Low levels of von Willebrand markers associate with high serum IgM levels and improve with response to therapy, in patients with Waldenström macroglobulinaemia

Waldenström macroglobulinaemia (WM) is a rare lymphoma characterized by the accumulation of malignant IgM-secreting lymphoplasmacytic cells in the bone marrow and other organs (Swerdlow *et al*, 2016). Recurrent *MYD88* and *CXCR4* mutations define the genomic landscape of WM (Hunter *et al*, 2017). Acquired von Willebrand disease (VWD) has been described in patients with WM (Mazurier *et al*, 1980), and a retrospective study suggested that high von Willebrand factor (VWF) levels might be prognostic in patients with WM (Hivert *et al*, 2012). However, the clinical characteristics and outcomes to therapy of WM patients with low levels of VWF antigen (VWF:Ag), VWF activity (Ristocetin cofactor; VWF:RCo) and factor VIII (FVIII) have not been extensively studied.

We carried a retrospective study in newly diagnosed WM patients seen at our institution who were tested for VWF:Ag, VWF:RCo and FVIII levels (VW markers). Low VW markers levels were defined as below 50%. Logistic regression models were fitted to evaluate the association of clinical factors and low VW markers. We also evaluated the association of response to therapy on VW markers using linear regression models. Categorical response to therapy was defined using current guidelines (Owen *et al*, 2013). *MYD88* mutation was evaluated using polymerase chain reaction techniques and *CXCR4* mutations using Sanger sequencing (Hunter *et al*, 2014; Xu *et al*, 2014). *MYD88* and *CXCR4* genotyping has been routinely performed at our centre since 2013, but it is not available in all patients.

A total of 320 WM patients were tested due to reported signs of bleeding or easy bruising, of whom 49 (15%) showed low levels of VW markers. The median serum IgM

Table I. Patient's characteristics and logistic regression models evaluating the association of clinical factors and low levels of VW markers in Waldenström macroglobulinaemia.

Patient characteristics	Total (n = 320)	Normal VW markers (n = 271)	Low VW markers (n = 49)	P-value
Age ≥65 years	170 (53%)	148 (55%)	22 (45%)	0·21
Male sex	180 (56%)	148 (55%)	32 (65%)	0·17
WBC count >6 × 10^9/l	149 (47%)	135 (50%)	14 (29%)	0·006
Haemoglobin <100 g/l	67 (21%)	53 (20%)	14 (29%)	0·15
Platelet count <100 × 10^9/l	15 (5%)	14 (5%)	1 (2%)	0·34
Bone marrow ≥50%	146 (46%)	123 (45%)	23 (47%)	0·84
Serum IgM <30 g/l	173 (54%)	169 (62%)	4 (8%)	<0·001
Serum IgM 30–59·99 g/l	108 (38%)	85 (31%)	23 (47%)	
Serum IgM ≥60 g/l	39 (12%)	17 (6%)	22 (45%)	
MYD88 mutation*	120/125 (96%)	95/99 (96%)	25/26 (96%)	0·96
CXCR4 mutation*	57/125 (46%)	37/99 (37%)	20/26 (77%)	<0·001

	Univariate analysis		Multivariate analysis	
Regression analyses	OR (95% CI)	P-value	OR (95% CI)	P-value
Age ≥65 years	0·67 (0·37–1·25)	0·21	0·52 (0·25–1·08)	0·08
Male sex	1·56 (0·83–2·95)	0·17	1·18 (0·55–2·52)	2·52
WBC count >6 × 10^9/l	0·40 (0·21–0·78)	0·007	0·69 (0·32–1·49)	0·35
Haemoglobin <100 g/l	1·65 (0·83–3·28)	0·16	1·38 (0·57–3·35)	0·48
Platelet count <100 × 10^9/l	0·38 (0·05–2·98)	0·36	0·40 (0·04–4·17)	0·44
Bone marrow ≥50%	1·06 (0·58–1·96)	0·84	0·61 (0·29–1·30)	0·20
Serum IgM 30–59·99 g/l	11·4 (3·83–34·1)	<0·001	11·8 (3·88–36·1)	<0·001
Serum IgM ≥60 g/l	54·7 (16·9–177·3)	<0·001	53·9 (15·5–187·5)	<0·001
MYD88 mutation*	1·05 (0·11–9·84)	0·96		
CXCR4 mutation*	5·59 (2·06–15·2)	0·001		

