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N-Glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression

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Abstract

Our group previously demonstrated that M-protein light chain (LC) glycosylation can be detected on routine MASS-FIX testing. Glycosylation is increased in patients with immunoglobulin light chain amyloidosis (AL) and rarely changes over the course of a patient's lifetime. To determine the rates of progression to AL and other plasma cell disorders (PCDs), we used residual serum samples from the Olmsted monoclonal gammopathy of undetermined significance (MGUS) screening cohort. Four-hundred and fourteen patients with known MGUS were tested by MASS-FIX, and 25 (6%) were found to have glycosylated light chains (LCs). With a median follow-up of surviving patients of 22.2 years, the 20-year progression rates to a malignant PCD were 67% (95% CI 29%, 84%) and 13% (95% CI 9%, 18%) for patients with and without glycosylated LCs, respectively. The risk of progression was independent of Mayo MGUS risk score. The respective rates of progression to AL at 20-years were 21% (95% CI 0.0, 38%) and 3% (95% CI 0.6%, 5.5%). In summary, monoclonal LC glycosylation is a potent risk factor for progression to AL, myeloma, and other PCDs, an observation which could lead to earlier diagnoses and potentially reduced morbidity and mortality.

INTRODUCTION

Pathogenic glycosylation of proteins has been implicated in various hematological malignancies, often with prognostic associations.[1] It has been postulated that glycosylation also has a pathogenic effect on immunoglobulin light chains (LCs) and that glycosylated LCs could be more prone to be amyloidogenic.[2] We demonstrated previously that monoclonal LCs of patients with kappa restricted immunoglobulin light chain amyloidosis

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CONTRIBUTIONS: AD and DLM designed the study. AD and DRL analyzed, interpreted the data and edited the manuscript. BM performed the LC-MS experiments. Other authors contributed intellectual content and review of this manuscript.

CONFLICT OF INTEREST: David Murray and Surendra Dasari have intellectual property rights to the MASS-FIX assay and patents. The other authors have no conflicts related to this work.

(AL) are N-glycosylated at a rate nearly 13-fold higher than the kappa LCs of patients with monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), and assorted plasma cell disorders (PCDs)[3, 4] and that N-glycosylation of monoclonal LCs starts at the MGUS stage, predating the diagnosis of AL amyloidosis by years to decades.[5]

These observations prompted the hypothesis that the finding of an N-glycosylated monoclonal LC as part of a diagnosis of MGUS heightens the risk for progression to AL. This post-translational modification is easily recognized with routine use of MALDI-TOF mass spectrometry, our standard method for detecting monoclonal proteins at the Mayo Clinic.[6, 7] Since we had previously demonstrated that the patterns seen on routine MALDI-TOF, which we had presumed was N-glycosylation, were indeed N-glycosylation through both deglycosylation experiments[4] and cDNA sequencing demonstrating somatic mutations to N-glycosylation consensus sequences (manuscript in progress), for the purposes of this manuscript, the N-glycosylation patterns seen on routine MALDI-TOF were deemed sufficient to call N-glycosylation for the purpose of this study. Using the Olmsted MGUS cohort, a well characterized population of patients screened for MGUS as part of an epidemiologic study,[8] we set out to define the rate of progression of N-glycosylated MGUS to AL and other PCDs.

METHODS

The samples and participants were taken from the Olmsted County MGUS epidemiology project.[8] In brief, this was a study in which residents of Olmsted County over the age of 50 years of age as of 1995 were invited to participate. Samples were analyzed from 21,463 of 28,038 enumerated residents. In that study 3.2% were found to have MGUS by serum protein electrophoresis screening.[8] A follow-up study employing remaining baseline samples using the FreeLite assay to screen for monoclonal gammopathies revealed that the actual prevalence of MGUS including LC MGUS was 4.2%.[9] Residual samples within 2 years of diagnosis were available for testing for 414 of the positive patients with MGUS, and this comprised our study population. This study was approved by the Mayo Clinic Institutional Review Board in accordance with the Declaration of Helsinki.

