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Associations of serum cytokines and chemokines with the risk of incident cancer in a prospective rheumatoid arthritis cohort

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ABSTRACT

Objectives: We aimed to assess whether serum cytokine/chemokine concentrations predict incident cancer in RA patients.

Methods: Data from cancer-free enrollees in the Veterans Affairs Rheumatoid Arthritis (VARA) Registry were linked to a national VA oncology database and the National Death Index (NDI) to identify incident cancers. Seventeen serum cytokines/chemokines were measured from enrollment serum and an overall weighted cytokine/chemokine score (CK score) was calculated. Associations of cytokines/chemokines with all-site, lung, and lymphoproliferative cancers were assessed in Cox regression models accounting for relevant covariates including age, sex, RA disease activity, and smoking.

Results: In 1216 patients, 146 incident cancers (42 lung and 23 lymphoproliferative cancers) occurred over 10,072 patient-years of follow-up with a median time of 4.6 years from enrollment (cytokine/chemokine measurement) to cancer incidence. In fully adjusted models, CK score was associated with a higher risk of all-site (aHR 1.32, 95% CI 1.01–1.71, $p < 0.001$), lung (aHR 1.81, 1.40–2.34, $p = 0.001$), and lung/lymphoproliferative (aHR 1.54 [1.35–1.75], $p < 0.001$) cancer. The highest quartile of CK score was associated with a higher risk of all-site (aHR 1.91, 0.96–3.81, $p = 0.07$; p-trend = 0.005), lung (aHR 8.18, 1.63–41.23, $p = 0.01$; p-trend < 0.001), and lung/lymphoproliferative (aHR 4.56 [1.84–11.31], $p = 0.001$; p-trend < 0.001) cancer. Thirteen of 17 individual analytes were associated with incident cancer risk.

Conclusion: Elevated cytokine/chemokine concentrations are predictive of future cancer in RA patients, particularly lung and lymphoproliferative cancers. These results suggest that the measurement of circulating cytokines/chemokines could be informative in cancer risk stratification and could provide insight into future cancer prevention strategies in RA, and possibly individuals without RA.

1. Introduction

Patients with rheumatoid arthritis (RA), an autoimmune disease characterized by chronic inflammation preferentially affecting the joints, have a higher risk of developing cancer, specifically lymphoproliferative and lung cancers [1]. Given established associations

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between RA disease activity and cancer risk [2], it is speculated that chronic inflammation and immune system perturbations occurring in RA may disrupt normal immunosurveillance that protects against the development of a malignancy [3]. Pro-inflammatory cytokines and chemokines, which serve critical messenger roles in the immune system, are altered in both RA and cancer [4,5], further suggesting shared pathogenic mechanisms.

With the growing body of evidence implicating inflammation and immune dysregulation in cancer pathogenesis, there have been efforts in the general population to utilize measures of inflammation for cancer prediction. C-reactive protein (CRP), an acute phase reactant, has been found to be predictive of cancer incidence, particularly lung cancer, and cancer survival [6,7]. Evaluation of specific cytokines in the general population has identified IL-6 as an independent predictor of poorer survival among lung cancer patients [8]. Secondary analyses of a large double-blind randomized controlled trial of canakinumab (an inhibitor of IL-1β) in patients with a history of previous myocardial infarction confirmed these prior observational findings, with hsCRP and IL-6 concentrations being higher in those who went on to develop cancer [9]. More importantly, treatment with canakinumab significantly reduced lung cancer incidence, lung cancer mortality, and overall cancer mortality [9]. These findings suggest that targeting pro-inflammatory cytokines/chemokines may be a method to not only perform cancer risk stratification, but also to reduce the risk of developing cancer. Patients with chronic inflammatory diseases, such as RA, may be those most likely to benefit from such strategies.

Because of the pivotal role cytokines/chemokines play in chronic inflammatory diseases such as RA [10], patients with chronic inflammatory diseases may be those most likely to benefit from such screening or therapeutic strategies. Additionally, analyses of the predictive potential of these measures for cancer risk in chronic inflammatory diseases may differ from that in the general population. We previously evaluated whether cytokine/chemokine concentrations were predictive of cancer mortality in a cohort of U.S. Veterans with RA. We observed associations between concentrations of these biomarkers with both all-cancer mortality and lung cancer mortality, independent of other risk factors [11]. At the time, only cancer-related deaths were available, raising the important question as to whether these prior observations were due to associations with higher rates of incident cancer or rather associations with tumor progression. Since this initial report, we have established requisite data linkages enabling the capture of non-fatal incident cancers. Therefore, the aim of this study was to investigate the association between cytokine/chemokine concentrations and incident cancer in U.S. Veterans with RA. We hypothesized that higher cytokine/chemokine concentrations would be associated with an increased risk of all cancer and lung cancer.

