Clinical and clonal characteristics of monoclonal Immunoglobulin M-associated type I cryoglobulins

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Abstract

Monoclonal immunoglobulin M-associated type I cryoglobulinaemia is poorly characterised. We screened 534 patients with monoclonal IgM disorders over a 9-year period and identified 134 patients (24%) with IgM type I cryoglobulins. Of these, 76% had Waldenström macroglobulinaemia (WM), 5% other non-Hodgkin lymphoma (NHL) and 19% IgM monocloncal gammopathy of undetermined significance (MGUS). Other clinically relevant IgM-associated disorders co-existed in 31%, more frequently in MGUS vs WM or NHL (72% vs 22%/29%, p<0.001). The majority of those with cryoglobulins and coexistent cold agglutinin disease (CAD)/syndrome(CAS) had the molecular characteristics of a CAD clone: wild type-*MYD88* in 80% (p<0.0001). A half of all patients had active manifestations at cryoglobulin detection: most commonly vasomotor (22%), cutaneous (16%), peripheral neuropathy (22%) and clinical hyperviscosity (12; 9%). Those with underlying MGUS were more likely to be symptomatic of cryoglobulinaemia. 16/134 required treatment for cryoglobulinaemia at a median of 38 days (range 6-239) from cryoglobulin detection. At a median follow up of 2.5 years (range 0-9.9), 2-year cryoglobulinaemia-treatment free survival was 81% (95% confidence interval [CI] 72-87%), 2-year overall survival was 92% (95% CI 85-96%). Age was the only predictor of overall survival. Predictors of cryoglobulinaemia-related treatment or death were hyperviscosity (HR 73.01 95% CI 15.62-341.36, p<0.0001) and cutaneous involvement (HR 2.95 95% CI 1.13-7.71, p=0.028) at cryoglobulin detection. Type 1 IgM cryoglobulinaemia is more prevalent than previously described in patients with IgM gammopathy and should be actively sought and treated when appropriate.

Introduction

Type I cryoglobulinaemia is defined by the presence of monoclonal immunoglobulins which precipitate at temperatures below 37°C and redissolve on warming. Type I cryoglobulins of IgM monoclonal protein (M-protein) are distinct from IgG isotype in that they may arise in the context of underlying Waldenström macroglobulinemia (WM), other non-Hodgkin lymphoma (NHL) or an IgM monoclonal gammopathy of undetermined significance (MGUS). This is in contrast to type II and III mixed cryoglobulins, which are largely due to hepatitis C infection, hepatitis B, HIV or autoimmune conditions. Symptomatic cryoglobulinaemia with IgM MGUS is one of the entities termed IgM monoclonal gammopathy of clinical significance (MGCS). IgM MGCS includes important conditions like cold agglutinin disease, anti-MAG neuropathy, Schnitzler's syndrome and light chain (AL) amyloidosis; and have distinct manifestations of disease related to the monoclonal protein itself beyond the tumour bulk, which is often minimal. Typically bone marrow tumour infiltrate is low (<10%) however the circulating IgM M-protein is behaves in a highly pathogenic way, contributing to IgM-mediated symptoms (Khwaja, D'Sa et al. 2022). All IgM MGCS have distinct clinical phenotypes but their co-existence is not well described, nor symptom overlap.

The incidence and clinical outcomes of patients with type I IgM cryoglobulins are poorly characterised. When first defined, approximately 10% of monoclonal IgM conditions were reported to have cryoglobulins and presenting symptoms were noted in 80% (Brouet, Clauvel et al. 1974). However more recent data demonstrate a variable proportion (x-x%?) of symptomatic cases likely reflecting the practice of cryoglobulinaemia screening (Harel, Mohr et al. 2015, Zhang, Cao et al. 2020). Previous studies examining type I cryoglobulins have grouped type I IgG and IgM cohorts together (Kolopp-Sarda, Nombel et al. 2019) and have not delineated the relative clinical characteristics, symptom burden or treatment delivery. Fewer than 40 patients with solely IgM gammopathy were included in each study.

Given the notable prevalence of such patients in our specialist practice, we reviewed our cohort in detail and posit that this entity is more prevalent than currently assumed. The proportion of patients with symptomatic vs asymptomatic disease is unclear from retrospective series. We report here incidence, clinical characteristics and treatment outcome of patients with IgM-associated type I cryoglobulins from a systematically screened cohort of patients from two IgM specialist centres and co-existence with other MGCS.

