Prediagnosis biomarkers of insulin-like growth factor-1, insulin, and interleukin-6 dysregulation and multiple myeloma risk in the Multiple Myeloma Cohort Consortium

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Insulin-like growth factor-1 (IGF-1), insulin, and IL-6 are dysregulated in multiple myeloma pathogenesis and may also contribute to multiple myeloma etiology. To examine their etiologic role, we prospectively analyzed concentrations of serologic markers in 493 multiple myeloma cases and 978 controls from 8 cohorts in the Multiple Myeloma Cohort Consortium. We computed odds ratios (ORs) and 95% confidence intervals (CIs) for multiple myeloma per 1-SD increase in biomarker concentration using conditional logistic regression. We examined heterogeneity by time since blood collection (≤ 3, 4- ≤ 6, and > 6 years) in stratified models. Fasting IGF binding protein-1 concentration was associated with multiple myeloma risk within 3 years (OR, 95% CI per 1-SD increase: 2.3, 1.4-3.8, *P* = .001) and soluble IL-6 receptor level was associated within 6 years after blood draw ($OR_{\leq 3\ years}$, 95% CI, 1.4, 1.1-1.9, *P* = .01; $OR_{4-\leq 6\ years}$, 95% CI, 1.4, 1.1-1.7, *P* = .002). No biomarker was associated with longer-term multiple myeloma risk (ie, > 6 years). Interactions with time were statistically significant (IGF binding protein-1, *P*-heterogeneity = .0016; sIL6R, *P*-heterogeneity = .016). The time-restricted associations probably reflect the bioactivity of tumor and microenvironment cells in transformation from monoclonal gammopathy of undetermined significance or smoldering multiple myeloma to clinically manifest multiple myeloma.

Introduction

Multiple myeloma is a malignancy of mature plasma cells that was expected to account for 21 700 new cancer diagnoses and 10 710 cancer deaths in the United States in 2012.[1] The average 5-year relative survival for United States multiple myeloma patients is only 41% among patients diagnosed from 2001 to 2007.[2] Present knowledge of the etiology of multiple myeloma is inadequate for the development of prevention strategies. Established risk factors include age, African ancestry, male sex, and a family history of multiple myeloma or its precursor states, monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma.[2-4] Obesity is the first potentially modifiable established risk factor for multiple myeloma.[5,6] Of note, obesity is also associated with a higher risk of MGUS,[7] a condition that precedes all diagnoses of multiple myeloma.[8] However, the biologic mechanisms for the associations of obesity with MGUS and multiple myeloma have not been determined.

Obesity is associated with dysregulation of several biologic pathways, such as insulin-like growth factor-1 (IGF-1) and insulin.[9] Obesity is also a state of chronic inflammation, resulting in part from adipocyte secretion of the proinflammatory cytokine

Table 1. Selected characteristics of the study population by cohort (Pooled, ATBC, and HPFS)

	Pooled		ATBC[31]		HPFS[30]	
	Cases	Controls	Cases	Controls	Cases	Controls
No. of persons	493	978	46	92	29	58
Mean age at blood draw, y (SD)	63.3 (7.1)	63.3 (7.2)	58.6 (4.7)	58.5 (4.7)	62.7 (9.4)	62.6 (9.5)
Sex, N (%)						
Male	172 (34.9)	344 (35.2)	46 (100)	92 (100)	29 (100)	58 (100)
Female	321 (65.1)	634 (64.8)	NA	NA	NA	NA
Race, N (%)						
White	432 (87.6)	860 (87.9)	46 (100)	92 (100)	26 (89.7)	55 (94.8)
Black	32 (6.5)	62 (5.3)	NA	NA	1 (3.5)	NA
Asian	12 (2.4)	25 (2.6)	NA	NA	NA	NA
Other	17 (3.4)	31 (3.2)	NA	NA	2 (6.9)	3 (5.2)
Mean BMI at blood draw, kg/m^2 (SD)	27.4 (5.2)	27.2 (5.0)	26.7 (2.8)	26.2 (3.8)	26.3 (3.3)	26.0 (3.2)
< 18.5, N (%)	2 (0.4)	8 (0.8)	NA	NA	9 (31.0)	24 (41.4)
< 18.5-25, N (%)	163 (33.1)	342 (35.0)	10 (21.7)	37 (40.2)	15 (51.7)	27 (46.6)
< 25-30, N (%)	215 (43.6)	406 (41.5)	29 (63.0)	42 (45.7)	4 (13.8)	4 (6.9)
30+, N (%)	110 (22.3)	212 (21.7)	7 (15.2)	13 (14.1)	1 (3.5)	3 (5.2)
Missing, N (%)	3 (0.6)	10 (1.0)	NA	NA	NA	NA
Type of blood sample, N (%)						
Serum	70 (14.2)	140 (14.3)	46 (100)	92 (100)	NA	NA
Plasma, heparin	200 (40.6)	392 (40.1)	NA	NA	NA	NA
Plasma, EDTA	202 (41.0)	404 (41.3)	NA	NA	29 (100)	58 (100)
Plasma, sodium citrate	21 (4.3)	42 (4.3)	NA	NA	NA	NA
Fasting status, N (%)						
Fasting	354 (71.8)	709 (72.5)	39 (84.8)	78 (84.8)	21 (72.4)	42 (72.4)
Not fasting	139 (28.2)	269 (27.5)	7 (15.2)	14 (15.2)	8 (27.6)	16 (27.6)
Mean blood draw to diagnosis, y (range)	6.6 (0.3-18.1)	6.5 (−0.5 to 18.2)	9.8 (1.3-18.1)	9.8 (1.3-18.2)	6.6 (0.5-12.2)	6.6 (0.4-12.3)
≤ 3, N (%)	92 (18.7)	170 (17.9)	4 (8.7)	8 (8.7)	8 (28.6)	15 (27.3)
4- ≤ 6, N (%)	150 (30.6)	303 (31.8)	10 (21.7)	18 (19.6)	5 (17.9)	10 (18.2)
> 6, N (%)	249 (50.7)	479 (50.3)	32 (69.6)	66 (71.7)	15 (53.6)	30 (54.6)
Mean peripheral blood biomarker concentrations (SD)						
IGF-1, ng/mL	150 (52.5)	146.4 (49.4)	170.3 (38.7)	150.9 (44.5)	189.6 (49.2)	168.8 (46.2)
IGFBP-3, ng/mL	4490 (1203)	4423 (1170)	3859 (941)	3402 (1053)	4531 (1110)	4267 (991)
IGF-1/IGFBP-3 molar ratio	0.1 (0.03)	0.1 (0.03)	0.2 (0.04)	0.2 (0.03)	0.2 (0.03)	0.1 (0.02)
IGFBP-1, ng/mL	32.2 (24.9)	30.3 (32.3)	21.9 (14.9)	25.2 (14.7)	34.9 (29.5)	28.8 (19.9)
C-peptide, ng/mL	2.1 (1.5)	2.1 (1.6)	1.9 (0.8)	1.7 (1.1)	2.4 (1.1)	2.6 (1.6)
CRP, mg/L	3.5 (5.6)	3.7 (5.8)	4.1 (6.8)	4.2 (8.3)	1.7 (1.6)	1.3(1.4)
IL-6, pg/mL	2.2 (2.3)	2.5 (4.5)	3.0 (2.9)	3.7 (5.5)	1.7 (1.5)	1.5 (1.2)
sIL6R, pg/mL	41 871 (15 713)	39 278 (10 923)	36 502 (9674)	36 318 (8881)	43 084 (15 111)	36 087 (8244)

