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A prospective study of circulating chemokines and angiogenesis markers and risk of multiple myeloma and its precursor

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Abstract

Background: Experimental and clinical studies have implicated certain chemokines and angiogenic cytokines in multiple myeloma (MM) pathogenesis. To investigate whether systemic concentrations of these markers are associated with future MM risk and progression from its precursor, monoclonal gammopathy of undetermined significance (MGUS), we conducted a prospective study within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.

Methods: We measured concentrations of 45 immunologic and pro-angiogenic markers in sera from 241 MM cases, 441 subjects with non-progressing MGUS, and 258 MGUS-free controls using Luminex-based multiplex assays and ELISA. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using multivariable logistic regression. We also evaluated absolute risk of progression using weighted Kaplan-Meier estimates.

Results: Pre-diagnostic levels of six markers were significantly elevated among MM cases compared with MGUS-free controls using a false discovery rate of 10% (EGF, HGF, Ang-2, CXCL12, CCL8, and BMP-9). Of these, three angiogenesis markers were associated with future progression from MGUS to MM: EGF (fourth vs. first quartile: OR=3.01, 95% CI 1.61 to 5.63; $P_{\text{trend}}=0.00028$), HGF (2.59, 1.33 to 5.03; $P_{\text{trend}}=0.015$) and Ang-2 (2.14, 1.15 to 3.98; $P_{\text{trend}}=0.07$). A composite angiogenesis biomarker score substantially stratified risk of MGUS progression to MM beyond established risk factors for progression, particularly during the first 5 years of follow-up (AUCs of 0.71 and 0.64 with and without the angiogenesis marker score, respectively).

Conclusions: Our prospective findings provide new insights into mechanisms involved in MM development and suggest that systemic angiogenesis markers could potentially improve risk stratification models for MGUS patients.

Introduction

Multiple myeloma (MM) is a B cell malignancy characterized by the accumulation of malignant plasma cells in the bone marrow. Despite recent advancements in treatment, the prognosis for MM remains unfavorable, with an estimated 5-year relative survival rate of 52% in the United States (1). MM is consistently preceded by a largely asymptomatic precursor condition called monoclonal gammopathy of undetermined significance (MGUS), which is typically characterized by detection of a monoclonal (M)-protein in serum and by measuring serum levels of kappa and lambda free light-chains (2-4). An abnormal serum free light-chain (sFLC) ratio in the absence of detectable M-protein is defined as light-chain (LC) MGUS, which can progress to LC MM (5). The likelihood of progression from MGUS to MM varies by certain characteristics of MGUS including the amount and type of M-protein and free light-chain levels/ratio, ranging from an estimated 0.3% progression per year for LC MGUS to approximately 1.5% per year for high-risk MGUS (3,4). Improving the ability to identify MGUS patients at highest and lowest risk of progression could better inform clinical decision making regarding the frequency of follow-up testing and whether patients should be referred for more intensive procedures (e.g., bone marrow biopsy, skeletal imaging) (6).

Severe immune dysregulation is a strong risk factor for MM, with clearly elevated risks among solid organ transplant recipients taking immunosuppressive medications and HIV/AIDS patients (7-10). It is also suspected that subclinical variation in immune system function may influence risk in the general population. Mechanistic and clinical studies suggest that certain chemokines and pro-angiogenic growth factors play an important role in MM pathogenesis, and may hold promise as therapeutic targets (11-14). To our knowledge, however, no prospective

studies have investigated the relationships between these markers and future MM risk and progression to MM among individuals with MGUS.

To address this research gap, we conducted a nested case-control study within the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial measuring levels of chemokines, pro-angiogenic cytokines and other immunologic markers in banked sera from three study groups: subjects newly diagnosed with MM during follow-up, subjects with MGUS who have not progressed to MM, and MGUS-free subjects. Our aims were to investigate marker associations with (1) future MM risk and (2) future progression from MGUS to MM. For selected markers, we additionally evaluated the potential improvement in prediction of absolute risk of progression from MGUS to MM beyond factors in the current Mayo risk stratification model (2-4).