2. Patients and methods

2.1. Study participants

Participants in this study were enrolled in the Veterans Affairs Rheumatoid Arthritis (VARA) Registry [12], a multicenter prospective cohort study of U.S. Veterans with RA initiated in 2003. Participants fulfilled the 1987 American College of Rheumatology (ACR) RA classification criteria [13]. Eligible participants were those with 0an available cytokine measurement from a prior study [14] and without a history of cancer prior to VARA enrollment (including non-melanoma skin cancer). This study was approved by the institutional review board at the Omaha VA and each participating site, and all patients provided informed consent.

Upon VARA enrollment, clinical and demographic variables collected included age, sex, self-reported race, smoking status (current, former, never), comorbid conditions, ACR core disease activity measures, 28-joint Disease Activity Score (DAS28) [15], Multidimensional Health Questionnaire score (MDHAQ) [16], and disease-modifying antirheumatic drug (DMARD) use. Using comorbid conditions collected by the enrolling investigator, the Rheumatic Disease Comorbidity Index (RDCI) was calculated [17]. Serum was collected from each patient at the time of enrollment and banked for subsequent analysis. From banked serum, a second-generation commercial anti-cyclic citrullinated peptide (CCP) antibody, rheumatoid factor (RF), and high sensitivity C-reactive protein (hsCRP) were measured as previously reported [18].

2.2. Cancer identification

To identify cancers among VARA participants, the VARA registry was linked with both the VA oncology raw domain (containing detailed cancer data as part of Veterans Health Administration efforts to maintain a cancer registry) residing within the VA Corporate Data Warehouse (CDW) as well as the National Death Index (NDI). VA cancer registry data has been reported to capture >90% of all cancers occurring in the VA health care system after 1995, with a resulting prevalence of cancers that mirrors the general U.S. population [19,20]. Specific oncologic data accessed included date of cancer diagnosis, anatomic site as defined by International Classification of Diseases for Oncology (ICD-O-3), histology, and stage. Linkage with the NDI was established through the Center of Excellence for Suicide Prevention, Joint Department of VA and Department of Defense Mortality Data Repository (http://vaww.virec.research.va.gov/Mortality/Overview.htm) extracted through 12/31/2014. Within the NDI, cancer was identified by International Classification of Diseases (ICD) 10th edition codes C00-D49 on death certificates. In addition to using the aforementioned cancer database to exclude participants with a previous history of cancer, we also excluded participants with diagnostic codes for cancer (ICD-9 140.xx-239.xx and ICD-10 C00.xx-D49.xx) from inpatient and outpatient encounters in the VA CDW that occurred before VARA enrollment.

2.3. Cytokine/Chemokine measurement

Baseline serum cytokine/chemokine measurements were performed as part of a prior study [14]. Briefly, these were measured from banked serum that was obtained upon enrollment into VARA using a BioRad Human cytokine 17-plex bead-based assay. The dilution buffer was modified with heterophilic blocking reagent to prevent RF interference. The 17 analytes examined included: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1β (MIP-1β), and tumor necrosis factor alpha (TNF-α). An overall CK score was calculated from the log-transformed, normalized, and weighted individual analytes to estimate an overall cytokine/chemokine concentration, as previously described [11,21]. Individual analyte concentrations and the CK score were log transformed for normality and additionally categorized into quartiles given that no specific clinically meaningful cut-points have been established for these measures.

2.4. Statistical analysis

Baseline characteristics of the study cohort were compared between quartiles of cytokine/chemokine score using chi-square and ANOVA tests. CK score was compared across tumor stage using Kruskal Wallis test. Primary analyses evaluated associations between baseline CK score and incident cancer risk using Cox proportional hazards models. Follow-up was initiated at the registry enrollment date and continued until incident cancer development or censoring due to death or end of the study period (4/1/2018). Multivariable Cox regression models were constructed adjusting for age and sex, as well as fully adjusted models that also included race, smoking status, comorbidity burden by the RDCI score, DAS28, MDHAQ, RF concentration, methotrexate use, and biologic DMARD use. Covariates were selected based on their potential to

act as confounders to the association between these inflammatory parameters and cancer, determined through exploratory analyses and prior work [11,22]. DAS28, MDHAQ, methotrexate use, and biologic DMARD use were allowed to vary over time, while other covariates were fixed at enrollment values. Missing DAS28 and MDHAQ values were imputed using the last observation carried forward method (<13% of DAS28 and <12% of MDHAQ values). The primary outcome was the development of any cancer, excluding non-melanoma skin cancer and non-invasive cancers. In secondary analyses, we evaluated lung cancer risk, lung and lymphoproliferative cancer risk, and all cancers including non-melanoma skin and non-invasive cancers. In lung cancer and lymphoproliferative cancer specific analyses, patients were censored at the time of diagnosis of an alternative cancer. Given the smaller number of incident lung and lymphoproliferative cancers, the multivariable models included a more parsimonious set of covariates: age, sex, race, smoking status, and DAS28.

