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# Serum fibrinogen is not elevated in patients with myasthenia gravis

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Myasthenia gravis (MG) is an autoimmune, autoantibody-mediated disease characterized by fatigable weakness of skeletal muscles. MG is a heterogeneous disease that currently lacks a robust biomarker for diagnosing all subtypes. Residual serum fibrinogen was found to be elevated 1000-fold in patients with MG in one study and posited to represent a universal diagnostic biomarker for MG. We set out to confirm elevated serum fibrinogen in patients with all subtypes of MG. We employed multiple methodologies to compare fibrinogen levels between MG patients and controls, using samples from independent cohorts. With enzyme-linked immunosorbent assay (ELISA), fibrinogen levels in sera from MG patients were not significantly different from controls. And in plasma samples, MG patients had a significantly lower amount of fibrinogen compared to controls. Using liquid chromatography-mass spectrometry (LC-MS), the abundance of serum fibrinogen- $\alpha$  was not elevated in patients compared to controls. Our results do not support serum fibrinogen to be a diagnostic biomarker for MG and underscore the need for replication of novel findings to ensure our common goal of identifying effective biomarkers for MG.

**Keywords** Myasthenia Gravis, Biomarker, Fibrinogen, Acetylcholine receptor antibody, Muscle-specific kinase antibody

Myasthenia gravis (MG) is an antibody-mediated autoimmune disease characterized by disrupted signal transmission at the neuromuscular junction (NMJ), leading to disabling skeletal muscle weakness<sup>1,2</sup>. The majority of MG patients have antibodies against the nicotinic acetylcholine receptors (AChR) at the NMJ, and the remaining patients have antibodies targeting other NMJ proteins, including muscle-specific tyrosine kinase (MuSK) and lipoprotein-related protein 4 (LRP4), or are seronegative for all these autoantibodies<sup>2,3</sup>. The diagnosis of MG is confirmed through typical clinical presentation, detection of serum autoantibodies, or electrophysiological studies<sup>4</sup>. However, a quarter of patients wait over a year from symptom onset to diagnosis, which results from several diagnostic challenges, including rarity of the disease, variable clinical manifestations, variable antibody detection methods, and occurrence of multiple disease serotypes<sup>5–7</sup>. Therefore, a robust biomarker for diagnosing all subtypes of MG is needed<sup>2,8</sup>.

Hussain et al. identified residual serum fibrinogen as a potential universal biomarker for MG through the use of mass spectrometry, immunoblotting, and enzyme-linked immunosorbent assay (ELISA) and found levels to be over a magnitude higher in MG patients compared to normal controls and subjects with rheumatoid arthritis (RA)<sup>9</sup>. Fibrinogen is a soluble protein that is typically assayed in plasma and plays an essential role in blood clot formation<sup>10,11</sup>. The fibrinogen protein found in circulation is made up of two sets of three polypeptide chains, named fibrinogen- $\alpha$ , fibrinogen- $\beta$ , and fibrinogen- $\gamma^{10,12}$ . Beyond its role in hemostatic clotting, fibrinogen has also been recognized as an immunoinflammatory signaling molecule with an ability to bind to a range of immune cells and affect cell functions<sup>11,12</sup>. Plasma fibrinogen levels are elevated in some diseases, mainly cardiovascular diseases, and fibrinogen has been implicated as a proinflammatory mediator in autoimmune diseases, including RA and multiple sclerosis<sup>12,13</sup>.

The underlying mechanism for the increased residual serum fibrinogen levels observed in MG patients is not clear given the present understanding of MG pathophysiology and activity of fibrinogen. This raises a question:

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Is fibrinogen a diagnostic biomarker for MG? In this study, we aimed to evaluate whether serum fibrinogen is elevated in patients with MG.