Methods

Study population

The design of the PLCO Cancer Screening Trial has been reported (15). The current investigation leveraged data from a larger screening study of MGUS in PLCO (16), described in the Supplemental Methods. A schematic diagram outlining the design of the current nested case-control study is shown in Supplemental Figure 1. Briefly, MGUS status was assessed in banked sera from MM cases diagnosed during follow-up and a large sample (N=5,916) of participants who did not develop MM or other hematologic malignancies, selected through stratified random sampling based on age, sex, and race. Our study included 241 newly diagnosed MM cases with available banked serum samples (185 of which were ‘progressors’ with MGUS characterized at the time of selection), 441 stable non-IgM or LC MGUS that had not progressed to MM through

up to 19 years of follow-up, and 258 MGUS-free controls. Cases of IgM MGUS were not included in this investigation, as this isotype generally progresses to a different malignancy, Waldenström macroglobulinemia (3,4). MGUS-free controls were matched to MM cases on age at blood collection (± 2 years), sex, race (non-Hispanic white, black, other), date and study year of phlebotomy, and number of freeze-thaw cycles. For participants with detected MGUS, we selected the earliest available banked serum sample at which MGUS was identified for use in this study. For MM cases without pre-diagnostic information on MGUS (N=56), we selected the earliest available serum sample. The PLCO Cancer Screening Trial was approved by Institutional Review Boards at the National Cancer Institute and the 10 study centers, and all participants provided written informed consent.

Immune marker measurements

Serum levels of 46 chemokines and angiogenesis markers were measured using three Millipore multiplex bead-based assay panels, and two soluble CD activation markers (sCD27, sCD30) were measured using standard ELISA methods. Samples were tested in duplicate for the angiogenesis panel and ELISA assays, and in single wells for the chemokine panels. We excluded three markers with >90% of test samples below the lower limit of quantification (LLOQ; CCL21, FGF-1, and IL-3), leaving 45 markers for analysis. For these remaining markers, measurements below the LLOQ were assigned half of the LLOQ value. Levels were detectable in >90% of samples for most markers (N=29, 64%), and assays demonstrated good reproducibility; the median coefficient of variation was 8.5%, and all intraclass correlation coefficients were ≥ 0.8 (Supplemental Table 1).

Statistical analysis

We used polytomous logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (CI) relating concentrations of immunologic markers and risks of MGUS and MM relative to MGUS-free controls; all models were adjusted for age, sex, race, study and calendar year of phlebotomy, and study center. Adjustment for body mass index (BMI) did not materially impact our results, and as such BMI was not included in our final statistical models. Markers were analyzed as continuous variables after $\log_2$ transformation such that risk estimates correspond to a doubling in marker levels. Associations with MM were considered statistically significant if the two-sided *P*-value was <0.05 and the Benjamini-Hochberg false discovery rate (FDR) was $<10\%$; FDR calculations were based on a total of 45 markers evaluated in the primary analyses.(17) Further categorical analyses of the top markers were performed with category cut points based on the quartiles among controls. We also conducted analyses stratified by sex, MGUS type (LC vs. heavy-chain non-IgM MGUS), time from blood collection to MM diagnosis based on median follow-up (<8 years, ≥ 8 years), and sensitivity analyses restricted to non-Hispanic whites.

For markers associated with MM risk, we also evaluated associations with progression among those with MGUS who later developed MM versus those with stable MGUS that did not progress to MM during follow-up. We computed ORs and 95% CIs using unconditional logistic regression models adjusted for the same factors as above, and further adjusted for established risk factors for progression modeled as a 3-category variable based on the current Mayo risk stratification model: LC MGUS, low risk non-IgM MGUS (M-protein concentration not elevated, normal sFLC ratio), and intermediate/high risk non-IgM MGUS (M-protein concentration ≥ 15 g/L and/or sFLC ratio <0.26 or >1.65). (2-4) For these analyses, quartile cut

points were assigned based on the distribution among the referent group of those with stable MGUS that did not progress to MM. We also conducted analyses stratified by sex and time from detection of MGUS/marker measurements to MM diagnosis (<5 years, ≥ 5 years).

We estimated the cumulative risk of progression from MGUS to MM (complement of Kaplan-Meier survival estimates) by weighting the nested case-control sample to represent the PLCO screening arm participants using the R package NestedCohort. The weights for membership in the nested case-control study depended on 48 cells crossing MGUS/MM status, age (55-59, 60-64, 65-69, and ≥ 70 years), sex, and race/ethnicity. A composite angiogenesis marker score was developed using a logistic regression model of MGUS progression in relation to $\log_2$ -transformed concentrations of EGF, HGF, and Ang-2. This score is the sum of the $\log_2$ marker concentration multiplied by its regression coefficient. Scores were dichotomized into the top tertile versus the two lower tertiles to compare absolute risks of progression. We assessed the improvement in predictions of absolute risk beyond the current Mayo risk stratification model by including the composite angiogenesis score versus only the 3-category MGUS variable defined above using Mean Risk Stratification (MRS), which estimates the average change in MM risk revealed by the angiogenesis score (18). MRS interprets the area-under-the-curve (AUC) in light of disease prevalence; a high AUC with low MM prevalence might have less risk stratification than a low AUC with high MM prevalence.

Statistical analyses were performed using Stata version 15.0 (College Station, TX) and R version 3.5.