Percentages and counts may not sum to the total because of rounding and/or missing data.
NA indicates not applicable.

IL-6.[10] IGF-1, insulin, and IL-6 are potent growth factors in multiple myeloma and promote cell survival and migration.[11-17] As such, molecules in these growth factor pathways have been explored as potential targets for novel therapies to treat multiple myeloma.[18] It is plausible that dysregulation of IGF-1, insulin, and IL-6 also contributes to the etiology of multiple myeloma and underlies the association of obesity with multiple myeloma.

Therefore, to evaluate the relations between IGF-1, insulin, and IL-6 dysregulation and multiple myeloma risk, we report the first prospective measurement of peripheral blood markers of IGF-1 bioactivity (total IGF-1 and IGF binding protein-1 [IGFBP-1], and IGFBP-3, which modulate access of IGF-1 to its tissue receptors[19]), insulin secretion (C-peptide[19]), and IL-6 bioactivity and inflammation (IL-6, the soluble IL-6 receptor [sIL6R]; ie, CD126), which binds circulating IL-6 and enhances its ability to bind target cells,[20] and C-reactive protein (CRP), an acute phase protein secreted by the liver in response to IL-6.[21] We prospectively examined these markers with risk of multiple myeloma in the Multiple Myeloma Cohort Consortium, a collaboration among 8 large population-based cohorts affiliated with the National Cancer Institute Cohort Consortium (http://epi.grants.cancer.gov/Consortia/cohort.html).

Methods

Study population

The participants in the present analysis derive from 8 large, actively followed cohorts with archived prediagnosis peripheral blood samples in the Multiple Myeloma Cohort Consortium (Tables 1-3). Collaborating cohorts include the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, the Health Professionals Follow-up Study (HPFS), the Melbourne Collaborative Cohort Study (MCCS), the Nurses' Health Study (NHS), the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), the Singapore Chinese Health Study (SCHS), the Women's Health Initiative (WHI), and the Women's Health Study (WHS). The details of each cohort's design and methods are published in detail elsewhere.[22-32] Information on lifestyle, medical history, and other items of interest was obtained from study questionnaires. Newly reported diagnoses or deaths because of multiple myeloma were confirmed by medical record review and/or by linkage to registries. Informed consent was obtained from participants in writing or (for HPFS and NHS participants) was implied by the return of the completed enrollment questionnaires. Written informed consent for biomarker studies was obtained on study enrollment or at the time of blood collection. The original cohort study protocols were approved

Table 2. Selected characteristics of the study population by cohort (MCCS, NHS, and PLCO)

