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TO THE EDITOR:

Sialic acid–bearing paraproteins are implicated in heparin-like coagulopathy in patients with myeloma

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Plasma cell dyscrasias, including multiple myeloma (MM), have been associated with acquired bleeding diathesis.[1,2] Rarely, this coagulopathy can be due to the presence of circulating heparin-like anticoagulant.[3-5] We present 2 patients with MM with hemorrhagic complications due to the presence of heparin-like anticoagulant that we attribute to increased negatively charged sialic acid (SA)-bearing monoclonal paraproteins.

Patient 1

A 73 year-old man with relapsed immunoglobulin G (IgG)λ MM on elotuzumab, lenalidomide, and dexamethasone presented with severe epistaxis leading to a hemoglobin drop to 5 g/dL, necessitating packed red blood cell transfusion and local measures to control epistaxis. Platelet count was 35 000 per microliter, requiring platelet transfusion. No other hemostatic agents were used. He had prolonged activated partial thromboplastin time (APTT) and prothrombin time (PT), as well as dilute Russell viper venom (dRVV) screening time; these did not correct on 1:1 mixing with pooled normal plasma (PNP), suggesting the presence of an inhibitor. The dRVV confirmatory test provided no evidence for lupus-like anticoagulant activity. Testing of individual coagulation factors for prolonged PT and APTT workup did not reveal any coagulation factor deficiencies. Notably, thrombin time (TT) was prolonged, with normal reptilase time (RT), a pattern attributed to heparin or direct thrombin inhibitor (DTI) effects; however, no source of exposure to exogenous heparin/DTI was identified. His hepatic transaminases, bilirubin, and alkaline phosphatase were within the normal reference range. Measured serum M-spike and λ free light chain (FLC) levels were at their highest since the initial MM diagnosis 5 years earlier (Figure 1A-B). For his aggressive disease, he had received multiple biochemotherapeutic regimens, including autologous stem cell transplant (ASCT) 11 months postdiagnosis. Interestingly, prior to the placement of a central venous catheter in preparation for ASCT, his screening APTT and PT were prolonged (66 seconds and 14.2 seconds, respectively), although they were not evaluated further.

The occurrence of the bleeding and coagulopathy at the time of MM relapse was at the peak of monoclonal paraprotein and FLC concentrations, leading us to hypothesize that the elevated monoclonal protein could be the cause of the heparin-like anticoagulant effects. To investigate further, we retrieved archived sera obtained from the patient at 4 time points, including at MM diagnosis and following the bleeding episode. Paraproteins were purified from the sera using camelid-derived single domain antibody coated beads (CaptureSelect; Thermo Fisher Scientific) specific for κ or λ light chain (LC) or for IgG, IgM, or IgA heavy chains.[6] Eluted reduced immunoglobulins were analyzed by matrix-assisted laser desorption/ionization time-of-flight

