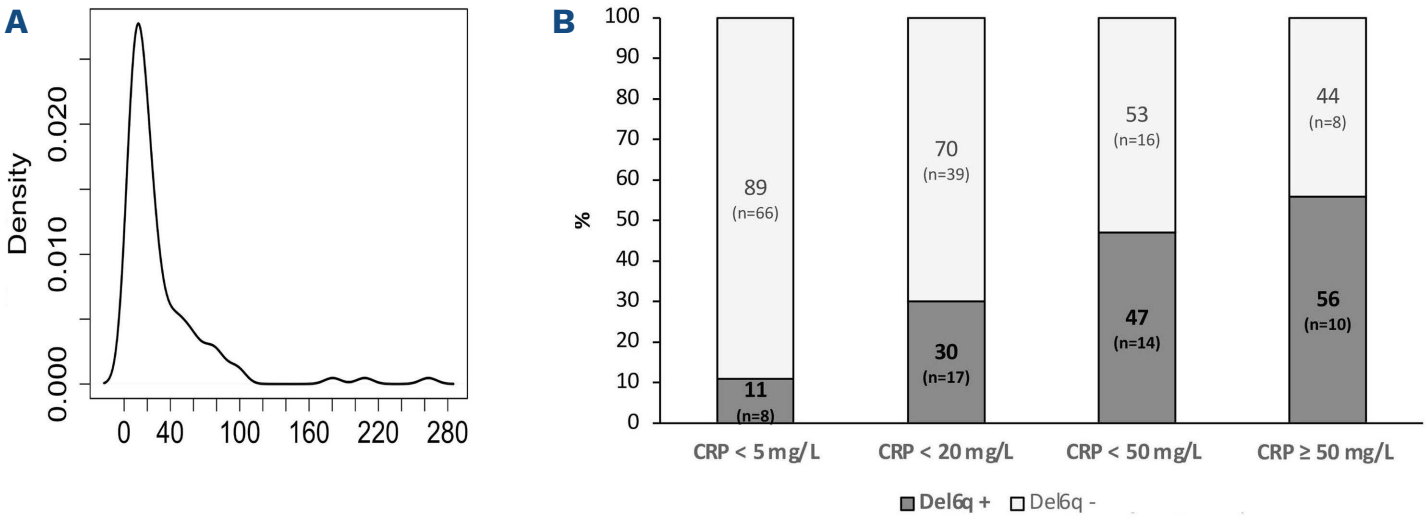

Figure 1. Description of inflammatory patients. (A) C-reactive protein (CRP) distribution (density plot), (B) proportion of 6q deletion (del6q) patients according to CRP levels, (C) correlation between CRP and hematological response.

respectively). The frequencies of main cytogenetic abnormalities and gene alterations in the whole cohort are represented in the *Online Supplementary Table S1*. The most frequent mutations were identified in *MYD88* (90%), *CXCR4* (24%), *MLL2/KMT2D* (11%), *ARID1A* (10%), *TP53* (7%), and *CD79A/B* (7%). Main cytogenetic abnormalities identified by karyotype and/or fluorescence *in situ* hybridization (FISH) were, in decreasing order of frequency, del6q (28%), tri4 (11%), tri12 (7%) and del17p (7%). Complex karyotype (CK) was observed in 17% of cases. *TP53* abnormalities (either del17p and/or *TP53* mutation) were present in 11% of 190 evaluable patients. In univariate analysis, del6q was the only cytogenetic or molecular abnormalities to be significantly associated with inflammatory status (Table 1), with del6q occurring in 39% of inflammatory *versus* 11% of non-inflammatory WM ($P<10^{-3}$, Table 1). The proportion of del6q patients increased along with CRP values (Figure 1B). The level of CRP as a continuous variable was significantly associated with the presence of del6q (hazard ratio [HR] 1.014, 95% confidence interval [CI]: 1.004-1.027; $P=0.015$) meaning the probability of del6q increased by 14% for a one-point increase in CRP (IC95%; 95% CI: 0.4-2.7; *Online Supplementary Table S2*). Eighty-three percent of del6q patients had CRP values ≥5 mg/L compared to 49% non-del6q WM patients. In multivariate analysis, only del6q ($P=0.02$) and albumin ($P<10^{-2}$) were found to be significantly associated with inflammatory status, whereas lymphadenopathies and anemia were not (Table 1).

Inflammatory WM patients more often required treatment (85% *vs*. 64%, $P<10^{-3}$) (Table 1) but this did not translate in TFS difference (Figure 2A). Types of therapies were comparable between the two subgroups except for a slightly higher proportion of WM patients receiving rituximab monotherapy in the non-inflammatory subgroup (12% *vs*. 5%). Mean CRP values were significantly lower after 1L treatment in inflammatory WM (Table 1) and correlated with IgM response (Figure 1C). We did neither observe any difference in terms of response to 1L therapy nor PFS (Table 1; Figure 2B; 5-year PFS of 52% and 65% for inflammatory and non-inflammatory group respectively). Median OS was 10 years in the inflammatory group *versus* not reached in the non-inflammatory group, however it did not reach statistical significance ($P=0.06$, Figure 2C). Survival analyses according to CRP groups (5-19, 20-49 and ≥50 mg/L) or as a continuous variable did not yield sig-

Table 1. Patients characteristics associated with inflammatory status.