	MCCS[23]		NHS[22,25]		PLCO[26]	
	Cases	Controls	Cases	Controls	Cases	Controls
No. of persons	55	110	39	71	96	192
Mean age at blood draw, y (SD)	59.5 (7.4)	59.5 (7.4)	59.4 (6.0)	58.3 (5.9)	63.9 (5.2)	63.8 (5.0)
Sex, N (%)						
Male	31 (56.4)	62 (56.4)	NA	NA	60 (62.5)	120 (62.5)
Female	24 (43.6)	48 (43.6)	39 (100)	71 (100)	36 (37.5)	72 (37.5)
Race, N (%)						
White	55 (100)	110 (100)	39 (100)	71 (100)	87 (90.6)	174 (90.6)
Black	NA	NA	NA	NA	4 (4.2)	8 (4.2)
Asian	NA	NA	NA	NA	2 (2.1)	4 (2.1)
Other	NA	NA	NA	NA	3 (3.1)	6 (3.1)
Mean BMI at blood draw, kg/m^2, (SD)	27.4 (4.2)	26.8 (3.9)	26.1 (4.7)	25.4 (4.5)	28.4 (6.0)	27.5 (4.7)
$<$ 18.5, N (%)	1 (1.8)	NA	1 (2.6)	NA	NA	2 (1.0)
$<$ 18.5-25, N (%)	16 (29.1)	38 (34.6)	16 (41.0)	36 (50.7)	27 (28.1)	53 (27.6)
$<$ 25-30, N (%)	24 (43.6)	50 (45.5)	14 (35.9)	20 (28.2)	41 (42.7)	95 (49.5)
30+, N (%)	14 (25.5)	22 (20.0)	6 (15.4)	10 (14.1)	28 (29.2)	40 (20.8)
Missing, N (%)	NA	NA	2 (5.1)	5 (7.0)	NA	2 (1.0)
Type of blood sample, N (%)						
Serum	NA	NA	NA	NA	NA	NA
Plasma, heparin	55 (100)	110 (100)	39 (100)	71 (100)	96 (100)	192 (100)
Plasma, EDTA	NA	NA	NA	NA	NA	NA
Plasma, sodium citrate	NA	NA	NA	NA	NA	NA
Fasting status, N (%)						
Fasting	42 (76.4)	84 (76.4)	27 (69.2)	56 (78.9)	NA	NA
Not fasting	13 (23.6)	26 (23.6)	12 (30.8)	15 (21.1)	96 (100)	193 (100)
Mean blood draw to diagnosis, y (range)	8.2 (1.1-16.8)	8.2 (1.2-16.8)	9.4 (1.5-16.0)	9.8 (2.8-16.1)	6.4 (1.2-12.3)	6.4 (1.2-12.5)
$\leq$ 3, N (%)	6 (10.9)	10 (9.1)	2 (5.3)	1 (1.9)	9 (9.4)	17 (8.9)
4- $\leq$ 6, N (%)	14 (25.5)	30 (27.3)	6 (15.8)	10 (18.9)	37 (38.5)	75 (39.1)
$>$ 6, N (%)	35 (63.6)	70 (63.6)	30 (79.0)	42 (79.3)	50 (52.1)	100 (52.1)
Mean peripheral blood biomarker concentrations (SD)						
IGF-1, ng/mL	158.9 (52.9)	161.5 (50.2)	151.5 (51.2)	151.3 (47.4)	159.9 (58.5)	158.1 (52.0)
IGFBP-3, ng/mL	4777 (1404)	4872 (1170)	5300 (1299)	5327 (1293)	4708 (1285)	4660 (1069)
IGF-1/IGFBP-3 molar ratio	0.1 (0.03)	0.1 (0.03)	0.1 (0.02)	0.1 (0.03)	0.1 (0.03)	0.1 (0.03)
IGFBP-1, ng/mL	34.4 (23.6)	25.4 (15.2)	20.9 (13.1)	24.4 (15.4)	NA	NA
C-peptide, ng/mL	1.7 (1.2)	1.5 (0.8)	2.2 (1.4)	2.5 (1.7)	2.8 (1.8)	3.0 (2.5)
CRP, mg/L	3.1 (7.8)	2.3 (3.0)	2.6 (3.8)	2.8 (3.9)	3.4 (4.8)	3.7 (6.0)
IL-6, pg/mL	1.7 (1.4)	2.4 (3.6)	2.2 (2.9)	1.6 (1.5)	2.3 (1.7)	2.4 (2.3)
sIL6R, pg/mL	42 252 (11 293)	41 908 (10 234)	42 793 (10 563)	40 909 (10 879)	43 495 (11 683)	41 909 (10 816)

Percentages and counts may not sum to the total because of rounding and/or missing data.
NA indicates not applicable.

by the institutional review board or human subjects committee at each host institution. The protocol for the present study, conducted in accordance with the Declaration of Helsinki, was also approved by the institutional review board of each collaborating institution or was considered within the scope of the original study protocol and participant informed consent.

Blood specimen collection

The peripheral blood samples used for the present studies were collected from study participants at baseline and/or repeat visits to the study centers (ATBC, MCCS, PLCO, SCHS, WHI) or by mailing phlebotomy kits to cohort members who had indicated a willingness to participate (HPFS, NHS, WHS). The detailed methods used for the collection and processing of cohort participants' blood samples are published elsewhere.[22-32]

Case definition

We included all cohort participants with an archived blood sample and a confirmed incident primary diagnosis of multiple myeloma (ICDA = 203, ICD-O-2 = 9731, 9732, 9830, or ICD-O-3 = 9731-9734). Date of diagnosis was restricted to cases at least 3 months after the date of blood draw and through 2006. A potential case was excluded if the multiple myeloma diagnosis date was unknown, or if the patient had a history of cancer other than nonmelanoma skin cancer before the multiple myeloma diagnosis date.

Control selection

For each eligible multiple myeloma case, 2 persons with an archived peripheral blood sample and no history of cancer (other than nonmelanoma skin cancer) as of the respective case's diagnosis date were matched on cohort of origin, month and year of birth ($\pm$ 12 months), sex, race (white, black, Asian, other), type of blood sample (serum or plasma with heparin, EDTA, or sodium citrate), month and year of blood collection ($\pm$ 2 months), time of day of blood draw ($\pm$ 2 hours), and (when available) fasting status at blood draw ($<$ 8, $\geq$ 8 hours since the last meal).

Biomarker measurement

The aliquots from matched sets of multiple myeloma patients and controls were assayed together in the same batch and ordered randomly within triplet sets. Blinded repeat quality control (QC) samples amounting to $\sim$ 10% of the overall sample count were placed randomly in each batch of study samples. The laboratory technicians were blinded to the status of the samples.