Samples were run on MASS-FIX as follows.[6, 7] The immuno-enrichment was performed as previously described by adding 10 μL aliquot of serum to 50μL agarose beads coupled with one of the single domain antibodies specific for heavy chains (HC) of IgM, IgA, IgG and LC of κ or λ constant domains (Thermo Fischer Scientific), washed, reduced, spotted, and analyzed separately on MALDI-TOF-MS (Microflex LT, Bruker). The spectra from each five immuno-enrichment of each sample were overlaid, and LC m/z distribution was visually inspected for the presence of peaks in $[M+1H]^{1+}$ and $[M+2H]^{2+}$ using in house developed software and categorized patient's monoclonal LC as either N-glycosylated or not N-glycosylated with no knowledge of their follow-up status.

Demographics were taken from the time of the date of the MGUS diagnosis. P values for differences between continuous variables were calculated using two-sample t-tests and nominal variables using chi-square tests. Survival and progression rates were calculated according the Kaplan-Meier method. The cumulative incidence of progression accounting

for the competing risk of death was calculated using the method of Putter, et al.[10] The association of progression and glycosylation was evaluated using Cox proportional hazards regression. All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Of the 414 individuals included in this study, 25 patients (6%) had N-glycosylation of their MGUS light chains. Demographics and baseline characteristics are shown in Table 1. Patients without and with N-glycosylated monoclonal proteins were similar in terms of age, gender, hemoglobin, serum creatinine, M-spike, involved FLC, and involved to uninvolved FLC. The N-glycosylation group was more likely to have an abnormal FLC ratio and to have a lower urine total protein. Sixty-four percent (16/25) of the N-glycosylated group had a kappa restricted monoclonal gammopathy, which was no different from the group without N-glycosylation. Overall, there was no difference in MGUS risk[11] between the two groups.

As of July 31, 2019, there were 11 AL progressions across the groups, 50 other PCD progressions (Table 2), and 324 deaths. Overall, there were more progressions in the N-glycosylated group 48% (12/25) versus 10% (38/389). With a median follow-up of surviving patients of 21.9 years among patients diagnosed with MGUS, Kaplan-Meier estimates at 20 years for progression to a malignant PCD was 66.6% (95% CI 28.9%, 84.3%) for patients with N-glycosylation of their monoclonal LCs and 13.4% (95% CI 8.9%, 17.7%) for patients without N-glycosylation of their monoclonal LCs (Figure 1a). The respective cumulative incidence of progression to a malignant PCD at 20 years accounting for competing risk of death was 50.2% (95% CI 33.7, 74.8) and 9.4% (95% CI 6.9%, 12.8%) (Figure 1b). The relative risk of progression was 6.4 (95% CI 3.3, 12.4) on univariate analysis and 7.8 (95%CI 4.0, 15.3) on multivariable analysis including the Mayo MGUS risk score[11] (Table 3).

The incidence of AL at 20 years was 21.4% (95% CI 0.0, 38.2%) for patients with glycosylated monoclonal LCs and 3.1% (95% CI 0.6%, 5.5%) for patients without glycosylation (Figure 1c). The cumulative incidence of AL amyloidosis accounting for competing risk of death was 16.7% (95% CI 6.8%, 40.9%) and 1.9% (95% CI 0.9%, 3.9%), respectively (data not shown). Excluding AL progressions, estimates at 20 years for progression to a malignant PCD were 89.4% (95% CI 85.7%, 93.3%) for patients with N-glycosylation of their monoclonal LCs and 47.6% (95% CI 24.6%, 92.2%) for patients without N-glycosylation of their monoclonal LCs.

None of the patients with N-glycosylated LCs who progressed to PCDs other than amyloidosis was specifically tested for AL amyloidosis with Congo red staining of their tissues. It is notable that 2 of the patients with N-glycosylation who progressed to a lymphoproliferative disorder both had cold agglutinin disease, a finding that complements another interesting observation made by our group regarding increased rates of N-glycosylation among patients with cold-agglutinin disease.[12]

DISCUSSION

This study confirmed our hypothesis that patients with glycosylated LCs have a higher likelihood of developing AL over time with a hazard ratio of 10.1 (95% CI 2.9, 34.7), an observation that could be rationalized biochemically.[13–17] The finding that the risk of progression to other PCDs was also higher among patients with N-glycosylated LCs was unexpected. Could the higher progression rate among non-AL PCD patients be a function of latent AL causing symptoms bringing patients with myeloma or other PCDs to medical attention for AL symptoms, which were not recognized as such, but attributed to the other PCD? Alternatively, LC glycosylation might reflect the increase in inflammation seen with aging and in some autoimmune diseases.[18] Therefore, these patients could represent a biologically older group with higher levels of immune dysregulation.

