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Chapter 5

High prevalence of the MYD88 L265P mutation in IgM anti-MAG paraprotein associated peripheral neuropathy

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Immunoglobulin M (IgM) anti-myelin-associated-glycoprotein (MAG) paraprotein-associated peripheral neuropathy (anti-MAG PN) is the most frequent type of paraprotein-associated neuropathy. It typically presents as a chronic demyelinating disorder with progressive ataxia, tremor and sensory disturbance.(1) By definition, IgM paraproteinemia and high titer anti-MAG antibodies are present. Up to 50% of patients develop significant disability. Progressive disease-related disability is considered an indication to start treatment. However, there is no consensus on the optimal treatment approach and a high clinical need for effective therapies(1).

IgM paraproteinemia is the hallmark of Waldenström's Macroglobulinemia (WM) and IgM Monoclonal Gammopathy of Unknown Significance (MGUS). WM is an indolent B-cell malignancy with lymphoplasmacytic differentiation typically localized in the bone marrow (BM), while IgM MGUS is considered a premalignant condition, defined as asymptomatic IgM paraproteinemia with <10% BM infiltration by lymphoplasmacytic cells. The term "IgM related disease" is reserved for IgM MGUS with symptoms that are attributable to the paraprotein, such as cryoglobulinaemia, cold agglutinin disease and indeed IgM related neuropathy(2).

Recently, a recurrent somatic point mutation of the myeloid-differentiation-factor 88 (*MYD88*) gene, leading to an amino-acid change from leucine to proline (L265P), has been reported in the vast majority (>90%) of WM patients and approximately 50% of IgM MGUS patients. The mutation is absent in healthy donors, multiple myeloma and non-IgM MGUS (3). MYD88 is an adaptor protein of the interleukin-1R and toll-like-receptor signalling pathways that ultimately lead to activation of nuclear-factor- κ B and Janus-kinase/signal-transducer and activator-of-transcription-3. The *MYD88 L265P* mutation results in aberrant activation of these pathways and is considered the central driver mutation of WM and a diagnostic signature of the disease. In addition, MYD88 status impacts the efficacy of Ibrutinib, an oral Bruton's-Tyrosine-Kinase (BTK) inhibitor and the only drug specifically approved for WM. Ibrutinib is markedly less effective in WM patients with wild-type *MYD88* than in those with *MYD88 L265P*. Among the IgM-related disorders, the incidence of *MYD88 L265P* has been studied in individuals with cold agglutinin disease and cryoglobulinemia, and found to be absent in both conditions, suggesting a distinct pathophysiology different from WM. Thus far, the mutational status of *MYD88* has not been studied in patients with anti-MAG PN.

Our study comprises 20 patients with anti-MAG PN. Inclusion criteria were: presence of IgM paraprotein in the serum; a positive serum IgM MAG-antibody test of at least 1500 Bühlmann titer units (BTU); a clinical diagnosis of anti-MAG neuropathy by a neurologist specialized in peripheral nerve disorders, including electromyography (EMG) with signs of demyelination; availability of a BM sample. Eligible patients were identified at four centres in the Netherlands (UMCU, AZN, and AMC, 11 patients) and the UK (UCLH, 9 patients). The study was conducted in accordance with the Declaration of Helsinki.

MYD88 L265P mutation analysis was performed at two centres (UCLH and AMC) on DNA extracted from either stored BM aspirates or trephine biopsy specimens, using an allele specific PCR as described previously (4), allowing for reproducible detection of as little as 1% tumour DNA diluted in wild-type DNA. In samples that tested negative for the mutation, the presence of a B-cell clone was assessed using multiplex-PCRs for the detection of B-cell receptor rearrangements including combined application of IGH and IGK tubes based on the BIOMED-2 consensus. Anti-MAG-antibody testing was performed per standard care using a commercially available ELISA (Bühlmann-Laboratories, Switzerland). Additional titration above that level was available for 12 patients. Multicolor flow cytometry including a pan-B-cell panel for the detection of clonal B-cells was available for 11 patients.