Markers of IGF-1 dysregulation and hyperinsulinemia. Peripheral blood concentration of total IGF-1, IGFBP-1 and IGFBP-3, and C-peptide were measured by ELISA. Reagents for the total IGF-1, IGFBP-1, and IGFBP-3 ELISAs were purchased from Diagnostic System Laboratories of Beckman Coulter. Reagents for the C-peptide ELISA were purchased from

Table 3. Selected characteristics of the study population by cohort (SCHS, WHI, and WHS)

	SCHS[24,27]		WHI[32]		WHS[29]	
	Cases	Controls	Cases	Controls	Cases	Controls
No. of persons	10	19	197	394	21	42
Mean age at blood draw, y (SD)	66.5 (6.6)	66.4 (6.7)	66.4 (6.5)	66.5 (6.5)	58.7 (6.9)	58.6 (6.7)
Sex, N (%)						
Male	6 (60.0)	12 (63.2)	NA	NA	NA	NA
Female	4 (40.0)	7 (36.8)	197 (100)	394 (100)	21 (100)	42 (100)
Race, N (%)						
White	NA	NA	160 (81.2)	320 (81.2)	19 (90.5)	38 (90.5)
Black	NA	NA	26 (13.2)	52 (13.2)	1 (4.8)	2 (4.8)
Asian	10 (100)	19 (100)	NA	NA	NA	2 (4.8)
Other	NA	NA	11 (5.6)	22 (5.6)	1 (4.8)	NA
Mean BMI at blood draw, kg/m^2 (SD)	23.7 (2.6)	23 (3.0)	27.8 (5.7)	28.2 (5.7)	25.7 (4.1)	26.6 (4.4)
< 18.5, N (%)	NA	2 (10.5)	NA	4 (1.0)	NA	NA
< 18.5-25, N (%)	8 (80.0)	11 (57.9)	66 (33.5)	128 (32.5)	11 (52.4)	15 (35.7)
< 25-30, N (%)	2 (20.0)	6 (31.6)	82 (41.6)	146 (37.1)	8 (38.1)	20 (47.6)
30+, N (%)	NA	NA	49 (24.9)	116 (29.4)	2 (9.5)	7 (16.7)
Missing, N (%)	NA	NA	NA	NA	NA	NA
Type of blood sample, N (%)						
Serum	NA	NA	24 (12.2)	48 (12.2)	NA	NA
Plasma, heparin	10 (100)	19 (100)	NA	NA	NA	NA
Plasma, EDTA	NA	NA	173 (87.8)	346 (87.8)	NA	NA
Plasma, sodium citrate	NA	NA	NA	NA	21 (100)	42 (100)
Fasting status, N (%)						
Fasting	10 (100)	19 (100)	197 (100)	394 (100)	18 (85.7)	36 (85.7)
Not fasting	NA	NA	NA	NA	3 (14.3)	6 (14.3)
Mean blood draw to diagnosis, y (range)	2.5 (0.3-5.2)	2.5 (0.2-5.1)	5.2 (0.5-11.1)	5.2 (−0.5 to 11.3)	6.1 (2.2-9.8)	6.1 (2.2-9.8)
≤ 3, N (%)	6 (60.0)	11 (57.9)	51 (25.9)	98 (25.2)	6 (28.6)	10 (23.8)
4- ≤ 6, N (%)	4 (40.0)	8 (42.1)	69 (35.0)	140 (36.0)	5 (23.8)	12 (28.6)
> 6, N (%)	NA	NA	77 (39.1)	151 (38.8)	10 (47.6)	20 (47.6)
Mean peripheral blood biomarker concentrations (SD)						
IGF-1, ng/mL	136.2 (43.9)	133.2 (44.1)	136.5 (48.5)	133.2 (46.7)	112.4 (31.4)	134.7 (43.5)
IGFBP-3, ng/mL	4456 (1213)	4356 (1427)	4356 (1059)	4313 (1046)	3825 (744)	4152 (901)
IGF-1/IGFBP-3 molar ratio	0.1 (0.03)	0.1 (0.03)	0.1 (0.03)	0.1 (0.03)	0.1 (0.03)	0.1 (0.03)
IGFBP-1, ng/mL	71.0 (47.0)	64.0 (137.9)	33.2 (24.7)	32.0 (26.6)	31.0 (14.6)	28.2 (26.3)
C-peptide, ng/mL	5.7 (5.6)	5.1 (2.2)	1.7 (0.8)	1.7 (0.8)	2.0 (1.1)	2.1 (1.1)
CRP, mg/L	1.0 (1.2)	1.2 (1.9)	3.9 (5.3)	4.5 (6.4)	4.7 (8.7)	3.7 (4.8)
IL-6, pg/mL	2.4 (2.1)	1.9 (2.0)	2.2 (2.6)	2.7 (5.8)	NA	NA
sIL6R, pg/mL	65 025 (70 369)	30 364 (6864)	40 665 (12 232)	38 537 (11 544)	NA	NA

Percentages and counts may not sum to the total because of rounding and/or missing data.
NA indicates not applicable.

Millipore Corporation. Only fasting blood samples were tested for IGFBP-1 concentration.[19] The lowest detectable concentration was 19 ng/mL IGF-1, 1.0 ng/mL IGFBP-1, 2.1 ng/mL IGFBP-3, and 0.2 ng/mL C-peptide.

Markers of IL-6 dysregulation or inflammation. Peripheral blood concentrations of IL-6, sIL6R, and CRP were assessed using commercially available ELISA kits (Quantikine HS Human IL-6 Immunoassay and Quantikine HS Human IL-6 sR Immunoassay, R&D Systems) or a high-sensitivity nephelometric/turbidimetric assay (HPQ High Sensitive CRP reagents from DiaSorin; read on a Hitachi 917 analyzer) according to the manufacturer's directions. The limit of detection was 0.039 pg/mL IL-6, 6.5 pg/mL sIL6R, and 0.05 mg/L CRP.

