Soluble CD14 and CD14 Polymorphisms in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Soluble CD14 (sCD14) is involved in innate immune responses and has been implicated to play a pathogenic role in inflammatory diseases including rheumatoid arthritis (RA). No studies have identified the specific factors that influence sCD14 expression in RA. We used cross-sectional data to evaluate the relationship of sCD14 concentrations in RA with measures of disease activity and severity. We hypothesized that sCD14 concentrations would be elevated in subjects with greater RA disease severity and markers of disease activity, compared to subjects with lower disease activity. We also examined whether well-defined polymorphisms in CD14 are associated with sCD14 expression in RA.

Methods. Soluble CD14 concentrations were measured using banked serum from patients with RA (n = 1270) and controls (n = 186). Associations of patient factors including demographics, measures of RA disease activity/severity, and select CD14 single-nucleotide polymorphisms (SNP) with sCD14 concentration were examined in patients with RA using ordinal logistic regression.

Results. Circulating concentrations of sCD14 were higher in patients with RA compared to controls ($p < 0.0001$). Factors significantly and independently associated with higher sCD14 levels in patients with RA included older age, being white (vs African American), lower body mass index, elevated high sensitivity C-reactive protein, and higher levels of disease activity based on the Disease Activity Score (DAS28). There were no significant associations of CD14 tagging SNP with sCD14 level in either univariate or multivariable analyses.

Conclusion. Circulating levels of sCD14 are increased in RA and are highest in patients with increased levels of RA disease activity. In the context of RA, sCD14 concentrations also appear to be strongly influenced by specific patient factors including older age and race but not by genetic variation in CD14. (J Rheumatol First Release Sept 15 2011; doi:10.3899/jrheum.110378)

Key Indexing Terms:

SOLUBLE CD14 | RHEUMATOID ARTHRITIS | ACUTE-PHASE RESPONSE
DISEASE SEVERITY | DISEASE ACTIVITY | POLYMORPHISMS

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CD14 and Toll-like receptors (TLR) are pattern-recognition receptors expressed on a variety of inflammatory cells, playing a central role both in host defense and in the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis (RA)[1,2]. CD14 is expressed as a membrane-bound protein and in a circulating soluble form (sCD14). Together with lipopolysaccharide (LPS) and LPS-binding protein, sCD14 forms a trimolecular ligand that interacts with the cell-surface TLR4/MD-2 receptor complex[3]. Engagement of this complex leads to activation of innate host defense mechanisms, stimulating the elaboration of numerous proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin 1ß (IL-1ß)[4], 2 of the primary mediators of inflammation implicated in the pathogenesis of RA.

In addition to its role in TLR binding, it has been suggested that expression of sCD14 represents an acute-phase protein response[5]. Consistent with this, serum sCD14 concentrations have been reported to be elevated in a number of inflammatory conditions including RA[6,7], periodontal disease[8], crystal-induced arthritis[5], reactive arthritis[5], atopic

dermatitis[9], systemic lupus erythematosus[10], and Kawasaki disease[11]. Moreover, declines in sCD14 concentration have been shown to correlate with treatment response in a prospective study examining TNF-α inhibition in a limited number of patients with RA[5].

While these observations support a potential role of sCD14 in disease pathogenesis, factors influencing the expression of sCD14 in RA have not been well defined. Specifically, no large-scale comprehensive studies have examined the relationship of sCD14 concentrations with measures of RA disease activity and/or severity. Such a study could provide insight by identifying those disease manifestations in RA most closely associated with sCD14 acute-phase responses. If sCD14 levels should prove to be associated with RA disease activity and severity, it raises the question whether polymorphisms for the gene encoding CD14 are associated with sCD14 levels in patients with RA. Indeed, single-nucleotide polymorphisms (SNP) in *CD14* (5q31) have been correlated with circulating concentrations of sCD14 in diverse populations such as women of reproductive age[12], infants[13], patients with cardiovascular disease[14], and healthy persons[15]. However, no studies have demonstrated whether genetic variations in *CD14* influence sCD14 concentrations in RA.

