ARTHRITIS & RHEUMATOLOGY
Vol. 68, No. 10, October 2016, pp 2394–2402
DOI 10.1002/art.39735

Associations of Circulating Cytokines and Chemokines With Cancer Mortality in Men With Rheumatoid Arthritis

Bryant R. England,[1] Jeremy Sokolove,[2] William H. Robinson,[2] Geoffrey M. Thiele,[1] Apar K. Ganti,[1] Harlan Sayles,[1] Kaleb Michaud,[3] Liron Caplan,[4] Lisa A. Davis,[5] Grant W. Cannon,[6] Brian Sauer,[6] Namrata Singh,[7] E. Blair Solow,[8] Andreas M. Reimold,[8] Gail S. Kerr,[9] Pascale Schwab,[10] Josh F. Baker,[11] and Ted R. Mikuls[1]

Objective. **To examine the potential of circulating cytokines and chemokines as biomarkers of cancer mortality risk in patients with rheumatoid arthritis (RA).**

Methods. **Male participants in the Veterans Affairs RA registry were followed up from the time of enrollment until death or December 2013. Cytokines and chemokines were measured in banked serum obtained at the time of enrollment, using a bead-based multiplex assay, and a previously developed cytokine score was calculated. Vital status and cause of death were determined through the National Death Index. Associations of cytokines with cancer mortality were examined using multivariable competing-risks regression.**

Results. **Among 1,190 men with RA, 60 cancer deaths (30 of which were attributable to lung cancer) occurred over 5,307 patient-years of follow-up. The patients had a mean age of 64.5 years, had established disease (median duration 8.7 years), were seropositive for rheumatoid factor (81%) or anti–cyclic citrullinated peptide antibody (77%), and frequently had a history of smoking (82% current or former). Seven of 17 analytes examined were individually associated with cancer mortality. The cytokine score was associated with overall cancer (subhazard ratio [SHR] 1.42, 95% confidence interval [95% CI] 1.08–1.85) and lung cancer (SHR 1.86, 95% CI 1.57–2.19) mortality in multivariable analyses. Those in the highest quartile of cytokine scores had a >2-fold increased risk of overall cancer mortality ($P = 0.039$) and a 6-fold increased risk of lung cancer mortality ($P = 0.028$) relative to the lowest quartile. A synergistic interaction between current smoking and high cytokine score was observed.**

Conclusion. **Serum cytokines and chemokines are associated with cancer and lung cancer mortality in men with RA, independent of multiple factors including age, smoking status, and prevalent cancer.**

Cause of death in patients with rheumatoid arthritis (RA) mirrors that in the general population, with cardiovascular disease, cancer, and respiratory disease representing the leading causes (1). A modest increased incidence of cancer has been observed in RA, with particular overrepresentation of lung cancer and lymphoma (2). Shared risk factors such as tobacco smoking may account

Dr. England's work was supported by a Rheumatology Research Foundation Resident Research Preceptorship award. Dr. Baker's work was supported by VA Clinical Science Research and Development Career Development award IK2-CX000955. Dr. Mikuls' work was supported by a VA Clinical Science Research and Development Merit award.

[1]Bryant R. England, MD, Geoffrey M. Thiele, PhD, Apar K. Ganti, MD, MS, Harlan Sayles, MS, Ted R. Mikuls, MD, MSPH: Veterans Affairs Nebraska−Western Iowa Health Care System and University of Nebraska Medical Center, Omaha; [2]Jeremy Sokolove, MD, William H. Robinson, MD, PhD: VA Palo Alto Health Care System and Stanford University, Palo Alto, California; [3]Kaleb Michaud, PhD: Veterans Affairs Nebraska−Western Iowa Health Care System and University of Nebraska Medical Center, Omaha, and National Data Bank for Rheumatic Diseases, Wichita, Kansas; [4]Liron Caplan MD, PhD: Denver VA Medical Center and University of Colorado, Denver; [5]Lisa A. Davis, MD, MSCS: Denver VA Medical Center, University of Colorado, and Denver Health Medical Center, Denver, Colorado; [6]Grant W. Cannon, MD, Brian Sauer, PhD: VA Salt Lake City Health Care System and University of Utah School of Medicine, Salt Lake City; [7]Namrata Singh, MD: Iowa City VA Health Care System and University of Iowa, Iowa City; [8]E. Blair Solow, MD, MSCS, Andreas M. Reimold, MD: Dallas VA Medical Center and University of Texas Southwestern Medical Center, Dallas; [9]Gail S. Kerr, MD: Washington DC VA Medical Center and Georgetown−Howard Universities Center for Clinical and Translational Science, Washington, DC; [10]Pascale Schwab, MD: Portland VA Health Care System and Oregon Health and Sciences University, Portland; [11]Josh F. Baker, MD, MSCE: Philadelphia VA Medical Center and University of Pennsylvania, Philadelphia.

Address correspondence to Ted R. Mikuls, MD, MSPH, 983025 Nebraska Medical Center, Omaha, NE 68198-3025. E-mail: tmikuls@unmc.edu.

Submitted for publication January 1, 2016; accepted in revised form April 21, 2016.

for the increased incidence of lung cancer (3), while chronic systemic inflammation accompanying persistent RA disease activity has been associated with an increased incidence of lymphoma (4). In addition to an increased cancer incidence, some studies have demonstrated increased cancer mortality in RA (5,6).