Statistical analysis

To diminish the influence of cohort-related variability in laboratory results on a pooled analyses across participating cohorts, we performed a statistical "cohort adjustment" to the original laboratory values for each biomarker before other statistical analyses, using methods developed by Rosner et al.[33,34] Briefly, for each biomarker, we used multivariable linear regression in the pooled controls to model the association of cohort with the natural log-transformed concentration, with adjustment for age (years) and sex. The computational steps by which we obtained a cohort-specific correction factor from the model output and applied the correction factor to the original laboratory values by cohort are detailed in supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). All subsequent analyses of the biomarker data, including the evaluation of the QC sample results, were performed using the cohort-adjusted biomarker concentrations.

We computed coefficients of variation (CV%) and intraclass correlation coefficients in the QC samples to assess the performance of the assays within and across the laboratory batches and study populations. We defined extreme outlier values as those with a cohort-adjusted concentration higher than 3 interquartile ranges above the 75th percentile cut-point in the pooled controls. Samples with extreme outlier values were omitted from analyses of the given analyte. We assigned the value of one-half the assay limit of detection to study samples with a recorded concentration below the assay limit for IGFBP-1 (4 samples) or C-peptide (2 samples). We computed pairwise Spearman partial correlation coefficients among the pooled controls for each possible pair of markers, using the natural log transformation of the cohort-adjusted concentration, with adjustment for the matching factors and for body mass index (BMI, kg/m^2) at blood collection.

To assess the association of a given biomarker with risk of multiple myeloma, we obtained odds ratios (ORs) and 95% confidence intervals (CIs) from conditional logistic regression models that were performed in the pooled study population. We modeled each analyte individually and examined the molar ratio of total IGF-1 to IGFBP-3 concentration. We modeled the incremental change in risk of multiple myeloma per SD

increase in the concentration of a given biomarker using the SD calculated among the pooled control samples. We also explored a possible dose-response by modeling quartile of a given biomarker level, using quartile categories defined from the marker's distribution in the pooled control samples. *P* values for statistical tests of linear trend were obtained from models that included an ordinal variable defined from the median of the corresponding quartile. The first statistical models included only one biomarker variable. We subsequently explored the independence of the markers that demonstrated an association with multiple myeloma in multimarker models. All models were conditioned on the matching factors and controlled for potential confounding by BMI at blood collection (kg/m^2).

To explore the influence of time since blood collection on the association of the biomarkers with multiple myeloma risk, we performed conditional logistic regression models within strata defined by interval from blood collection to the matched case diagnosis date (≤ 3, 4- ≤ 6, > 6 years). The follow-up intervals were chosen to achieve as much statistical balance as possible while also using relatively intuitive whole-year cut-points. Specifically, we first dichotomized the follow-up interval at 6 years (close to the population median interval) and then further divided the earliest 6-year period in half. To assess the statistical significance of the observed heterogeneity of biomarker-multiple myeloma associations by follow-up time interval, we used likelihood ratio tests that compared the BMI-adjusted main effects model to a model that also included a cross-product term for biomarker concentration and years of follow-up. We also evaluated potential effect measure modification by BMI at blood draw (< 25, ≥ 25 kg/m^2), sex, and race (white, black, Asian, other).

Results

We observed very good assay reproducibility as characterized by assay CV% computed in the pooled QC samples: IGF-1 (7%), IGFBP-1 (8%), IGFBP-3 (5%), C-peptide (13%), IL-6 (13%), sIL6R (8%), and CRP (12%). Study-specific CV% varied only slightly from the pooled data values and did not indicate any population-specific limitations to assay reproducibility (data not shown). Intraclass correlation coefficients were > 0.90 for all analytes (data not shown). Records from at most 5% of cases and controls were omitted from any given analysis because of outlier biomarker values. The largest number of omissions because of outlier values occurred in the IL-6 (20 cases, 45 controls) and CRP analysis (19 cases, 53 controls); few outlier values were observed for the remaining analytes (range, 0-7 cases, 0-17 controls). Biomarker concentrations did not vary systematically by sample type among controls (data not shown).

The pooled study population included 493 persons with confirmed, incident diagnoses of multiple myeloma and 978 matched controls (Table 1). IGFBP-1 concentrations were measured only in blood samples with known fasting status (ie, ≥ 8 hours since last meal at time of blood draw: 354 cases, 709 controls). The distributions of age, sex, race, fasting status, and timing of blood draw were comparable among cases and controls because of the matched design. BMI at blood draw also did not differ notably between cases and controls (Table 1).

Using conditional logistic regression models, we observed that fasting prediagnosis concentration of IGFBP-1 was positively associated with risk of multiple myeloma (Table 4). For each SD increase in IGFBP-1 concentration, the risk of multiple myeloma increased $\sim 20\%$ (OR, 95% CI, 1.2, 1.0-1.5; $P = .08$). Of interest, we observed a highly significant interaction of IGFBP-1 concentration with years from blood collection to case diagnosis (*P*-heterogeneity = .0016). When modeled within intervals of time from blood collection to case diagnosis, the association of IGFBP-1 concentration with multiple myeloma was restricted to the 3 years after blood draw (OR, 95% CI per SD increase: 2.3, 1.4-3.8, $P = .001$; Table 4). No association of fasting IGFBP-1 concentration with multiple myeloma was apparent for cases diagnosed in the follow-up intervals of 4- ≤ 6 or > 6 years after blood draw. In analyses of quartile of fasting IGFBP-1 level, compared with persons with IGFBP-1 concentrations in the lowest quartile (Q1), those with concentrations in the highest quartile (Q4) had a $> 250\%$ increase in risk of a multiple myeloma diagnosis within 3 years of blood draw (OR, 95% CI, 3.6, 1.4-9.5; *P*-trend = .002). In contrast, quartile of IGFBP-1 was not associated with risk of a multiple myeloma diagnosis 4- ≤ 6 or > 6 years after blood draw. Peripheral blood levels of total IGF-1 (supplemental Table 1), IGFBP-3 (supplemental Table 2), the molar ratio of IGF-1: IGFBP-3 concentration (supplemental Table 3), and C-peptide (supplemental Table 4) were not significantly associated with risk of multiple myeloma in any follow-up interval.