We used cross-sectional data to evaluate the relationship of sCD14 concentrations in RA with measures of disease activity and severity. We hypothesized that sCD14 concentrations would be elevated in subjects with greater RA disease severity and markers of disease activity, compared to subjects with lower disease activity. Finally, we also examined whether well-defined polymorphisms in *CD14* are associated with sCD14 expression in RA.

MATERIALS AND METHODS

Study subjects. Patients with RA included U.S. veterans enrolled in the Veterans Affairs Rheumatoid Arthritis (VARA) registry[16,17] with enrollment sites at 11 VA Medical Centers located in Birmingham, AL, Brooklyn, NY, Dallas, TX, Denver, CO, Iowa City, IA, Jackson, MS, Little Rock, AR, Omaha, NE, Portland, OR, Salt Lake City, UT, and Washington, DC. The registry received institutional review board approval at each site and all patients provided informed written consent prior to enrollment. Patients with RA satisfied the 1987 American College of Rheumatology classification criteria[18]. To allow comparisons with patients, we also examined sCD14 concentrations in a convenience sample (n = 186) that included healthy controls (n = 127) and individuals with chronic obstructive pulmonary disease (COPD; n = 59). Veteran (n = 48) and nonveteran (n = 79) healthy controls were recruited as part of 2 separate investigations examining disease risk factors in RA[19] and COPD[20]. Healthy controls were volunteers lacking systemic inflammatory diseases including RA and COPD. Smoking status data were not universally available for controls. Veterans with COPD were identified using pulmonary function testing, and all satisfied the Gold classification criteria[21]. Patients with COPD were chosen as disease controls based on studies implicating CD14 in the pathogenesis of chronic airway disease[22,23]. The data available for controls were limited to age at enrollment, sex, and race/ethnicity (white vs other).

Characteristics of patients with RA. In addition to collecting serum and DNA samples at enrollment, VARA includes standardized clinical data measured as part of routine care. Enrollment variables include diagnostic criteria (such as the presence of subcutaneous nodules), comorbidity (described below), cigarette smoking status (never, former, or current), sociodemographics (education, race/ethnicity, age, sex), body mass index (BMI), date of RA diagnosis, and medication use including prednisone, methotrexate (the most commonly used disease-modifying antirheumatic drug) and anti-TNF-α therapy. Anticitrullinated protein antibodies were measured on banked serum using a second-generation ELISA (Diastat, Axis-Shield Diagnostics, Dundee, Scotland; positivity ≥ 5 U/ml). Rheumatoid factor (positivity ≥ 15 IU/ml) and high sensitivity C-reactive protein (hsCRP, mg/l) were determined by nephelometry (Siemens Healthcare Diagnostics, Munich, Germany). The presence of HLA-DRB1 shared-epitope (SE) containing alleles was determined using banked DNA as described[24]. Additional measures collected at enrollment included tender and swollen joint counts (range 0–28), erythrocyte sedimentation rate (ESR; mm/h), pain (range 0–10), a 10-item multidimensional Health Assessment Questionnaire score (MD-HAQ; range 0–3)[25], and patient and physician global well-being scores (100-mm visual analog scales). A 4-variable Disease Activity Score based on 28 joints (DAS28) was calculated[26] and categorized based on values consistent with remission (< 2.6), low (≥ 2.6 and < 3.2), moderate (≥ 3.2 and < 5.1), or high (≥ 5.1) disease activity[27]. Comorbidity was examined as a cumulative count (range 0–9) based on the presence of diagnostic codes for diabetes mellitus, ischemic heart disease, hypertension, cerebrovascular disease, chronic kidney disease, hyperlipidemia, depression, interstitial lung disease, and COPD. Results of formal pulmonary function testing were not available for RA cases.

sCD14 was measured from banked serum using a commercial ELISA with units measured in ng/ml (R&D Systems, Minneapolis, MN, USA). Given its skewed distribution, sCD14 concentrations in patients with RA were subsequently examined in quartiles (< 1617 ng/ml, 1617 to < 1952 ng/ml, 1952 to < 2340 ng/ml, ≥ 2340 ng/ml).

