Association of Immune Marker Changes With Progression of Monoclonal Gammopathy of Undetermined Significance to Multiple Myeloma

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Risk of progression from monoclonal gammopathy to multiple myeloma is not constant: a prospective study with serially collected samples

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KEY POINTS

Question. Do changes in serum immune markers over time predict progression from monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma?

Findings. Individuals with low-risk/intermediate-risk MGUS can convert to high-risk MGUS and progress to multiple myeloma within 5 years; the same result was found in light-chain MGUS. Evolving monoclonal proteins, serum free light chains, and immunosuppression were associated with progression.

Meaning. Our finding that clinical risk categories can change over time prior to multiple myeloma diagnosis supports annual blood testing and risk assessment for all individuals with MGUS or light-chain MGUS.

TWEET

Prospective study shows risk of progression from monoclonal gammopathy to myeloma is not constant: supports annual blood testing & risk re-assessment

ABSTRACT

Importance. Multiple myeloma (MM) is consistently preceded by monoclonal gammopathy of undetermined significance (MGUS). Risk models that estimate the risk of progression from MGUS to MM use data from a single time point, usually initial work-up.

Objective. To longitudinally investigate dynamics of serum immune markers over time in stable versus progressive MGUS.

Design. This prospective study followed participants in the screening arm of the National Cancer Institute Prostate, Lung, Colorectal and Ovarian (NCI-PLCO) Cancer Screening Trial (N = 77,469) who had a diagnosis of progressing MGUS (n = 187) or stable MGUS (n = 498), including light-chain subtype. For each participant we obtained all available serially stored pre-diagnostic serum samples ($N_{samples}$ = 3266). We conducted cross-sectional and longitudinal analyses of serum markers in relation to progression.

Setting. The NCI-PLCO Cancer Screening Trial is a large population-based randomized trial investigating how screening affects cancer-related mortality and secondary endpoints.

Participants. Adults aged 55 to 74 randomized from 1992 to 2011.

Main Outcomes and Measures. Serum protein and monoclonal immunoglobulins, serum free light chains (sFLCs), and serum light chains within each immunoglobulin class were measured.

Results. In cross-sectional modeling, progressive MGUS risk factors were IgA isotype ($OR_{adjusted}$ = 1.80; P = .038), ≥15g/L monoclonal spike ($OR_{adjusted}$ = 23.5; P < .001), skewed (<0.1 or >10) sFLC ratio ($OR_{adjusted}$ = 46.4; P < .001), and severe immunoparesis (≥2 suppressed uninvolved immunoglobulins) ($OR_{adjusted}$ = 19.1; P < .001); progressive light-chain MGUS risk factors were skewed sFLC ratio ($OR_{adjusted}$ = 44.0; P < .001) and severe immunoparesis ($OR_{adjusted}$ = 48.6; P < .001). From the cross-sectional analyses, we developed a scoring system for the longitudinal analysis, finding that individuals with low-risk/intermediate-risk MGUS can convert to high-risk MGUS and progress to MM within 5

years; the same result was found in light-chain MGUS. Evolving monoclonal proteins, sFLCs, and immunosuppression were associated with progression.

Conclusions and Relevance. Our finding that clinical risk categories can change over time prior to MM diagnosis supports annual blood testing and risk assessment for patients with MGUS or light-chain MGUS.

INTRODUCTION

Worldwide over 120,000 individuals are diagnosed annually with multiple myeloma.[1] This malignancy is characterized by the proliferation of abnormal monoclonal plasma cells derived from B-cells in the bone marrow. Frequently, there is invasion of the adjacent bone, which destroys skeletal structures and causes bone pain and fractures in more than 80% of newly diagnosed patients.[2] The abnormal plasma cells can produce monoclonal-(M)-proteins and circulating free light-chains (FLCs) detectable in peripheral blood. Approximately 15% to 20% of newly diagnosed patients with multiple myeloma secrete FLCs only.[2] Renal impairment; commonly caused by FLCs or hyperviscosity from excessive amounts of M-protein in the blood, affects up to 40% of newly diagnosed patients.[3] Multiple myeloma is one of the few malignancies with a defined precursor condition, monoclonal gammopathy of undetermined significance (MGUS), which is detectable in peripheral blood.[4] Similarly, the light-chain subtype of multiple myeloma is preceded by light-chain MGUS.[4]

Recent population-based screening studies have shown that MGUS can start at age 30 years[5,6] and is found in 2.3% of individuals 50 years or older.[6] Because there is no established screening for MGUS in the general population, it is typically diagnosed incidentally during medical work-ups for other reasons (such as infectious, neurologic, or rheumatologic symptoms, or as part of life-insurance blood screening).[7] Long-term retrospective follow-up studies of individuals diagnosed with MGUS show 0.5% to 1% annual risk of progressing to multiple myeloma.[7-9] Risk models using data from a single time point (usually the initial work-up) have been proposed to estimate the risk of progression from MGUS to multiple myeloma.[7,9] This modeling has led to development of clinical consensus guidelines recommending annual peripheral blood monitoring of serum protein markers and other assays for patients with intermediate-risk and high-risk MGUS.[10] Smaller retrospective studies suggest that evolving changes in M-protein levels may be associated with progression[11-13]; however, in clinical practice, most patients are counseled based on their risk profile at initial work-up. Limited information is

available regarding the risk of progression from light-chain MGUS to light-chain multiple myeloma and clinical guidelines for this condition are lacking.[14]

We conducted the first prospective study to longitudinally investigate the dynamics of serum immune markers in individuals with myeloma precursor disease. Within the screening arm of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (N = 77,469), we evaluated serially collected serum samples from participants with progressing MGUS and stable MGUS, including light-chain subtype disease. We show that the risk profiles for individuals with MGUS and light-chain MGUS can change over time, reflected in conversion from low-risk/intermediate-risk MGUS to high-risk MGUS. Also, we provide longitudinal data on evolving serum immune markers in progressing patients, including markers of malignant plasma cells and markers of humoral host immunity. Our study provides several new insights of direct relevance for clinical monitoring and counseling of patients.