We previously calculated a cancer-specific standardized mortality ratio (SMR) of 1.50 in a cohort of men with established RA (7). Notably, RA patients in disease remission at the time of enrollment did not have an increased risk of cancer mortality (SMR 0.97). This suggests that RA-specific measures, particularly those reflecting disease activity, may carry prognostic information for cancer outcomes. Thus, more complete characterization of systemic inflammation may offer clearer prognostic information with regard to cancer mortality. Our previous study identified well-established risk factors for cancer mortality (low body mass index [BMI], prevalent cancer, smoking) but did not identify novel prognostically useful clinical factors (7). Clearly, better mechanisms to identify RA patients at risk of cancer and cancer mortality are needed. Whether biomarkers could supplement clinical measures in the identification of cancer risk and subsequent outcomes in RA is unknown.

Cytokine and chemokine dysregulation is critical to the pathogenesis of both RA (8) and cancer, this latter relationship through a process termed immunoediting (9). The immunoediting hypothesis proposes that the immune system both eliminates tumors and shapes tumor immunogenicity. At the cellular level, cytokines and chemokines influence angiogenesis, apoptosis, cellular proliferation, growth, and invasion, all of which are key mechanisms underlying tumorigenesis and metastasis (10). The prognostic value of measuring serum cytokine levels in patients with cancer is an area of active study. In lung cancer, for instance, elevated pretreatment serum measurements of interleukin-6 (IL-6), interferon-γ (IFNγ), vascular endothelial growth factor, and IL-1β are predictive of increased mortality (11–14). Similar associations between cytokine concentrations and mortality risk have been observed in other cancer types (15).

Although cytokines and chemokines contribute to the pathogenesis of both RA and cancer, no studies to date have examined the prognostic potential of serum cytokines and chemokines in RA populations. The aim of this study was to assess the predictive ability of serum cytokine and chemokine concentrations for cancer mortality in a cohort of patients with established RA. We hypothesized that higher baseline serum concentrations of proinflammatory cytokines and chemokines would predict cancer mortality independent of other potential confounders.

PATIENTS AND METHODS

Study participants. The study participants were enrollees in the Veterans Affairs Rheumatoid Arthritis (VARA) registry, which has been well described previously (16). Briefly, VARA is a longitudinal, observational study of US veterans with RA that was initiated in 2003, with participation from 12 rheumatology clinics at VA medical centers across the US. All patients fulfilled the 1987 American College of Rheumatology RA classification criteria (17), had disease onset after 18 years of age, and provided informed consent. This study was approved by the institutional review board at each site and the VARA Scientific and Ethics Advisory Committee.

A total of 1,421 patients had cytokine measurements available during the study period (Figure 1). Women were excluded, because they comprised a small proportion of the total cohort (9% [n = 127]) and observed deaths (3% [n = 10]). If the enrollment covariates utilized in multivariable analyses were missing, the most proximate nonmissing values were imputed (covariates for the Disease Activity Score in 28 joints [DAS28 (18); n = 134] and prednisone use [n = 59]). Patients for whom data were unavailable (data for BMI were missing in 70 patients, for prednisone use in 21 patients, for the DAS28 in 8 patients, for the Multidimensional Health Assessment Questionnaire score [MD-HAQ (19)] in 4 patients, and for rheumatoid factor [RF] in 1 patient) were excluded.

Patients were followed up longitudinally from the time of enrollment until death or December 31, 2013. Vital status and cause of death were determined through linkage with the National Death Index (National Center for Health Statistics, US Department of Health and Human Services). Primary cause of death was coded according to the International Classification of Diseases, Tenth Revision; Chapter II codes (C00−D48) were assigned to cancer. Specific causes of cancer death were assigned using Healthcare Cost and Utilization Project Clinical Classifications Software (HCUP-CCS).

Variables. Data on age, self-reported race/ethnicity, education level, date of RA diagnosis, smoking status (current, former, never), and BMI were collected at study enrollment. At each visit, data on medications, MD-HAQ, and DAS28 were collected. The DAS28 was categorized into remission, low, moderate, and high disease activity states (20). Anti−cyclic citrullinated peptide (anti-CCP) antibody, RF, and high-sensitivity C-reactive protein (hsCRP) were measured in banked serum obtained at the time of enrollment (21). Comorbid conditions, including prevalent cancer, were aggregated for the 12 months prior to enrollment from VA national administrative data, using the HCUP-CCS from the Agency for Healthcare Quality and Research, and an overall comorbidity score was calculated using the Rheumatic Disease Comorbidity Index (RDCI) (22).

Cytokines and chemokines were measured in banked serum obtained at enrollment, using a Bio-Plex Pro Human Cytokine 17-plex Assay (Bio-Rad) run on a Luminex 200 system (23,24). Data processing was performed with Bio-Plex Manager software, and analyte concentrations were interpolated from standard curves. Seventeen analytes were examined: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, IFNγ, monocyte chemoattractant protein 1, macrophage inflammatory protein 1β (MIP-1β), and tumor necrosis factor (TNF).