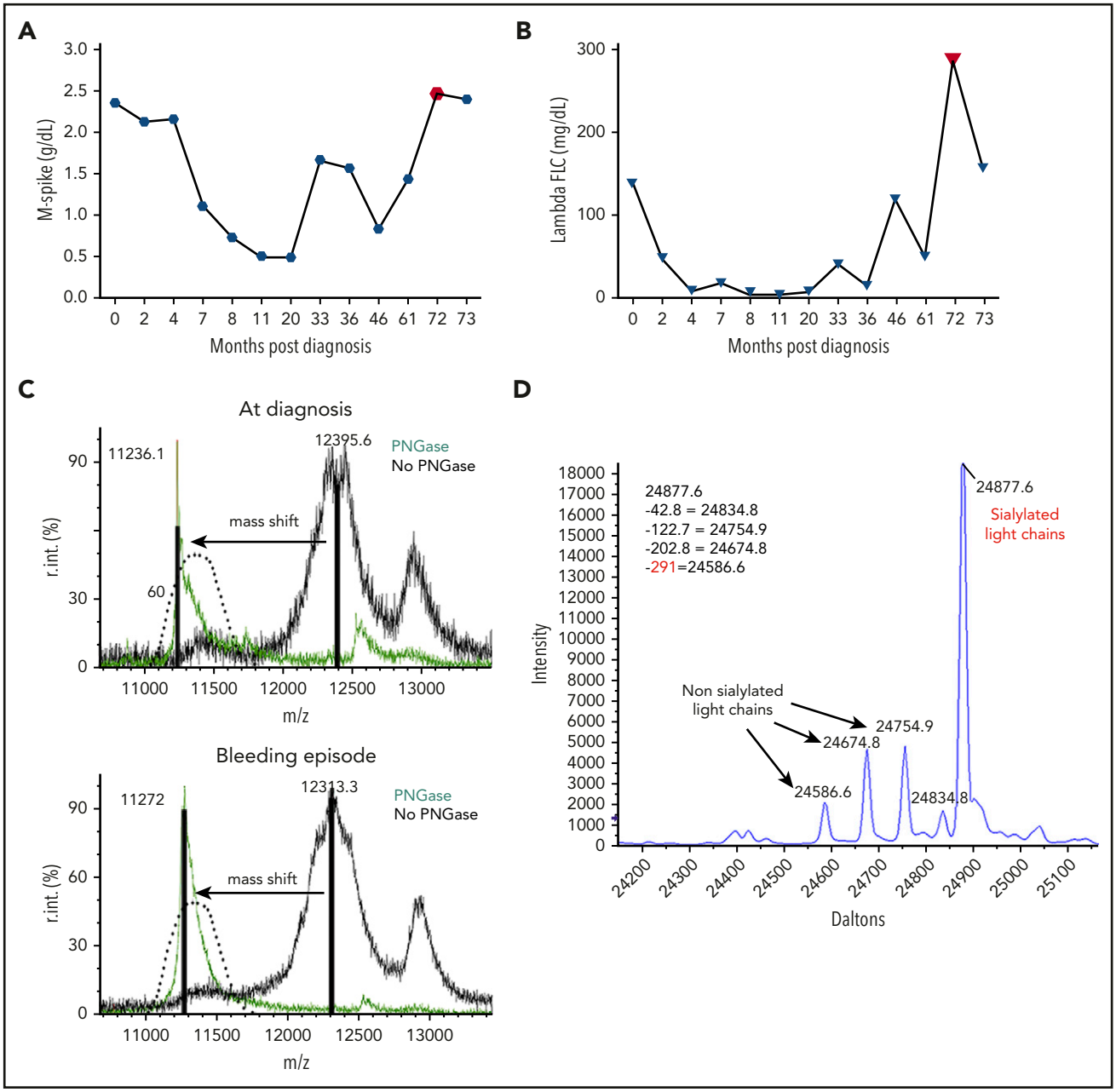
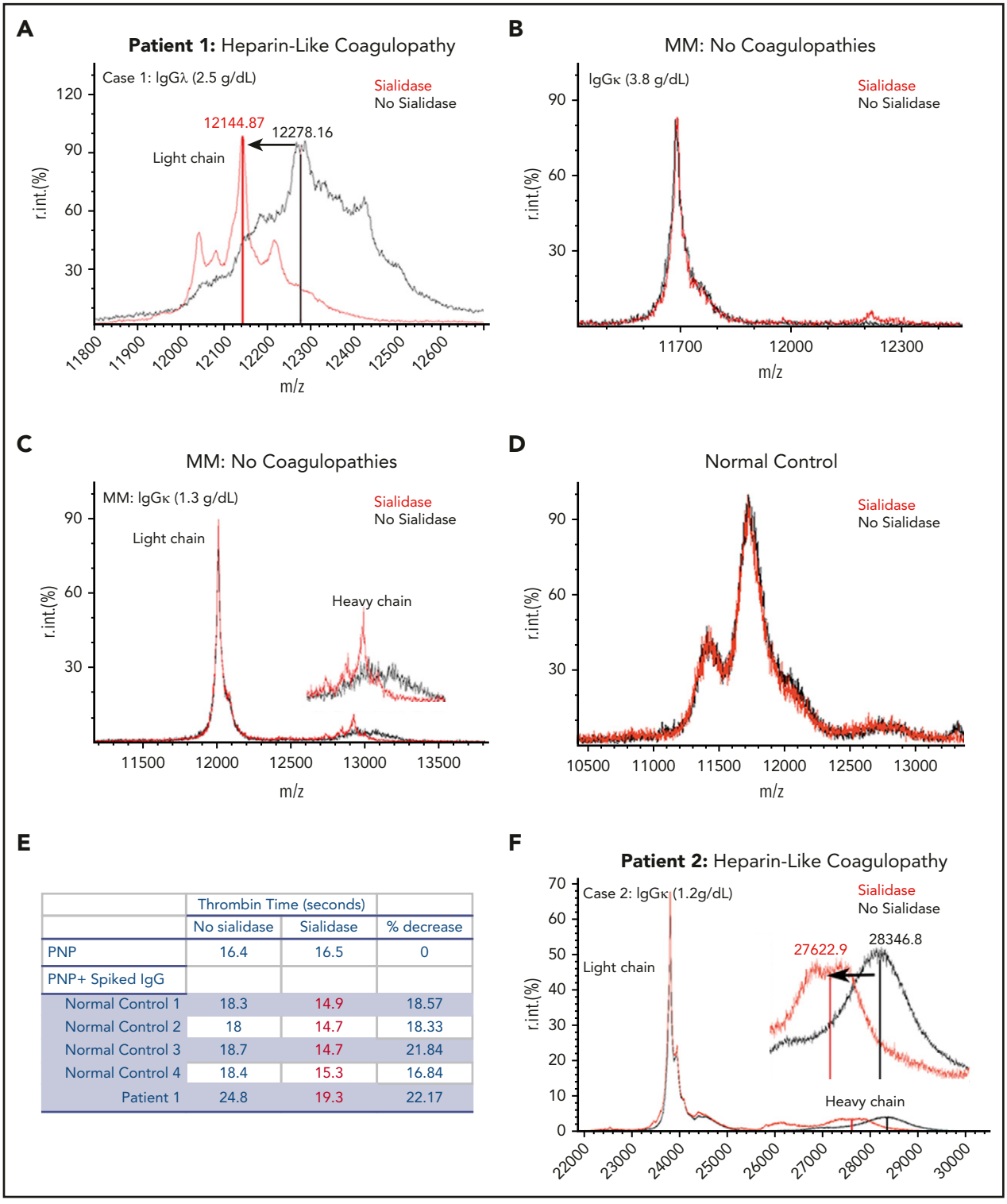