Methods

Consecutive adult patients were screened for the presence of cryoglobulins between 2013 and 2022 from clinical databases at two specialist referral centres (University College London Hospital, UCLH, United Kingdom and Amsterdam UMC, Netherlands). One-hundred and thirty-four patients with type I IgM cryoglobulins were identified and included in this analysis. Data cut-off was 30th December 2022. Incidence of type I IgM cryoglobulins was estimated as a proportion of the total number of tests performed at the UCLH Centre for WM and Related Disorders.

The presence of a cryoglobulin was confirmed by laboratory testing in accordance with standard laboratory procedures (Sargur, White et al. 2010). Samples were taken in prewarmed tubes and transported at 37°C, separated by centrifugation for 10 minutes at 2500 rpm and sample split into two aliquots (one at 4°C and one at 37°C). Half of the serum was incubated at 37°C and analysed by capillary zone electrophoresis (Sebia, France) to identify the presence of a M-protein. Half of the serum was incubated for 7 days at 4°C and inspected daily for the formation of a cryoprecipitate. If a precipitate was observed which was not present in the 37°C sample, it was washed three times in saline at 4°C, resuspended in saline and warmed to 37°C to redissolve it. Analysis of the cryoprecipitate was performed by electrophoresis and immunofixation (Sebia, France). Cryocrit (measure of relative volume of precipitate as a proportion of the total serum volume) was not routinely measured.

All patients had a baseline bone marrow assessment. When appropriate, *MYD88* mutation testing was performed using a Plentiplex MYD88 L265P real-time PCR assay. *CXCR4* mutation was

tested by direct Sanger sequencing or next generation sequencing using the Archer Myeloid VariantPlex assay (methodology switch in September 2021).

Collected data included histopathological category of the underlying IgM disorder (WM, IgM MGUS or NHL) according to WHO 4 criteria, concurrent presence of other MGCS: cold agglutinin disease/syndrome (CAD/CAS), anti-MAG polyneuropathy, AL amyloidosis, Schnitzler syndrome, CANOMAD; any presence of Bing-Neel syndrome (BNS) in those with WM. Symptoms at detection of cryoglobulin were reviewed: cutaneous manifestations (ulcers, purpura, livedo reticularis or racemose), vasomotor symptoms (acrocyanosis, Raynaud's syndrome, cold intolerance), renal biopsy-proven cryoglobulinaemia, symptomatic hyperviscosity and clinical peripheral neuropathy (biopsy not required). For those who had treatment, the indication for treatment (cryoglobulin-related or other indication), details of treatment administered, outcomes (clinical complete response, improvement, no improvement, worsening) and survival were collected.

The primary end point was cryoglobulin-treatment free survival calculated as time from cryoglobulin detection to treatment for cryoglobulinaemia or death of any cause. The secondary endpoint was overall survival (OS) calculated as time from cryoglobulin detection to death. Cause of death was determined from death certification, post-mortem data when available, communication with patient's physician and review of medical records. Data were collected in compliance with national and/or local regulations and data transfer agreements used where required.

Patients who had not experienced an event were censored at last follow-up. Estimates were generated using the Kaplan-Meier method and groups were compared using Cox regression and the log-rank test. Backwards selection with p=0.05 for inclusion was used for multivariable analyses. Baseline characteristics were compared using χ^2 or Fisher's exact tests (categorical variables) or Wilcoxon Mann-Whitney/Kruskal-Wallis tests (continuous variables). All statistical analyses were conducted using STATA v16.1 (STATAcorp, Texas).

Results

Patient characteristics

534 samples were screened for cryoglobulins between 2013-2022. Of these, 176 (33%) were positive for any cryoglobulin (type I, II, III); 134 (25%) had type I IgM cryoglobulins.