Clinical characteristics, including neurological findings and relevant biochemical parameters, as well as the results of the mutation analysis are summarized in **table 1**. All patients had the typical clinical picture of sensorimotor polyneuropathy and an EMG consistent with demyelinating polyneuropathy with a prolonged distal motor latency. All but one patients had a very low bone marrow tumour load well below 10% and histologically consistent with MGUS. The *MYD88 L265P* mutation was detected in 12/20 patients (60%). Of the 8 patients (40%) that tested negative for the mutation, the presence of a B-cell clone was confirmed by rearrangement testing in 2 patients. In the other 6 patients that tested negative for the mutation, a clonal B cell population could not be detected. None of the clinical characteristics were significantly different between patients with and without the mutation. Disease severity and response to treatment could not be assessed in relation to the mutation due to the lack of consensus-based scoring instruments, and the clinical heterogeneity within the cohort.

Our study demonstrates that the *MYD88 L265P* mutation is highly prevalent in a cohort of well-characterized anti-MAG PN patients. The detected mutational rate of 60% most likely represents an underestimate since the tumour load was generally very low, as shown by the low quantity of clonal B-cells as detected by flow cytometry and negative B-cell receptor rearrangement-based clonality studies in 7/9 mutation negative patients. This indicates a very low frequency of neoplastic B cells in these samples, which may have precluded *MYD88 L265P* detection. Indeed, the reported prevalence of *MYD88 L265P* in the general IgM MGUS population is highly variable (10-87%) which most likely relates to sensitivity issues in the setting of a low clonal B-cell burden. Whether the presence of the mutation is predictive for clinical course or response to treatment needs further study in a large, preferably prospective cohort using consensus based response criteria (5).

Our results establish that, contrary to other IgM-MGUS related disorders, but in line with asymptomatic IgM MGUS and WM, the majority of anti-MAG PN cases contain the *MYD88 L265P* mutation. Furthermore, like B-cells in WM, anti-MAG PN B-cells have been shown to represent post-germinal center memory B-cells. Taken together, these

Table 1: Clinical characteristics and MYD88 L265P status of 20 anti-MAG PN patients¹

Sexe (M/F)	Age	MYD88 L265P mutation	B-cell clonality testing	Percentage bonemarrow infiltration based on histology	Percentage bonemarrow infiltration based on flow cytometry	Serum IgM level (g/L)	Titrated MAG level above 1500 if available (BTU)	Subjective duration of disease (years)	EMG based duration of disease (years)
M	64	Negative	Negative	Negative	Negative	1,7	n.t.	0	0
M	67	Negative	Negative	Negative	Negative	2,3	n.t.	8	8
M	76	Negative	Negative	Negative	Negative	8,1	>7000	1	0
F	66	Negative	Negative	Negative	<1%	5,4	>70.000	1	0
M	62	Negative	Negative	Negative	n.t.	4	>70.000	1	0
M	73	Negative	Negative	Negative	Negative	2,3	n.t.	11	1
F	72	Negative	Positive	<5%	n.t.	9	>7000	3	0
M	71	Negative	Positive	Negative	1%	5,7	>70.000	6	4
M	72	Positive	Negative	Negative	n.t.	<3	>70.000	1	0
F	43	Positive	Negative	<5%	n.t.	7	>70.000	11	0
M	70	Positive	Negative	Negative	n.t.	<3	>7000	4	3
F	74	Positive	Negative	Negative	n.t.	<3	>70.000	11	5
M	62	Positive	Negative	<10%	n.t.	10	>70.000	12	4
M	71	Positive	Negative	25	n.t.	3	>70.000	1	0
M	66	Positive	Negative	Negative	2%	5	>70.000	26	9
M	64	Positive	Negative	<5%	3%	16	n.t.	11	6
F	79	Positive	Negative	Negative	4%	5,7	n.t.	13	13
F	59	Positive	Negative	Negative	n.t.	4,7	n.t.	5	5
F	75	Positive	Negative	<10%	5%	3,16	n.t.	4	0
M	70	Positive	Negative	2%	Negative	0,6	n.t.	3	1

¹ Duration of disease at the time of bonemarrow sampling based on subjective onset of symptoms

BTU: Bühlmann Titer Units. N.t.: Not tested

data strongly suggest a pathogenetic link of anti-MAG PN with WM. This supports the initiation of clinical trials for anti-MAG PN using agents that have proven efficacy in WM patients. Specifically, novel oral agents with little (neuro-) toxicity such as the BTK inhibitors or second generation proteasome-inhibitors could be of great interest in the treatment of anti-MAG PN.

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