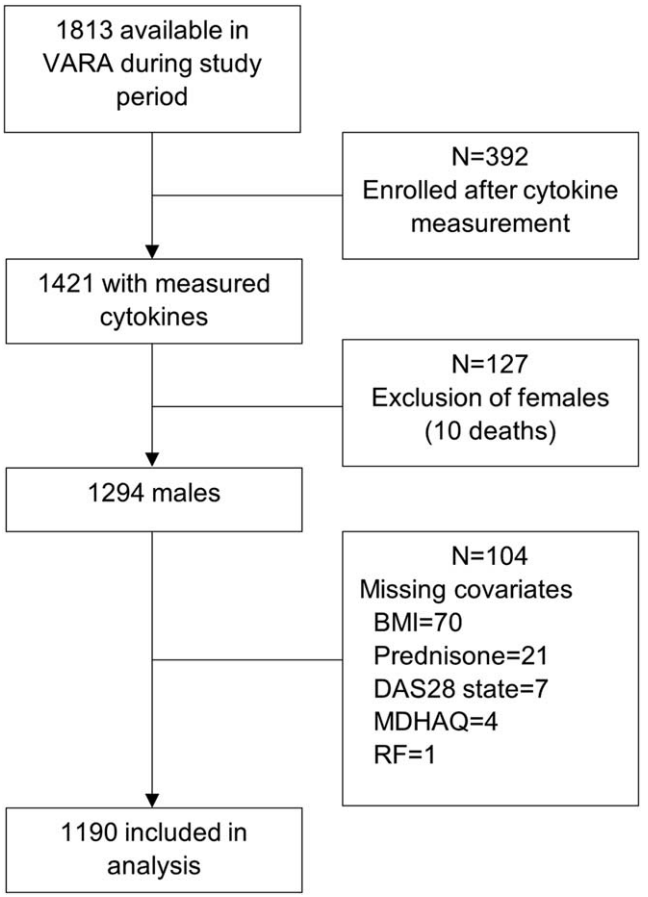

Figure 1. Patient selection. During the study period, 1,813 patients were enrolled in the Veterans Affairs Rheumatoid Arthritis Registry (VARA), and 1,421 patients had cytokine and chemokine measurements. Female patients (n = 127) were excluded, because they comprised only 9% of the cohort and <3% of all deaths. Patients who had missing covariates for the multivariable analyses (n = 104) were also excluded. A total of 1,190 men were included in the analyses. BMI = body mass index; DAS28 = Disease Activity Score in 28 joints; MD-HAQ = Multidimensional Health Assessment Questionnaire; RF = rheumatoid factor.

Statistical analysis. Baseline characteristics and follow-up duration were compared between those who survived, those who died of cancer, and those who died of other causes, using chi-square, analysis of variance, and Kruskal-Wallis tests for descriptive purposes. Cytokines and chemokines were analyzed individually and as a composite measure, using a previously developed cytokine score (25); the individual analyses were adjusted for multiple testing with a conservative Bonferroni correction. Because of the conservative nature of this adjustment, we also report analytes with statistical significance before correction. The cytokine score was developed as a measure of overall inflammation and is calculated from the log-transformed, normalized, and weighted summation of individual cytokines with the following formula: $\sum_{CK=1}^{17} B_i \frac{\log(CKi)}{\mathrm{SD}(CKi)}$, where B_i are regression coefficients. Individual cytokines and the cytokine score were natural log−transformed to achieve a Gaussian distribution. The cytokine score was additionally modeled as an ordinal categorical variable by categorizing into quartiles.

We compared median cytokine scores among those who survived, those who died of cancer, and those who died of other causes, using the Kruskal-Wallis and Dunn's post hoc tests. Subhazard ratios (SHRs) and 95% confidence intervals (95% CIs) were used to characterize age-adjusted and multivariable associations between cytokines and cytokine score with cancer mortality and cause-specific cancer mortality, generated using competing-risks regression based on the methods described by Fine and Gray (26) (Stata command stcrreg) to account for obscuring of the associations by other causes of death (27,28). Multivariable analyses included covariates identified by comparisons of baseline characteristics among survivors, those who died of cancer, and those who died of other causes, as well as from previous work in this cohort (7). Covariates examined included age, race, smoking status, enrollment BMI category, RDCI score, prevalent cancer, visit frequency, MD-HAQ, RF titer, nodules, enrollment DAS28 status, enrollment disease-modifying antirheumatic drugs (DMARDs; using hierarchical model), and enrollment prednisone use. The DMARD hierarchical model was (in ascending order) no DMARDs, non-methotrexate DMARDs, methotrexate, and biologic agents, with non-methotrexate DMARDs serving as the reference group. We found no evidence for collinearity among covariates and cytokine score by correlation coefficients or variance inflation factors. Standard error adjustment was applied to all models to account for clustering by site (n = 9). Analyses were conducted using Stata version 14.

Previous work in this cohort demonstrated that current smoking, but not former smoking, influenced cytokine expression relative to never smoking (29). We specifically addressed this finding in 2 analyses. First, we adjusted solely for current smoking by modeling current smokers referent to a combined group of never and former smokers. Second, we modeled an interaction term between smoking status (current versus former and never) and cytokine score.

In further sensitivity analyses, we excluded patients with prevalent cancer at enrollment (HCUP-CCS categories 11–43) to assess whether an association between cytokine score and cancer was independent of cancer diagnosis. We investigated the temporal relationship between cytokine measurement and cancer mortality, to determine whether an association was explained by imminent death, by excluding participants who died within 12 months of cytokine measurement. To assess whether other markers of inflammation explained the association, hsCRP measured in banked serum was added to the multivariable model.

RESULTS

Baseline characteristics of the patients. The men with RA who were included in this study (n = 1,190) had a mean ± SD age of 64.5 ± 10.6 years, had established disease (median duration 8.7 years; interquartile range [IQR] 2.9–18.7), were frequently smokers (81.9% current or former), were predominantly white (79.2%), and the majority were seropositive for

Table 1. Baseline characteristics of the men with RA who survived, died of cancer, or died of other causes*