CD14 genotypes. For genotyping purposes, the complete coding region of *CD14*, intronic sequence, about 6 kb of 5' genomic, and 2 kb of 3' genomic were resequenced from DNA obtained from 23 European Americans as part of the Innate Immunity Program in Genomic Applications[28]. A total of 17 SNP were identified; 15 of these had a minor allele frequency > 10%. From these 15 SNP a tagging strategy, based on bins of polymorphic sites that exceeded 10% minor allele frequency and a within-bin linkage disequilibrium (LD) exceeding 0.7, was employed using publicly available software[29]. This algorithm resulted in 4 bins, nomenclature relative to translation start site: *CD14*/–260, rs2569190; *CD14*/–651, rs5744455; *CD14*/–1720, rs2915863; and *CD14*/–2838, rs2569193. Aliases relative to the transcription start site are *CD14*/–159, *CD14*/–550, *CD14*/–1619, and *CD14*/–2737, respectively.

Genomic DNA was extracted from whole blood using a QiaAmp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). DNA samples were genotyped using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom Inc., San Diego, CA, USA). Multiplex polymerase chain reaction assays and associated extension reactions were designed using SpectroDesigner software (Sequenom). Primer extension products were loaded onto a 384-element chip with a nanoliter pipetting system (Sequenom) and analyzed with a MassArray mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The resulting mass spectra were analyzed for peak identifications using SpectroTyper RT 4.0 software (Sequenom). For genotyping quality control, Hardy-Weinberg calculations were performed to ensure that each marker was within the expected allelic population equilibrium. Genotyping data were not available for controls.

Statistical analyses. Soluble CD14 concentrations were compared by patient groups using the nonparametric Mann-Whitney U test. Concentrations of sCD14 for patients with RA were classified into 4 levels based on quartiles. Correlations of baseline acute-phase responses (ESR, hsCRP) with sCD14 quartiles were computed for patients with RA using Spearman rank correlations. Associations of different patient characteristics with sCD14 concentrations were examined using the cumulative odds ordinal logistic regression model[30], where sCD14 quartile was treated as the

ordinal response variable. Corresponding OR and 95% CI of increased sCD14 (higher quartile relative to lower quartile) associated with each independent variable were calculated. Using this approach, for example, OR of 1.3 corresponds to 30% higher odds of being in a higher quartile (top quartile vs lower 3 quartiles or top 2 quartiles vs lower 2 quartiles or top 3 quartiles vs the lowest quartile). The Brant test[31] was used to test the proportional odds assumption that regression lines for the comparison of categories are parallel for each variable considered; this assumption was satisfied for all variables examined in the ordinal regression models.

A multivariable model was generated using backwards stepwise ordinal logistic regression to identify sociodemographic and disease-related factors independently associated with sCD14 concentration (clinical model). Given their significance in univariate analyses coupled with associations reported in other populations[8], both age and race were forced into the clinical model. Joint counts and ESR were not examined in multivariable analyses since they are component measures of the DAS28[26]. To account for colinearity in patient-reported outcomes, we examined associations of the Routine Assessment of Patient Index Data (RAPID-3), a composite disease activity measure that incorporates the MD-HAQ, pain, and patient global well-being[32]. To further assess associations of *CD14* SNP with level of sCD14, we generated separate multivariable models by sequentially adding the different *CD14* tagging SNP to the clinical model (clinical model plus single polymorphism). Using a Bonferroni correction, SNP associations were considered to be statistically significant at a value of $p < 0.0125$ (0.05/4 SNP). Because OR for minor allele homozygotes and heterozygotes were similar within each SNP, genetic associations were limited to binary comparisons (presence vs absence of minor allele for each SNP). Two-way multiplicative interactions between each tagging *CD14* SNP and race were also examined in the multivariable models. Analyses were completed using Stata v10.1 (StataCorp, College Station, TX, USA).