### Methods

#### Human subjects

Sera were accessed from multiple biobanks (Table 1). (1) The George Washington Laboratory for MG Research patient and control sera were used for ELISA and immunoblotting. (2) Additional patient sera for ELISA were provided from the Rare Disease Clinical Research Network dedicated to MG (MGNet). (3) The biospecimen bank of the MGTX clinical trial (NS42685) housed at George Washington University was used for liquid chromatography–mass spectrometry (LC-MS) analysis<sup>14</sup>. Plasma for fibrinogen content were derived from the Duke University biospecimen bank.

For all studies, MG subjects were required to have a clinical diagnosis of MG and confirmed by either elevated autoantibody levels or neurophysiological testing. Control subject participation required an absence of autoimmune disease and no treatment with an immunotherapy within 12 months or a vaccination within 1 month of blood draw. All subjects were older than 18 years and had provided informed consent. All subject blood collection centers received George Washington University and local institutional review boards' or ethics committees' approvals, and all methods were performed in accordance with relevant guidelines and regulations.

### Blood sample processing

Blood samples for the MGTX study were collected in serum separator vacutainers with no serum separator gel, packed immediately in a cushioned container with an external cold pack, and shipped by overnight delivery to

	ELISA and immunoblotting, serum	LC-MS	ELISA, plasma
MG patients	N=51	N=86	N=20
Age, years, mean ± SD	58.4±18.9	$35.9 \pm 13.0$	$54.4 \pm 20.1$
Age, years, range	19-83	18-63	20-87
Female, n (%)	26 (51%)	62 (72%)	12 (60%)
EOMG, n (%)	19 (37%)	n/a	9 (45%)
LOMG, n (%)	32 (63%)	n/a	11 (55%)
MG Serotype, n (%)			
Anti-AChR antibody positive	30 (59%)	86 (100%)	18 (90%)
Anti-MuSK antibody positive	10 (20%)	-	-
Seronegative	11 (21%)	-	2 (10%)
MGFA Classification, n (%)			
Remission	3 (6%)	-	7 (35%)
Ι	19 (37%)	-	2 (10%)
II	21 (41%)	58 (67%)	1 (5%)
III	6 (12%)	26 (30%)	6 (30%)
IV	1 (2%)	2 (2%)	4 (20%)
Unknown	1 (2%)	-	-
Treatments, n			
Prednisone	37	66	1
Rituximab	9	-	-
Azathioprine	11	-	-
Tacrolimus	2	-	-
Mycophenolate	12	-	-
Pyridostigmine	31	-	8
IVIg	21	-	-
Cyclosporine	-	-	-
None	5	20	12
Controls	N=14	N=37	N=10
Age, years, mean ± SD	46.8±16.4	$43.5 \pm 13.5$	$52.2 \pm 15.6$
Age, years, range	23-71	23-73	29-74
Female, n (%)	10 (71%)	26 (70%)	4 (40%)

**Table 1**. Cohort clinical and demographic characteristics. AChR, acetylcholine receptor; ELISA, enzymelinked immunosorbent assay; EOMG, early onset myasthenia gravis; IVIg, intravenous immunoglobulin; LC-MS, liquid chromatography-mass spectrometry; LOMG, late onset myasthenia gravis; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK, muscle-specific tyrosine kinase; SD, standard deviation the National Cancer Center in Frederick, Maryland. Upon arrival, specimens were immediately processed to sera and frozen at -80 °C until thawed for the first time for mass spectrometry.

At George Washington University and MGNet centers, blood was collected in serum separator vacutainers with separator gel. The tube was inverted gently six times and then allowed to clot for at least 30 min but no more than two hours. Serum was then isolated by centrifugation at room temperature and 1200 g for 10 min. Serum was aliquoted into cryovials and stored at -80 °C. George Washington University serum samples were thawed on ice and used in experiments within 30 min of thawing for ELISA or immunoblotting. MGNet serum samples were shipped with cold packs, and stored at 4 °C until used in ELISA.

Plasma collection at Duke University entailed blood collection in EDTA tubes and the tubes gently inverted several times to ensure proper mixing of the blood, followed by centrifugation at 4 °C and 2500 g for 15 min. Processing occurred within three hours of blood draw. The plasma was transferred into a 15mL conical tube. A second centrifugation at 4 °C and 2500 g for 15 min was conducted to further clarify the plasma. Approximately 1 mL of plasma was aliquoted into cryovials and then stored at -80 °C until analysis.