Characteristic	Overall (n = 1,190)	Survived (n = 915)	Other death (n = 215)	Cancer death (n = 60)	*P*
Age, mean ± SD years	64.5 ± 10.6	62.6 ± 10.2	71.3 ± 9.2	69.1 ± 9.3	<0.001
Disease duration, median (IQR) years	8.7 (2.9–18.7)	8.1 (2.5–17.5)	11.6 (4.6–24.2)	10.6 (3.3–19.3)	<0.001
Follow-up duration, mean ± SD years	4.5 ± 2.7	4.9 ± 2.7	3.1 ± 1.8	3.1 ± 1.8	<0.001
White race	79.2	77.0	87.4	81.7	0.003
High school or higher†	81.9	83.1	75.7	83.3	0.054
Smoking status					0.036
Current	27.6	28.5	20.9	36.7	
Former	54.3	53.1	59.1	55.0	
Never	18.2	18.4	20.0	8.3	
BMI category					<0.001
<20 kg/m^2	4.9	2.6	12.1	13.3	
20–25 kg/m^2	24.9	23.1	31.6	28.3	
25–30 kg/m^2	39.0	39.7	36.7	36.7	
30–35 kg/m^2	21.4	24.0	12.6	13.3	
>35 kg/m^2	9.8	10.6	6.9	8.3	
RDCI, mean ± SD (0–9 scale)	2.4 ± 1.7	2.3 ± 1.7	2.8 ± 1.5	2.7 ± 1.7	<0.001
Cancer	14.5	14.4	12.1	25.0	0.042
DAS28 status					0.11
Remission	22.8	24.3	17.2	20.0	
Low	15.1	15.4	18.1	16.7	
Moderate	39.2	38.5	40.5	46.7	
High	22.9	21.9	28.8	16.7	
Anti-CCP antibody positive‡	77.3	78.0	75.3	73.3	0.53
RF positive	80.5	80.0	83.3	78.3	0.51
RF titer, mean ± SD units/ml	335 ± 670	324 ± 657	371 ± 745	390 ± 573	0.45
MD-HAQ score, mean ± SD (0–3 scale)	0.96 ± 0.60	0.91 ± 0.59	1.10 ± 0.61	1.08 ± 0.63	<0.001
High-sensitivity CRP, mean ± SD mg/liter	1.3 ± 2.0	1.1 ± 1.8	1.8 ± 2.5	1.9 ± 2.9	<0.001
Nodules	35.3	32.1	49.3	33.3	<0.001
Prednisone treatment	42.4	40.2	52.6	40.0	0.004
DMARD treatment					0.29
None	12.4	11.8	12.1	21.7	
Other	19.8	19.2	22.8	16.7	
Methotrexate	39.9	40.1	40.5	35.0	
Biologic agent	28.0	28.9	24.7	26.7	

* Except where indicated otherwise, values are the percent. IQR = interquartile range; BMI = body mass index; RDCI = Rheumatic Disease Comorbidity Index; DAS28 = Disease Activity Score in 28 joints; anti-CCP = anti–cyclic citrullinated peptide; RF = rheumatoid factor; MD-HAQ = Multidimensional Health Assessment Questionnaire; CRP = C-reactive protein; DMARD = disease-modifying antirheumatic drug.
† Data were missing for 67 patients.
‡ Data were missing for 1 patient.

RF (80.5%) or anti-CCP (77.3%) (Table 1). There were 5,307 patient-years of follow-up, with 275 total deaths and 60 deaths attributed to cancer (Table 2). Lung cancer was the most frequent cause of cancer death (n = 30), followed by lymphoma and leukemia (n = 9).

Compared with those who survived, patients who died were older, had longer disease duration, shorter follow-up, were more frequently underweight, had greater comorbidity, higher MD-HAQ scores, and higher hsCRP values (Table 1). Those who died of cancer had higher rates of current smoking and prevalent cancer. Those who died of other causes were more often white, more likely to have subcutaneous nodules, and more likely to be receiving prednisone.

Association of cytokines and chemokines with cancer mortality. In both age-adjusted and multivariable analyses, 7 of 17 analytes measured were associated with cancer mortality (Figure 2); significant analytes included IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, and MIP-1β. After Bonferroni correction for multiple testing, IL-1β, IL-6, and IL-8 remained associated with cancer mortality. Likewise, the cytokine score was higher in those who died of cancer (median 12.3 units; IQR 6.1–31.2) versus other causes (median 8.6 units; IQR 4.8–20.6, $P < 0.001$) and survivors (median 7.3 units; IQR 4.4–18.8, $P < 0.001$). In both age-adjusted (SHR 1.38, 95% CI 1.04–1.82) and multivariable analysis (SHR 1.42, 95% CI 1.08–1.85), continuous cytokine score was

Table 2. Cause of death in men with rheumatoid arthritis during 5,307 patient-years of follow-up*

Cancer	60 (21.8)
Lung	30
Lymphoma/leukemia	9
Prostate	4
Pancreatic	3
Other	14
Cardiovascular	86 (31.3)
Respiratory	47 (17.1)
Musculoskeletal	14 (5.1)
External (accidents)	12 (4.4)
Infection	11 (4.0)
Other	45 (16.4)
Total	275

* Values are the number (%).

associated with cancer mortality (Table 3). Compared with RA patients in the lowest quartile, we found those in the highest cytokine score quartile to have a >2-fold increased risk of cancer mortality (multivariable SHR 2.23, 95% CI 1.04–4.80, $P = 0.039$). Trend test across quartiles approached but did not reach statistical significance ($P = 0.059$). In contrast to cancer mortality, there were no associations between cytokine score and all-cause (HR 1.12, 95% CI 0.93–1.35), cardiovascular (SHR 0.98, 95% CI 0.84–1.15), or respiratory (SHR 1.07, 95% CI 0.80–1.43) mortality after multivariable adjustment.

Association of the cytokine score with lung cancer mortality. For cancer-specific associations, only lung cancer mortality was observed with enough frequency to be investigated separately. In both age-adjusted (SHR 1.78, 95% CI 1.52–2.10) and multivariable analyses (SHR 1.86, 95% CI 1.57–2.19), cytokine score as a continuous variable was associated with lung cancer mortality (Table 3). We found that those in the highest quartile of cytokine score had a 6-fold higher risk of lung cancer mortality compared with those in the lowest quartile (multivariable SHR 6.37, 95% CI 1.23–33.11, $P = 0.028$). A trend test across quartiles was statistically significant ($P = 0.004$).