METHODS

Study Population and Design

The prospective NCI-PLCO Cancer Screening Trial has previously been described in detail.[15] The selection of screening-arm participants for the current investigation is summarized in **Figure 1** and in the Supplementary Methods. Briefly, we selected serial pre-diagnostic serum samples from 208 multiple myeloma cases diagnosed during follow-up, and samples from the latest available study year for 5916 subjects who never received a diagnosis of multiple myeloma or another hematologic malignancy. In the latter group, we identified individuals with non-IgM MGUS or light-chain MGUS who did not progress to multiple myeloma or light-chain multiple myeloma and selected all earlier available serum samples from these individuals.

Participants provided written informed consent in accordance with the Declaration of Helsinki at the trial baseline. The PLCO Cancer Screening Trial was conducted according to a protocol approved by the

Institutional Review Boards (IRBs) of the NCI and the 10 screening centers, and the current investigation was approved by the NCI Special Studies IRB. The primary outcome of the analysis was progression from MGUS to multiple myeloma or from light-chain MGUS to light-chain multiple myeloma.

Laboratory Tests

For all subjects, pre-diagnostic serum samples (0.5 mL) stored at –70°C were thawed and blindly processed and analyzed in an identical fashion (**Figure 1**, footnote of **Table 1**).

Statistical Methods

Differences in characteristics between cases and controls were assessed using Fisher exact and chi-square tests.

Cross-Sectional Marker Analysis

First, we analyzed the risk of progression in relation to each marker using the pre-diagnostic measurements from the time point most proximal to multiple myeloma diagnosis date (for cases) or selection date (for non-progressing MGUS and light-chain MGUS controls). We included serum immune markers proposed in prior studies.[7,9] We estimated odds ratios (ORs) and 95% confidence intervals (CIs) for associations with case status, using logistic regression models with and without adjustment for sex, race, study center, and age and calendar year at most proximal blood draw.

Patterns of Serum Markers Over Time

Using the results from the cross-sectional analyses, we developed a scoring system. For MGUS, the following variables were defined as risk factors for progression: monoclonal-(M)-spike with IgA isotype, M-spike concentration ≥15 g/L, sFLC ratio <0.1 or >10, and immunoparesis (≥1 uninvolved immunoglobulins below lower level of normal). In the scoring system for light-chain MGUS, sFLC ratio <0.1 or >10 and immunoparesis were used as risk factors. The score was defined as the total number of risk factors for each individual blood sample. Scores for select individuals are shown in **Table 2**.

We also assessed marker changes over time in a subset of patients (see Supplementary Methods for details on the models).[16,17] In brief, we selected MGUS patients who progressed to multiple myeloma (cases) and MGUS patients who did not progress (controls) and matched them in a 1:1 control-to-case ratio on the basis of age (±6 years), sex, and race. For each case, we identified the sample most proximal to multiple myeloma diagnosis and matched a control of similar age as the case at the blood draw most proximal to cohort exit. Then we assigned the age of multiple myeloma diagnosis of the case as age of selection for the control. Matched controls had to be free of multiple myeloma as of the time of the diagnosis of their corresponding case. For three cases no match could be identified, resulting in 159 cases and 156 matched controls. Using the same matching criteria, for each progressing light-chain MGUS (cases) we selected two stable light-chain MGUS (controls) (28 cases, 56 matched controls). For all cases and controls we used all samples available before multiple myeloma diagnosis date and selection date, respectively. We then fit models to the log-transformed marker data that allowed the slopes for time to vary for cases and non-cases by including interaction terms of case-status with time. P-values for significance for these interaction terms are presented as evidence of differences in marker trajectories over time for cases (progressors) and controls (non-progressors).

RESULTS

For our study, we identified 187 individuals who progressed from non-IgM MGUS/light-chain MGUS to multiple myeloma/light-chain multiple myeloma and 498 individuals whose diagnosis remained non-IgM MGUS/light-chain MGUS without progression to multiple myeloma through ≤16 years of follow up (**Figure 1; eTable 1**). For each participant we obtained all available serially stored pre-diagnostic serum samples, collecting 3266 samples in total.

Risk Factors and Patterns of Progression among Individuals with non-IgM MGUS

Cross-Sectional Modeling

Associations with progression from non-IgM MGUS to multiple myeloma for immunoglobulin isotype, concentration of the M-spike, skewed sFLC ratio, and immunoparesis from the time point most proximal to diagnosis or selection are shown in **Table 1**. Compared to individuals with IgG isotype, those with IgA isotype had a modest but statistically significant increased risk of progression to multiple myeloma ($OR_{adjusted}$=1.80; 95% CI, 1.03-3.13; 28% vs. 17%; *P*=.038). Subjects who had M-spike concentration ≥15 g/L were more than 23 times more likely to develop multiple myeloma compared to those with a lower concentration of the protein ($OR_{adjusted}$=23.5; 95% CI; 8.9-61.9; 41% vs. 4%; *P*<.001). We also evaluated risk of progression among subjects with altered sFLC ratios just outside the published reference range of 0.26 to 1.65 [7,8] as well as further outside the range. Compared to individuals with a sFLC ratio within the normal reference range, those with a sFLC ratio <0.1 or >10 were 46 times more likely to develop multiple myeloma ($OR_{adjusted}$=46.4; 95% CI, 18.4-117; 41% vs. 4%; *P*<.001). Finally, we assessed the risk of progression by severity of the immunoparesis, as defined by the number of suppressed uninvolved immunoglobulins (IgG, IgA, and/or IgM). Compared to those with no evidence of immunoparesis, individuals with two suppressed uninvolved immunoglobulins were much more likely to progress to multiple myeloma ($OR_{adjusted}$=19.1; 95% Cl, 7.5-48.3; 29% vs. 3%; *P*<.001). As described above in the Methods section, we defined a risk score based on the identified risk factors (**Table 2**). Based on our clinical risk score, we plotted longitudinal patterns of progression to multiple myeloma (**eFigure 1, Panel A**).