	Univariate			Multivariate	
	Non-Inflammatory WM (N=103)	**Inflammatory WM (N=119)**	***P***	**OR (95 CI)**	***P***
Clinical data					
Age at diagnosis, years, mean (range)	64.3 (28.4-86.6)	64.6 (35.1-88.2)	1.00		
Male, N (%)	61/103 (59)	86/119 (72)	0.77		
Lymphadenopathies, N (%)	14/101 (14)	38/118 (32)	**0.04**	2.2 (0.7-8.0)	0.21
Splenomegaly, N (%)	8/100 (8)	17/118 (14)	1.00		
Hyperviscosity syndrome, N (%)	6/101 (6)	10/118 (9)	1.00		
Past history of dysimmune conditions, N (%)	11/92 (12)	6/110 (5)	1.00		
Biological data					
M spike, g/dL, mean (range)	16.0 (0.1-71.0)	17.8 (1.0-58.0)	1.00		
Hb <11 g/dL, N (%)	31/66 (47)	75/101 (74)	**$<10^{-2}$**	1.6 (0.6-4.4)	0.32
Platelets < 100 x 10^9/L, N (%)	9/66 (14)	15/100 (15)	1.00		
Medullary infiltration %, mean (range)	43 (10-97)	33 (0-95)	0.16		
CRP, mg/L, mean (range)	0.4 (0-4.4)	30.9 (5.0-263.0)	NR		
Albumin, g/L, mean (range)	40.4 (31.0-50.5)	36.3 (15.0-45.6)	**$<10^{-5}$**	**0.8 (0.7-0.9)**	**$<10^{-2}$**
β2 microglobulin, mg/L, mean (range)	2.5 (1.2-12.0)	3.9 (1.5-33)	0.07		
IPSSWM: low; intermediate; high, N (%)	18/56 (32); 16/56 (29); 22/56 (39)	14/85 (16); 23/85 (27); 48/85 (56)	1.00		
Cytogenetic/molecular biology					
6q deletion, N (%)	8/74 (11)	41/104 (39)	**$<10^{-3}$**	**4.4 (1.4-16.0)**	**0.02**
TP53 abnormalities, N (%)	6/79 (8)	14/111 (13)	1.00		
Complex karyotype, N (%)	9/68 (13)	18/91 (20)	1.00		
MYD88 mutation, N (%)	65/71 (92)	83/93 (89)	1.00		
CXCR4 mutation, N (%)	18/66 (27)	19/91 (21)	1.00		
Follow-up					
Need for treatment initiation, N (%)	66/103 (64)	101/119 (85)	**<10-2**	**NA**	
ORR*, N (%); VGPR+CR, N (%)	38/64 (59); 12/64 (19)	61/96 (64); 20/96 (21)	1.00		
CRP after 1L treatment, mg/L, mean (range)	1.0 (0-16)	5.5 (0-69)	0.08		

*ORR=CR+VGPR+PR. WM: Waldenström's macroglobulinemia; OR: odds ratio; CI: confidence interval; ORR: overall response rate; VGPR: very good partial response; CR: complete response; 1L: fist-line; *P*: *P* value; PR: partial reponse; DLBCL: diffuse large B-cell lymphoma; CRP: c-reactive protein; IPSSWM: International Prognostic Scoring System of (WM). Subgroup comparisons were performed using Chi-square of Fisher's exact test for categorical variables, and Student's *t* test for continuous variables. Multiple hypothesis correction was performed with Holm's method for univariate analysis. A multivariate logistic regression was performed for multivariate analysis.

nificant differences (data not shown and *Online Supplementary Table S3*). Univariate and multivariate analyses of clinical and biological variables associated with TFS, PFS and OS in the entire cohort are summarized in the *Online Supplementary Table S3*. Prognostic factors associated with shorter OS included ISSWM ($P<10^{-2}$), anemia ($P=0.02$),