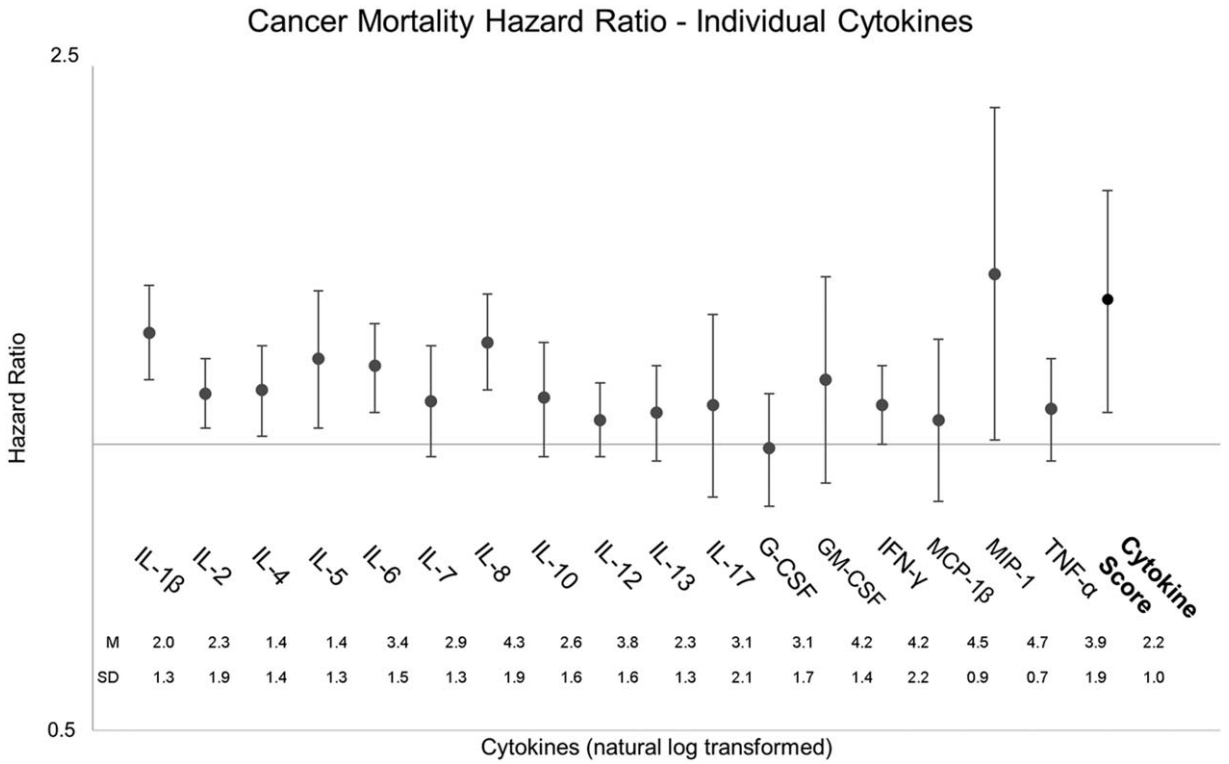

Figure 2. Forest plot of the associations between individual cytokines and chemokines with cancer mortality in a multivariable model. All analytes were modeled as natural log−transformed continuous variables. The mean (M) and SD are shown below each analyte. The cytokine score, a composite measure of individual cytokine values, is shown for comparison. Seven of the 17 analytes were significantly associated with cancer mortality: interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-8, and macrophage inflammatory protein 1 (MIP-1). Covariates were age, race, smoking status, enrollment body mass index category, visit frequency, Rheumatic Disease Comorbidity Index, cancer, Multidimensional Health Assessment Questionnaire, rheumatoid factor titer, subcutaneous nodules, enrollment Disease Activity Score in 28 joints, enrollment disease-modifying antirheumatic drugs, and enrollment prednisone. Vertical bars show the 95% confidence interval. G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte−macrophage CSF; IFNγ = interferon-γ; MCP-1β = monocyte chemotactic protein 1β; TNF = tumor necrosis factor.

Table 3. Age-adjusted and multivariable associations of the cytokine score with cancer and lung cancer mortality in the men with RA*

Model	Cancer		Lung cancer	
	Age-adjusted	Multivariable	Age-adjusted	Multivariable
Cytokine score, continuous†	1.38 (1.04–1.82) (0.025)	1.42 (1.08–1.85) (0.011)	1.78 (1.52–2.10) (<0.001)	1.86 (1.57–2.19) (<0.001)
Cytokine score, quartiles				
Quartile 1	Reference	Reference	Reference	Reference
Quartile 2	1.25 (0.73–2.14)	1.10 (0.60–2.00)	2.71 (0.43–17.17)	2.64 (0.54–12.79)
Quartile 3	0.98 (0.31–3.09)	0.72 (0.26–2.05)	2.92 (0.38–22.43)	2.32 (0.32–16.91)
Quartile 4	2.40 (1.06–5.41)	2.23 (1.04–4.80)	7.23 (1.21–43.27)	6.37 (1.23–33.11)
P for trend	0.041	0.059	<0.001	0.004

* The multivariable model included age, race, smoking status, enrollment body mass index category, visit frequency, Rheumatic Disease Comorbidity Index, cancer, Multidimensional Health Assessment Questionnaire, rheumatoid factor titer, subcutaneous nodules, enrollment 28-joint Disease Activity Score status, enrollment disease-modifying antirheumatic drugs, and enrollment prednisone. Values are the hazard ratio (95% confidence interval) (*P*).
† Natural log−transformed.