#### **ELISA**

A human fibrinogen ELISA kit (ab241383, Abcam) was used to quantify fibrinogen in serum and plasma samples collected from subjects. All ELISAs were performed in accordance with the manufacturer's instructions, and using all antibodies and reagents provided in the kit. Sera and plasma ELISAs were performed as separate experiments. For both MG and control subjects, sera were diluted 1:2,000 and plasma were diluted  $1:1 \times 10^6$  to fit within the kit's detection range. A standard curve was constructed using a series of recombinant fibrinogen standards. Standards and samples were run in duplicate. Absorbance at 450 nm was measured and the average absorbances of the sample duplicates, after subtracting the blank control value, were used to interpolate fibrinogen concentrations from the standard curve.

### **SDS-PAGE and Western blot**

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot were performed to evaluate fibrinogen- $\alpha$  in serum. One  $\mu$ L of each serum sample was mixed with 4  $\mu$ L phosphate-buffered saline (PBS) and 5  $\mu$ L loading buffer (2x Laemmli buffer + 100 mM of  $\beta$ -mercaptoethanol + 4% SDS). The plasma sample was first diluted 1:500 in PBS, then 1 µL of the dilution was mixed with 4 µL PBS and 5 µL loading buffer. Sample mixtures were heated at 95 °C for 5 min, before cooling to room temperature. Ten µL of sample mixtures were loaded into gel wells, and a protein ladder (#1610373, Bio-Rad) was loaded into one well. Samples were resolved on 4-15% tris-glycine gels before transfer to a 0.45 µm pore size polyvinylidene difluoride transfer membrane (IPVH09120, Millipore). Ponceau S stain of the membrane was used to visualize protein loading and transfer (Supplemental Figure S1). The membrane was washed (immersed in PBS+0.05% Tween20 for 10 min with shaking, repeated 3 times), then incubated in blocking buffer (PBS + 5% milk) overnight at 4 °C with shaking. The membrane was washed, then incubated for 2 h with shaking in anti-fibrinogen- $\alpha$  primary antibody (SC-166968, Santa Cruz Biotechnology) diluted 1:200 in blocking buffer per manufacturer's instruction. The membrane was then washed before incubation for 1 h with shaking in anti-mouse IgG HRP conjugate secondary antibody (#40120, Alpha Diagnostics Intl.) diluted 1:2,000 in blocking buffer per manufacturer's instruction. The membrane was washed, then incubated for 10 min with enhanced chemiluminescence (ECL) substrate solution (#1705060S, Bio-Rad). Results were visualized within 20 min with a GeneGnome imaging system (Syngene).

#### Serum proteomics by LC-MS

The sera abundance of each fibrinogen subunit, fibrinogen- $\alpha$ , fibrinogen- $\beta$ , and fibrinogen- $\gamma$ , was determined via LC-MS performed at Nelson Scientific Labs LLC, using methods previously described<sup>15</sup>. In short, sera protein extraction was performed and the resulting samples were transferred to MS vials for UPLC-ESI-Q-TOF-MS analysis. Five  $\mu$ L of each sample was pooled as a pooled quality control (Pooled QC) and 20  $\mu$ L of each sample was used for individual sample preparation. Samples were prepared [enriched, digested, purified] using ENRICH-iST Kit (PreOmics<sup>\*</sup>). Each sample was injected into a PepSep<sup>tom</sup> (25 cm x 75  $\mu$ m, 1.5  $\mu$ m, Bruker Scientific, USA) HPLC column and passed through a PepMap<sup>tom</sup> cartridge trap column (Thermo Scientific<sup>tom</sup>) upon entry to the mass spectrometer. Mass spectrometry was conducted using a nanoElute<sup>\*</sup> 2 instrument (Bruker Scientific). Pooled QC peptides were used to get peptide fractions using Pierce<sup>tom</sup> High pH Reversed-Phase Peptide Fractionation Kit for creating the DDA spectral library. The DDA library was used to quantify sample proteins in DIA mode in FragPipe (v21.1 Nesvilab). Protein quantification of each sample was expressed as abundance values relative to the respective protein's pooled QC value. The resulting label free quantitation (LFQ) data was log-transformed using the Perseus-type imputation method in FragPipe-Analyst.