Changes of Marker Values Over Time

First, we used information for all available serum samples from multiple myeloma cases (n=159) and matched controls (n=156). Specifically, we plotted the M-spike concentration and the involved sFLC concentration from individual samples on the y-axis against the years prior to multiple myeloma diagnosis (for cases) and selection (for controls) on the x-axis, respectively (**Figure 2, Panels A and B;**

e**Figure 2**). Increasingd M-spike concentration (*P*=.065 for linear term and *P*=.017 for quadratic term), increasing involved sFLC levels (*P*=.012), and an increasing number of suppressed uninvolved immunoglobulins (*P*<.0001) were associated with higher risk of progression to multiple myeloma.

Second, to investigate patterns of serum markers over time we conducted another longitudinal analysis (see **Figure 1**). These analyses were limited to individuals with at least one pre-diagnostic serum sample within 2 years of diagnosis and at least 3 serial samples. For MGUS patients without progression to multiple myeloma, we required at least 8 years of follow-up. Our final selection for these analyses included 43 multiple myeloma cases and 108 controls with non-progressing MGUS. Twenty-three of the 43 selected MGUS case patients who progressed to multiple myeloma (53%) had a high-risk score prior to multiple myeloma diagnosis, while only 1 of 108 non-progressing MGUS controls had a high-risk score (*P*<.001) (**Figure 3, Panel A**). Furthermore, among MGUS case patients who progressed and had a high-risk score prior to multiple myeloma diagnosis, 16 (70%) had preceding blood samples with low-risk and/or intermediate-risk scores. Among the progressors, a high-risk score was detected up to 5 years prior to multiple myeloma diagnosis (**Figure 3, Panel A**). For progressors without a high-risk score, 9 had available stored blood 1 year prior to multiple myeloma diagnosis; 5 were intermediate-risk and 4 were low-risk (**Figure 3, Panel A; eTable 2**). Of the progressors, 9 (21%) fulfilled the diagnostic blood-based criteria for smoldering multiple myeloma (i.e., M-spike concentration ≥30 g/L)[18] and 6 (14%) fulfilled the diagnostic blood-based criteria for multiple myeloma (i.e., sFLC ratio ≥100 and involved sFLC concentration ≥10 mg/dL)[19] prior to clinical multiple myeloma diagnosis; none of the non-progressors fulfilled these criteria (*P*<.00001) (**eTable 2**). Blood-based criteria for multiple myeloma were detected up to 5 years prior to its diagnosis (**Figure 3, Panel A**). Severe immunoparesis (two uninvolved immunoglobulins below lower level of normal) was found in pre-diagnostic blood samples of 18 of the 43 (42%) progressors and 4 of 108 (4%) non-progressors (*P*<.00001) (**eTable 2**).

Risk Factors and Patterns of Progression among Individuals with Light-Chain MGUS

Cross-Sectional Modeling

Among individuals with light-chain MGUS, those with highly skewed sFLC ratio (defined as >10 or <0.1) at the time point most proximal to diagnosis/selection were much more likely to progress to light-chain multiple myeloma compared to those whose sFLC ratio was less skewed ($OR_{adjusted}$=44.0; 95% CI 14.2-136; 75% vs. 6%; *P*<.001; Table 2). Similar to our findings among MGUS case patients, we found more severe immunoparesis in light-chain MGUS cases who progressed to light-chain multiple myeloma, compared to controls who remained stable ($OR_{adjusted}$=48.6; 95% CI 9.55-248; 36% vs. 2%; *P*<.001). Based on our clinical risk score (**Table 2**), we plotted longitudinal patterns of progression to light-chain multiple myeloma (e**Figure 1, Panel B**).

Changes of Marker Values Over Time

First, we used all available serum samples from light-chain multiple myeloma cases (n=28) and matched light-chain MGUS controls (n=56) and plotted the involved sFLC concentration from individual samples by number of years prior to multiple myeloma diagnosis (for cases) and selection (for controls) (**Figure 2, Panels C and D**). Increased involved sFLC levels (*P*=.0004) and increased number of suppressed uninvolved immunoglobulins (*P*=.0007) were associated with higher risk of progression to multiple myeloma.

The second longitudinal analysis (see **Figure 1**) was limited to individuals with at least one pre-diagnostic serum sample within 2 years of diagnosis and at least 3 serial samples. For MGUS patients without progression to light-chain multiple myeloma, we required at least 8 years of follow-up. Our final selection for these analyses included 10 light-chain multiple myeloma cases and 120 light-chain MGUS subjects. Seven of the 10 (70%) light-chain MGUS patients who progressed to light-chain multiple

myeloma had a high-risk score prior to light-chain multiple myeloma diagnosis; only 1 of 120 who did not progress (<1%) had high-risk score (*P*<.00001) (**Figure 3, Panel B**). Furthermore, among light-chain MGUS case patients who progressed and had high-risk status prior to light-chain multiple myeloma diagnosis, 5 (71%) had preceding blood samples with low-risk and/or intermediate-risk score. Among the progressors, high-risk status was detected up to 5 years prior to multiple myeloma diagnosis (**Figure 3, Panel B**). Blood samples were available for two individuals who were not at high risk before they progressed; they had intermediate-risk and low-risk, respectively, 1 year prior to multiple myeloma diagnosis (**Figure 3, Panel B; eTable 2**). Six (60%) of the light-chain MGUS case patients who progressed met the diagnostic blood-based criteria for light-chain multiple myeloma prior to their clinical multiple myeloma diagnosis; one of the non-progressors (<1%) also did (*P*<.00001) (**eTable 2**). Blood-based criteria for multiple myeloma were detected up to 5 years prior to multiple myeloma diagnosis. Severe immunoparesis was found in pre-diagnostic blood samples of 7 of 10 (70%) progressors and 3 of 120 (2%) non-progressors (*P*<.00001) (**eTable 2**).