Baseline characteristics are outlined in Table 1. Of the total 134 patients (78 male, 56 female) identified, 102 (76%) had underlying WM, 7 had other NHL (5 marginal zone lymphoma, 2 chronic lymphocytic leukaemia; 5%) and remaining IgM MGUS (25; 19%). There was a kappa light-chain predominance (84%). *MYD88*^{L265P} was detected in 59/65 (91%) of those with WM and 3/15 (20%) of MGUS cases. *CXCR4* was mutated in 7/29 (24%) of WM cases. Those with MGUS had the lowest M-protein concentration at cryoglobulin detection (4g/l vs 14g/l for WM, p<0.0001; vs 10g/l for NHL, p=0.005). Of all patients with a type I cryoglobulin, 42 (31%) had a co-existent other IgM MGCS or BNS: CAD/CAS (20; 15%), anti-MAG antibody (7; 5%), non-cryoglobulinaemic glomerulopathy (3; 2%), CANOMAD syndrome (3; 2%), Schnitzler syndrome (1; 1%), AL amyloidosis (1; 1%), BNS (8; 6%). A significantly higher proportion of cryoglobulin positive patients with IgM MGUS (compared to WM or NHL) had CAD/CAS and anti-MAG antibodies (p<0.0001 and p=0.026, respectively). The majority of those with cryoglobulins and coexistent CAD/CAS had characteristics of a CAD clone: *MYD88* wild type in 80% (p<0.0001). Of those co-existent CAD/CAS, 12/20 were symptomatic: most had cutaneous or vasomotor manifestations (11/12) and a 1/3 experienced digital gangrene and necrosis (4/12).

Median M-protein at cryoglobulin detection was 12g/l (range 0-63) and lowest in the MGUS cohort vs WM vs NHL (4g/l vs 14g/l vs 10g/l, p=0.0001). Median time to detection of cryoglobulins was 13 months (range -4 – 442 months) after diagnosis of the underlying IgM disorder and longer in those with WM compared with MGUS or NHL (17 vs 2 vs 7 months, p=0.031). Of the entire cohort, the minority (7%; 9/134) had cryoglobulins detected prior to the diagnosis of the underlying IgM disorder at a median of 1 (range 0-4) month.

Symptoms

A half of patients had active manifestations of cryoglobulinemia at cryoglobulin detection. The most common features were vasomotor in 30 (22%) (acrocyanosis, Raynaud's, cold intolerance), cutaneous in 21 (16%) (skin ulcers, purpura, necrosis, livedo reticularis/racemose), peripheral neuropathy 29 (22%), clinical hyperviscosity 12 (9%), arthralgia 10 (7%), cryoglobulinaemic glomerulopathy 2 (1%). The cutaneous changes included skin ulceration in 11 and in 8 cases digital gangrene and autoamputation. Those with underlying MGUS were more likely to be symptomatic compared with WM or NHL (MGUS vs WM/NHL, p=0.041) and have cutaneous manifestations (vs WM/NHL, p= 0.041). Two asymptomatic patients with raised plasma viscosity without clinical manifestations of clinical hyperviscosity was considered as a spuriously elevated level due to assay interference from the presence of cryoglobulin therefore not categorised as having hyperviscosity. There were no cases of cardiorespiratory symptoms related to cryoglobulinaemia. Of those with clinical hyperviscosity, median plasma viscosity was above the level of detection >7 mPa (range 1.65 to >7mPa). Thrombotic events were noted in 14 (10%) patients: venous in 11 (8%) and cerebral arterial in 4 (3%).

Outcomes

Sixteen patients required treatment for cryoglobulinaemia at a median of 38 days (range 6-239) from cryoglobulin detection. Fifteen patients received chemoimmunotherapy and one additional patient declined chemoimmunotherapy and was treated with intermittent plasma exchange. Indications for treatment were hyperviscosity alone (8; 50%), hyperviscosity with cutaneous/vasomotor (2; 13%), cutaneous alone (3; 19%), cutaneous and peripheral neuropathy (1; 6%), cryoglobulinaemic glomerulopathy (2; 13%). Responses are shown in Table 3. Symptoms of hyperviscosity resolved completely when treated with combination of plasma exchange and chemoimmunotherapy but only a temporary improvement was noted in one patient who declined chemoimmunotherapy. In those with cutaneous involvement there was clinical improvement and stabilisation of renal function in those with renal involvement.

At a median follow up of 2.5 years (range 0-9.9), median OS and cryoglobulinaemia-treatment free survival was not reached. 2-year cryoglobulinaemia-treatment free and overall survival were 81% (95% confidence interval [CI] 72-87%) and 92% (95% CI 85-96%), respectively. Age was the only predictor of OS (per year, hazard ratio [HR] 1.09 95% CI 1.04 – 1.13, p<0.001). Underlying diagnosis, symptoms, *MYD88* mutation status, presence of IgM-associated disorders did not predict OS in this cohort.