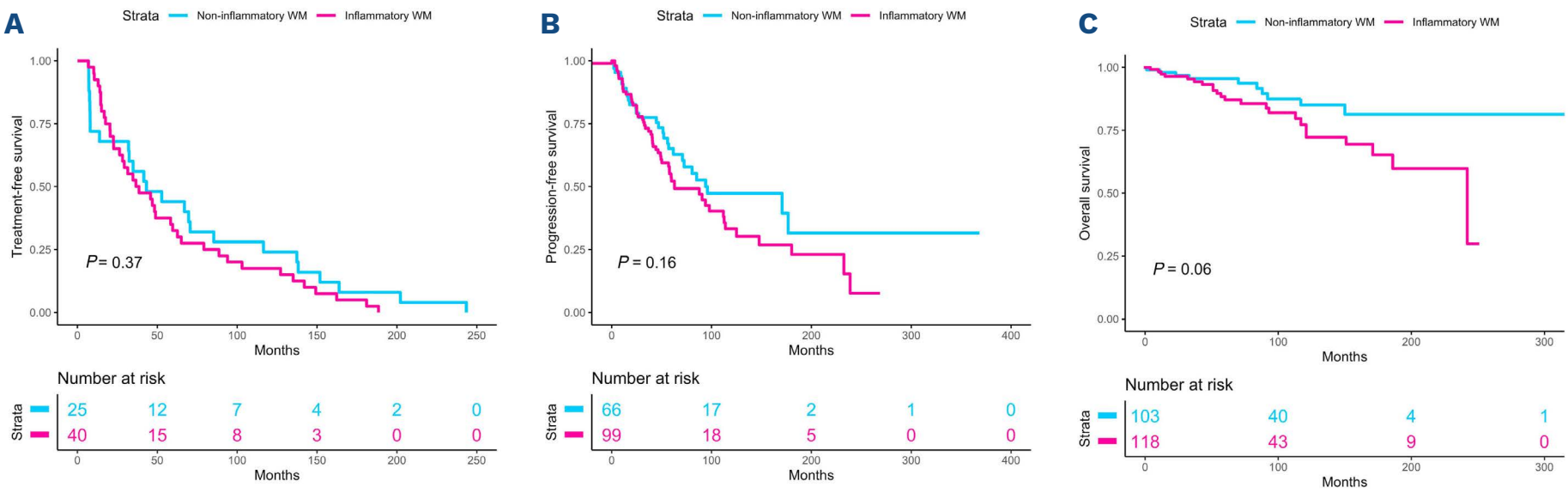

Figure 2. Outcomes according to inflammatory and non-inflammatory subgroups. (A) Treatment-free survival, (B) progression-free survival and (C) overall survival according to inflammatory (pink) and non-inflammatory (blue) subgroups. Survival comparisons were performed using log-rank test and shown with Kaplan-Meier curves. All *P* values were 2-sided, with $P<0.05$ indicating statistical significance. Statistical analyses were performed with R 3.6.3 (Foundation for Statistical Computing, Vienna, Austria).

tri4 ($P=0.03$), del6q ($P<10^{-2}$) and *TP53*abn ($P<10^{-2}$), while only *TP53*abn ($P=0.04$) retained significant pejorative impact in multivariate analysis.

In this large retrospective WM cohort, considering a positivity threshold of 5 mg/L, we observed WM-associated inflammation in more than half of the cases. Inflammatory status was significantly associated with anemia and consequently more frequent need for treatment initiation. Inflammatory status was not associated with higher IgM spike or BM infiltration, which is consistent with the hypothesis of cytokine-mediated anemia in this context. One of the main findings of our work was the strong association between inflammation and del6q, corroborating two previous retrospective studies focusing on del6q status.[11,12] It is of particular interest given that the minimally deleted region of 6q chromosome in WM contains *IBTK*, *HIVEP2* and *TNFAIP3* genes, negative regulators of NF-κB[13] which mediates IL-6 production. Furthermore, it has been shown that TNFAIP3 specifically exerts an inhibitory effect on the L265P mutated MYD88 receptor,[14] allowing us to postulate that one of the mechanisms through which del6q is associated with inflammation is the loss of *TNFAIP3*. However, more than half of the inflammatory patients from our cohort did not harbor del6q, indicating that other mechanisms are at play.

While inflammatory WM patients more frequently required therapy, we did not observe significant association with TFS, response to therapy nor PFS. A trend for poorer OS was observed although not significant ($P=0.06$). Inflammation has many deleterious effects, such as fostering malnutrition, cardiovascular disease and cancer. Rare cases of WM associated with AA amyloidosis have been described.[15] The sharp decrease in CRP values at the end of 1L treatment in our cohort shows that hematological and inflammatory responses were well correlated. Although our study did not explore this hypothesis, it could be speculated that persistence of elevated CRP after 1L therapy could contribute to morbi-mortality in these patients. This raises the question of early therapy initiation and/or complementary therapies directed against either the WM clone or IL-6 in order to prevent long-term consequences of chronic inflammation. Indeed, IL-6 plays an autocrine role in lymphoplasmacytic differentiation[9] and an anti-tumoral effect of anti-IL6 therapy has been demonstrated in a WM murine model in which anti-IL6 receptor antibodies were associated with a decrease in tumoral syndrome and M spike.[16]

In conclusion, our work highlights clinical and biological specificities of WM patients with chronic inflammation, notably a higher prevalence of del6q, more frequent need for therapy initiation and a trend for poorer OS, which will have to be confirmed by further studies. These findings may have implications for the understanding of inflammation in WM as well as for further therapeutic developments.

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Disclosures

No conflicts of interest to disclose.

Contributions

NF, VL and DRW designed the research. NF and DRW analyzed data and wrote the manuscript. NF, JC, CBG, DK, NG, CB, FNK, EC and DRW performed experiments. NF, MB, CBG, DK, NG, VM, NJ, MO, SC, FNK, VL and DRW recruited patients. All authors reviewed and approved the manuscript.

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Data-sharing statement

Due to the nature of this research, participants of this study did not agree to their data being shared publicly, so supporting data is not available.

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