Among the markers of IL-6 dysregulation and inflammation, sIL6R was most strongly associated with multiple myeloma risk. Multiple myeloma risk increased $\sim 20\%$ per 1-SD increase in sIL6R concentration (OR, 95% CI, 1.2, 1.1-1.3, $P = .0005$; Table 5). As for concentration of IGFBP-1, we observed a statistically significant interaction of sIL6R level with time from blood draw to multiple myeloma diagnosis (*P*-heterogeneity = .016). The association of sIL6R with multiple myeloma appeared to be restricted to the 6 years after blood draw ($OR_{\leq 3\ years}$, 95% CI per 1-SD increase: 1.4, 1.1-1.9, $P = .01$; $OR_{4-\leq 6\ years}$, 95% CI per 1-SD increase: 1.4, 1.1-1.7, $P = .002$) and was stable across that follow-up window. Quartile of sIL6R concentration demonstrated a statistically significant linear trend of increased risk of multiple myeloma with increasing sIL6R concentration in the 6 years after blood draw (≤ 3 years, *P*-trend = .04; 4- ≤ 6 years, *P*-trend = .01). Within 3 years after blood draw, Q3 (vs Q1: OR, 95% CI, 2.1, 0.9-5.0) and Q4 concentrations of sIL6R (vs Q1: 2.2, 1.0-5.3) were strongly associated with multiple myeloma risk, whereas only Q4 concentrations were significantly associated with risk of a multiple myeloma diagnosis 4- ≤ 6 years after blood draw (vs Q1: OR, 95% CI, 1.9, 1.0-3.3). sIL6R levels were not associated with multiple myeloma risk more than 6 years after blood draw. Concentrations of IL-6 (supplemental Table 5) and CRP (supplemental Table 6) were not significantly associated with multiple myeloma in any follow-up time interval.

We next evaluated the independence of the associations of IGFBP-1 and sIL6R concentration with multiple myeloma risk. The mutually adjusted ORs that we observed across the entire follow-up period (per 1-SD increase, $OR_{IGFBP\text{-}1}$: 1.2, 95% CI, 1.0-1.5; OR_{sIL6R}: 1.3, 95% CI,1.1-1.4), and within 3 years (per 1-SD increase, $OR_{IGFBP\text{-}1}$: 2.0, 95% CI, 1.2-3.4; OR_{sIL6R}: 1.3, 95% CI, 1.0-1.8) and 4- ≤ 6 years after blood draw (per 1-SD increase, $OR_{IGFBP\text{-}1}$: 1.2, 95% CI, 0.8-1.8; OR_{sIL6R}: 1.5, 95% CI,1.2-2.0) were similar to those observed in the single-marker models. In the mutually adjusted models, IGFBP-1 and sIL6R concentrations were not associated with risk of a multiple myeloma diagnosis > 6 years after blood draw (data not shown). Further adjustment for biomarkers that were strongly correlated with IGFBP-1 or sIL6R in the study population (supplemental Table 7) did not change the observed associations of IGFBP-1 or sIL6R with multiple myeloma risk in any follow-up interval (data not shown).

We next assessed possible variation in the association of markers across strata of BMI, sex, and race. We did not observe heterogeneity in the association of the serologic markers with multiple myeloma by BMI at blood draw (< 25, ≥ 25 kg/m^2), race

Table 4. Association of cohort-corrected fasting peripheral blood concentration of IGFBP-1 (ng/mL) with risk of multiple myeloma, in the pooled study population and by year from blood draw to multiple myeloma diagnosis

Fasting IGFBP-1 concentration, ng/mL*	Case	Control	OR (95% CI)†	*P*‡
Per SD increase (SD = 32.29 ng/mL)§				
Pooled population	354	709	1.2 (1.0-1.5)	.08
By years, blood draw to multiple myeloma diagnosis				
≤ 3	76	141	2.3 (1.4-3.8)	.001
4- ≤ 6	104	213	1.1 (0.8-1.6)	.50
> 6	170	329	1.0 (0.7-1.4)	.83
Per quartile increase‖				
Pooled population	354	709		
Q1	80	176	1.0 (ref)	
Q2	72	178	0.9 (0.6-1.3)	
Q3	94	176	1.2 (0.8-1.8)	
Q4	107	171	1.4 (0.9-2.1)	.04
By years, blood draw to multiple myeloma diagnosis				
≤ 3	76	141		
Q1	14	44	1.0 (ref)	
Q2	14	37	1.0 (0.4-2.6)	
Q3	18	34	1.7 (0.7-4.2)	
Q4	30	26	3.6 (1.4-9.5)	.002
4- ≤ 6	104	213		
Q1	24	51	1.0 (ref)	
Q2	16	54	0.6 (0.3-1.3)	
Q3	31	49	1.3 (0.6-2.7)	
Q4	33	59	1.2 (0.6-2.6)	.28
> 6	170	329		
Q1	42	74	1.0 (ref)	
Q2	42	84	0.8 (0.5-1.5)	
Q3	45	89	0.9 (0.5-1.7)	
Q4	41	82	1.0 (0.5-1.8)	.83

*Measurement of IGFBP-1 concentration was restricted to blood samples known to be collected ≥ 8 hours after the last meal.