Results

Demographic characteristics of study participants are shown in Table 1. A greater proportion of the MGUS cases were black relative to MM cases and controls, as expected based

on the sampling strategy for MGUS screening which over-sampled black participants, as described. Other demographic characteristics were generally similar for MM, MGUS, and controls. Among MM cases, the median time from sample collection to diagnosis was 8.3 years (range <1-19 years).

Figure 1 summarizes the *P*-values for tests of association with MM risk relative to MGUS-free controls for all 45 markers modeled as continuous variables. We identified six markers – four angiogenic cytokines/growth factors (EGF, HGF, Ang-2, BMP-9) and two chemokines (CXCL12, CCL8) – that were statistically significantly associated with MM risk using a FDR of 10%. These markers were uncorrelated or only modestly correlated with one another or with characteristics of MGUS (M-protein concentration, sFLC levels and ratio) (Supplemental Table 2). As shown in Table 2, all six markers were positively associated with MM risk. These associations remained for cases diagnosed ≥ 8 years after blood collection for all markers except CCL8 (Supplemental Table 3) and did not materially differ by sex (results not shown).

In analyses comparing stable MGUS cases and MGUS-free controls (Table 2), we observed strong associations with the chemokines CXCL12 and CCL8, although tests for trend were also statistically significant for HGF and Ang-2. While results for LC MGUS and non-IgM MGUS were not significantly different, associations with CXCL12, HGF, and Ang-2 were more apparent among those with non-IgM MGUS and CCL8 was more strongly associated with LC MGUS (Supplemental Table 4). Comparisons of marker levels among stable MGUS and MM cases, respectively, versus MGUS-free controls are shown in Supplemental Figure 2.

When we compared MM ‘progressors’ and stable MGUS, we observed an increased risk of future progression to MM among those with high levels of the angiogenic cytokines EGF

(fourth quartile vs. first: OR=3.01, 95% CI 1.61 to 5.63, $P_{\text{trend}}=2.8 \times 10^{-4}$), HGF (2.59, 1.33 to 5.03, $P_{\text{trend}}=0.015$), and Ang-2 (2.14, 1.15 to 3.98, $P_{\text{trend}}=0.07$; Table 3). Associations with HGF and Ang-2 were particularly strong for MM cases that progressed within 5 years of blood draw relative to those diagnosed later ($P_{\text{heterogeneity}} \leq 0.0036$), whereas associations with EGF did not appear to vary by follow-up time. Risk estimates were generally similar in magnitude for sensitivity analyses restricted to non-Hispanic whites and stratified by sex (data not shown).

Figure 2 shows the absolute risk of progression from MGUS to MM for three established MGUS risk groups (LC MGUS, low risk non-IgM MGUS, and intermediate/high risk non-IgM MGUS) and a composite angiogenesis marker score based on EGF, HGF, and Ang-2. In each risk group, risk of 15-year progression was increased among those with an angiogenesis score in the top tertile vs. those in lower tertiles (LC MGUS: 4.2% vs 1.0%, $P=0.016$; low-risk non-IgM MGUS: 8.2% vs. 3.5%, $P=0.056$; intermediate/high-risk non-IgM MGUS: 18.3% vs. 8.9%, $P=0.015$). Differences in progression risk were particularly notable during the first five years of follow-up (LC MGUS: 2.6% vs 0.2%, $P=0.012$; low-risk non-IgM MGUS: 2.3% vs. 0.6%, $P=0.10$; intermediate/high-risk non-IgM MGUS: 8.3% vs. 1.4%, $P=0.0006$).

In the first 5 years of follow-up, the overall AUC for progression from MGUS to MM increased from 0.64 (using risk strata in the current Mayo model) to 0.71 after incorporating the composite angiogenesis marker score. Within individual MGUS risk strata, the AUCs for the angiogenesis score at 5 years of follow-up ranged from 0.65 to 0.78, with 52%-83% of MM diagnosed during this time period occurring among those with high angiogenesis score (Table 4). The ability of the angiogenesis score to predict progression between 5-10 years of follow-up was attenuated; compared to the first 5 years, the AUCs declined from 0.65-0.78 to 0.54-0.58, the

fraction of MM with a high angiogenesis score declined from 52%-83% to 36%-43%, and the MRS declined from 0.11%-0.61% to 0.07%-0.18% (Table 4).

The composite angiogenesis marker score performed better for risk prediction than any of the three angiogenesis markers modeled individually (Supplementary Figure 3) and yielded similar findings using alternate cut points (e.g., top quartile vs. lower quartiles; Supplementary Figure 4).