Figure 1. Characterization of monoclonal protein over the course of disease. Line graphs of measured serum M-spike (A) and λ FLC levels (B) in patient 1 at different testing time points. Diagnosis = 0 months; red point at 72 months is the bleeding time point. (C) Overlaid MALDI-TOF/MS LC $[M+2H]^{2+}$ spectra of immunopurified IgG of patient 1 at diagnosis (upper panel) and bleeding (lower panel) time points. λLCs (vertical black lines) are of a greater mass than predicted normal range (dotted lines). Arrows show mass shifts from untreated IgG (black graph) to PNGase F–treated IgG (green graph), indicating the cleavage of *N*-linked glycan structures by PNGase F. (D) Deconvoluted λLC mass spectra generated by high-resolution MS of immunopurified IgG. Arrows point to sialylated and nonsialylated forms; 291 Da corresponds to SA.

(MALDI-TOF) mass spectrometry (MS) (microflex LT; Bruker, MA). The resulting spectra were consistent with an IgGλ paraprotein, and all time points had congruent mass/charge (*m/z*) values, indicating persistence of the same plasma cells clone. Notably, the λLCs were of higher *m/z* than the expected typical mass range (Figure 1C). We immunopurified IgG from sera collected at diagnosis and bleeding time points and treated it overnight with PNGase F (New England Biolabs, MA) to remove *N*-linked glycans. PNGase F treatment reduced the λLC mass by 2000 Da, to 2100 Da, indicating the presence of *N*-linked glycans on the LC (Figure 1C). To further characterize these glycans, purified IgG was analyzed using liquid chromatography coupled to high-resolution electrospray ionization time-of-flight MS (TripleTOF 5600; Sciex).[7,8] Deconvoluted spectra revealed monoclonal LCs with δ masses of 291 Da, corresponding to SA (Figure 1D). Like heparin, the highly negatively charged SA containing LCs may bind positively charged motifs present on thrombin and antithrombin, mimicking heparin-like anticoagulant in vitro and potentially in vivo. This effect may be concentration dependent and might be most pronounced at high levels of monoclonal paraproteinemia.



	Thrombin Time (seconds)		
	No sialidase	Sialidase	% decrease
PNP	16.4	16.5	0
PNP+ Spiked IgG			
Normal Control 1	18.3	14.9	18.57
Normal Control 2	18	14.7	18.33
Normal Control 3	18.7	14.7	21.84
Normal Control 4	18.4	15.3	16.84
Patient 1	24.8	19.3	22.17

Figure 2. Properties of the monoclonal protein that altered the coagualtion profile. (A) Overlaid MALDI-TOF/MS LC $[M+2H]^{2+}$ spectra of sialidase-treated and untreated immunopurified IgG from patient 1. Arrow indicates mass shift of sialidase-treated IgG. Mass spectra similar to (A) of 2 patients with myeloma with no bleeding or coagulation abnormalities (B-C) and a normal healthy control (D). Note the absence of mass shifts in LC. (C) Smaller heavy chain mass shifts were noted with sialidase treatment (inset). (E) Table of clot-based TT measured in sialidase-treated or untreated PNP and PNP spiked with sialidase-treated or untreated immunopurified IgG from sera of patient 1 and 4 normal controls, at 1:10 ratio. The percentage decrease in TT with sialidase treatment is shown. (F) MALDI-TOF spectra of sialidase-treated or untreated immunopurified IgA from patient 2. Arrow shows a clear mass shifts in sialidase-treated IgA heavy chain (inset). No LC mass shift was noted with sialidase treatment.