These data support the recommendation that patients with apparent MGUS (or any other PCD) and N-glycosylation discovered on routine MASS-FIX be screened for AL with a minimum of an AL review of systems, a Congo red of the bone marrow and of the fat, an NT-proBNP, and a test for albuminuria.[19] Follow-up among these patients with MGUS (or smoldering PCDs) would be in line with that recommended for high-risk MGUS.

In summary, the recognition that glycosylated monoclonal light chains pose a risk for progression could lead to earlier diagnoses of AL and other PCDs, which could translate into reduced morbidity and mortality.

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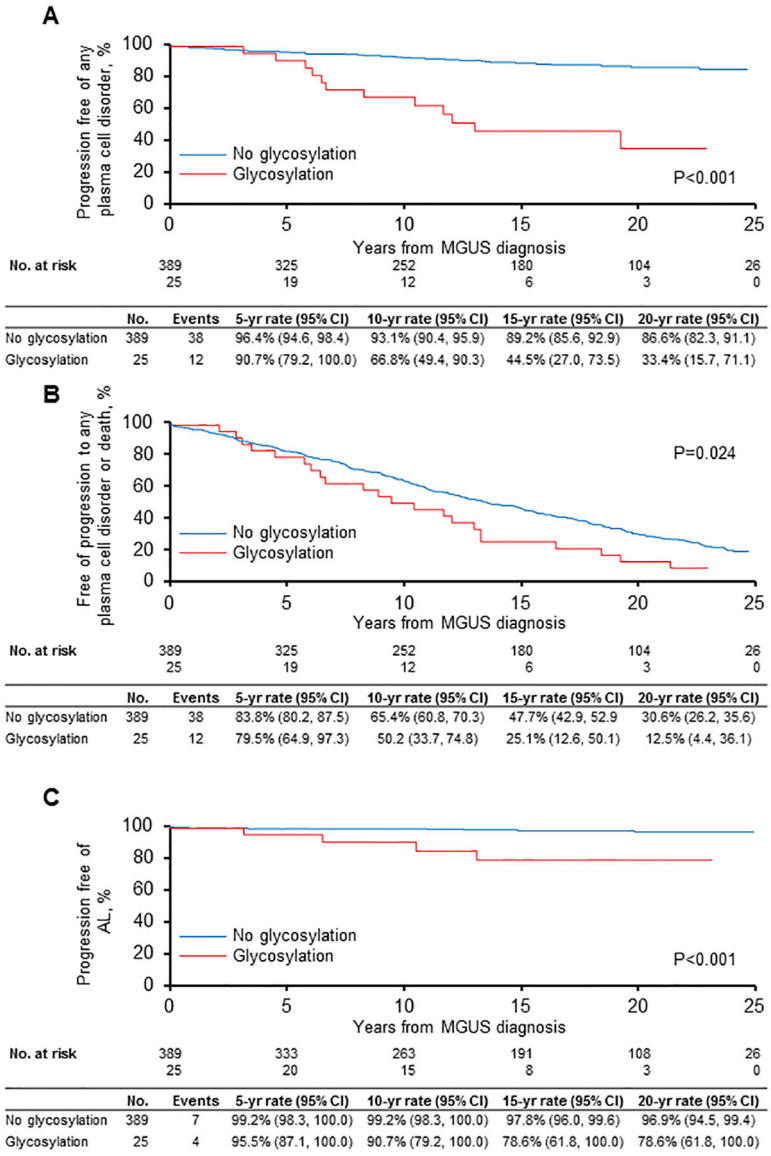

Figure 1.

Rates of progression based on N-glycosylation status of the monoclonal light chains

a. Progression to any plasma cell disorder or lymphoproliferative disorder

b. Progression to any plasma cell disorder lymphoproliferative disorder with competing risk of death

c. Progression to AL amyloidosis

Table 1.