Discussion

This study is, to our knowledge, the largest prospective investigation of circulating chemokines and pro-angiogenic cytokines in relation to risk of MM and the first to evaluate whether these markers are associated with future progression from MGUS to MM. In case-control analyses we observed strong positive associations with future MM risk for four pro-angiogenic cytokines/growth factors (EGF, HGF, Ang-2, and BMP-9) and two chemokines (CXCL12 and CCL8) after accounting for multiple comparisons. Of the six markers associated with future MM risk, we observed particularly strong associations with progression from MGUS to MM in relation to three pro-angiogenic cytokines (EGF, HGF, and Ang-2). Notably, a composite angiogenesis marker score based on levels of these three markers substantially improved our ability to predict absolute risk of progression from MGUS to MM beyond criteria in the current Mayo risk stratification model,(2-4) particularly during the first five years of follow-up. Two recent prospective studies with fewer MM cases (N=76 and 65, respectively) identified several immune markers that were inversely associated with MM risk (19,20); however, these studies did not evaluate most of the markers that we found to be positively associated with MM risk and MGUS progression to MM, and other overlapping markers (e.g., FGF-2, VEGF, TGF- α) did not reach statistical significance in our analyses.

Increased bone marrow angiogenesis plays a well-established role in MGUS progression to MM (12-14), and the transition to a vascular phase of tumor growth has been defined as an ‘angiogenic switch’ in the equilibrium between positive and inhibitory regulators of angiogenic activity that is characteristic of progression to active MM (21-24). Elevated angiogenesis markers – including those associated with future progression from MGUS to MM in this study – have also been linked to poorer prognosis among MM patients (13,22,25,26), and, more recently, with response to treatment (27). The EGF family of proteins plays a critical role in bone biology and have been studied as myeloma growth factors (28,29). EGF enhances proliferation and migration in bone marrow stromal cells and directly targets the pro-inflammatory cytokine IL-6 (30), which has been shown to influence MM pathogenesis (31). Increased expression of HGF and Ang-2 has been correlated with more advanced disease stage and poorer prognosis (13,25,26,32,33); elevated levels of these markers may reflect hypoxia and enhanced angiogenic activity in the bone marrow microenvironment, which contributes to disease progression and tumor dissemination (12). Although increased expression of VEGF in the bone marrow is thought to play an important role in MM pathogenesis (34), we did not observe associations with circulating levels of VEGF for MM risk or MGUS progression to MM; *ex vivo* platelet degranulation can affect VEGF measurements in serum (35), which may have limited our ability to detect associations with this marker.

Our findings suggest that a composite angiogenesis marker score may improve risk stratification for progression from MGUS to MM based on the existing Mayo model (2-4). Interestingly, we observed statistically significant differences in progression to MM between subjects with high versus low levels of the dichotomized biomarker score among those with intermediate/high-risk non-IgM MGUS as well as for those with low-risk LC MGUS, suggesting

potential improvements in stratification at both ends of the current risk spectrum. Across MGUS risk strata, the ability of the angiogenesis score to predict progression to MM was most apparent during the first 5 years of follow-up, and was attenuated ≥ 5 years after assessment of MGUS and the angiogenesis markers. Assessment of longitudinal changes in these angiogenesis markers could potentially improve our ability to discriminate the risk of progression over a longer period of follow-up. Furthermore, we note that if our findings are confirmed in a validation set, these markers could potentially be used to improve existing risk stratification models for progression from MGUS to MM, and as such may have clinical utility for better informing decisions about the frequency and type of follow-up testing over time.

Whereas the associations with progression from MGUS to MM were most apparent for these angiogenesis markers, we found that the chemokines CXCL12 and CCL8 were most strongly associated with MGUS, suggesting that these markers may reflect mechanisms involved at the early stages of MM development. CXCL12 is secreted by mesenchymal stromal cells and other cell types in the bone marrow, and it binds to the CXCR4 receptor, which is expressed on myeloma cells (12). Findings from experimental studies suggest that this chemokine supports the migration, adhesion, and homing of myeloma cells to the bone marrow (12,36). CCL8 is also produced by bone marrow stromal cells and it targets the CCR2 receptor, another chemokine receptor expressed by myeloma cells (37). Experimental data for CCL8 are less robust but suggest that this chemokine may also be involved in bone marrow homing of myeloma cells (11,37).

A unique feature of this study is the availability of information on MGUS status for the majority of MM cases and the inclusion of stable MGUS that did not progress to MM during follow-up. This allowed us to directly investigate markers associated with progression from

MGUS to MM and assess their potential utility for improving existing risk stratification models. While we used a conservative approach to control for multiple comparisons, and there is strong biologic plausibility for the observed associations, confirmation of these findings in other prospective studies is warranted. In this investigation we utilized data on MGUS and measured chemokines and angiogenesis markers in samples at a single time point prior to MM diagnosis/selection; future longitudinal investigations using measurements in serial samples collected over time may provide greater insights into whether marker trajectories predict MGUS progression to MM. Limitations of the current study include the small number of MM cases for certain subgroup analyses (e.g., progression from LC MGUS to LC MM, and in analyses stratified by follow-up time) and the absence of specimens to characterize clinically distinct MM tumor subtypes (e.g., hyperdiploid vs. non-hyperdiploid, *IGH* translocations) or identify cases of smoldering MM. Efforts to validate our findings in larger datasets with more detailed information on clinical characteristics of MM would be informative.