### **Statistical analysis**

Prior to the initiation of the study, we performed two power analyses, keeping everything the same except between group variance. We used squared Cohen's d as a surrogate for group variance. 0.8 is considered a large effect, so the first iteration is  $0.8^2 = 0.64$ , and this leads to an estimate of 13 per group (MG vs. Controls). We then used Cohen's d = 1, which is a larger effect and estimated 9 for each group. Given the 1000-fold difference in fibrinogen levels identified by Hussain et al., we could be confident with our sample numbers to identify a difference.

To test for differences between control and MG groups, we fitted linear models to data representing each of the five outcome variables: fibrinogen in serum, fibrinogen in plasma, fibrinogen- $\alpha$ , fibrinogen- $\beta$ , and fibrinogen- $\delta$ . The same methodology was applied to test for differences in serum fibrinogen between sera collected from George Washington University and MGNet centers. Full logistic model parameter estimates can be found in

Supplemental Tables S1 and S2. We log-transformed each variable and fitted models to the log-transformed data. We reported and visualized parameter estimates on the original, back-transformed response scale. We fitted all models as functions of group, which we categorized as controls and MG. We performed two-tailed hypothesis tests for the group coefficient in each model using 5% alpha. Residual diagnostic plots confirmed that log-transformations resulted in homoscedastic error. We fitted and visualized models using the R statistical computing environment (R Core Team, 2021). Data and code used to produce this analysis are available in the GitHub project repository, https://github.com/seanmlee/MG\_fibrinogen.

### Results

### Study cohort

The characteristics of MG and control subjects are summarized in Table 1. Subject race and additional MG clinical information can be found in Supplemental Table S3. MG cohorts from The George Washington Laboratory for MG Research, MGNet, and the Duke University biospecimen bank included patients on a variety of medications. The biospecimen bank of the MGTX clinical trial cohort were either on prednisone or no medication<sup>14</sup>. MGTX patients' age onset was not collected, however all patients enrolled were within three years of diagnosis.

### Residual serum fibrinogen amounts by ELISA and immunoblotting are not different between MG patients and controls

Serum fibrinogen in MG patients was not different compared to controls (Incidence Rate Ratio (IRR) = 1.17 (95% CI: 0.81-1.70); p=0.389) (Fig. 1). The MG group had a slightly higher mean serum fibrinogen level; however, the difference was not significant and all but two of the MG datapoints are within the range of variation of the control group. Serum fibrinogen amounts determined via ELISA in this study are comparable to the extremely low levels of fibrinogen expected in serum, due to the removal of clotting factors during the serum collection process<sup>16</sup>. The one MG serum sample outlier (fibrinogen concentration = 9569.42ng/mL) was from the MGNet cohort, seronegative, IIIa MGFA classification, and on immunosuppressive therapy. Due to the differences in handling the George Washington University and MGNet serum samples, we analyzed the fibrinogen amounts obtained between the two groups. Serum fibrinogen amounts did not differ between sera collected from George Washington University and MGNet centers (Incidence Rate Ratio (IRR) = 0.79 (95% CI: 0.50-1.24); p=0.297).

We selected four MG patients and four controls used in the ELISA for further analysis by SDS-PAGE and Western blot to detect fibrinogen- $\alpha$  in sera. We exploited the sensitivity of ECL for detection given the expected amount of fibrinogen separated in the serum to be ~ 1ng. One MG plasma sample was randomly chosen for inclusion in the immunoblotting as a positive control, and was diluted to contain comparable fibrinogen levels to the serum samples. The resulting blot revealed that serum fibrinogen- $\alpha$  was similarly detectable in both the MG and control serum samples (Fig. 2). Fibrinogen products are consistent with Hussain, et al. Fibrinogen in the plasma was at a smaller protein size than in sera, potentially due to the lower total protein loaded affecting migration.