DISCUSSION

In 2009, using data from the PLCO cancer screening trial, we conducted the first prospective screening study showing that multiple myeloma is consistently preceded by MGUS.[20] Here, we designed a longitudinal study to investigate dynamics of serum immune markers in individuals with MGUS. We provide novel insights by showing that the individual patient's risk of progression from MGUS to multiple myeloma is not constant over the years, and the same is true for individuals with light-chain MGUS. This study based on prospectively collected samples helps us to better understand the findings of prior retrospective follow-up studies. Indeed, previously reported 0.5% to 1% annual risks of progressing from MGUS to multiple myeloma[7-9] reflect the average risk among all MGUS cases, but are not applicable to individual patients. In fact, in the current study we found that individual patients' clinical risk categories can convert. Our data indicate that individuals with low-risk or intermediate-risk

MGUS can convert to high-risk MGUS and progress to multiple myeloma within a 5-year window. The same conversion patterns are true for individuals with light-chain MGUS. This finding is of direct clinical relevance and supports annual blood tests for all individuals diagnosed with MGUS or light-chain MGUS, and importantly, yearly assessment of a patient's clinical risk status.

It has been proposed, based on clinical observations, that evolving changes in monoclonal protein levels are associated with progression.[11-13] We found that significantly increasing M-spike and involved sFLC concentrations were detectable in MGUS progresssors more than 5 years prior to multiple myeloma diagnosis; progressing light-chain MGUS case patients similarly had significantly increasing involved sFLC concentrations over time. When we combined individual markers into a risk score for progression, 53% of progressing MGUS patients but only 1 of 108 non-progressing MGUS patients had a high-risk score. Similarly, we found evidence of high-risk status in 70% of the progressing light-chain MGUS cases but only 1 of 120 non-progressing light-chain MGUS controls. Importantly, most patients who developed multiple myeloma after a preceding state of high-risk MGUS had converted from low-risk/intermediate-risk stages within 5 years prior to multiple myeloma diagnosis; similar patterns were found in light-chain MGUS progressors.

There are no previous data on the rate of smoldering multiple myeloma development on the pathway to multiple myeloma. Here, using serum markers prior to formal diagnosis of multiple myeloma, we found that only 21% of the MGUS progressors fulfilled the blood-based criteria for smoldering multiple myeloma[18] and 14% fulfilled the criteria for multiple myeloma.[19] Of the light-chain MGUS progressors, 60% fulfilled the blood-based criteria for light-chain multiple myeloma prior to formal diagnosis.[19] These blood-based criteria were detectable up to 5 years prior to formal diagnosis of both multiple myeloma and light-chain multiple myeloma. However, we did not have access to bone marrow biopsy results, so we could not evaluate data on plasma cell percentage.

The biology of multiple myeloma is complex, and DNA sequencing studies show that the mutational landscape is already altered in earlier stages of the disease process.[21] Although there are only limited data available, it has been proposed that the host biology plays a role in the regulation of stable precursor disease versus progression.[7,9,22] In our cross-sectional analysis, immunosuppression (one or more suppressed uninvolved immunoglobulins) was found in 58% of MGUS progressors compared to 20% of MGUS non-progressors. In the light-chain MGUS subjects, immunosuppression was found in 54% and 12% of progressors and non-progressors, respectively. In MGUS and light-chain MGUS subjects, those with severe immunosuppression (two or more suppressed uninvolved immunoglobulins) had a significantly elevated risk of progression (progressors versus non-progressors: 29% versus 3%; and 18% versus 2%, respectively). Future studies should investigate biological interactions between non-tumor and tumor cells in relation to progression.

In summary, our data show that individuals with low-risk or intermediate-risk MGUS can convert to high-risk MGUS and progress to multiple myeloma within 5 years; the same is true for light-chain MGUS. Our results are clinically relevant and support annual blood tests for all individuals diagnosed with MGUS or light-chain MGUS.

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Table 1. Serum protein markers associated with progression: cross-sectional analysis

	Non-progressing MGUS (n = 281)	Progressing MGUS to multiple myeloma (n = 159)			Non-progressing light-chain MGUS (n = 217)	Progressing light-chain MGUS to light-chain multiple myeloma (n = 28)		
Study Status	**Control**	**Case**	**OR_{UA} (95% CI)**	**OR_A[a] (95% CI)**	**Control**	**Case**	**OR_{UA} (95% CI)**	**OR_A[a] (95% CI)**
Immunoglobulin isotype					N/A			
IgG	216 (77%)	109 (69%)	Ref	Ref				
IgA	47 (17%)	45 (28%)	**1.90 (1.19-3.03)**	**1.80 (1.03-3.13)**				
Biclonal	18 (6%)	5 (3%)	0.55 (0.20-1.52)	0.49 (0.15-1.61)				
M-spike concentration					N/A			
<15 g/L	178 (96%)	88 (58%)	Ref	Ref				
≥15 g/L	8 (4%)	62 (41%)	**15.68 (7.19-34.17)**	**23.50 (8.93-61.86)**				
FLC ratio								
0.26 to 1.65	185 (66%)	37 (23%)	Ref	Ref				
0.1 to 0.26 or >1.65 to 10	86 (31%)	57 (36%)	**3.31 (2.04-5.39)**	**3.45 (1.93-6.16)**	205 (94%)[c]	7 (25%)[c]	Ref[c]	Ref[c]
<0.1 to >10	10 (4%)	65 (41%)	**32.5 (153-69.0)**	**46.4 (18.4-117)**	12 (6%)	21 (75%)	**51.3 (18.2-144)**	**44.0 (14.2-136)**
Immunoparesis[b]								
0	211 (80%)	64 (42%)	Ref	Ref	190 (88%)	13 (46%)	Ref	Ref
1	45 (17%)	45 (29%)	**3.30 (2.00-5.43)**	**3.01 (1.66-5.47)**	22 (10%)	5 (18%)	**3.32 (1.08-10.2)**	**3.98 (1.07-14.8)**
2	7 (3%)	45 (29%)	**21.2 (9.11-49.3)**	**19.1 (7.52-48.3)**	5 (2%)	10 (36%)	**23.2 (8.70-98.2)**	**48.6 (9.55-248)**

Laboratory tests: All assays were conducted at The Binding Site (Birmingham, UK). Serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE) were performed on agarose gel (Hydrasys and Hydragel; Sebia, Norcross, GA). Levels of serum free light chains (sFLCs) were determined in all study samples using Freelite assays, and serum light-chains within each immunoglobulin class (i.e., IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, and IgMλ) were measured using Hevylite assays; these assays were performed on a SPAPLUS turbidimeter.