Predictors of cryoglobulinaemia-related treatment or death on univariate analysis were any symptom of cryoglobulinaemia at cryoglobulin detection (HR 2.82 95% CI 1.25-6.40, p=0.013), hyperviscosity (HR 35.07 95% CI 13.03-94.38, p<0.0001), cutaneous involvement (HR 3.47 95% CI 1.60-7.57, p=0.002), and renal involvement (HR 12.17 95% CI 2.73-54.29, p=0.001). On multivariable analysis, independent predictors of cryoglobulinaemia-related treatment or death were hyperviscosity (HR 73.01 95% CI 15.62-341.36, p<0.0001) and cutaneous involvement (HR 2.95 95% CI 1.13-7.71, p=0.028) at cryoglobulin detection alone (Table 2).

Discussion

Few studies report specifically on type I IgM cryoglobulins, with most literature conducted on type II disease, which is largely due to hepatitis C (Roccatello, Saadoun et al. 2018). Hence, the true morbidity of IgM type I cryoglobulinemia remains underestimated. We report the largest series of patients exclusively with IgM type I cryoglobulins. Over half the patients in the current cohort were symptomatic. The most prominent features of symptomatic cryoglobulinaemia were cutaneous, vasomotor (skin mottling +/- subcutaneous oedema with reduced functionality), peripheral neuropathy and clinical hyperviscosity. Crucially, those with underlying MGUS and consequently, lower monoclonal protein concentration, were more likely to be symptomatic of cryoglobulins. As expected, those with coexistent MGCS had a lower burden of bone marrow infiltrate. The true prevalence and incidence of cryoglobulinaemia amongst patients with monoclonal IgM disorders is not established. The national WMUK Rory Morrison Registry of over 1300 patients entered by 25 UK

centres with WM or associated conditions showed a prevalence of 7% (Uppal, Khwaja et al. 2023) although this does not include patients who were screened for cryoglobulins and excludes those with other NHL. In our cohort, a fifth of patients with IgM M-protein systemically screened had type I cryoglobulins detected. Two series from China and France characterised 26 patients each with IgM type I cryoglobulinaemia. In these series, patients with IgM disorders were not universally screened so a denominator cannot be estimated. Of those with positive cryoglobulins detected, asymptomatic disease was present in 8-35% (Harel, Mohr et al. 2015, Zhang, Cao et al. 2020), a much smaller proportion than we report (50%). The proportion of those presenting with asymptomatic disease is therefore likely underreported alongside unidentified symptomatic patients.

The underlying bone marrow clone as well as the molecular profile assessments remain limited in type I cryoglobulinaemia; particularly in relation to clinical or therapeutic outcomes. The molecular characteristics of bone marrow clones in cryoglobulinaemia have not been previously examined. We show that type I cryoglobulinaemia may present in the setting of distinct clonal disorders (*MYD88* mutated WM/MGUS clone and *MYD88* unmutated CAD/NHL clone), variable bone marrow infiltration and around a third have additional co-existent IgM MGCS. In our cohort, cryoglobulinaemia presented with small clonal infiltrates (<10% infiltrate, MGUS) or with overt lymphoma (WM or NHL). There was a predominant kappa light chain restriction as in CAD (Berentsen, Ulvestad et al. 2006), anti-MAG neuropathy (Allain, Thonier et al. 2018) and Schnitzler syndrome (de Koning 2014).The majority in our cohort had underlying *MYD88* mutated WM/MGUS. The patients with CAD or underlying NHL, had a *MYD88* unmutated clone. WM is characterised by lymphoplasmacytic marrow infiltration. *MYD88* L265P mutation is detected in >90% patients. IgM MGCS is typically characterised by a low bone marrow infiltrate (Khwaja, D'Sa et al. 2022). CAD (Randen, Trøen et al. 2014) is now recognised as a distinct small B-cell *MYD88* negative clone (Alaggio, Amador et al. 2022). The clonal/mutation status in AL amyloidosis, Schnitzler syndrome and MAG neuropathies (Vos, Notermans et al. 2018) remain unclear with *MYD88* mutation reported in some cases.