### Serum fibrinogen- $\alpha$ was not different between MG patients and controls, but fibrinogen- $\beta$ and fibrinogen- $\gamma$ levels were lower in MG patients than controls

By using LC-MS, we determined the protein fragments of fibrinogen in MG and control serum samples. Abundance of fibrinogen- $\alpha$  was not significantly different between MG patients and controls (IRR=0.99 (95% CI: 0.98–1.01); p=0.349), although the abundance was lower in MG patients compared to controls. The MG group had significantly lower protein abundance compared to the control group for fibrinogen-ß (IRR=0.95 (95% CI: 0.92–0.98); p=0.002) and fibrinogen- $\gamma$  (IRR=0.96 (95% CI: 0.93–0.99); p=0.003) (Fig. 3).

### Plasma fibrinogen amounts by ELISA are lower in MG patients compared to controls

MG patients had significantly lower amounts of plasma fibrinogen compared to the controls (IRR=0.66 (95% CI: 0.46–0.96); p=0.032) (Fig. 4). Average plasma fibrinogen amounts for both groups determined via ELISA in this study are within the expected range for normal physiological conditions, which is around 2,000–4,000 µg/ mL, with both groups demonstrating outliers<sup>12</sup>.

### Discussion

We found that fibrinogen levels in sera and plasma from MG patients were not elevated compared to controls using multiple sample sources and methodologies. Using ELISA, there was not a significant difference in serum fibrinogen levels between groups. Both MG and control sera exhibited detectable amounts of fibrinogen- $\alpha$  shown by SDS-PAGE and Western blot with ECL detection. With LC-MS, there was no difference between MG and control groups in abundance of serum fibrinogen- $\alpha$ , and MG patients had a significantly lower abundance of serum fibrinogen- $\beta$  and fibrinogen- $\gamma$  compared to controls. MG patients had a significantly lower amount of plasma fibrinogen compared to controls as determined by ELISA.

Hussain et al. utilized serum samples from the Canadian BioSample Repository (CBSR), composed of MG patients, RA patients, and normal controls. We expect that serum was collected by methods that were standard and in common with the biobanks utilized in our study. The group used serum separator tubes without resin, which was the case in the MGTX study samples, while the other biorepositories used tubes with resin. The MG group included subjects across the adult age spectrum, disease severity, and serotypes with the majority being AChR-antibody positive. In a shotgun proteomic analysis of 18 serum samples (6 MG, 6 RA, 6 control) and a validation proteomic analysis (31 MG, 18 RA, 30 control), fibrinogen- $\alpha$ , fibrinogen- $\beta$ , and fibrinogen- $\gamma$  were found to be higher in abundance by over an order of magnitude in MG patients compared to RA patients and controls. SDS-PAGE and Western blot for fibrinogen- $\alpha$  demonstrated the presence of fibrinogen- $\alpha$  in MG sera



**Fig. 1**. Fibrinogen in serum determined via ELISA. Estimated mean fibrinogen in serum (larger circle and square) from linear model, as a function of group (control and MG). Smaller circles and squares represent data points. Mean serum fibrinogen (ng/mL) [95% CI]: Control=886.64 [643.40, 1,221.82], MG=1,037.37 [879.83, 1,231.20].



Fig. 2. Detection of fibrinogen- $\alpha$  in serum via SDS-PAGE and Western blot. Cropped image of membrane displays the molecular mass range of fibrinogen- $\alpha$ . Original blot is presented in Supplemental Figure S2. MG and control sera both exhibited detectable amounts of fibrinogen- $\alpha$ .