Because of previously observed interactions between these laboratory measurements and current smoking [11], stratified analyses based on smoking history (current vs. former/never) were completed. Sensitivity analyses included additional adjustments for factors that have been associated with cytokine/chemokine expression and cancer risk. These included enrollment BMI [23], a history of alcohol use disorder identified by ICD codes prior to VARA enrollment [24], and hsCRP at enrollment. Additional sensitivity analyses included an age^2 term, to account for a non-linear association between age and cancer, and excluded participants who developed cancer within the first twelve months following enrollment (also the time of cytokine/chemokine measurement). Standard errors were adjusted for clustering by site in all models. All analyses were completed within the VA Informatics and Computing Infrastructure using Stata v15 (StataCorp, College Station, TX). Data is available upon reasonable request and ethical approval.

3. Results

3.1. Baseline characteristics

At the time of the initial study [14], cytokine/chemokine measurements were completed on the entire cohort of 1468 participants. Participants with cancer prior to enrollment were excluded (n = 252), resulting in a cohort of 1216 participants in the present analyses. The study cohort was predominately male (89.4%), Caucasian (77.1%), had a mean ± standard deviation (SD) age of 62.8 ± 11.5 years at time of enrollment, and frequent smoking history (29.0% current, 50.3% former) (Table 1). Mean RA disease duration was 11.6 years (SD 11.3), and the majority were seropositive for RF (79.9%) or anti-CCP antibody (77.8%). Higher quartiles of CK score were significantly associated with older age, current smoking, anti-CCP antibody and RF positivity/concentration, DAS28, and MDHAQ (Table 1).

3.2. Incident cancers

Over 10,072 patient-years of follow-up, 146 incident cancers (excluding non-melanoma skin and non-invasive) occurred with a median time to diagnosis of 4.6 years (interquartile range [IQR] 2.7–6.9) from registry enrollment (and cytokine measurement). Most cancers (n = 103) were identified from VA oncology data. Lung cancer was the most frequent site (n = 42), followed by prostate (n = 26) and lymphoproliferative (n = 23) cancers (Table 2). Cancer stage at diagnosis was available for 80 cancer cases with 21 Stage I, 21 Stage II, 13 Stage III, and 25 being Stage IV.

3.3. Association of cytokines/chemokines with all cancer incidence

When evaluated as a continuous variable, CK score (per 1 log-adjusted unit) was significantly associated with incident cancer following adjustment for age and sex (adjusted hazard ratio [aHR] 1.34; 95% CI 1.22–1.48; p < 0.001) as well as following adjustment for all

Table 1
Baseline characteristics by quartile of cytokine score (n = 1216).

	Overall	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value
Demographics & other health factors						
Age, years	62.8 (11.5)	61.7 (12.6)	65.0 (11.2)	61.6 (10.6)	63.1 (11.2)	<0.001
Male sex, %	89.4	87.2	92.4	86.8	91.1	0.06
White race, %	77.1	78.6	73.0	75.3	81.6	0.06
≥HS education, %	84.6	87.3	83.1	84.8	83.3	0.47
Smoking Status, %						<0.001
Current	29.0	18.8	27.3	32.9	37.2	
Former	50.3	57.6	50.0	47.7	46.1	
Never	20.6	23.7	22.7	19.4	16.8	
BMI (kg/m^2)	28.4 (5.6)	28.6 (5.9)	28.5 (5.0)	28.3 (5.3)	28.1 (6.0)	0.71
RDCI score	1.9 (1.5)	1.7 (1.5)	1.9 (1.4)	1.9 (1.5)	2.0 (1.6)	0.09
RA characteristics						
RA duration, years	11.6 (11.3)	11.7 (11.0)	11.8 (12.0)	11.5 (11.3)	11.3 (10.8)	0.95
RF positive, %	79.9	70.3	74.4	86.1	88.8	<0.001
RF conc. (IU/ml)	327 (646)	101 (165)	164 (254)	386 (615)	656 (1003)	<0.001
CCP positive, %	77.8	68.7	74.3	84.1	84.2	<0.001
CCP conc. (U/ml)	272 (447)	187 (356)	261 (437)	321 (474)	318 (498)	<0.001
DAS28	4.0 (1.6)	3.6 (1.4)	3.9 (1.5)	4.2 (1.6)	4.3 (1.8)	<0.001
MDHAQ	0.9 (0.6)	0.8 (0.6)	0.9 (0.6)	1.0 (0.6)	1.0 (0.6)	0.02
DMARD, %	83.1	87.5	82.2	82.2	80.3	0.10
MTX, %	54.1	57.8	56.9	53.2	48.6	0.11
bDMARD*, %	29.6	32.6	25.3	31.3	29.3	0.23
Prednisone, %	42.3	38.8	42.0	40.9	47.5	0.18