This current cohort demonstrated that majority of symptomatic patient with cyroglobulinaemia have low clonal burden. As the monoclonal protein (and not the clone) is the driver of the disease, the attainment of a deep response is probably desirable due to the potential of even low level protein continuing to cause symptomatic disease. Whilst standard regimes used in WM are often the first line treatment here, the trade-off is treatment-related toxicity from chemoimmunotherapy. The optimum agents and number of cycles needs clarification in prospective studies. The molecular characteristics of the underling clone in WM are proving significant in impacting the degree and depth of response to therapies (Tam, Opat et al. 2020). Whilst we can report MYD88/CXCR4 mutations, this study is unable to comment on response in relation to the mutational status. More detailed studies are needed in the setting of cryoglobulinaemia to gain insight as to how to direct therapies beyond aiming for a swift response. The corollary is the nature of the response from targeted therapies such as BTK inhibitors; ibrutinib was disappointingly ineffective in the AL amyloidosis setting (Pika, Hegenbart et al. 2018) perhaps related to the lack of deep haematological responses. Further studies are needed to assess the effect of BTKi (first and next generations) in the IgM cryoglobulin setting.

The presenting symptoms of cryoglobulinemia, whilst well described in the literature, are often subtle and can affect a number of organ systems. It is important for relevant specialists to have an awareness of cryoglogulinaemia as potential underlying cause of patients with severe, unexplained or progressive symptoms such as (but not limited to) fatigue, skin changes, hyperviscosity, neuropathy and cold intolerance. In this series, 16 patients had the above symptoms correctly correlated as a result of identifying the cryoglobulin by screening. The other critical observation was the co-existence of two unrelated IgM related problems in a single patient – cryoglobulinaemia with either CAD/CAS or anti-MAG antibody positivity or BNS. This is an important observation and the presence of a co-existent cryoglobulin can help to delineate attributable symptoms as clinical symptoms which may not be fully explained by the primary condition and, more importantly, may have significant treatment implications. For example, both CAD and cryoglobulinaemia have frequent circulatory manifestations including acrocyanosis, Raynaud's and ulceration (Berentsen, Barcellini et al. 2020) and there is no

consensus on grading these symptoms, nor are these easily distinguishable. Importantly, therapeutic options vary according to the underlying pathology, with complement inhibition now approved for CAD (Röth, Barcellini et al. 2021). However, complement inhibitors do not improve circulatory symptoms (Röth, Barcellini et al. 2021, Röth, Berentsen et al. 2022) and may in fact worsen them, so the detection of a concurrent cryoglobulin is important. For example, in a critical situation of digital ischaemia, plasma exchange may be required- something that is not routinely employed in the CAD setting.

Both cryoglobulinaemia and CAD are considered to be monoclonal gammopathies of thrombotic significance due to intravascular occlusion from type I cryoglobulins, which is distinct mechanism from the intravascular haemolysis associated with thrombosis in CAD (Gkalea, Fotiou et al. 2023). No studies have formally defined the burden of venous thromboembolism in patients with cryoglobulinaemia and it is unclear what proportion of patients with CAD have cryoglobulins. 10% had thrombotic (venous and arterial) events in our cohort, which is lower than CAD alone, which is reported at 30% (Broome, Cunningham et al. 2020) although our cohort may have insufficient numbers to confirm this. Future work is required to assess the interaction of vascular occlusion and haemolysis in these disorders.

The decision to treat symptomatic patients with cryoglobulinaemia depends on the severity and progressiveness of the symptoms. A proportion of patients require treatment at an early stage (most <2 months from cryoglobulin detection in our cohort). It has been demonstrated that a minimal amount of measurable cryoglobulin may cause symptoms. In one study where two-thirds were symptomatic, 58% of patients with IgM type I cryoglobulinaemia demonstrated a cryocrit of <1%, which was a significantly greater proportion than in IgG cryoglobulinaemia (Zhang, Cao et al. 2020). Symptom burden is likely to be temperature-dependent, and not associated with cryocrit (Harel, Mohr et al. 2015). Independent predictors of treatment for cryoglobulinaemia alone or death in our cohort were hyperviscosity or cutaneous manifestations. These are previously unreported findings.