Data are given as No. (%) unless otherwise noted. Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; M-spike, monoclonal spike; NA, no association; OR_{A}, odds ratio adjusted; OR_{UA}, odds ratio unadjusted; sFLC, serum free light chain (kappa/lambda).

[a]Odds ratios and 95% CIs are adjusted for sex, age at most proximal blood draw (continuous), race (non-Hispanic White, non-Hispanic Black, and others [Hispanic, Asian, and Pacific Islanders]), calendar year of most proximal blood draw (1995-1998, 1999-2002, 2003-2006), and study center (coded as Upper Midwest [Wisconsin and Minnesota], West/South [Colorado, Hawaii, Missouri, Utah, and Alabama] and East [Georgetown, Detroit, and Pittsburgh]).

[b]Immunoparesis is the number of uninvolved immunoglobulins (IgG, IgA, or IgM) below lower level of normal. Models for light-chain secreting multiple myeloma and light-chain MGUS cases were not adjusted for race owing to small numbers of non-white light-chain multiple myeloma cases. Analysis was performed in SAS version 9.4.

[c] FLC ratio = 0.1 to 10, no exclusion.

[d] Immunoparesis = 2-3.

Table 2. Adverse markers for progression and definitions of high-risk, intermediate-risk, and low-risk status for MGUS and light-chain MGUS, respectively

MGUS

Variable	Adverse marker	Points
M-spike isotype	IgA	1
M-spike concentration	≥15 g/L	1
sFLC ratio (kappa/lambda)	<0.1 or >10	1
Immunoparesis	Uninvolved immunoglobulins below lower level of normal	1 or 2

MGUS risk-category, for progression to multiple myeloma	SCORE
Low-risk MGUS	0 to 1
Intermediate-risk MGUS	2
High-risk MGUS	3 or higher

Light-chain MGUS

Variable	Adverse marker	Points
sFLC ratio (kappa/lambda)	<0.1 or >10	1
Immunoparesis	Uninvolved immunoglobulins below lower level of normal	1, 2 or 3

Light-chain MGUS risk-category, for progression to light-chain multiple myeloma	SCORE
Low-risk light-chain MGUS	0 to 1
Intermediate-risk light-chain MGUS	2
High-risk light-chain MGUS	3 or higher

Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; M-spike, monoclonal spike; sFLC, serum free light chain.

FIGURE LEGENDS:

Figure 1. Study design and selection of cases and controls

[a] A case-control study was undertaken within the Prostate, Lung, Colorectal and Ovarian (NCI-PLCO) Cancer Screening Trial study population, which has previously been described in detail (see Methods section).

[b] Of the 208 multiple myeloma subjects diagnosed during follow-up who had one or more stored pre-diagnostic serum samples, we identified 159 with non-IgM MGUS and 28 with light-chain MGUS prior to diagnosis (the multiple myeloma and light-chain multiple myeloma case groups, respectively). Of the 208, 21 had their last serial blood draw conducted up to 9 years (median: 7 years) prior to multiple myeloma diagnosis; there was no M-spike or abnormal sFLC patterns detectable in those samples, and these subjects were excluded. Among the 5916 subjects never diagnosed with multiple myeloma or another hematologic malignancy, we identified 281 with non-IgM MGUS and 217 with light-chain MGUS. Thus, our final sample for pre-planned cross-sectional analyses included 159 multiple myeloma cases, 28 light-chain multiple myeloma cases, 281 MGUS subjects, and 217 light-chain MGUS subjects.

[c] For these longitudinal analyses, we selected multiple myeloma cases and controls with MGUS that did not progress and matched them in a 1:1 control-to-case ratio on the basis of age (±6 years), sex, and race. For each case, we identified the sample most proximal to multiple myeloma diagnosis and matched a control of similar age as the case at the blood draw most proximal to cohort exit. Then we assigned the age of multiple myeloma diagnosis of the case as age of selection for the control. Matched controls had to be free of multiple myeloma as of the time of the diagnosis of their corresponding case. For three cases no match could be identified, resulting in 159 multiple myeloma cases and 156 matched controls. Using the same matching criteria, for each light-chain multiple myeloma case we selected two controls with light-chain MGUS that did not progress (28 cases, 56 matched controls).

[d] For these longitudinal analyses, we required available stored pre-diagnostic serum samples and at least one serum sample within 2 years of diagnosis; because our study was designed to study patterns of serum markers, the subjects were required to have at least a 3-year period with serial samples. For the subjects with MGUS or light-chain MGUS without progression to multiple myeloma or light-chain multiple myeloma, to make sure they were non-progressing during the collection of serum samples, we required at least 8 years of follow-up without a diagnosis of multiple myeloma or light-chain multiple myeloma. Also, since we focused on patterns of serum markers, subjects with MGUS or light-chain MGUS without progression were required to have at least a 3-year period with serial samples. Our final selection for these analyses included 43 multiple myeloma cases, 10 light-chain multiple myeloma cases, 108 MGUS subjects, and 120 light-chain MGUS subjects, respectively.

Table 2. Adverse markers for progression and definitions of high-risk, intermediate-risk, and low-risk status for MGUS and light-chain MGUS, respectively

Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; M-spike, monoclonal spike; sFLC, serum free light chain.