p-value by chi-square or ANOVA.
Values mean (SD) unless otherwise noted.
*97% of bDMARDs were TNFi.
Abbreviations: HS, high-school; BMI, body mass index; RDCI, Rheumatic Disease Comorbidity Index; RA, rheumatoid arthritis; SE, shared epitope; RF, rheumatoid factor; CCP, anti-cyclic citrullinated peptide antibody; DAS28, Disease Activity Score in 28 joints; MDHAQ; multidimensional Health Assessment Questionnaire; DMARD, disease-modifying anti-rheumatic drug; MTX, methotrexate.

Table 2
Primary site of incident cancers.

Site	N (% of all cancers)
Lung/Bronchus	42 (28.8)
Prostate	26 (17.8)
Lymphoproliferative	23 (15.8)
Pancreas	8 (5.5)
Skin (melanoma)	6 (4.1)
Colon	5 (3.4)
Stomach	5 (3.4)
Liver	4 (2.7)
Bladder	3 (2.1)
Esophagus	3 (2.1)
Kidney	2 (1.4)
Larynx	2 (1.4)
Palate	2 (1.4)
Thyroid	2 (1.4)
Tongue	2 (1.4)
Unknown	2 (1.4)
Other *	7 (4.4)
Total	**146 (100)**

Other includes the following with n = 1: brain, breast, cervical/uterine, ovarian, rectal, small intestine.

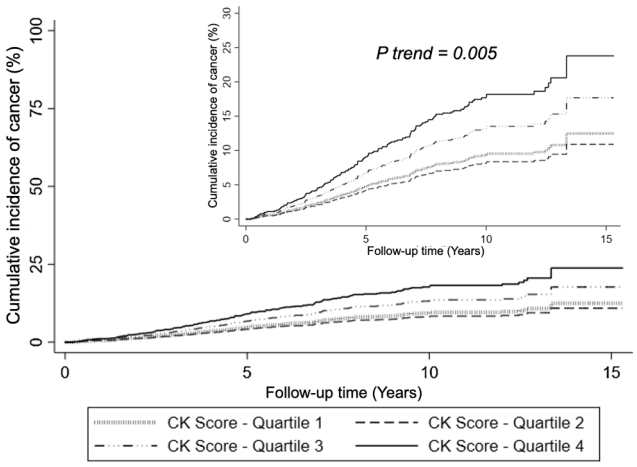


Fig. 1. Cumulative incidence of cancer by cytokine score quartiles. Predicted cumulative incidence of cancy by cytokine (CK) score quartiles from multivariable Cox regression models. Quartile 1 represents the lowest cytokine score values while quartile 4 represents the highest cytokine score values. P for trend across quartiles of cytokine score in fully adjusted model.

covariates (aHR 1.35; 95% CI 1.20–1.52; p < 0.001) (Table 3). The highest quartile of CK score trended towards an association with incident cancer (Table 3 & Fig. 1; Q4 vs. Q1 aHR 1.91, 95% CI 0.96–3.81), although this did not achieve statistical significance (p = 0.07). However, a test of trend across increasing CK score quartiles was highly significant (p = 0.005). Thirteen individual analytes were associated with higher risk of cancer when examined individually in fully adjusted models. These included IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-17, G-CSF, GM-CSF, MIP-1β, TNF-α, all of which had similar effect sizes (Fig. 2**A**). Results were less precise when examined as quartiles, though the highest quartile of 9 individual analytes remained significantly associated with cancer development (IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-17, G-CSF, GM-CSF). Patients in the highest quartile of these 10 analytes had 1.3 to 2.4-fold increased risk of incident cancer relative to those in the lowest quartile for the same analyte (data not shown).

3.4. Association of cytokines/chemokines with lung and lymphoproliferative cancer incidence

Associations between CK score and incident cancer were stronger when examining lung cancer specifically. As a continuous variable, the CK score was associated with incident lung cancer in both age and sex adjusted (aHR 1.93; 95% CI 1.44–2.58; p < 0.001) and fully adjusted (aHR 1.81; 95% CI 1.40–2.34; p = 0.001) models (Table 3). Patients in the highest quartile of CK score demonstrated an 8-fold higher risk of lung cancer relative to those in the lowest quartile (Q4 vs. Q1 HR 8.18; 95% CI 1.63–41.23; p = 0.011). Of the 17 analytes measured, 15 were individually associated with an increased risk of lung cancer (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-17, GM-CSF, IFN-γ, MCP-1, MIP-1β, TNF-α) (Fig. 2**B**). Associations between analyte quartiles (Q4 vs. Q1) and lung cancer were imprecise, though effect sizes were substantially greater than for all cancers (range aHR 1.81 to 16.51 for the 13 significantly associated measures). These measures were IL-1β, IL-2, IL-5, IL-6, IL-7, IL-10, IL-12(p70), IL-13, IL-17, GM-CSF, MCP-1, MIP-1β, and TNF-α.