Smoking status and the cytokine score. Modeling current smokers relative to combined never and former smokers, cytokine score remained associated with overall cancer mortality (SHR 1.40, 95% CI 1.03–1.91). An interaction term between high/low cytokine score (dichotomized at the 75th percentile, given the strongest association with cancer mortality) and smoking status (current versus former/never) demonstrated a synergistic interaction for cancer mortality risk (*P* for interaction = 0.024). Relative to individuals with a low cytokine score and former/never smoking, the highest overall cancer mortality risk was observed in those who had a high cytokine score and current smoking, followed by those with a high cytokine score plus former/never smoking, and those with a low cytokine score and current smoking (Figure 3).

Sensitivity analyses. A total of 173 patients had prevalent cancer at the time of enrollment, with 41 dying during follow-up (15 cancer deaths). After exclusion of these patients, the association between cytokine score and cancer mortality was stronger than in the original model (SHR 1.61, 95% CI 1.40–1.87, $P < 0.001$). Within 12 months of enrollment, 34 deaths occurred, with 8 attributed to cancer. Excluding these participants, cytokine score remained associated with cancer mortality (SHR 1.48, 95% CI 1.09–2.02, $P = 0.011$). The addition of hsCRP to the multivariable model did not affect the association between cytokine score and cancer mortality (SHR 1.41, 95% CI 1.09–1.83, $P = 0.008$).

DISCUSSION

In the current study, we observed higher serum cytokine and chemokine concentrations in RA patients who subsequently died of cancer; to our knowledge, this study is the first to demonstrate such an association. Notably, the observed associations were independent of multiple other known cancer risk factors, including age, prevalent cancer, arthritis severity, and smoking, and they were not observed with other causes of death. We found that 7 of the 17 cytokine analytes examined yielded significant associations with cancer mortality, and that none showed "protective" effects. The cytokine score, which serves as a sum of individual cytokine values, rendered robust associations that were similar in direction and of slightly greater magnitude compared with most of the individual analytes. In addition to supporting the potential use of the cytokine score (or a similarly conceived composite measure) as an informative biomarker in RA, these data support the existence of a global association of circulating concentrations of cytokines and chemokines with cancer mortality, rather than an association that is driven by a single analyte.

The present findings are consistent with those from prior studies in cancer patients showing the potential for serum cytokine measurements to serve as prognostic markers for cancer mortality (11–15). In contrast to those studies, our study demonstrated an association between cytokines and cancer mortality in RA patients without prevalent cancer. Our findings further support the link of high inflammatory disease activity between RA and cancer, as previously demonstrated by Baecklund et al in their study of longitudinal disease activity and lymphoma risk (4). A striking similarity between the Baecklund study and ours is the dramatic increased risk among those in the highest strata, as defined by cytokine status, of "inflammation." Although a proinflammatory state in the lungs has been proposed as an explanation for the increased risk of lung cancer in RA (3), to our knowledge, this is the first study to support a link between inflammation and solid tumors in RA patients. In a non-RA population, Siemes et al demonstrated an association between CRP and incident solid malignancy, with the association being strongest in

