Circulating Adiponectin Levels Differ Between Patients with Multiple Myeloma and its Precursor Disease

Jonathan N. Hofmann[1], Sham Mailankody[2], Neha Korde[2], Ye Wang[3], Nishant Tageja[4], Rene Costello[4], Adriana Zingone[4], Malin Hultcrantz[2], Michael N. Pollak[3], Mark P. Purdue[1], and Ola Landgren[2*]*

Objective: An increased risk of multiple myeloma (MM) has been observed among individuals with low prediagnostic circulating levels of adiponectin, a metabolic hormone that is typically underexpressed among those with overweight or obesity. To assess whether adiponectin may influence myeloma development or progression to frank MM, circulating adiponectin levels were compared across patients with different stages of MM and its precursor, monoclonal gammopathy of undetermined significance (MGUS).
Methods: Adiponectin was measured in 213 patients with MGUS, smoldering MM, or fully developed MM. Differences in adiponectin levels across patient groups were assessed using multivariate linear regression.
Results: Relative to MGUS patients, adiponectin levels were statistically significantly lower among those with smoldering and fully developed MM, both overall (16%-20% decrease; $P = 0.048$) and among those with IgG/IgA isotypes (26%-28% decrease; $P = 0.004$). Among MGUS patients, adiponectin levels were significantly lower for those with the higher-risk IgM isotype compared with those who had IgG/IgA isotypes (42% decrease; $P = 0.036$).
Conclusions: The findings of this study, the largest to investigate adiponectin levels in patients with different stages of MM and the first to evaluate associations with clinical characteristics, suggest that reduced expression of adiponectin may be associated with progression from MGUS to MM.

Introduction

Obesity has recently been classified by the International Agency for Research on Cancer as an established risk factor for multiple myeloma (MM), an incurable plasma cell malignancy (1). However, the biological mechanisms underlying this association have yet to be fully elucidated. In a recent pooled investigation of seven cohorts in the National Cancer Institute Cohort Consortium (2), we found that future risk of developing MM was lower among individuals with high circulating levels of adiponectin, a hormone with anti-inflammatory and insulin-sensitizing properties that is typically underexpressed in individuals with overweight or obesity. These results are consistent with evidence from experimental studies that host-derived adiponectin is tumor suppressive and a potential novel therapeutic target for MM and associated bone disease (3,4). Taken together, these findings suggest that adiponectin may play an important role in MM pathogenesis.

MM is consistently preceded by monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic, premalignant plasma cell disorder characterized by the presence of a monoclonal (M)-protein in serum. Several clinical characteristics are associated with the likelihood of progression from MGUS to clinically manifest MM and related lymphoproliferative malignancies, including the quantitative levels and type of serum M-protein (i.e., immunoglobulin [Ig] M vs. IgG or IgA), kappa and lambda free light chain (FLC) levels and ratio, the presence of immunoparesis, and the percentage of clonal plasma cells in the bone marrow (5,6). There is considerable interest in identifying novel biomarkers that can provide insights into the underlying mechanisms of progression from MGUS to MM and improve risk prediction models (7).

Data on the role of adiponectin in progression from MGUS to smoldering multiple myeloma (SMM) and frank MM are limited. To

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA. Correspondence: Jonathan N. Hofmann (hofmannjn@mail.nih.gov) [2] Myeloma Service, Memorial Sloan Kettering Cancer Center, New York, New York, USA [3] Oncology Department, McGill University and Segal Cancer Centre, Jewish General Hospital, Montreal, Quebec, Canada [4] Multiple Myeloma Section, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA.

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*Mark P. Purdue and Ola Landgren contributed equally to this work.

TABLE 1 Selected characteristics of study participants[a]

	MGUS	SMM	MM
n	84 (100)	104 (100)	25 (100)
Mean age at blood draw, y (SD)	63.4 (11.4)	61.1 (10.1)	61.1 (14.1)
Sex			
Female	42 (50)	41 (39)	11 (44)
Male	42 (50)	63 (61)	14 (56)
Race			
White	67 (80)	92 (88)	19 (76)
Black	14 (17)	10 (10)	5 (20)
Other	3 (4)	2 (2)	1 (4)
Mean BMI at blood draw, kg/m^2 (SD)	28.1 (4.7)	29.2 (6.6)	27.9 (5.2)
Immunoglobulin type			
IgG	56 (67)	71 (68)	15 (60)
IgA	15 (18)	21 (20)	7 (28)
IgM	8 (10)	1 (1)	0 (0)
Biclonal[b]	4 (5)	2 (2)	0 (0)
Light chain	1 (1)	9 (9)	3 (12)
Mean M-spike concentration, g/L (SD)	0.7 (0.5)	1.7 (1.0)	2.8 (1.6)
Serum free light chain ratio			
<0.26	4 (5)	26 (25)	9 (36)
0.26-1.65	41 (49)	8 (8)	1 (4)
>1.65	39 (46)	68 (67)	15 (60)
Mean percentage of clonal plasma cells in the bone marrow (SD)	7.3 (1.7)	21.4 (14.7)	52.9 (26.2)
Mayo risk stratification score			
0	37 (44)	0 (0)	
1	34 (40)	44 (42)	
2	13 (15)	54 (52)	
3	0 (0)	6 (6)	
Spanish risk stratification score			
0		15 (14)	
1		29 (28)	
2		60 (58)	
Median concentration of total adiponectin, μg/mL (IQR)[c]	13.0 (7.3-21.0)	10.9 (7.2-16.5)	11.4 (5.6-18.1)