There were insufficient lymphoproliferative cancers to evaluate them alone. Therefore, we combined lung and lymphoproliferative cancers since both cancers are closely tied to RA (1). CK score was significantly associated with the risk of lung or lymphoproliferative cancer (aHR 1.54, 95% CI 1.35–1.75; p < 0.001) (**Supplemental** Table 1). The highest quartile of CK score had a 4.6-fold higher risk of lung or lymphoproliferative cancer (aHR 4.56, 95% CI 1.84–11.31; p = 0.001).

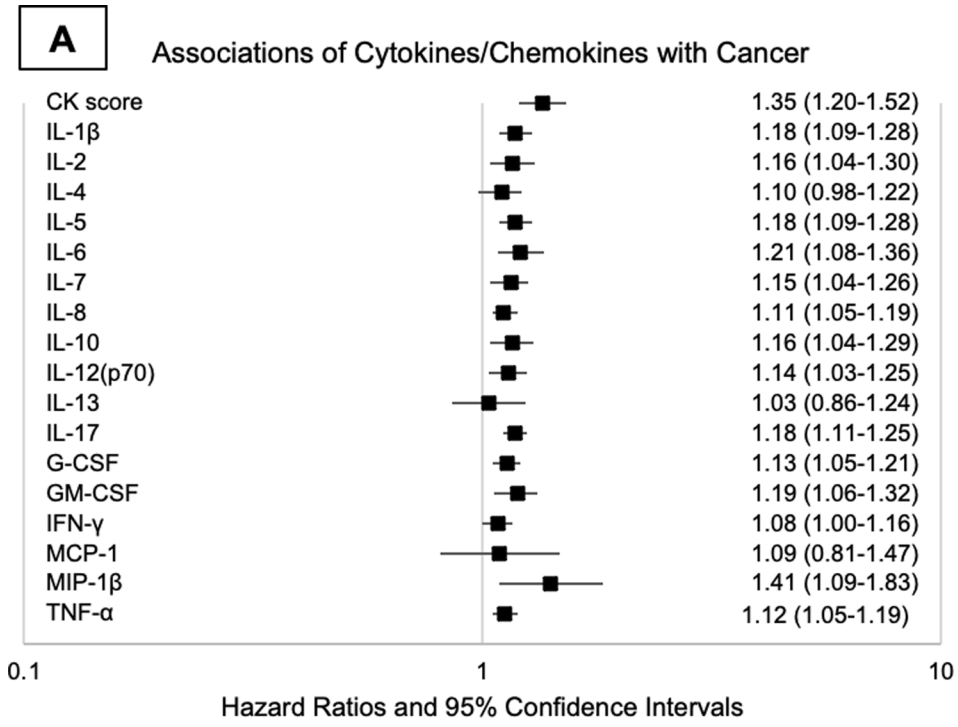
3.5. Association of cytokines/chemokines with tumor stage

Of the 80 participants developing incident malignancy for whom staging data was available at the time of diagnosis, 23 of these were incident lung cancer. For all site cancers, there was no association between CK score and tumor stage (p = 0.74 by Kruskal-Wallis). Similar results were observed in analyses limited to those with lung cancer (p =

Table 3
Associations of cytokine score with incident cancer.

	All Cancer* (n = 146)		Lung Cancer (n = 42)	
Model	Age and Sex Adjusted	Fully Adjusted†	Age and Sex Adjusted	Fully Adjusted‡
CK Score, Continuous	1.34 (1.22–1.48); <0.001	1.35 (1.20–1.52); <0.001	1.93 (1.44–2.58); <0.001	1.81 (1.40–2.34); <0.001
CK Score, Quartiles				
Quartile 1	Reference	Reference	Reference	Reference
Quartile 2	1.01 (0.44–2.35); 0.98	0.87 (0.35–2.16); 0.77	4.80 (0.84–27.54); 0.08	3.69 (0.62–22.19); 0.15
Quartile 3	1.59 (0.91–2.76); 0.10	1.42 (0.79–2.55); 0.24	6.06 (1.28–28.73); 0.02	4.56 (0.94–22.22); 0.06
Quartile 4	2.01 (1.05–3.87); 0.03	1.91 (0.96–3.81); 0.07	10.58 (2.14–52.21); 0.004	8.19 (1.63–41.29); 0.01
P-trend	0.001	0.005	<0.001	<0.001