**MYD88* and *CXCR4* mutational status was not included in the multivariate analysis (n = 125 observations).

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level in patients with low VW markers was 56·6 g/l (range 18–103 g/l) and 22·7 g/l (range 0·25–110 g/l) in patients with normal VW markers ($P < 0·001$). Patients with low VW markers were more likely to have serum IgM levels 30–59·99 and ≥60 g/l (Table I). In the univariate logistic regression analysis, serum IgM level 30–59·99 and ≥60 g/l were associated with increased odds of low VW markers. In the multivariate logistic regression analyses, only serum IgM levels 30–59·99 and ≥60 g/l were independently associated with increased odds of low VW markers (Table I).

In the subset of patients who had genomic testing ($n = 125$), patients with low VW markers were more likely to carry *CXCR4* mutations (77% vs. 37%, $P < 0·001$) than patients with normal VW markers. Of these, 44 patients (77%) had nonsense (NS) and 13 (22%) had frameshift (FS) mutations. Univariate analysis associated *CXCR4* mutations with higher odds of low VW markers (odds ratio [OR] 5·59, 95% confidence interval [CI] 2·06–15·2, $P = 0·001$). The odds of VWD were higher in *CXCR4* NS-mutated patients (OR 7·85, 95% CI 2·81–22·0; $P < 0·001$) than on *CXCR4* FS-mutated patients (OR 0·86, 95% CI 0·09–7·81; $P = 0·89$). After adjusting for serum IgM level, *CXCR4* mutations were independently associated with higher odds of low VW markers (OR 4·07, 95% CI 1·31–12·7, $P = 0·016$), and *CXCR4* NS patients had higher odds of VWD (OR 4·67, 95% CI 1·46–14·9; $P = 0·01$) than *CXCR4* FS patients (OR 1·24, 95% CI 0·10–14·8; $P = 0·86$).

Of 18 patients with low VW markers who received therapy for WM, six patients (33%) received proteasome inhibitors/rituximab, 5 (28%) alkylators/rituximab, 5 (28%) ibrutinib and 2 (11%) rituximab alone. At best response, the median serum IgM level decreased, from 62·41 g/l (range 33·47–103 g/l) to 22·74 g/l (range 4·06–61 g/l), the median VWF:Ag level had increased, from 21% (range 5–49%) to 64% (range 15–200%), the median VWF:RCo level had increased, from 23% (range 9–41%) to 58% (range 19–196%), and the median FVIII level had increased, from 21% (9–43%) to 57% (30–183%) ($P < 0·001$ for all comparisons). Linear regression models associating change in serum IgM and change in VW markers are shown in Fig 1. The association between change in serum IgM and change in VW markers remained significant after adjusting for baseline VWF:Ag, VWF:RCo and FVIII levels, and after removing outlier observations (data not shown). Three patients (17%) achieved very good partial response (VGPR), 6 (33%) partial response (PR), 8 (44%) minor response (MR) and 1 (11%) stable disease. After therapy, 14 patients (78%) had normalized VW markers. In patients achieving VGPR, PR and MR, changes in serum IgM were −90%, −66% and −34%, while VWF:Ag changes were +700%, +225% and +95%, VWF:RCo changes were +427%, +173% and +102%, and FVIII changes were +650%, +261% and +95%, respectively ($P < 0·001$ for trend in each category).