Figure 2, Panels A and B. Progression from MGUS to multiple myeloma, average M-spike concentration over time

Figure 2, Panels C and D. Progression from light-chain MGUS to light-chain multiple myeloma, average involved sFLC concentration over time

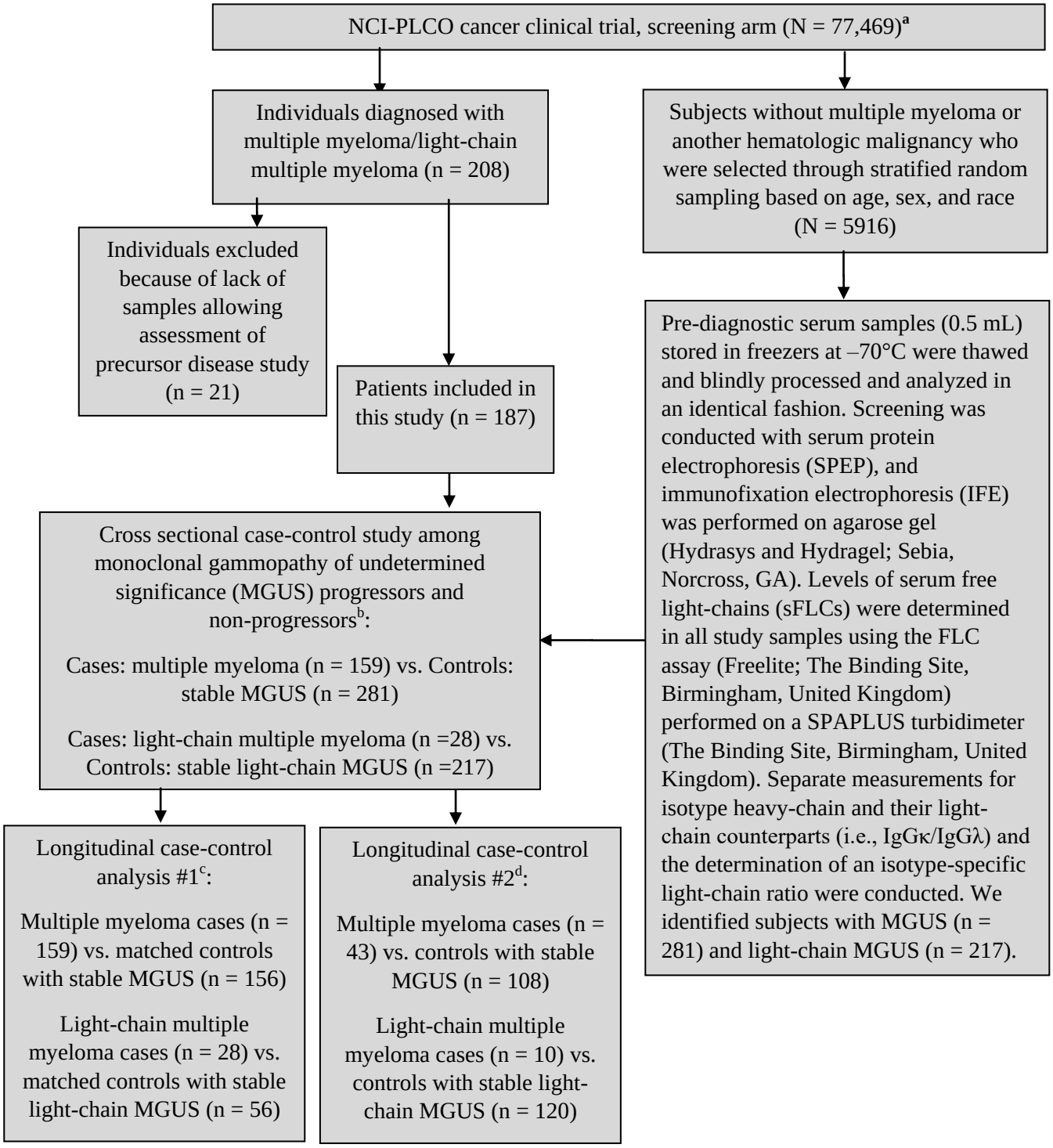
Figure 3, Panel A. Progression from MGUS to multiple myeloma: longitudinal analysis of risk scores among selected progressors and selected non-progressors

Footnote: Based on the risk-score presented in Table 2, we used the score to assess and label individual blood samples as follows:: score 0 to 1 = low-risk MGUS (green); score 2 = intermediate-risk MGUS (yellow); score 3 or higher = high-risk MGUS (red). Each series of boxes represents a unique patient; each box represents a unique blood sample; and the X-axis represents number of years prior to multiple myeloma diagnosis (for cases) and number of years prior to selection (for controls). Each box includes a number that represents the risk score. *Fulfilled the blood-based criteria for smoldering multiple myeloma (i.e., M-spike concentration ≥30 g/L); and ** fulfilled the blood-based criteria for multiple myeloma (i.e., sFLC ratio ≥100 and involved sFLC concentration ≥10 mg/dL).

Figure 3, Panel B. Progression from light-chain MGUS to light-chain multiple myeloma: longitudinal analysis of risk among progressors and non-progressors

Footnote: Based on the risk-score presented in Table 2, we used the score to assess and label individual blood samples as follows: score 0 to 1 = low-risk MGUS (green); score 2 = intermediate-risk MGUS (yellow); score 3 or higher = high-risk MGUS (red). Each series of boxes represents a unique patient; each box represents a unique blood sample; and the X-axis represents number of years prior to multiple myeloma diagnosis (for cases) and number of years prior to selection (for controls). Each box includes a number that represents the risk score. ** Fulfilled the blood-based criteria for multiple myeloma (i.e., sFLC ratio of ≥100 and involved sFLC concentration of ≥10 mg/dL).

Figure 1. Study design and selection of cases and controls



[a] A case-control study was undertaken within the Prostate, Lung, Colorectal and Ovarian (NCI-PLCO) Cancer Screening Trial study population, which has previously been described in detail (see Methods section).