In summary, this prospective investigation of MM, its precursor MGUS, and MGUS-free controls identified six circulating immunologic markers associated with future MM risk, offering new insights into mechanisms involved in MM development. In particular, our findings provide novel evidence that systemic markers of angiogenesis are predictive of increased future risk of progression from MGUS to clinically manifest MM. Additional prospective studies are needed to replicate our findings; if confirmed, these markers could substantially improve our ability to identify MGUS patients at high and low risk of progression and may help inform decision making regarding clinical management of MGUS.

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Conflicts of Interest

OL has received research funding from: National Institutes of Health (NIH), U.S. Food and Drug Administration (FDA), Multiple Myeloma Research Foundation (MMRF), International Myeloma Foundation (IMF), Leukemia and Lymphoma Society (LLS), Perelman Family Foundation, Rising Tides Foundation, Amgen, Celgene, Janssen, Takeda, Glenmark, Seattle Genetics, Karyopharm; Honoraria/ad boards: Adaptive, Amgen, Binding Site, BMS, Celgene, Cellectis, Glenmark, Janssen, Juno, Pfizer; and serves on Independent Data Monitoring Committees (IDMCs) for clinical trials lead by Takeda, Novartis, Janssen. The other authors declare no competing financial interests.

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Table 1. Demographic characteristics of MGUS and MM cases and MGUS-free controls*

Characteristic	Controls	MGUS	Multiple myeloma
Overall	258	441	241
Age at blood draw, median (IQR)	65 (59-69)	68 (62-72)	64 (59-68)
Sex			
Female	89 (35%)	133 (30%)	84 (35%)
Male	169 (66%)	308 (70%)	157 (65%)
Race			
Non-Hispanic white	235 (91%)	271 (61%)	219 (91%)
Black	12 (5%)	143 (32%)	12 (5%)
Other	11 (4%)	27 (6%)	10 (4%)
MGUS subtype			
IgG		205 (46%)	107 (58%)
IgA		42 (10%)	45 (24%)
Biclonal		17 (4%)	5 (3%)
Light chain		177 (40%)	28 (15%)

* Reported as frequency (%) unless otherwise noted.

Table 2. Odds ratios (95% confidence intervals) for selected markers and risk of MGUS and MM in relation to MGUS-free controls*

Marker (pg/mL)	N _{controls}	MGUS		Multiple myeloma	
		N	OR (95% CI)	N	OR (95% CI)
EGF					
<41.9	65	110	1.00 (ref)	41	1.00 (ref)
41.9-72.7	65	79	0.83 (0.50 to 1.36)	30	0.99 (0.53 to 1.84)
72.8-135.8	64	121	1.17 (0.72 to 1.90)	52	1.73 (0.97 to 3.10)
>135.8	64	131	1.01 (0.62 to 1.64)	118	3.12 (1.80 to 5.42)
P _{trend}			0.79		2.9x10 ⁻⁶
Continuous [†]	258	441	1.06 (0.94 to 1.19)	241	1.52 (1.31 to 1.76)
HGF					
<456.5	66	118	1.00 (ref)	39	1.00 (ref)
456.5-579.0	64	78	0.69 (0.41 to 1.15)	39	1.13 (0.62 to 2.07)
579.1-750.3	64	99	0.90 (0.55 to 1.48)	69	2.26 (1.28 to 4.00)
>750.3	64	146	1.53 (0.95 to 2.44)	94	2.94 (1.69 to 5.11)
P _{trend}			0.018		2.5x10 ⁻⁵
Continuous [†]	258	441	1.30 (1.02 to 1.67)	241	1.89 (1.42 to 2.51)
Ang-2					
<1508.7	66	84	1.00 (ref)	33	1.00 (ref)
1508.7-2193.7	64	92	1.00 (0.60 to 1.65)	56	1.70 (0.94 to 3.08)
2193.8-3103.9	64	110	1.16 (0.70 to 1.91)	55	1.71 (0.95 to 3.08)
>3103.9	64	155	1.54 (0.95 to 2.49)	97	3.00 (1.71 to 5.26)
P _{trend}			0.04		1.8x10 ⁻⁴
Continuous [†]	258	441	1.20 (0.97 to 1.47)	241	1.57 (1.24 to 1.98)
CXCL12					
<1117.0	65	74	1.00 (ref)	39	1.00 (ref)
1117.0-1451.3	65	91	1.25 (0.75 to 2.11)	50	1.27 (0.71 to 2.27)
1451.4-1780.5	64	114	1.84 (1.10 to 3.07)	54	1.56 (0.87 to 2.77)
>1780.5	64	162	2.36 (1.44 to 3.88)	98	2.62 (1.52 to 4.52)
P _{trend}			2.4x10 ⁻⁴		2.6x10 ⁻⁴
Continuous [†]	258	441	1.45 (1.15 to 1.84)	241	1.71 (1.27 to 2.30)
CCL8					
<23.1	64	67	1.00 (ref)	38	1.00 (ref)
23.1-32.6	66	114	1.79 (1.07 to 3.00)	64	1.74 (0.98 to 3.08)
32.7-43.4	64	107	1.96 (1.17 to 3.30)	67	1.86 (1.05 to 3.30)
>43.4	64	153	2.11 (1.26 to 3.52)	72	2.08 (1.17 to 3.70)
P _{trend}			0.011		0.021
Continuous [†]	258	441	1.51 (1.18 to 1.93)	241	1.55 (1.17 to 2.04)
BMP-9					
<101.3	65	131	1.00 (ref)	40	1.00 (ref)
101.3-146.9	65	101	0.70 (0.43 to 1.14)	40	0.92 (0.51 to 1.67)
147.0-218.8	64	104	0.75 (0.46 to 1.21)	63	1.66 (0.95 to 2.90)
>218.8	64	105	0.93 (0.57 to 1.51)	98	2.31 (1.34 to 3.99)
P _{trend}			0.94		4.1x10 ⁻⁴
Continuous [†]	258	441	0.93 (0.82 to 1.06)	241	1.29 (1.09 to 1.52)