†ORs and 95% CIs were calculated in conditional logistic regression models that were stratified on matched set and further adjusted for BMI at blood draw (kg/m^2). Persons with outlier IGFBP-1 values (ie, cohort-corrected levels > 121.68 ng/mL) and those with missing BMI data were excluded.

‡The *P* values associated with the per-SD ORs and CIs are Wald *P* values estimated in the corresponding conditional logistical regression models. The *P* values associated with the quartile-based results are from trend tests performed in conditional logistic regression models that were identical to those run for the quartile variables (ie, stratified on matched set and further adjusted for BMI at blood draw; kg/m^2). Persons with outlier values and those with missing BMI data were excluded.

§The SD was obtained from the distribution of cohort-corrected values in the pooled fasting controls.

‖Quartile of IGFBP-1 level was determined from the distribution of cohort-corrected values among the pooled controls.

(white, black, Asian, other), or sex (data not shown). We had insufficient statistical power to examine these potential interactions separately by time to diagnosis.

Discussion

The present study is the first from the Multiple Myeloma Cohort Consortium and, to our knowledge, the first prospective evaluation of peripheral blood markers of IGF-1, insulin, and IL-6 dysregulation in the etiology of multiple myeloma. Peripheral blood concentration of IGFBP-1 was positively associated with risk of multiple myeloma in the 3 years after blood draw. Concentration of sIL6R was independently associated with multiple myeloma risk in the 6 years after blood collection. The heterogeneity of the biomarker associations with multiple myeloma by follow-up time interval was statistically significant. In contrast, the findings did not appear to vary by BMI at blood draw or other potential effect modifiers, but we were unable to evaluate interactions within the follow-up intervals in which the biomarker associations were strongest. None of the biomarkers was associated with multiple myeloma risk > 6 years after blood collection, and no associations were apparent for concentration of total IGF-1, IGFBP-3, the IGF-1/IGFBP-3 molar ratio, C-peptide, CRP, or IL-6.

We hypothesized that persons with up-regulated IGF-1 and insulin signaling would have a greater risk of multiple myeloma; the hypothesis is plausible because those hormones are potent promoters of the growth, survival, and migration of multiple myeloma cells according to studies performed in human myeloma cell lines.[11,12,15-17] IGFBP-1 concentration is usually inversely correlated with IGF-1 and insulin levels[19,35,36]; thus, the positive association that we observed between IGFBP-1 and multiple myeloma risk is contrary to expectation. Further, we found no association with multiple myeloma for the remaining IGF-1 and insulin markers.

We note that the concentrations of IGFBP-1 (and other biomarkers) that we analyzed were measured in peripheral blood samples and probably reflect secretion by multiple cell types in tissue(s) of origin that cannot be identified with the data available to the study. Nonetheless, because the IGFBP-1 association was restricted to the 3 years immediately after blood draw, it is plausible that the finding reflects in part the presence of a nascent tumor and the highly bioactive tumor microenvironment associated with multiple myeloma pathogenesis.[37,38] We did not have information on M-protein or other clinical phenomena in our study participants, but we suspect that many of the participants who developed multiple myeloma within 3 years of blood collection had undetected smoldering multiple myeloma or progressing MGUS at blood draw. In this regard, our findings for IGFBP-1 are somewhat consistent with clinical observations that multiple myeloma and MGUS patients have lower circulating IGF-1 levels than healthy

Table 5. Association of cohort-corrected peripheral blood concentration of sIL6R (pg/mL) with risk of multiple myeloma, in the pooled study population and by year from blood draw to multiple myeloma diagnosis

sIL6R concentration, pg/mL	N		OR (95% CI)*	*P*†
	Case	Control		
Per SD increase (SD = 10 402.77 pg/mL)‡				
Pooled population	469	926	1.2 (1.1-1.3)	.0005
By years, blood draw to multiple myeloma diagnosis				
≤ 3	86	158	1.4 (1.1-1.9)	.01
4- NA ≤ 6	142	287	1.4 (1.1-1.7)	.002
> 6	238	457	1.0 (0.9-1.2)	.62
Per quartile increase§				
Pooled population	469	926		
Q1	100	231	1.0 (ref)	
Q2	105	232	1.0 (0.7-1.4)	
Q3	118	232	1.2 (0.8-1.6)	
Q4	146	231	1.5 (1.1-2.0)	.01
By years, blood draw to multiple myeloma diagnosis				
≤ 3	86	158		
Q1	16	47	1.0 (ref)	
Q2	12	34	1.2 (0.4-3.1)	
Q3	25	36	2.1 (0.9-5.0)	
Q4	33	41	2.2 (1.0-5.3)	.04
4- ≤ 6	142	287		
Q1	29	68	1.0 (ref)	
Q2	28	88	0.8 (0.4-1.4)	
Q3	33	65	1.2 (0.6-2.2)	
Q4	52	66	1.9 (1.0-3.3)	.01
> 6	239	456		
Q1	54	109	1.0 (ref)	
Q2	65	105	1.3 (0.8-2.0)	
Q3	59	125	1.0 (0.6-1.5)	
Q4	61	117	1.1 (0.7-1.7)	.96

*ORs and CIs were calculated in conditional logistic regression models that were stratified on matched set and further adjusted for BMI at blood draw (kg/m^2). Persons with outlier sIL6R values (ie, cohort-corrected levels > 88 888.83 pg/mL) and those with missing BMI data were excluded.

†The *P* values associated with the per-SD ORs and CIs are Wald *P* values estimated in the corresponding conditional logistical regression models. The *P* values associated with the quartile-based results are from trend tests performed in conditional logistic regression models that were identical to those run for the quartile variables (ie, stratified on matched set and further adjusted for BMI at blood draw; kg/m^2). Persons with outlier values and those with missing BMI data were excluded.