**Fig. 3**. Serum abundance of fibrinogen peptides determined via LC-MS. Estimated mean fibrinogen protein relative abundance (larger circle and square) from linear models, as a function of group (control and MG). Mean serum fibrinogen- $\alpha$ , fibrinogen- $\beta$ , and fibrinogen- $\delta$  (relative abundance) [95% CI], respectively: Control = 20.01 [19.82, 20.23], 13.70 [13.36, 14.05], 14.20 [13.89, 14.52]; MG = 19.81 [19.77, 20.04], 13.02 [12.84, 13.27], 13.63 [13.43, 13.83].

but was undetectable in control samples. Similarly, ELISA of fibrinogen resulted in amounts of sera fibrinogen, represented as unitless values relative to the MG group, to be *over a magnitude* greater in the MG group compared to the control group. We used specimens from over 157 patients, the majority with AChR antibodies, but also 10 MuSK-antibody positive and 12 seronegative patients.

A mechanism that could connect elevated serum fibrinogen to MG pathogenesis is unclear. MG is a chronic autoimmune disease in which autoantibodies target proteins at the NMJ, disrupting signal transmission across the NMJ and inducing skeletal muscle weakness<sup>1,17</sup>. Fibrinogen is a soluble glycoprotein that is produced in the liver and circulates in the blood<sup>10</sup>. Following activation of the coagulation cascade, fibrinogen is converted to fibrin to form blood clots<sup>10,18</sup>. In blood, fibrinogen levels are assessed in plasma because serum isolation induces clot formation which then leads to removal of the vast majority of fibrinogen<sup>16</sup>. Fibrinogen, aside from its function in hemostasis, has also demonstrated an ability to act as an inflammatory regulator through binding with immune cells<sup>11,12</sup>. These binding interactions have shown to induce a variety of effects on immune cells, including activating inflammatory pathways, which has been linked to autoimmune diseases like RA and multiple sclerosis<sup>12,13</sup>.

We acknowledge the discrepancy between our study and that of Hussain and colleagues. Our study was initiated to validate the findings in Hussain, et al. using the methods described with the expectation that a scientific publication provides sufficient methodological detail to allow replication. We acknowledge that Hussain et al. did not use a serum resin separator, but this was also the case for the MGTX study samples. All our samples were collected in a manner to allow clotting to occur, as expected of serum samples. Although the Hussain et al. article states the collection of blood in "vacutainer red top no additive tubes (Becton Dickenson)", no other description of treatment of serum samples used in the study were provided. We and Hussain et al. used identical ELISA kits and no description of deviation from manufacturer's protocol. Hussain et al. performed one experiment indicating that incubation of their serum at room temperature for two hours led to loss of detection of fibrinogen in MG patients, but the sera used in our study were kept at 4 °C after thawing. Therefore, we doubt that our methods would have led to loss of fibrinogen.

Our results do not support serum fibrinogen to be a diagnostic biomarker for MG. Our work stresses the need for replication and validation of novel findings to ensure our common goal of identifying effective biomarkers for MG.



**Fig. 4**. Fibrinogen in plasma determined via ELISA. Estimated mean fibrinogen in plasma (larger circle and square) from linear model, as a function of group (control and MG). Mean plasma fibrinogen ( $\mu$ g/mL) [95% CI]: Control = 3,826.33 [2,861.53, 5,116.44], MG = 2,525.38 [2,069.01, 3,120.44].

### Data availability

The datasets generated and analyzed during the current study are available in the GitHub project repository, https://github.com/seanmlee/MG\_fibrinogen. Proteomics data analyzed during the current study are also availa ble in the Panorama repository, https://panoramaweb.org/GWU/project-begin.view.