[a]Reported as frequency (%) unless otherwise noted. Missing values were excluded from percentages.
[b]Among the biclonal MGUS patients, two had IgG and IgM, one had IgA and IgM, and another had IgG and IgA. Both of the biclonal SMM patients had IgG and IgM.
[c]$P = 0.14$, Kruskal-Wallis test.
MGUS, monclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma; MM, multiple myeloma. IQR, interquartile range.

assess the stage of MM development at which adiponectin's protective effects may act, we conducted an investigation comparing circulating adiponectin levels across patients with different stages of MM and its precursor disease.

Methods

Our investigation included patients with MGUS, SMM, and MM who were enrolled in two clinical studies between April 2010 and July 2013 (ClinicalTrials.gov identifiers NCT01109407 and NCT01402284). We measured levels of total and high-molecular-weight (HMW) adiponectin in serum samples collected at baseline from 213 patients (84 with MGUS, 104 with SMM, and 25 with MM). The assays were performed in duplicate using standard enzyme-linked immunosorbent assay (ELISA) methods with reagents purchased from R&D Systems, Inc. (Minneapolis, Minnesota). The lower limit of detection was 3.9 ng/mL for both total and HMW adiponectin. Samples from MGUS, SMM, and MM patients were distributed across all batches. Based on blinded replicate samples dispersed across all batches, the overall coefficients of variation were 5.7% for total adiponectin and 7.1% for HMW adiponectin. Because total and HMW adiponectin levels were almost perfectly correlated (Spearman $\rho = 0.99$), we only report the results for total adiponectin.

Differences in circulating levels of total adiponectin by stage of MM development (SMM and MM vs. MGUS) were assessed using

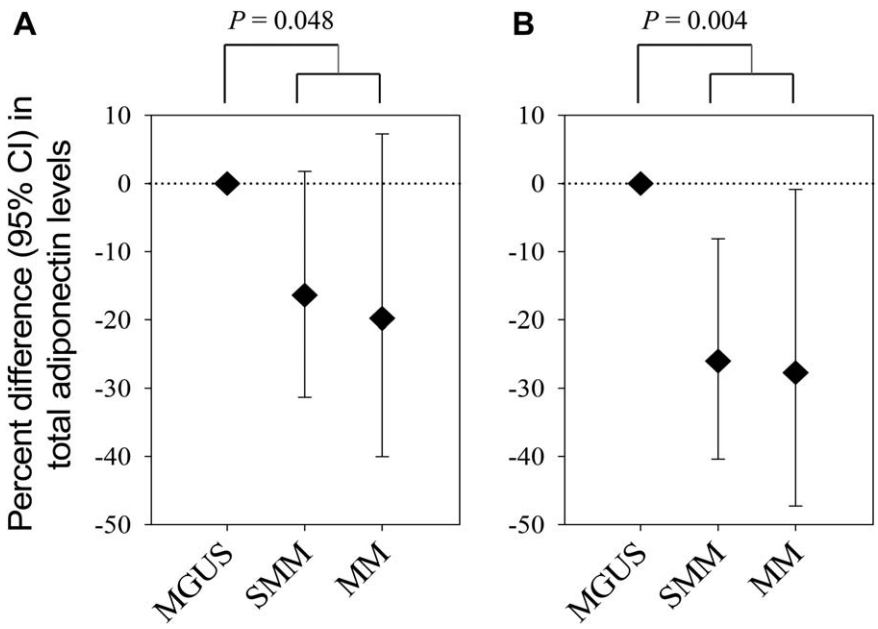

Figure 1 Estimated percent difference (95% CI) in circulating total adiponectin levels in patients with MGUS vs. those with SMM and MM (**A**) overall and (**B**) among those with IgG or IgA isotypes. Results are based on multivariable linear regression models adjusted for age, sex, race, and BMI. Adiponectin levels were significantly lower among SMM/MM patients compared with MGUS patients (overall, $P = 0.048$; among those with IgG/IgA isotypes, $P = 0.004$).

multivariable linear regression models; adiponectin levels were natural log-transformed and modeled as a continuous outcome variable, and age, sex, race (white, black, other), BMI (continuous) were included as covariates. Similar analyses were performed after stratifying by patient characteristics (e.g., age, sex, BMI category), and we also evaluated associations with clinical parameters (e.g., Ig isotype, M-protein concentration, FLC ratio, and percentage of clonal plasma cells in the bone marrow) within each of the patient subgroups. Finally, we computed odds ratios (ORs) and 95% CIs using polytomous logistic regression models to compare risk across disease stages. In these analyses, we estimated the risk corresponding to a doubling of total adiponectin levels (i.e., a 1-unit increase on the log base 2 scale) after adjustment for the same set of covariates as above.