Patient characteristics[a]

Characteristic	No N-glycosylation (n=389)	N-glycosylation (N=25)	P
Days sample from MGUS dx	319 (−636, 729)	272 (0, 723)	0.778
Male gender, n (%)	192 (49)	14 (56)	0.52
Age, years	69.0 (39.0, 98.0)	69.0 (54.0, 87.0)	0.885
Hemoglobin, g/dL	13.8 (8.1, 17.4)	12.8 (9.3, 15.8)	0.143
Creatinine, mg/dL	1.1 (0.5, 5.7)	1.1 (0.9, 1.7)	0.788
Serum M-protein, g/L	6 (0, 29)	5 (0, 27)	0.759
Serum M-protein >=15 g/L, n (%)	92 (24.1)	6 (26.1)	0.828
Heavy chain isotype Ig G / IgA or IgM, n (%)	278 (72) / 111 (28)	20 (80) / 5 (20)	0.357
Light chain kappa, n (%)	241 (62)	16 (64)	0.838
iFLC, mg/L[b]	19 (1.0, 1650)	28 (9.0, 911)	0.217
iFLC/uFLC (n=216)[b]	1.5 (0.0, 258)	2.3 (0.1, 98.5)	0.230
Abnormal FLC ratio, n (%)[b]	112 (30.1)	12 (52.2)	***0.027***
Urine protein g/24 hours (n=68)	0.10 (0, 1.9)	0.10 (0.0, 0.2)	***0.028***
MGUS risk[11] group			0.506
Low	155 (42.7)	6 (28.6)	
Low-intermediate	133 (36.6)	11 (52.4)	
Intermediate	60 (16.5)	3 (14.3)	
High	15 (4.1)	1 (4.8)	

[a]Unless otherwise indicated, represented in median and range

[b]FLC missing at diagnosis in 22 patients

Table 2.

PCD progression diagnoses

	Not N-glycosylated N=389	N-glycosylated N=25	HR (95% CI)	p-value
No progression, n (%)	351 (90)	13 (52)	NA	NA
Progression to PCD, n (%)[a]	38 (10)	12 (48)	6.4 (3.3, 12.4)	<0.001
AL amyloidosis, n (%)	7 (1.8)	4 (16)	10.1 (2.9, 34.7)	<0.001
Multiple myeloma, n (%)	25 (6.4)	5 (20)	3.8 (1.4, 9.9)	0.007
Waldenstrom macroglobulinemia and other LPDs, n (%)	5 (1.3)	1 (4.0)	4.3 (0.5, 38.6)	0.192
Other LPDs with a monoclonal IgM, n (%)	1 (0.3)[a]	2 (8.0)[b]	33.1 (3.0, 365.2)	0.004

LPD, lymphoproliferative disorder; PCD, plasma cell disorder

[a]SLL. Antecedent rheumatoid arthritis

[b]Both with cold agglutinin disease; one of two with unexplained progressive pulmonary hypertension with right-sided heart failure diagnosed 3 years prior to his death increased LV filling pressures increased RVSP, pulmonary hypertension, atrial fibrillation, ascites requiring paracentesis. Patient was ever tested for AL.

Table 3.

Univariate and multivariate for progression and death

					Univariate		Multivariable	
Outcome	**Risk factor**	**Level**	**N**	**Events**	**HR (95% CI)**	**p-value**	**(95% CI)**	**p-value**
Progression to any PCD	Glycosylation	No glycosylation	389	38	Reference		Reference	
		Glycosylation	25	12	6.4 (3.3, 12.4)	<0.001	7.8 (4.0, 15.3)	<0.001
	Mayo Risk Group[11]	0 factors	161	11	Reference		Reference	
		Any 1 factor	144	13	1.4 (0.6, 3.2)	0.398	1.2 (0.5, 2.7)	0.648
		Any 2 factors	63	13	3.7 (1.6, 8.2)	0.002	3.2 (1.4, 7.2)	0.005
		Any 3 factors	16	10	13.3 (5.0, 31.4)	<0.001	13.4 (5.6, 32.0)	<0.001
Progression or death	Glycosylation	No glycosylation	389	307	Reference		Reference	
		Glycosylation	25	22	1.6 (1.1, 2.5)	0.027	1.6 (0.99, 2.5)	0.051
	Mayo Risk Group[11]	0 factors	161	118	Reference		Reference	
		Any 1 factor	144	117	1.2 (0.9, 1.5)	0.224	1.2 (0.9, 1.5)	0.267
		Any 2 factors	63	56	1.4 (1.03, 2.0)	0.033	1.4 (1.02, 1.9)	0.041
		Any 3 factors	16	15	1.7 (0.98, 2.9)	0.060	1.7 (0.98, 2.9)	0.060

Mayo risk factors for progression from MGUS to myeloma and related conditions:[11] free light chain ratio abnormal, M-spike ≥ 1.5, heavy chain isotype not IgG