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#### References

- Kaminski, H. J., Sikorski, P., Coronel, S. I. & Kusner, L. L. Myasthenia gravis: the future is here. J. Clin. Invest. 134, e179742. https: //doi.org/10.1172/JCI179742 (2024).
- Dresser, L., Wlodarski, R., Rezania, K. & Soliven, B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. J. Clin. Med. 10, 2235. https://doi.org/10.3390/jcm10112235 (2021).
- Lazaridis, K., Tzartos, S. J. Myasthenia gravis: autoantibody specificities and their role in MG management. Front. Neurol. 11, 596981. https://doi.org/10.3389/fneur.2020.596981 (2020).
- Andrapalliyal, N., Claytor, B. & Li, Y. Incidence and causes of overdiagnosis of myasthenia gravis. *Muscle Nerve.* 67, 464–468. https://doi.org/10.1002/mus.27774 (2023).
- Cortés-Vicente, E. et al. The impact of diagnosis delay on European patients with generalised myasthenia gravis. Ann. Clin. Transl Neurol. 11, 2254–2267. https://doi.org/10.1002/acn3.52122 (2024).
- 6. Kaminski, H. J. Chapter 390 in Goldman-Cecil Medicine (eds Goldman, L. & Cooney, K. A.) 2588–2593 (Elsevier, (2024).
- 7. Kwon, Y. N. et al. Clinical pitfalls and serological diagnostics of MuSK myasthenia gravis. *J. Neurol.* **270**, 1478–1486. https://doi.or g/10.1007/s00415-022-11458-4 (2023).
- Benatar, M., Cutter, G. & Kaminski, H. J. The best and worst of times in therapy development for myasthenia gravis. *Muscle Nerve*. 67, 12–16. https://doi.org/10.1002/mus.27742 (2023).
- 9. Hussain, F. S. et al. Residual serum fibrinogen as a universal biomarker for all serotypes of myasthenia gravis. *Sci. Rep.* **13**, 21229. https://doi.org/10.1038/s41598-023-47559-x (2023).
- 10. Weisel, J. W. Fibrinogen and fibrin. Adv. Protein Chem. 70, 247-299 (2005). doi: 0.1016/S0065-3233(05)70008-5.
- Luyendyk, J. P., Schoenecker, J. G. & Flick, M. J. The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood* 133, 511–520. https://doi.org/10.1182/blood-2018-07-818211 (2019).
- Davalos, D. & Akassoglou, K. Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol. 34, 43–62. https://d oi.org/10.1007/s00281-011-0290-8 (2012).
- Arbustini, E., Narula, N. & D'Armini, A. M. Fibrinogen: a circulating factor in search of its genetic architecture. *Circulation* 128, 1276–1280. https://doi.org/10.1161/CIRCULATIONAHA.113.005125 (2013).

- Wolfe, G. I. et al. Randomized trial of thymectomy in myasthenia gravis. N Engl. J. Med. 375, 511–522. https://doi.org/10.1056/N EJMoa1602489 (2016).
- Cheema, A. K. et al. Radiotherapy induces innate immune responses in patients treated for prostate cancers. *Clin. Cancer Res.* 29, 921–929. https://doi.org/10.1158/1078-0432.CCR-22-2340 (2023).
- Tuck, M. K. et al. Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group. J. Proteome Res. 8, 113–117. https://doi.org/10.1021/pr80054 5q (2009).
- 17. Gilhus, N. E. et al. Myasthenia gravis. Nat. Rev. Dis. Primers. 5, 30. https://doi.org/10.1038/s41572-019-0079-y (2019).
- Lippi, G., Favaloro, E. J., Franchini, M. & Guidi, G. C. Milestones and perspectives in coagulation and hemostasis. Semin Thromb. Hemost. 35, 9–22. https://doi.org/10.1055/s-0029-1214144 (2009).

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### Author contributions

Study conceptualization: T.A.B., H.J.K., L.L.K. Methodology: T.A.B., S.M.L., V.C.J, Y.L., K.G., J.C., H.J.K., L.L.K. Investigation/data acquisition: T.A.B., Y.L., J.C., K.G. Data analyses: T.A.B., S.M.L., K.G. Writing-original draft: T.A.B., S.M.L. Writing-editing and review: T.A.B., S.M.L., V.C.J, Y.L., K.G., J.C., H.J.K., L.L.K.

### Declarations

### Competing interests

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### Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/1 0.1038/s41598-025-97599-8.

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