Our study shows that 15% of WM patients tested had low VW markers. Given that not all patients were tested, the incidence reported here is probably an overestimate. Our

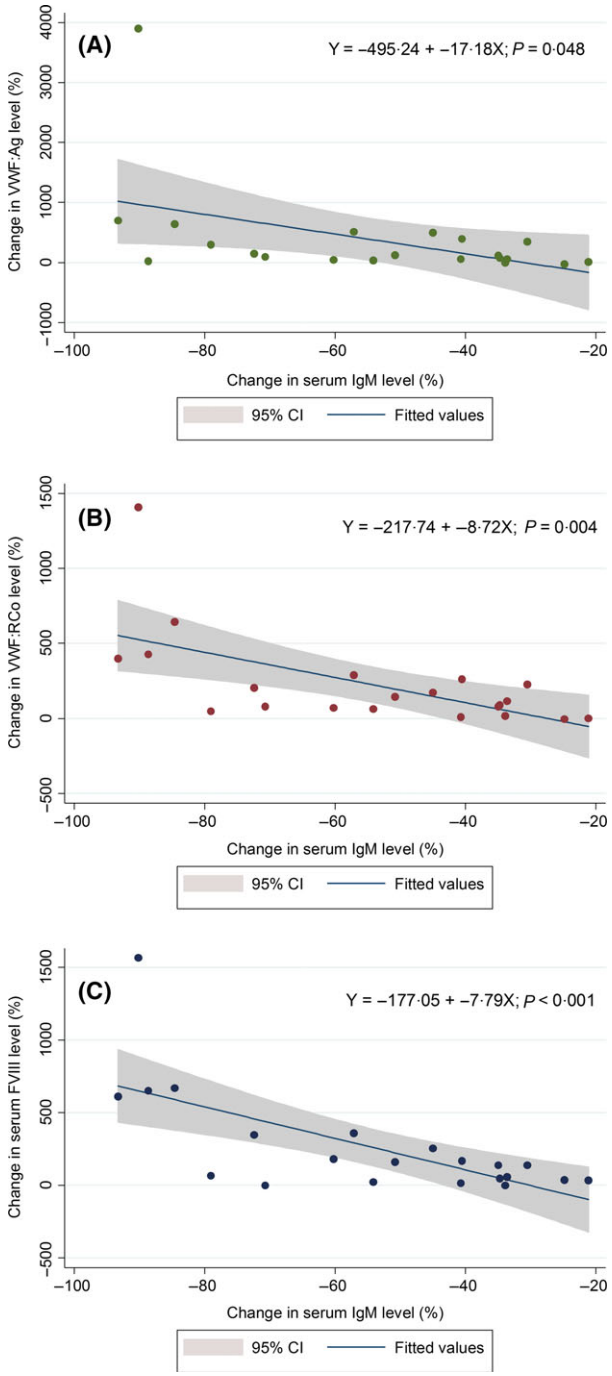

Fig 1. Linear regression models associating changes in serum IgM level and changes in levels of VW markers (A) von Willebrand factor antigen (VWF:Ag), (B) von Willebrand factor activity (VWF:RCo) and (C) factor VIII, in patients with Waldenström macroglobulinaemia who were treated and responded to therapy. [Colour figure can be viewed at wileyonlinelibrary.com]

study suggests that low VW markers were associated with higher serum IgM levels. However, not all patients with high IgM levels had low VW markers, suggesting that the development of low VW markers is not purely dependent on IgM levels. Therefore, other factors must contribute to the

development of low VW markers in WM patients, such as selective VWF adsorption on malignant cells, increased VWF proteolysis, production of anti-VWF antibodies and VWF and FVIII activation associated with increase in serum viscosity due to increased serum IgM level, or there could be interactions between VWF and FVIII with the IgM molecule itself (Michiels *et al*, 2001).

In a subset analysis, we identified increased odds of low VW markers in patients with *CXCR4* mutations. We had previously showed that *CXCR4* mutations were associated with hyperviscosity (Gustine *et al*, 2017). In the present study, *CXCR4* mutations were associated with 4-fold increased odds of low VW markers after adjusting for serum IgM levels. This finding deserves attention as it could have therapeutic implications. Ibrutinib is the only medication approved for the treatment of WM. In WM patients with *CXCR4* mutations, however, responses are slower and more superficial (Treon *et al*, 2015). Ibrutinib affects platelet aggregation and could increase the risk of bleeding in WM patients with low VW markers. We recommend screening for VW markers in WM patients with complaints of easy bruising or mucocutaneous bleeding. Finally, our study shows that response to therapy improves VW markers in WM patients. The degree of improvement correlates with the depth of the response.