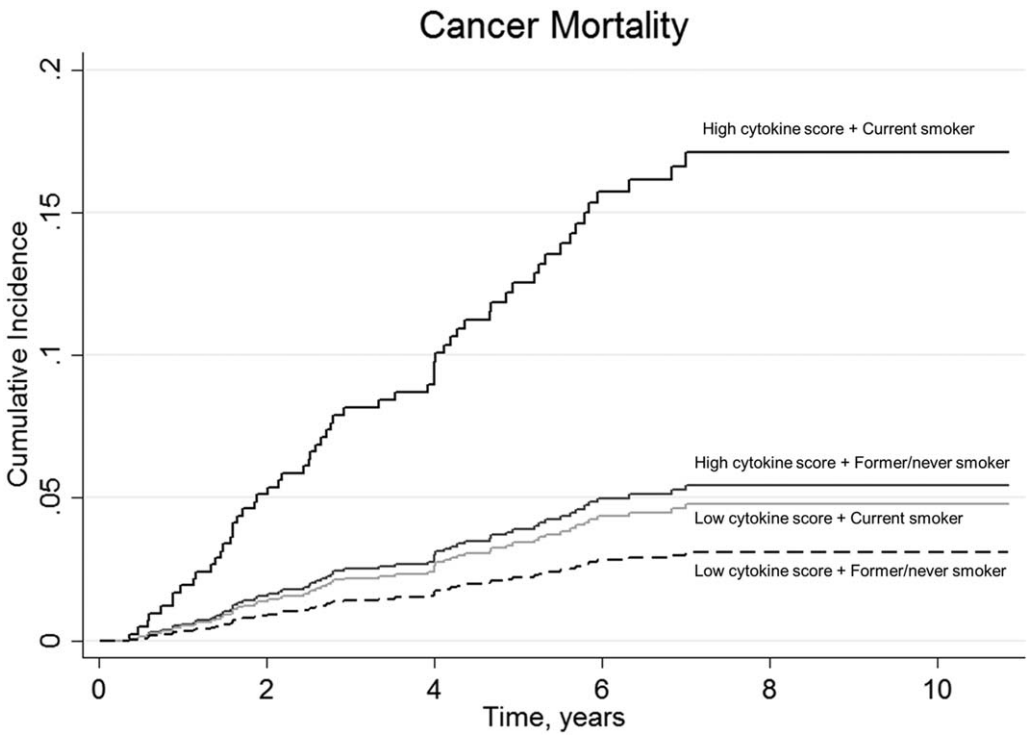

Figure 3. Cumulative incidence of cancer death according to cytokine score (≥75th percentile versus low) and smoking status (current versus former/never) in men with rheumatoid arthritis, as generated by multivariable competing-risks regression. An interaction term between cytokine score and smoking status was included in the model (P for interaction = 0.024). Subhazard ratios were 5.99 (95% confidence interval [95% CI] 3.72–9.65, $P < 0.001$) for current smoker and high cytokine score, 1.79 (95% CI 1.17–2.73, $P = 0.007$) for former or never smoker and high cytokine score, and 1.56 (95% CI 0.95–2.56, $P = 0.077$) for current smoker and high cytokine score. Former or never smokers with a low cytokine score served as referent. Covariates were age, race, enrollment body mass index category, visit frequency, Rheumatic Disease Comorbidity Index, cancer, Multidimensional Health Assessment Questionnaire, rheumatoid factor titer, subcutaneous nodules, enrollment Disease Activity Score in 28 joints, enrollment disease-modifying antirheumatic drugs, and enrollment prednisone treatment.

lung cancer (30), while Shiels et al observed an association between 11 inflammatory markers and incident lung cancer (31). In our study, adding hsCRP values from the same serum sample as that used to measure cytokines and chemokines did not attenuate the association between cytokine score and cancer mortality or lung cancer mortality (data not shown for lung cancer mortality). This suggests a benefit of analyzing specific cytokines and chemokines that contribute to a heightened level of inflammation and provides support for the role of cytokines and chemokines in cancer mortality.

Because of previous work in this cohort suggesting differential cytokine expression between current and never smokers (29), additional consideration of the influence of smoking status was necessary. Adjusting solely for current smoking did not influence the association between cytokine score and cancer mortality, but we observed evidence of a synergistic interaction between high cytokine score and current smoking status, with an approximate 6-fold increased risk relative to those who were former/never smokers with low cytokine scores. These data further support the need for aggressive smoking cessation counseling, an intervention that may be particularly important in patients with the highest circulating levels of the proinflammatory cytokines examined in this study. One potential explanation for this finding is local induction of reactive oxygen species (ROS) and heightened inflammation occurring when circulating inflammatory cytokines and tobacco smoke interact (32). ROS, with direct DNA-damaging effects, may contribute to the link between inflammation and carcinogenesis (33). However, even in individuals who were former or never smokers, higher cytokine scores remained associated with cancer mortality.

Our findings do not imply causation or identify the mechanism(s) linking cytokines and cancer mortality. However, there are biologic processes that support our findings. The immune system is intimately involved in tumorigenesis, with the immunoediting hypothesis underscoring the role that the immune system plays in shaping

tumor expression (9). Cytokines, including IL-1, IL-6, and TNF, contribute to metastasis by creating an inflammatory milieu with increased vascular permeability, angiogenesis, and extracellular matrix degradation from activated proteases (10,34). Metastasis accounts for ~90% of cancer mortality (10), suggesting that our findings could be attributable to an association of cytokines with the development of higher stage malignancies. A general trend toward higher cytokine concentrations with increasing disease stage has been previously reported (15). Unfortunately, data on staging of malignancies were not available for the present analyses. Although the assay used in this study allowed for the simultaneous measurement of multiple analytes, it is worth noting that these were originally selected for their putative role in RA and not cancer. Thus, it is quite conceivable that the addition of alternative cytokines and/or chemokines may improve the prognostic ability observed in our study. Due to the nature of cytokine dysregulation in RA, alternative cytokines and chemokines may better identify cancer mortality risk in other populations.

There are additional limitations to our study. First, we included only men with RA; therefore, generalizability to other populations may be limited. Cytokines and chemokines were measured at a single time point, and the time of measurement was not standardized in relation to RA disease duration, although further adjustment for disease duration did not meaningfully impact these results (data not shown). Cytokines and chemokines are dynamic molecules; it is not known how serial measurement or measuring at different stages in the RA disease course may influence the observed association. Finally, pack-year smoking history was not available in the cohort.

Despite these limitations, our study has a number of strengths. We studied male patients, a population that is historically understudied in RA and is known to have the highest absolute mortality rates (1). Our longitudinal study design, robust clinical and demographic information, and enriched comorbidity measures allowed for identification of and adjustment for many potential confounders in our analyses. We also demonstrated the advantages of using a multiplex assay to simultaneously examine the association of several cytokines and chemokines. Finally, we performed multiple sensitivity analyses investigating alternate explanations, and these results support the fidelity of our original findings.

In this study, we demonstrate associations of circulating cytokines and chemokines with overall cancer and lung cancer mortality in a cohort of men with established RA; these associations were independent of multiple potential confounders and independent of a known diagnosis of cancer. Our findings provide a critical first step toward identifying RA patients at high risk of cancer mortality. Further investigations examining the associations of cytokine and chemokine concentrations with incident cancer risk and stages of cancer at presentation, as well as investigations into mechanisms linking cytokines and cancer biology in RA, are warranted.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Mikuls had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. England, Mikuls.

Acquisition of data. England, Sokolove, Robinson, Caplan, Davis, Cannon, Sauer, Singh, Solow, Reimold, Kerr, Schwab, Baker, Mikuls.

Analysis and interpretation of data. England, Sokolove, Robinson, Thiele, Ganti, Sayles, Michaud, Mikuls.