Clinical hyperviscosity is typically considered in patients with very high IgM levels in the context of WM. However we demonstrate that this may present in patients with MGUS and NHL as well. In vitro, cryoglobulinaemia may interfere with laboratory assays of M-protein and plasma viscosity which may be falsely elevated (Keuren, Raijmakers et al. 2010), so clinical correlation is essential. Patients with clinical symptoms of hyperviscosity were included in our series and this most strongly predicted need for cryoglobulin-directed treatment including plasma exchange followed by chemoimmunotherapy (HR 41.95 95% CI 9.78-179.9). Unlike other series reporting cardiac or pulmonary involvement in 4% of IgM cases (Zhang, Cao et al. 2020), we did not observe this pattern of organ involvement and postulate these symptoms may related to hyperviscosity. Plasma exchange was employed as a temporising measure in the majority (11/16; 69%) of all treated for cryoglobulinaemia. Complete clinical responses were observed in all patients with hyperviscosity after establishment of chemoimmunotherapy subsequent to plasma exchange. Deferral of rituximab may be needed to avert IgM flare. The optimal choice of chemoimmunotherapy in this setting is uncertain and in this series determined by the underlying clonal disorder. Deep responses have been recommended in the setting of MGCS (Pratt, El-Sharkawi et al. 2022), but acknowledged that complete clonal eradication is difficult to achieve in IgM associated disorders.

Cutaneous symptoms were more prevalent in patients with underlying MGUS rather than WM or NHL and may be due to co-existent CAD. Symptoms of peripheral neuropathy were common but no patients were treated for this indication alone and no cases were biopsy proven. The diagnosis of IgM associated neuropathies requires careful workup (D'Sa, Kersten et al. 2017) particularly as peripheral neuropathy has been reported in up to 8% of those aged >55 years (E. Beghi 1995); coincidental associations need exclusion. The diagnosis of cryoglobulinaemic neuropathy is protean and may necessitate a nerve biopsy.

Our data are retrospective and have inherent limitations but do uncover a clinical burden that may otherwise persist. We were unable to accurately identify all baseline characteristics including

molecular status (*MYD88*/*CXCR4*) due to early lack of availability of such tests and the absence of indication for MGUS and NHL in routine practice. We calculated the estimate of incidence amongst patients with IgM-related disorders based on the referrals to the specialist clinic. As tertiary referral centres we acknowledge the selection bias of patients with complications of IgM related disorders. Ultimately these patients do exist; careful clinical evaluation is feasible in all centres and encouraged so as to trigger detection in more cases and enable appropriate management. Correlation with large population-based studies in the rare disease and corroboration with registry data will be important to estimate true prevalence and incidence.

This is the largest reported series describing the characteristics of IgM-associated type I cryoglobulinaemia. The prevalence of type I IgM cryoglobulins is around a fifth of cases of which xx are symptomatic.. A lower threshold for seeking out cryoglobulins is advisable, especially if patients experience relevant symptoms. Treatment is advisable for symptomatic patients, especially in those with symptomatic hyperviscosity and aggressive cutaneous involvement in a short time from diagnosis.