* All analyses were adjusted for age, sex, race, study year and calendar year of phlebotomy, and study center. Tests for trend were performed by assigning the value of the within-category median (among controls) and modeling as a continuous variable.

[†] Estimated risk corresponding to an approximate doubling in marker levels (1-unit increase in log₂-transformed levels).

Table 3. Odds ratios (95% confidence intervals) for selected markers and risk of progression from MGUS to MM*

Marker (pg/mL)	N _{MGUS}	Overall		<5 years to MM diagnosis		≥5 years to MM diagnosis		P _{het}
		N _{MM}	OR (95% CI)	N _{MM}	OR (95% CI)	N _{MM}	OR (95% CI)	
EGF								
<42.5	111	36	1.00 (ref)	10	1.00 (ref)	26	1.00 (ref)	
42.5-85.5	110	37	1.36 (0.71 to 2.64)	7	0.94 (0.31 to 2.79)	30	1.54 (0.76 to 3.15)	
85.6-149.5	111	35	1.20 (0.62 to 2.31)	9	1.10 (0.39 to 3.12)	26	1.23 (0.59 to 2.54)	
>149.5	109	77	3.01 (1.61 to 5.63)	32	3.62 (1.47 to 8.91)	45	2.71 (1.35 to 5.44)	
P _{trend}			2.8x10 ⁻⁴		0.0006		0.0056	
Continuous [†]	441	185	1.31 (1.12 to 1.54)	58	1.47 (1.15 to 1.87)	127	1.25 (1.05 to 1.49)	0.22
HGF								
<443.0	111	26	1.00 (ref)	3	1.00 (ref)	23	1.00 (ref)	
443.0-621.3	110	47	2.24 (1.12 to 4.46)	9	3.38 (0.82 to 14.0)	38	2.04 (0.99 to 4.21)	
621.4-812.7	110	54	2.69 (1.38 to 5.24)	14	6.16 (1.59 to 23.8)	40	2.16 (1.07 to 4.39)	
>812.7	110	58	2.59 (1.33 to 5.03)	32	12.02 (3.30 to 43.9)	26	1.33 (0.63 to 2.80)	
P _{trend}			0.015		3.9x10 ⁻⁶		0.76	
Continuous [†]	441	185	1.49 (1.09 to 2.04)	58	4.51 (2.59 to 7.84)	127	1.01 (0.72 to 1.43)	<0.001
Ang-2								
<1714.0	111	31	1.00 (ref)	7	1.00 (ref)	24	1.00 (ref)	
1714.0-2494.0	110	50	2.11 (1.12 to 3.96)	10	1.79 (0.60 to 5.40)	40	2.25 (1.14 to 4.45)	
2494.1-3545.7	110	43	1.36 (0.71 to 2.59)	15	2.05 (0.74 to 5.72)	28	1.15 (0.56 to 2.36)	
>3545.7	110	61	2.14 (1.15 to 3.98)	26	4.25 (1.60 to 11.3)	35	1.64 (0.82 to 3.27)	
P _{trend}			0.07		0.0017		0.52	
Continuous [†]	441	185	1.27 (0.98 to 1.64)	58	1.98 (1.33 to 2.95)	127	1.06 (0.79 to 1.41)	0.0036
CXCL12								
<1255.5	111	46	1.00 (ref)	9	1.00 (ref)	37	1.00 (ref)	
1255.5-1612.2	110	44	0.73 (0.40 to 1.35)	15	1.41 (0.53 to 3.72)	29	0.59 (0.30 to 1.15)	
1612.3-1924.6	110	38	0.68 (0.37 to 1.26)	16	1.91 (0.72 to 5.05)	22	0.46 (0.23 to 0.92)	
>1924.6	110	57	0.94 (0.52 to 1.71)	18	1.75 (0.67 to 4.59)	39	0.79 (0.41 to 1.52)	
P _{trend}			0.78		0.27		0.38	
Continuous [†]	441	185	1.02 (0.73 to 1.42)	58	1.37 (0.82 to 2.29)	127	0.93 (0.65 to 1.32)	0.16
CCL8								
<27.3	111	56	1.00 (ref)	11	1.00 (ref)	45	1.00 (ref)	
27.3-36.6	110	48	0.91 (0.51 to 1.61)	16	1.4 (0.57 to 3.45)	32	0.77 (0.41 to 1.45)	
36.7-50.3	110	43	0.91 (0.50 to 1.66)	15	1.63 (0.64 to 4.15)	28	0.75 (0.38 to 1.45)	