Values: hazard ratio (95% confidence interval); p-value. *excluding non-melanoma skin and in-situ cancers.
†Fully adjusted model includes age, sex, race, smoking status, Rheumatic Disease Comorbidity Index, rheumatoid factor concentration, 28-joint Disease Activity Score, Multidimensional Health Assessment Questionnaire, methotrexate use, and biologic use.
‡Fully adjusted model includes age, sex, race, smoking status, 28-joint Disease Activity Score.
Abbreviations: CK, cytokine score.



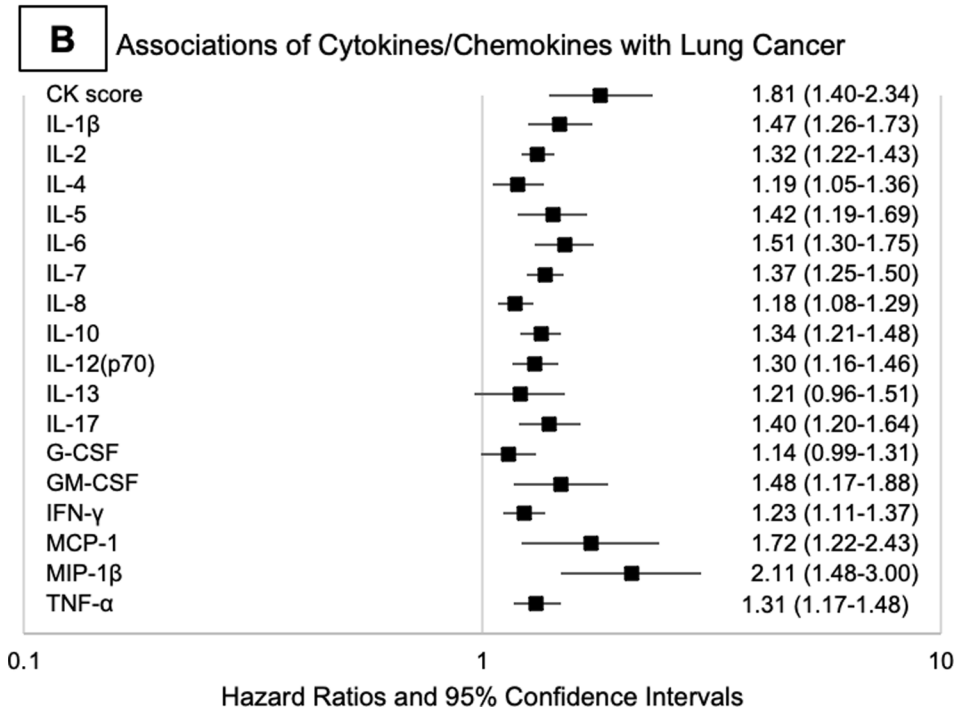


Fig. 2. Forest plots of the associations between individual cytokine/chemokine analytes and incident cancer. Associations of individual cytokine/chemokine concentrations with incident cancer is shown with accompanying hazard ratios and 95% confidence intervals. **A** depicts continuous cytokine concentrations (per 1 log-adjusted unit) in a fully adjusted model for all cancers, excluding non-melanoma skin and in-situ cancers. **B** depicts continuous cytokine concentrations (per 1 log-adjusted unit) in a fully adjusted model for lung cancer. Covariates in models include: age, sex, race, smoking status, Disease Activity Score in 28 joints (DAS28), Multidimensional Health Questionnaire (MD-HAQ)*, Rheumatic Disease Comorbidity Index (RDCI)*, rheumatoid factor concentration*, methotrexate use*, and biologic use* (* denotes variables not included in lung cancer models due to fewer number of lung cancers). Horizontal bars represent the 95% confidence interval. Abbreviations: IL = interleukin; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IFN-γ = interferon-γ; MCP-1 = monocyte chemotactic protein 1; MIP-1β = macrophage inflammatory protein 1β; TNF = tumor necrosis factor.

0.32 by Kruskal-Wallis).

3.6. Stratified analyses by smoking status

In subgroup analyses stratified by smoking status at enrollment (current vs. former/never smokers), associations between continuous CK score and incident cancer were similar in current smokers (aHR 1.45; 95% CI 1.17–1.80; p = 0.001) and former/never smokers (aHR 1.32; 95% CI 1.01–1.71; p = 0.04). When evaluating CK score quartiles, the association was numerically greater in current smokers (highest vs. lowest quartile of CK score in current smokers: aHR 3.48; 95% CI 0.92–13.22; p = 0.07; highest vs. lowest quartile of CK score in former/never smokers: aHR 1.50; 95% CI 0.74–3.05; p = 0.27). However, a multiplicative interaction term between the highest quartile of CK score and current smoking was not statistically significant (p = 0.21).