‡The SD was obtained from the distribution of cohort-corrected values in the pooled controls.

§Quartile of sIL6R level was determined from the distribution of cohort-corrected values among the pooled controls.

blood donors[39] and that IGF-1 and insulin concentrations typically diminish in persons with advanced cancers.[40]

In addition to IGFBP-1 levels, the concentration of sIL6R independently predicted a modest increase in multiple myeloma risk, consistent with the hypothesis that IL-6 up-regulation and/or chronic inflammation contributes to the etiology of multiple myeloma. sIL6R is a naturally occurring alternate form of the membrane-bound IL6R molecule (ie, CD126), which enhances the sensitivity of target cells, including multiple myeloma plasma cells, to IL-6.[20,41] sIL6R is elevated in peripheral blood samples from multiple myeloma and MGUS patients compared with healthy donors.[41,42] sIL6R levels were also correlated with known prognostic indicators and independently associated with survival in a study of 626 multiple myeloma patients.[42] The present study suggests that sIL6R level is also modestly associated with risk of a subsequent multiple myeloma diagnosis within 6 years of blood collection. As with the IGFBP-1 findings, the sIL6R association may in part reflect secretion of IL-6 and sIL6R in the microenvironment of a proliferating clone of plasma cells or autocrine secretion of these molecules by the tumor cells.[13,14,20] However, the latency period for multiple myeloma is not known, and the finding may also indicate an earlier contribution of IL-6 signaling to a physiologic milieu that favors the development of multiple myeloma. We did not observe an association of IL-6 level with multiple myeloma, possibly because of limited assay sensitivity or because cytokines have a shorter half-life and greater vulnerability to degradation in archived peripheral blood samples than markers like soluble receptor molecules.[43]

The considerable strengths of the present study include the availability of prediagnosis archived blood samples from multiple myeloma cases and matched control participants for what is, to our knowledge, the first prospective evaluation of the IGF-1, insulin, and IL-6 pathways in multiple myeloma etiology performed to date. With the matched design and use of statistical models that conditioned on the matching factors and adjusted for BMI, we controlled for potential confounding by known multiple myeloma risk factors. The matched design also diminished the influence of sample type-related or diurnal or seasonal variation in biomarker levels on the analysis. Nonetheless, we cannot rule out the possibility of residual confounding by unknown or poorly measured factors. The biomarkers were measured with standardized and well-validated assays by blinded technicians from experienced laboratories and demonstrated excellent reproducibility. We implemented reliable statistical methods to correct for cohort-related variability in the laboratory values and were thus able to pool the data across cohorts and maximize the statistical power for analysis.

The study also has limitations. First, we did not have information on clinical data to identify persons with MGUS or preclinical multiple myeloma at blood draw. We were not able to control for history of diabetes or use of hypoglycemic agents in the analysis.

Although those factors may influence circulating levels of the biomarkers we studied, it is not likely that they would explain the time-restricted positive associations that we observed. The pooled study population included an insufficient number of nonwhite participants for stable race-specific analyses. We are not aware of hypotheses of race-related differences in the activity of the IGF-1, insulin, or IL-6 pathways in multiple myeloma pathogenesis, but an ability to directly demonstrate consistency of the findings across races would be reassuring. We included only one blood sample per study participant; however, peripheral blood levels of the markers we measured have exhibited reasonable within-person temporal stability in published studies.[44-47] Because the blood samples in the present study were prospectively acquired, the errors introduced by the within-person variation of biomarker levels are likely to be nondifferential with regard to case status. Thus, our analysis may have underestimated the true association of a given biomarker with multiple myeloma. With insufficient statistical power to jointly examine interactions of follow-up interval and BMI with biomarker concentration in relation to multiple myeloma risk, the present analysis is inconclusive as to whether dysregulation of IGF-1, insulin, or IL-6 explains the association of obesity with multiple myeloma.[5,6] Lastly, the median follow-up interval for study participants was < 7 years. Although we did not observe biomarker associations with multiple myeloma risk > 6 years after blood collection, the present analysis could not evaluate whether IGF-1, insulin, or IL-6 dysregulation has an early role in multiple myeloma etiology.

In conclusion, the present study demonstrated that concentrations of IGFBP-1 and sIL6R are positively associated with risk of a multiple myeloma diagnosis within 3-6 years of blood collection. Current approaches to risk stratification in patients with MGUS use clinical parameters, such as serum M protein isotype and concentration and the free light chain ratio, to estimate a patient's risk of progression to malignancy.[48-50] If the present novel findings are confirmed in other populations, clinical studies in patients with MGUS or smoldering multiple myeloma would be warranted to explore whether IGFBP-1, sIL6R, or other related markers improve the ability of current models to identify the patients at higher risk of progression to multiple myeloma and who may most benefit from increased medical surveillance.[48,49]

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A listing of WHI investigators can be found at https://cleo.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf.

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Authorship

Contribution: B.M.B. designed the research and data analysis plan, analyzed and interpreted the data, and wrote the manuscript; M.L.N., J.E.B., N.R., and J.-M.Y. provided data, reviewed the analysis plan, interpreted the data, and reviewed the manuscript; B.R. and G.A.C. designed the research and data analysis plan, interpreted the data, and reviewed the manuscript; D.A., G.G.G., and J.V. provided and interpreted the data and reviewed the manuscript; Q.L., I.-M.L., M.P.P., and G.S. reviewed the analysis plan, interpreted the data, and reviewed the manuscript; K.C.A. and J.E.M. designed the research, interpreted the data, and reviewed the manuscript; M.P. and N.R. provided laboratory services, interpreted the data, and reviewed the manuscript; P.H., O.L., L.L., and R.B.W. interpreted the data and reviewed the manuscript; and all authors reviewed and approved of the final submitted manuscript.

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