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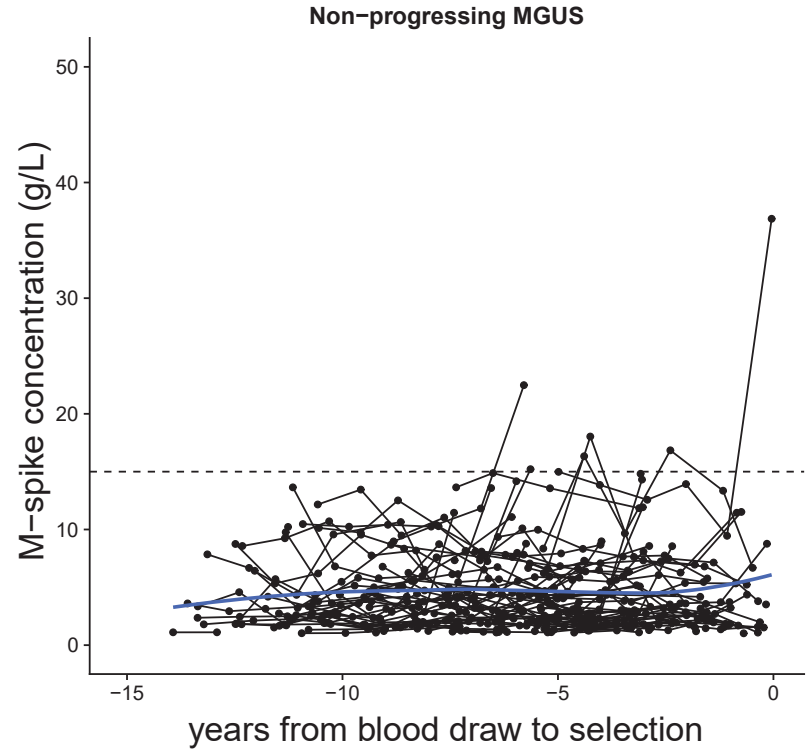
Non−progressing MGUS
M−spike concentration (g/L)
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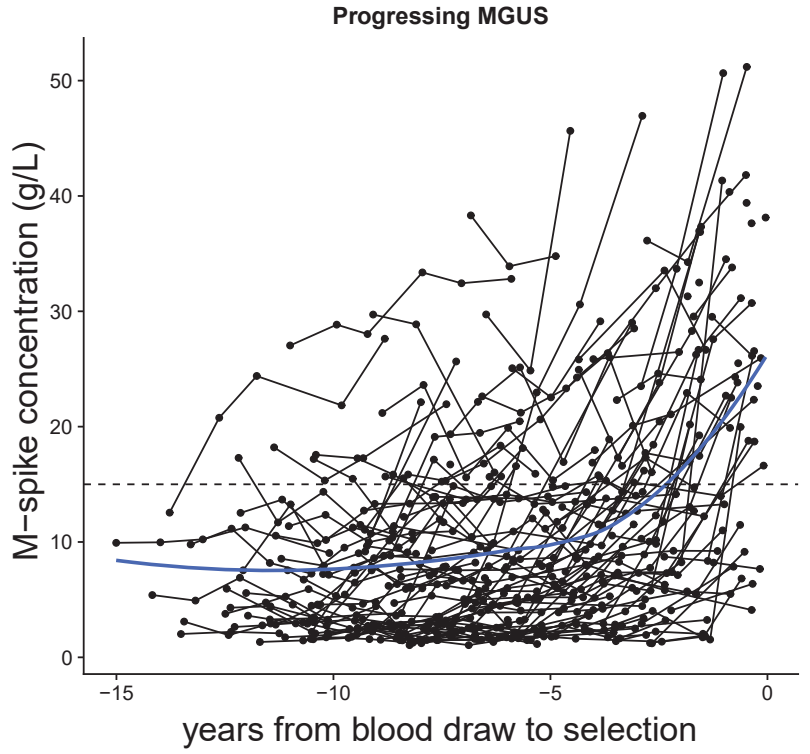
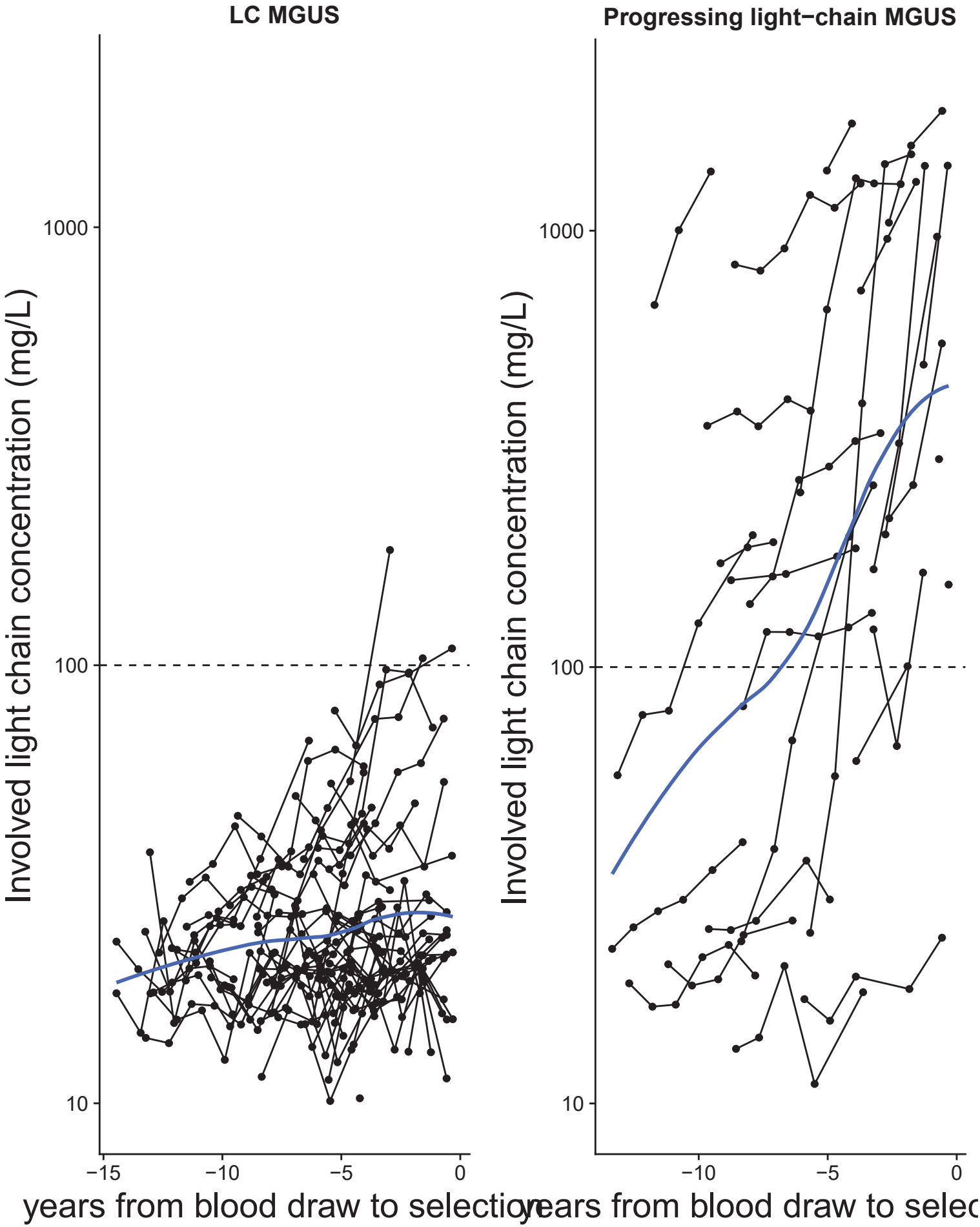
40
30
20
10
0
−15
−10
−5
0
years from blood draw to selection