3.7. Sensitivity analyses

Results of sensitivity analyses compared to the primary results are shown in Table 4. Additional adjustment for enrollment BMI (aHR 1.31; 95% CI 1.17–1.48; $p < 0.001$) and prior alcohol use disorders (aHR 1.35; 95% CI 1.21–1.51; $p < 0.001$) did not significantly alter findings. Inclusion of hsCRP did not attenuate associations of CK score with incident cancer (aHR 1.35; 95% CI 1.20–1.52; $p < 0.001$), and hsCRP concentrations were not significantly associated with cancer incidence (aHR 1.01; 95% CI 0.93–1.10; $p = 0.75$). Exclusion of those that developed cancer within 12 months of enrollment ($n = 11$) resulted in a similar association between CK score and cancer incidence (aHR 1.33; 95% CI 1.21–1.47; $p < 0.001$).

4. Discussion

The essential roles that inflammation and the immune system serve in cancer development continue to be elucidated. Harnessing this growing body of evidence, observational studies in the general population have leveraged measures of inflammation to better quantify cancer risk and a recent randomized controlled trial showed for the first time that cytokine inhibition (IL-1β) prevented lung cancer occurrence [9]. In RA, an autoimmune disease where cytokine perturbations are inherent to the disease process, we have previously shown that serum cytokine concentrations were predictive of cancer mortality [11]. Building upon those initial findings, we linked data from the VARA registry to a national VA oncology database and the NDI to identify both fatal and non-fatal incident cancers. This allowed us to test our hypothesis that cytokine/chemokine measures would be associated with increased cancer and lung cancer risk. Using a well-characterized registry of RA patients with these data linkages, coupled with multiplex cytokine measurements, adjustment for critical confounders (including age, smoking status, RA disease activity), and multiple sensitivity analyses, we found that higher cytokine/chemokine concentrations were associated with an increased risk of incident cancer and particularly heightened risk of lung and lymphoproliferative cancer. These data add to the growing literature supporting a direct effect of inflammation on cancer risk and suggest that these pro-inflammatory measures may be a valuable addition to cancer risk models as well as a potential therapeutic target, particularly in individuals with chronic inflammatory diseases.

In our study, we found cytokine/chemokine concentrations to be predictive of incident all-site, lung, and lymphoproliferative cancer. However, the associations between cytokine concentrations and lung cancer were the greatest in magnitude. This was most clearly apparent when contrasting cancer risk across quartiles of CK score. Compared to those with CK scores in the lowest quartile, individuals in the highest quartile of the CK score demonstrated a near 2-fold higher risk of all-site cancer, 4.6-fold higher risk of lung or lymphoproliferative cancer, and a more than 8-fold higher risk of lung cancer. While characterization of the predictive potential of CKs for other cancer sites, such as lymphoproliferative cancers alone [previously linked to RA disease activity (2)], would be informative, the limited numbers of other site-specific cancers prohibited such analyses.

To determine whether findings might be unique to a select number of cytokines, we also investigated the associations between individual analytes with cancer risk. Our findings were consistent with a more global inflammatory association, rather than suggesting an analyte-dependent association. Thirteen of the 17 individual analytes were significantly associated with cancer incidence, yielding effect sizes that were similar across measures. These findings were expected given similar findings of analyte-independent associations in our prior study of cancer mortality [11] in addition to recognizing the highly correlated nature of these cytokine measurements (data not shown).

Recently, IL-1β inhibition with canakinumab in patients with atherosclerosis was found to reduce lung cancer incidence and cancer-related mortality in the CANTOS trial [9]. In this same trial, IL-6 concentrations were higher in individuals who went on to develop lung cancer. Likewise, we also found IL-6 to be significantly associated with incident cancers of all types as well as lung cancer. In our study, patients with RA falling in the highest quartile of IL-6 measurements had a 1.6-fold higher risk of developing cancer during follow-up. Additionally, we found IL-1β to be associated with an increased risk of all cancer and lung cancer development. In addition to supporting the validity of our findings, this illustrates that cytokines key to RA pathogenesis (and targets of approved RA therapies) are associated with cancer incidence in this population. Whether targeting these molecules in RA may preferentially mitigate the risk of lung cancer or other cancers, is a compelling question that will require further studies to address.