Table 1. Baseline characteristics

Variable (% or range)	Total n=134	WM n=102	MGUS n=25	NHL n=7	p value
Age, median (range)	67 (35-90)	66 (39-90)	67 (35-89)	70 (49-81)	0.79
Gender					0.24
Male	78 (58)	62 (61)	11 (44)	5 (71)	
Female	56 (42)	40 (39)	14 (56)	2 (29)	
Light chain predominance					0.82
Kappa	111 (84)	83 (83)	22 (88)	6 (86)	
Lambda	21 (16)	17 (17)	3 (12)	1 (14)	
Molecular					
MYD88^{L265P}	62 (73)	59 (91)	3 (20)	0	<0.0001
MYD88 wild type	23 (27)	6 (9)	12 (80)	5 (100)	
CXCR4^{Mutated}	8 (24)	7 (24)	1 (33)	0	0.82
CXCR4 wild type	25 (76)	22 (76)	2 (67)	1 (100)	
M-protein, g/l	12 (0-63)	14 (0-63)	4 (0-20)	10 (0-58)	0.0001
Time to cryoglobulin detection, months	13 (-4-442)	17 (-4-442)	2 (-3-79)	7 (0-41)	0.031
Cryoglobulin detected prior to WM/MGUS/NHL diagnosis	9 (7)	6 (6)	3 (12)	0 (0)	0.42
Co-existent IgM disorder	42 (31)	22 (22)	18 (72)	2 (29)	<0.0001
CAD/CAS	20 (15)	8 (8)	10 (40)	2 (29)	0.0002
BNS	8 (6)	8 (8)	0	0	0.26
Anti-MAG antibody	7 (5)	3 (3)	4 (16)	0	0.026
Non-cryoglob. glomerulopathy	3 (2)	3 (3)	0	0	0.62
CANOMAD	3 (2)	1 (1)	2 (8)	0	0.11
Schnitzler	1 (1)	0	1 (4)	0	0.11
AL amyloidosis	1 (1)	0	1 (4)	0	0.10
Cryoglobulin symptoms	67 (50)	45 (44)	18 (72)	4 (57)	0.041
Cutaneous	21 (16)	10 (10)	8 (32)	3 (43)	0.0030
Ulcers	11 (8)	6 (6)	5 (20)	0	0.051
Purpura	12 (9)	5 (5)	5 (20)	3 (43)	0.007
Livedo reticularis/racemosa	2 (1)	2 (2)	0	0	0.73
Necrosis	5 (4)	2 (2)	3 (12)	0	0.060
Vasomotor	30 (22)	20 (20)	8 (32)	2 (29)	0.38
Acrocyanosis	13 (10)	7 (7)	4 (16)	2 (29)	0.086
Raynaud's	9 (7)	6 (6)	3 (12)	0	0.42
Cold symptoms*	20 (15)	12 (12)	6 (24)	2 (29)	0.18
Peripheral neuropathy	29 (22)	20 (20)	7 (28)	2 (29)	0.59
Sensory	19 (14)	13 (13)	4 (16)	2 (29)	0.49
Motor	1 (1)	1 (1)	0	0	0.85
Sensorimotor	9 (14)	6 (6)	3 (12)	0	0.42
Hyperviscosity	12 (9)	9 (9)	1 (4)	2 (29)	0.13
Arthralgia	10 (7)	6 (6)	3 (12)	1 (14)	0.45
Renal**	2 (1)	2 (2)	0	0	0.73
Cardiac or pulmonary	0	0	0	0	1

*includes cold urticaria and cold intolerance **biopsy proven cryoglobulinaemic glomerulopathy

Table 2. Predictors of cryoglobulinaemia-treatment free survival

	Univariable analysis		Multivariable analysis	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Age, per year	1.02 (0.99-1.05)	0.28	-	-
WM	1.33 (0.51-3.48)	0.57	-	-
MGUS	0.56 (0.17-1.86)	0.34	-	-
NHL	1.56 (0.36-6.76)	0.56	-	-
MYD88 mutated	0.95 (0.33-2.71)	0.93	-	-
MGCS	0.67 (0.30-1.54)	0.35	-	
Cryoglobulinaemia symptoms at cryoglobulin detection				
Any symptom	2.82 (1.25-6.40)	0.013	0.38 (0.08-1.78)	0.22
Cutaneous	3.47 (1.60-7.57)	0.002	2.95 (1.13-7.71)	0.028
Vasomotor	1.86 (0.84-4.14)	0.127	1.64 (0.61-4.43)	0.33
Neuropathy	1.57 (0.72-3.46)	0.26	2.90 (0.75-11.27)	0.12
Hyperviscosity	35.07 (13.03-94.38)	<0.0001	73.01 (15.62-341.36)	<0.0001
Arthralgia	0.82 (0.19-3.47)	0.78	0.95 (0.21-4.26)	0.94
Renal	12.17 (2.73-54.29)	0.001	0.14 (0.014-1.45)	0.22

Table 3. Indications and responses to first-line therapy for cryoglobulinaemia, n=16

Indication	n=16	Regimen	Best clinical response
Hyperviscosity	3 3 1 1	PEX + CIT (DRC, DR, Chlorambucil) CIT (BR, Vel/dex, R-cladrabine) PEX + Ibrutinib PEX	Complete Complete Complete Improved
Hyperviscosity + cutaneous/ vasomotor	2	PEX + FCR PEX + BR	Complete Complete
Cutaneous alone	2 1	PEX + DRC DRC	Improved Improved
Cutaneous + PN	1	DRC	Improved
Renal	2	PEX + DRC BR	Stable

CIT = chemoimmunotherapy

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