REFERENCES

1. Sokka T, Abelson B, Pincus T. Mortality in rheumatoid arthritis: 2008 update. Clin Exp Rheumatol 2008;5 Suppl 51:S35–61.
2. Simon TA, Thompson A, Gandhi KK, Hochberg MC, Suissa S. Incidence of malignancy in adult patients with rheumatoid arthritis: a meta-analysis. Arthritis Res Ther 2015;17:212.
3. Smitten AL, Simon TA, Hochberg MC, Suissa S. A meta-analysis of the incidence of malignancy in adult patients with rheumatoid arthritis. Arthritis Res Ther 2008;10:R45.
4. Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F, et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis Rheum 2006;54:692–701.
5. Ji J, Liu X, Sundquist K, Sundquist J. Survival of cancer in patients with rheumatoid arthritis: a follow-up study in Sweden of patients hospitalized with rheumatoid arthritis 1 year before diagnosis of cancer. Rheumatology (Oxford) 2011;50:1513–8.
6. Franklin J, Lunt M, Bunn D, Symmons D, Silman A. Influence of inflammatory polyarthritis on cancer incidence and survival: results from a community-based prospective study. Arthritis Rheum 2007;56:790–8.
7. England BR, Sayles H, Michaud K, Caplan L, Davis LA, Cannon GW, et al. Cause-specific mortality in male US veterans with rheumatoid arthritis. Arthritis Care Res (Hoboken) 2016;68:36–45.
8. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365:2205–19.
9. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–70.
10. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010;140:883–99.
11. Martin F, Santolaria F, Batista N, Milena A, Gonzalez-Reimers E, Brito MJ, et al. Cytokine levels (IL-6 and IFN-γ), acute phase response and nutritional status as prognostic factors in lung cancer. Cytokine 1999;11:80–6.
12. Dudek AZ, Mahaseth H. Circulating angiogenic cytokines in patients with advanced non-small cell lung cancer: correlation with treatment response and survival. Cancer Invest 2005;23:193–200.
13. Kim JW, Koh Y, Kim DW, Ahn YO, Kim TM, Han SW, et al. Clinical implications of VEGF, TGF-β1, and IL-1β in patients with advanced non-small cell lung cancer. Cancer Res Treat 2013;45:325–33.
14. Enewold L, Mechanic LE, Bowman ED, Zheng YL, Yu Z, Trivers G, et al. Serum concentrations of cytokines and lung

cancer survival in African Americans and Caucasians. Cancer Epidemiol Biomarkers Prev 2009;18:215–22.
15. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. Nat Rev Cancer 2008;8:887–99.
16. Mikuls TR, Reimold A, Kerr GS, Cannon GW. Insights and implications of the VA Rheumatoid Arthritis Registry. Fed Prac 2015;32:24–9.
17. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
18. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight−joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.
19. Pincus T, Summey JA, Soraci SA Jr, Wallston KA, Hummon NP. Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983;26:1346–53.
20. Van Gestel AM, Haagsma CJ, van Riel PL. Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. Arthritis Rheum 1998;41:1845–50.
21. Miriovsky BJ, Michaud K, Thiele GM, O'Dell JR, Cannon GW, Kerr G, et al. Anti-CCP antibody and rheumatoid factor concentrations predict greater disease activity in men with rheumatoid arthritis. Ann Rheum Dis 2010;69:1292–7.
22. England BR, Sayles H, Mikuls TR, Johnson DS, Michaud K. Validation of the Rheumatic Disease Comorbidity Index. Arthritis Care Res (Hoboken) 2015;67:865–72.
23. Sokolove J, Johnson DS, Lahey LJ, Wagner CA, Cheng D, Thiele GM, et al. Rheumatoid factor as a potentiator of anti-citrullinated protein antibody−mediated inflammation in rheumatoid arthritis. Arthritis Rheumatol 2014;66:813–21.
24. Sokolove J, Bromberg R, Deane KD, Lahey LJ, Derber LA, Chandra PE, et al. Autoantibody epitope spreading in the preclinical phase predicts progression to rheumatoid arthritis. PLoS One 2012;7:e35296.
25. Hughes-Austin JM, Deane KD, Derber LA, Kolfenbach JR, Zerbe GO, Sokolove J, et al. Multiple cytokines and chemokines are associated with rheumatoid arthritis-related autoimmunity in first-degree relatives without rheumatoid arthritis: studies of the Aetiology of Rheumatoid Arthritis (SERA). Ann Rheum Dis 2013;72:901–7.
26. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509.
27. Andersen PK, Geskus RB, de Witte T, Putter H. Competing risks in epidemiology: possibilities and pitfalls. Int J Epidemiol 2012;41:861–70.
28. Lau B, Cole SR, Gange SJ. Competing risk regression models for epidemiologic data. Am J Epidemiol 2009;170:244–56.
29. Cramb C, Sokolove J, Thiele GM, Kerr GS, Cannon GW, Reimold AM, et al. Smoking status is associated with inflammatory cytokine profile and disease activity in anti-citrullinated protein antibody positive rheumatoid arthritis: decreased inflammation and disease improvement with smoking cessation? [abstract]. Arthritis Rheum 2012;64 Suppl:S508.
30. Siemes C, Visser LE, Coebergh JW, Splinter TA, Witteman JC, Uitterlinden AG, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. J Clin Oncol 2006;24:5216–22.
31. Shiels MS, Pfeiffer RM, Hildesheim A, Engels EA, Kemp TJ, Park JH, et al. Circulating inflammation markers and prospective risk for lung cancer. J Natl Cancer Inst 2013;105:1871–80.
32. Barbieri SS, Zacchi E, Amadio P, Gianellini S, Mussoni L, Weksler BB, et al. Cytokines present in smokers' serum interact with smoke components to enhance endothelial dysfunction. Cardiovasc Res 2011;90:475–83.
33. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 2010;49:1603–16.
34. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009;9:798–809.