Progressing MGUS
M−spike concentration (g/L)
50
40
30
20
10
0
−15
−10
−5
0
years from blood draw to selection


LC MGUS
Progressing light−chain MGUS
Involved light chain concentration (mg/L)
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100
10
−15
−10
−5
0
years from blood draw to selection
years from blood draw to selec


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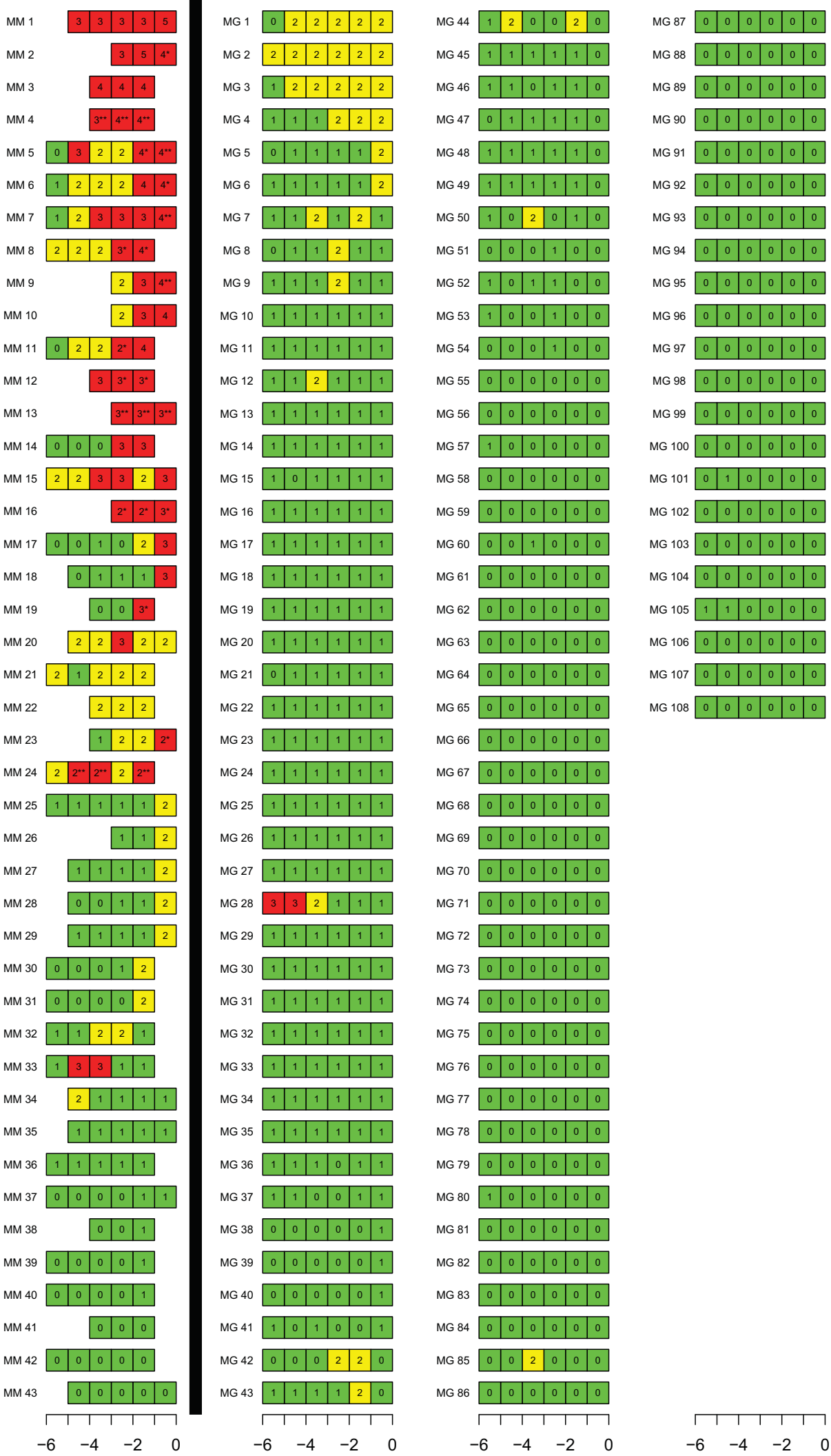
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