To confirm the presence of SA residues on the monoclonal immunoglobulins, immuno-purified IgG was treated with sialidase enzyme (Roche Diagnostics) overnight and analyzed with MALDI-TOF/MS. λLC in the sialidase-treated samples demonstrated a mass shift, indicating the presence of SA (Figure 2A), whereas treatment of sera collected from 3 patients with MM without

coagulation abnormalities, as well as from 4 normal controls, did not display any LC mass shifts (Figure 2B-D). Of note, occasional smaller shifts were noted on the immunoglobulin heavy chain peaks (Figure 2C).

To test the effect of LC SA on the action of thrombin and TT, purified IgG from serum collected at the bleeding time point was split and were either untreated or treated untreated with sialidase, and then spiked at a 1:10 ratio into PNP (Precision BioLogic). TT was consistently shortened in all plasma samples spiked with sialidase-treated IgG but not in controls (Figure 2E). The addition of sialidase enzyme to PNP did not alter the TT (Figure 2E). Based on the above findings, we hypothesize that the increased LC sialylation of the patient's monoclonal paraprotein contributed to the TT prolongation in the setting of a normal RT and the heparin-like activity in vitro and in vivo.

Patient 2

A 75-year-old woman, with relapsed IgAκ MM on melphalan, prednisone, and thalidomide, developed hemoptysis and respiratory failure soon after hospitalization for acute-on-chronic renal failure secondary to cast nephropathy. Her serum IgA M-spike was 1.2 g/dL with κ FLC of 924 mg/dL. She had prolonged APTT and dRVV screen not corrected on 1:1 mixing with PNP, suggesting the presence of an inhibitor. The PT was within the normal reference range. Testing of individual coagulation factors for prolonged APTT workup did not reveal any coagulation factor deficiencies. The TT was prolonged, with normal RT, suggesting heparin-like anticoagulant effects; however, she had no heparin/DTI exposure. Heparin anti-Xa activity was at the lowest level of detection (0.05 IU/mL). She was treated with high-dose Solu-Medrol, plasmapheresis, and hemodialysis for cast nephropathy. Because platelet transfusion and protamine sulfate were not effective in controlling her hemoptysis, recombinant factor VIIa (NovoSeven) was administered, with temporary improvement. She expired on day 15 following hospitalization.

Her archived serum samples were retrieved for analysis. Similar analysis of immunopurified paraprotein by MALDI-TOF/MS showed LC mass shift with PNGase F treatment (data not shown), but not with sialidase treatment, indicating the absence of SA on the LC (Figure 2F). However, the heavy chains showed a clear mass shift following sialidase treatment. The 23-aa-long hinge region of the IgA1 heavy chain molecule typically harbors 3 to 6 variably sialylated *O*-linked glycan structures.[9] We hypothesize that the abundantly sialylated IgA paraproteins with disease progression led to an exaggerated net negative charge and heparin-like activity that may have contributed to bleeding manifestation.[10]

The negatively charged SA residues present in the glycan structures linked to the circulating monoclonal immunoglobulins may function similarly to heparin by binding to antithrombin and potentiating its activity; alternatively, they may have direct antithrombin-like activity by binding to and inhibiting thrombin directly, with no effect on RT.[11] This is supported by the finding that sialidase-treated monoclonal paraproteins did not prolong the TT, as was noted with the untreated paraprotein. Mártinez-Mártinez et al presented an IgGλ MM patient with prolonged TT and normal RT and demonstrated paraprotein affinity to antithrombin.[12] In most reported cases, positively charged protamine sulfate has been the mainstay treatment to manage acute bleeding episodes[1,13]; however, it was not effective in patient 2. Chemotherapy has also been reported as essential in resolving the coagulopathy.[3,14-16]

In summary, we suggest that the increased net negative charge of heavily sialylated paraproteins may be one of the mechanisms for its heparin-like anticoagulant effects in MM patients.

Authorship

Contribution: A.S. designed and performed experiments and wrote the manuscript; P.L., R.L., J.T., and M.K. performed experiments; and J.S., A.A., and D.M. designed experiments and provided supervision.

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Footnotes

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Data sharing requests should be sent to David Murray (murray.david@mayo.edu).

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