>50.3	110	38	1.03 (0.55 to 1.95)	16	2.02 (0.78 to 5.22)	22	0.79 (0.39 to 1.60)	
P _{trend}			0.89		0.15		0.53	
Continuous [†]	441	185	0.90 (0.66 to 1.23)	58	1.22 (0.74 to 2.01)	127	0.81 (0.58 to 1.15)	0.13
BMP-9								
<94.4	111	27	1.00 (ref)	7	1.00 (ref)	20	1.00 (ref)	
94.4-144.1	110	35	1.19 (0.60 to 2.35)	8	0.84 (0.27 to 2.62)	27	1.41 (0.66 to 2.99)	
144.2-213.7	110	49	1.27 (0.67 to 2.40)	14	1.15 (0.41 to 3.25)	35	1.36 (0.67 to 2.77)	
>213.7	110	74	1.61 (0.87 to 3.00)	29	2.14 (0.83 to 5.57)	45	1.41 (0.70 to 2.84)	
P _{trend}			0.12		0.025		0.46	
Continuous [†]	441	185	1.18 (1.00 to 1.40)	58	1.26 (0.95 to 1.68)	127	1.16 (0.97 to 1.40)	0.59

* Adjusted for age, sex, race, study year and calendar year of phlebotomy, and study center, as well as a score variable based on established risk factors for progression (M-protein concentration, serum free light-chain ratio, and Ig type) with categories defined as LC MGUS, non-IgM MGUS with no risk factors, and non-IgM MGUS with abnormal sFLC ratio and/or elevated M-protein concentration.

† Estimated risk corresponding to an approximate doubling in marker levels (1-unit increase in log₂-transformed levels).

Table 4. Estimated absolute risk and measures of risk prediction for the angiogenesis marker score and progression from MGUS to multiple myeloma, stratified by characteristics of MGUS

Measure	Light-chain MGUS	Low risk non-IgM MGUS	Intermediate/high risk non-IgM MGUS
No. progressed/overall	28/205	47/193	110/228
5-year follow-up:			
Estimated absolute risk of progression (95% CI)			
Low angiogenesis score*	0.2% (0 to 0.7%)	0.6% (0.2 to 1.5%)	1.4% (0.7 to 2.9%)
High angiogenesis score*	2.6% (1.2 to 5.2%)	2.3% (1.0 to 5.2%)	8.3% (5.2 to 13.0%)
P-value	0.012	0.10	0.0006
AUC†	0.78	0.65	0.71
MRS/year‡	0.19%	0.11%	0.61%
NNtest¶	1,059	1,745	330
Sensitivity§	82.5%	51.5%	74.3%
Specificity	72.8%	77.8%	67.0%
10-year follow-up:			
Estimated absolute risk of progression (95% CI)			
Low angiogenesis score*	1.0% (0.5 to 1.9%)	2.2% (1.3 to 3.6%)	6.4% (4.3 to 9.4%)
High angiogenesis score*	4.2% (2.3 to 7.6%)	5.3% (2.8 to 9.9%)	15.3% (10.3 to 22.2%)
P-value	0.016	0.076	0.007
Predictiveness in years 5-10**:			
AUC†	0.58	0.57	0.54
MRS/year‡	0.07%	0.11%	0.18%
NNtest¶	2,902	1,904	1,096
Sensitivity§	43.1%	36.0%	41.3%
Specificity	72.7%	72.7%	66.8%
Total follow-up:			
Estimated absolute risk of progression (95% CI)			
Low angiogenesis score*	1.0% (0.5 to 1.9%)	3.5% (2.2 to 5.4%)	8.9% (6.2 to 12.7%)
High angiogenesis score*	4.2% (2.3 to 7.6%)	8.2% (4.7 to 14.3%)	18.3% (12.5 to 26.3%)
P-value	0.016	0.056	0.015
AUC†	0.67	0.59	0.59
MRS/year‡	0.13%	0.12%	0.30%
NNtest¶	1,529	1,607	660
Sensitivity§	61.1%	40.4%	50.6%
Specificity	72.8%	77.8%	66.9%