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Author contributions

JJC designed the study, analysed the study data and wrote the manuscript. JJC, TD, PS and SPT provided treatment and follow-up for patients on the study. JNG and KM collected the study data. ZRH, GY and LX performed genotyping for *MYD88* and *CXCR4* mutations. All authors read, critically reviewed and approved the final manuscript.

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References

Gustine, J.N., Meid, K., Dubeau, T., Hunter, Z.R., Xu, L., Yang, G., Ghobrial, I.M., Treon, S.P. & Castillo, J.J. (2017) Serum IgM level as predictor of symptomatic hyperviscosity in patients with Waldenstrom macroglobulinaemia. *British Journal of Haematology*, **177**, 717–725.

Hivert, B., Caron, C., Petit, S., Charpy, C., Fankam-Siaka, C., Lecocq, S., Zawadzki, C., Susen, S., Rusu, M., Duhamel, A., Tournilhac, O., Goudemand, J. & Morel, P. (2012) Clinical and prognostic implications of low or high level of von Willebrand factor in patients with Waldenstrom macroglobulinemia. *Blood*, **120**, 3214–3221.

Hunter, Z.R., Xu, L., Yang, G., Zhou, Y., Liu, X., Cao, Y., Manning, R.J., Tripsas, C., Patterson, C.J., Sheehy, P. & Treon, S.P. (2014) The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood*, **123**, 1637–1646.

Hunter, Z.R., Yang, G., Xu, L., Liu, X., Castillo, J.J. & Treon, S.P. (2017) Genomics, signaling, and treatment of Waldenstrom macroglobulinemia. *Journal of Clinical Oncology*, **35**, 994–1001.

Mazurier, C., Parquet-Gernez, A., Descamps, J., Bauters, F. & Goudemand, M. (1980) Acquired von Willebrand's syndrome in the course of Waldenstrom's disease. *Thrombosis and Haemostasis*, **44**, 115–118.

Michiels, J.J., Budde, U., van der Planken, M., van Vliet, H.H., Schroyens, W. & Berneman, Z. (2001) Acquired von Willebrand syndromes: clinical features, aetiology, pathophysiology, classification and management. *Best Practice and Research. Clinical Haematology*, **14**, 401–436.

Owen, R.G., Kyle, R.A., Stone, M.J., Rawstron, A.C., Leblond, V., Merlini, G., Garcia-Sanz, R., Ocio, E.M., Morra, E., Morel, P., Anderson, K.C., Patterson, C.J., Munshi, N.C., Tedeschi, A., Joshua, D.E., Kastritis, E., Terpos, E., Ghobrial, I.M., Leleu, X., Gertz, M.A., Ansell, S.M., Morice, W.G., Kimby, E. & Treon, S.P. (2013) Response assessment in Waldenstrom macroglobulinaemia: update from the VIth International Workshop. *British Journal of Haematology*, **160**, 171–176.

Swerdlow, S.H., Campo, E., Pileri, S.A., Harris, N.L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G.A., Zelenetz, A.D. & Jaffe, E.S. (2016) The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*, **127**, 2375–2390.

Treon, S.P., Tripsas, C.K., Meid, K., Warren, D., Varma, G., Green, R., Argyropoulos, K.V., Yang, G., Cao, Y., Xu, L., Patterson, C.J., Rodig, S., Zehnder, J.L., Aster, J.C., Harris, N.L., Kanan, S., Ghobrial, I., Castillo, J.J., Laubach, J.P., Hunter, Z.R., Salman, Z., Li, J., Cheng, M., Clow, F., Graef, T., Palomba, M.L. & Advani, R.H. (2015) Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *The New England Journal of Medicine*, **372**, 1430–1440.

Xu, L., Hunter, Z.R., Yang, G., Cao, Y., Liu, X., Manning, R., Tripsas, C., Chen, J., Patterson, C.J., Kluk, M., Kanan, S., Castillo, J., Lindeman, N. & Treon, S.P. (2014) Detection of MYD88 L265P in peripheral blood of patients with Waldenstrom's macroglobulinemia and IgM monoclonal gammopathy of undetermined significance. *Leukemia*, **28**, 1698–1704.