While pharmacologic targeting of cytokines shows promise for reducing cancer risk, an additional clinical application of our findings is the development of cancer risk models. The median time from cytokine/chemokine measurement to cancer development in our study approached 5 years and exclusion of cancers occurring in the first 12 months after cytokine/chemokine measurement did not meaningfully impact results. Therefore, it is unlikely we were simply detecting latent cancers that had yet to be detected clinically. Moreover, this implies that a single baseline measurement of multiplex cytokines may add predictive value to models including other known cancer risk factors (e.g. age, smoking history) in risk stratifying RA patients, aid in identifying those in need of additional or heightened cancer screening, and ultimately allow for earlier detection of cancers. If validated further in other populations (e.g. other chronic inflammatory diseases), it is possible that these cytokine measures could potentially serve as a screening tool in individuals without RA. Development and performance testing of these risk models will require future, prospective efforts and validation in external cohorts.

Our population was primarily composed of older, Caucasian males, which is representative of the individuals receiving treatment from the VA (National Center for Veterans Analysis and Statistics; https://www.va.gov/vetdata/). The generalizability to those outside of the Veteran population, where the majority of patients with RA are female, could be limited. A single multiplex cytokine/chemokine panel measurement at enrollment was used and these measures were selected based on a commercial multiplex panel with analytes recognized to be critical in RA pathogenesis. Whether longitudinal measurements or the application of a broader panel of inflammatory markers with analytes specifically selected for their role in cancer pathogenesis would enhance predictive capacity remain important questions and will mandate further investigation. Due to lags in the availability of NDI data, fatal cancers not recorded in the VA oncology data during the final study years may have been missed. However, most cancers were identified in the VA oncology data and any potential outcome misclassification is expected to be non-differential to cytokine/chemokine measures, which would result in a

Table 4
Association of cytokine score with incident cancer in sensitivity analyses.

Model	Hazard Ratio	95% CI	p-value
Primary analysis (from Table 3)	1.35	1.20–1.52	<0.001
Sensitivity analyses			
Including non-melanoma skin and in situ cancers	1.30	1.12–1.49	<0.001
Adjusting for body mass index	1.31	1.17–1.48	<0.001
Adjusting for prior alcohol use disorder	1.35	1.21–1.51	<0.001
Adjusting for enrollment hsCRP	1.35	1.20–1.52	<0.001
Adjusting for age^2	1.35	1.21–1.52	<0.001
Excluding cancers occuring in the first 12 months of follow-up	1.33	1.21–1.47	<0.001

Models adjusted for age, sex, race, smoking status, Rheumatic Disease Comorbidity Index, rheumatoid factor concentration, 28-joint Disease Activity Score, Multidimensional Health Assessment Questionnaire, methotrexate use, and biologic use. Abbreviations: hsCRP, high sensitivity C-reactive protein.

bias towards the null. Detailed smoking history beyond smoking status at registry enrollment was unavailable. However, current smoking has been most closely tied to higher serum cytokine concentrations [11], and no evidence of an interaction between current smoking and cytokines with cancer incidence was observed.

In conclusion, using a cohort of U.S. Veterans with RA and robust data linkages, we identified higher serum cytokine/chemokine concentrations to be associated with incident all-site, lung, and lung or lymphoproliferative cancer development. While biologic mechanisms remain to be elucidated and external validation is needed, these findings yield important implications for patients with RA and potentially to individuals without RA. These results suggest that multiplex measurement of cytokines/chemokines could be harnessed as a means of identifying patients with RA or other chronic inflammatory diseases at heightened risk for future malignancy, ultimately leading to improved screening strategies, personalized approaches to disease-modifying therapy, earlier cancer diagnosis, and improved long-term outcomes.

CRediT authorship contribution statement

Bryant R. England: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Megan Campany:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Harlan Sayles:** Software, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing. **Punyasha Roul:** Software, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing. **Yangyuna Yang:** Software, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing. **Apar Kishor Ganti:** Conceptualization, Writing - review & editing, Supervision. **Jeremy Sokolove:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing. **William H. Robinson:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing. **Andreas M. Reimold:** Resources, Data curation, Writing - review & editing. **Gail S. Kerr:** Resources, Data curation, Writing - review & editing. **Grant W. Cannon:** Resources, Data curation, Writing - review & editing. **Brian C. Sauer:** Resources, Data curation, Writing - review & editing. **Joshua F. Baker:** Resources, Data curation, Writing - review & editing. **Geoffrey M. Thiele:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing. **Ted R. Mikuls:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclosures

AMR speaker for Eli Lilly, participate in clinical tirals with Pfizer and Abbvie. GMT has participated in the Regeneron speakers bureau. AKG has served advisory roles for Genentech/Roche, AstraZenec, Cardinal Health, Jazz Pharmaceuticals, G1 Therapeutics, Blueprint Medicines, and Flagship Biosciences. All other authors have declared no conflicts of interest.

Appendix A. Supplementary data

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