This research was approved by the institutional review board at the National Cancer Institute's Center for Cancer Research, and all participants provided written informed consent.

Results

Characteristics of patients with MGUS, SMM, and MM are shown in Table 1; the distributions of these characteristics were generally similar across disease subgroups, although a slightly higher proportion of the MGUS patients were female compared with SMM and MM patients. Overall, we found that circulating total adiponectin levels were statistically significantly lower among patients with more advanced disease after adjustment for covariates ($P = 0.048$ for SMM/MM vs. MGUS; Figure 1). Compared to patients with MGUS, adiponectin levels were approximately 16% lower among SMM patients (95% CI: −31% to 2%) and 20% lower among those with MM (95% CI: −40% to 7%). We repeated these analyses after restricting the analysis to patients with the IgG and IgA isotypes, which typically progress to MM (whereas IgM MGUS progresses to Waldenström macroglobulinemia and light chain MGUS develops into light chain MM) (6). In these analyses, differences in adiponectin levels by disease stage were more apparent (compared with MGUS patients, adiponectin levels were 26% lower (95% CI:−40% to −8%) and 28% lower (95% CI: −47% to −1%) in SMM and MM patients, respectively ($P = 0.004$ for SMM/MM vs. MGUS). Analyses using logistic regression revealed that a doubling of total adiponectin levels was associated with statistically significant reduced odds of SMM/MM combined compared with MGUS (OR 0.68, 95% CI: 0.48 to 0.95); the corresponding ORs for SMM and MM were 0.68 (95% CI: 0.48 to 0.98) and 0.67 (95% CI: 0.40 to 1.12), respectively (Supporting Information Table S1).

The associations between adiponectin levels and disease stage did not differ by sex, age, or BMI category ($P_{int} \geq 0.23$), and results were similar when we restricted the analyses to non-Hispanic white patients (Supporting Information Table 2). Among MGUS patients, circulating adiponectin levels were significantly lower (−42%, 95% CI: −65% to −4%, $P = 0.036$) among patients with IgM MGUS compared to those with IgG or IgA MGUS; this difference is notable because the rate of progression to lymphoproliferative malignancies is typically higher for IgM MGUS than for non-IgM MGUS (6). No associations with adiponectin were observed for other clinical parameters in the MGUS, SMM, or MM patient subgroups, such as M-protein concentration, serum FLC ratio, or percentage of clonal plasma cells in the bone marrow (data not shown). Similarly, we did

not observe differences in adiponectin levels according to the assigned Mayo Clinic risk stratification score in MGUS patients (5) or for either the Mayo or Spanish scores in SMM patients (5,8).

Discussion

This study is, to our knowledge, the largest investigation of circulating adiponectin levels among patients with different stages of MM development, including the precursor condition MGUS, and the first to evaluate differences in adiponectin levels by immunoglobulin type and other clinical characteristics. We found that adiponectin levels were lower among patients with MM or SMM compared to those with MGUS both overall and among those with non-IgM disease. Our findings are generally consistent with a prior investigation that observed lower adiponectin levels among 20 MGUS patients who subsequently developed MM compared to 20 stable MGUS patients without MM progression (3). In that investigation, the association between low adiponectin levels and progression from MGUS to MM was apparent among women but not among men. We found that low adiponectin levels were associated with more advanced disease among women, although in our study a similar pattern of association was observed among men, and there was no evidence of heterogeneity in our findings by sex.

Mechanistic and animal studies have demonstrated that adiponectin induces apoptosis in malignant plasma cells and inhibits their survival and proliferation (3,4,9). Proposed antiproliferative mechanisms of adiponectin include activation of pathways related to suppression of lipogenesis (4) or inhibition of proinflammatory cytokines such as IL-6 or TNF-α and induced expression of anti-inflammatory cytokines such as IL-10 and IL-1RA (reviewed in Dalamaga and Christodoulatos (9)). Prior prospective epidemiologic studies have found an increased future risk of MM among those with low adiponectin levels, even after restricting to cases diagnosed ≥ 6 years after phlebotomy (2,10). These findings suggest that the relationship between adiponectin and MM may be etiologic in nature rather than being attributable to reverse causation, and altered adiponectin expression may partly explain the biological mechanisms of action through which obesity influences MM development. Low adiponectin levels have also been associated with risk of other hematological malignancies (9,11) as well as other obesity-related cancers (12-14).

In conclusion, our data suggest that reduced expression of adiponectin may be associated with progression from MGUS to clinically manifest MM. Although this is the largest such investigation to date, there were a limited number of patients in some strata, and, as such, our findings should be interpreted cautiously. Larger prospective studies of MGUS patients are needed to further elucidate the role of adiponectin in MM pathogenesis and to assess the potential utility of this biomarker in enhancing risk prediction models for progression from MGUS to MM.O

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