The power to detect associations between the *CD14* SNP of interest and sCD14 concentration was estimated, assuming that 30% and 50% of individuals carried the minor allele for each polymorphism[33]. We further assumed that 28%, 26%, 24%, and 22% of individuals without the minor allele fell respectively into the first, second, third, and fourth quartile of the sCD14 concentration. Based on the available RA sample size and a 2-tailed $\alpha = 0.0125$, we had 80% power to detect a minimal detectable OR of 1.40 and 1.48 with an ordinal logistic regression model with minor allele prevalence of 30% and 50%, respectively.

RESULTS

There were 1270 RA cases and 186 controls (59 individuals with COPD and 127 healthy controls) included in the analyses. Characteristics of patients with RA are summarized in Table 1. Reflecting demographic trends in the VA population nationally[34], patients with RA included predominantly older men (mean age 64 yrs; 90% men) who were white (78%), with longstanding RA disease duration at enrollment in the registry. Controls were also predominantly men (66%), slightly younger (mean age 56 ± 13 yrs), and almost exclusively white (93%). The frequency of select CD14 tagging SNP in patients with RA is summarized in Table 1 and is similar to that reported for individuals with Western European ancestry[35].

Circulating concentrations of sCD14 were higher in patients with RA compared to all controls combined ($p < 0.0001$; Table 2). Median sCD14 concentrations were 1952.3 ng/ml in patients with RA (interquartile range 1617.1 to 2340.0 ng/ml) and 1688.1 ng/ml in controls (interquartile range 1423.3 to 2023.9 ng/ml). Among controls, sCD14 concentrations were higher in individuals with COPD compared to healthy controls ($p = 0.0008$). Soluble CD14 levels in controls with COPD (median value 1925.8 ng/ml) approached those of patients with RA ($p = 0.54$ for RA vs COPD). Differences in sCD14 concentration (RA vs all controls) remained highly significant even after limiting the analysis to RA cases with normal hsCRP concentrations, < 3.0 mg/l ($p = 0.0009$), or RA cases with low disease activity, with DAS28 values < 3.2 ($p = 0.0004$).

In individuals with RA, there were moderate but highly significant correlations of sCD14 quartiles with baseline ESR ($r = 0.17$, $p < 0.001$) and hsCRP concentrations ($r = 0.34$, $p < 0.001$). In unadjusted analyses, factors associated with higher concentrations of sCD14 in patients with RA included older age, being white (vs African American), lower BMI, increased number of comorbidities, HLA-DRB1 SE positivity, and measures indicating increased RA disease activity [presence of subcutaneous nodules, elevated hsCRP (≥ 3 mg/l) and ESR, higher joint counts, and worse physician global scores, MD-HAQ and DAS28; Table 3].

In unadjusted analyses, there were borderline associations with sCD14 quartiles for the minor alleles in 2 of the 4 *CD14* tagging SNP examined, associations that were not statistically significant following adjustment for multiple comparisons. The minor allele of rs2569193 (*CD14*/–2838) trended toward a modest but nonsignificant association with higher levels of sCD14, whereas the minor allele of rs2915863 (*CD14*/–1720) showed a modest and nonsignificant inverse association (Table 3).