* The following equation was used to calculate the angiogenesis score:

$0.23 \times \log_2(\text{EGF}) + 0.21 \times \log_2(\text{HGF}) + 0.14 \times \log_2(\text{Ang-2})$. We defined a high angiogenesis score as score > 5.25 based on the cut point for the top tertile of the overall distribution; those in the lower tertiles were defined as having a low angiogenesis score.

† AUC, area under the curve (Receiver Operating Characteristic), for a binary test is the average of sensitivity and specificity.(38)

‡ MRS, mean risk stratification, is the average change in yearly MGUS progression rate to multiple myeloma revealed by the angiogenesis score.(18) Although AUC measures classification accuracy and MRS measures risk-stratification, AUC is related to MRS by the following equation: $\text{MRS} = 4 \times (\text{AUC} - 0.5) \times p \times (1 - p)$, where p is the yearly MGUS progression rate to multiple myeloma. Thus a group with a high AUC, but a low progression rate, might have less risk stratification than a low AUC but a high progression rate. For example, at 5-years of follow-up, light-chain MGUS has

a superior AUC to intermediate/high-risk non-IgM MGUS (0.78 vs. 0.71), but less risk stratification (MRS of 0.19% vs. 0.61% progression per year to multiple myeloma). This is because the yearly progression rate to multiple myeloma is much lower for light-chain MGUS vs. intermediate/high-risk non-IgM MGUS (0.17% vs. 0.74%).

[¶] NNtest (which is computed as $2/\text{MRS}$) is the number of individuals with MGUS who would need to be tested using the angiogenesis panel to identify one additional case of progression to multiple myeloma per year of follow-up beyond those that would be identified by random selection. For example, we estimate that during the first five years of follow-up, 330 individuals with intermediate/high-risk MGUS would need to be tested with the angiogenesis panel to identify one additional case of progression to multiple myeloma per year. Although the AUCs for the angiogenesis panel are lower for intermediate/high-risk MGUS than for light-chain MGUS, this panel is more efficient at finding those who will progress to multiple myeloma among the intermediate/high-risk MGUS. This is attributable to the higher rate of progression in this group, which is taken into account by NNtest (and MRS) but not AUC.

[§] The fraction of multiple myeloma that occurred in those in the highest tertile of angiogenesis score

^{||} The complement of the fraction of multiple myeloma that occurred in those in the bottom two tertiles of angiogenesis score

^{**} Reported values are restricted to the follow-up time period of 5-10 years after the blood draw at which MGUS and the immunologic markers were assessed.

Figure 1. Summary of P-values from tests of association with MM risk for all markers

modeled as continuous variables. The top six markers were statistically significantly associated with MM using a false discovery rate of 10%, and the top four markers met a Bonferroni-corrected $P < 0.0011$.

Figure 2. Estimated cumulative incidence of progression from MGUS to MM stratified by characteristics of MGUS and high vs. low levels of a composite angiogenesis marker score (based on levels of EGF, HGF, and Ang-2). MGUS groups were defined as follows: (A) LC MGUS ($N_{\text{progressed}}/N_{\text{overall}}=28/205$), (B) non-IgM MGUS with no risk factors for progression ($N_{\text{progressed}}/N_{\text{overall}}=47/193$), and (C) non-IgM MGUS with an elevated M-protein concentration (≥ 15 g/L) and/or abnormal sFLC ratio (<0.26 or >1.65) ($N_{\text{progressed}}/N_{\text{overall}}=110/228$). The composite angiogenesis score was computed as follows: $0.23 \cdot \log_2(\text{EGF}) + 0.21 \cdot \log_2(\text{HGF}) + 0.14 \cdot \log_2(\text{Ang-2})$. A high angiogenesis marker score was defined as score >5.25 based on the cut point for the top tertile of the overall distribution; those in the lower tertiles were defined as having a low angiogenesis score.

Figure 1

Figure 2