Results of multivariable ordinal regression are summarized in Table 4. Factors significantly and independently associated with higher sCD14 levels in the clinical model (model excluding CD14 genotypes) included older age, being white (vs African American), lower BMI, elevated hsCRP, and higher levels of disease activity based on the DAS28 composite measure. Associations of HLA-DRB1 SE positivity, comorbidity score, subcutaneous nodules, and MD-HAQ scores with sCD14 concentration were attenuated and were no longer significant after multivariate adjustment. After multivariate adjustment, African Americans were about 50% less likely than whites to have higher sCD14 levels. Elevations in hsCRP (≥ 3 mg/l) were associated with about a 2-fold increased likelihood of having higher sCD14 concentrations, while high levels of RA disease activity (DAS28 > 5.1 vs DAS28 < 2.6) were associated with about 70% to 80% increased likelihood of having a sCD14 concentration within a higher quartile.

Sequential adjustments for the 4 different CD14 tagging SNP did not substantially attenuate the associations of the aforementioned factors in the clinical model with sCD14 quartiles (Table 4). Of the SNP examined, only the minor allele of rs5744455 (*CD14*/–651) showed a borderline association with sCD14 concentration ($p = 0.044$), although this did not achieve significance (adjusted $\alpha = 0.0125$). We

Table 1. Characteristics of patients with rheumatoid arthritis (n = 1270) and controls (n = 186) at enrollment.

Characteristics	Mean (SD) or %	
	RA, n = 1270	Controls, n = 186
Demographic		
Age at enrollment, yrs (SD)	63.6 (11.4)	56.5 (12.5)
Male, %	90.5	67.4
Race, %		
White	77.6	93.0
African American	16.5	
Other	5.8	
≥ High school education, %	84.0	
Smoking status, %		
Never	20.3	
Former	52.0	
Current	27.7	
Body mass index, kg/m^2 (SD)	28.3 (5.6)	
Comorbidity count (0–9) (SD)	3.2 (1.8)	
RA-related measures at enrollment		
Disease duration at enrollment, yrs	12.2 (11.5)	
HLA-DRB1 SE-positive, %	71.7	
ACPA-positive, %	75.6	
Rheumatoid factor-positive, %	80.5	
Subcutaneous nodules, %	36.1	
hsCRP, mg/l	13.0 (20.1)	
hsCRP ≥ 3 mg/l, %	69.5	
ESR, mm/h	26.0 (22.2)	
Tender joint count (0 to 28)	5.0 (6.7)	
Swollen joint count (0 to 28)	4.1 (5.4)	
Pain (0 to 10)	4.5 (2.9)	
Physician global (0 to 100 mm)	36.4 (22.8)	
Patient global (0 to 100 mm)	41.1 (25.6)	
Multidimensional HAQ (0 to 3)	0.93 (0.60)	
DAS28 category, %		
< 2.6 (remission)	22.9	
≥ 2.6 and < 3.2 (low activity)	14.6	
≥ 3.2 and < 5.1 (moderate activity)	40.8	
≥ 5.1 (high activity)	21.8	
RAPID-3 score	2.6 (1.4)	
Prednisone use	43.2	
Methotrexate use	55.6	
Anti-TNF-α use	29.4	
CD14 genotypes		
rs2569190 (*CD14*/–260), %		
GG	30.0	
AG	49.4	
AA	20.7	
rs5744455 (*CD14*/–651), %		
CC	64.1	
CT	31.5	
TT	4.5	
rs2915863 (*CD14*/–1720), %		
TT	39.7	
CT	45.2	
CC	15.1	
rs2569193 (*CD14*/–2838), %		
GG	54.5	
AG	38.4	
AA	7.2	

SE: shared epitope; ACPA: anticitrullinated protein antibody; hsCRP: high sensitivity C-reactive protein; ESR: erythrocyte sedimentation rate; MD-HAQ: Multidimensional Health Assessment Questionnaire; DAS: Disease Activity Score; RAPID: Routine Assessment of Patient Index Data; TNF: tumor necrosis factor.

Table 2. Soluble CD14 (sCD14) concentrations in patients with rheumatoid arthritis (RA) and controls*.

Group	Median (interquartile range), ng/ml
RA, n = 1270	1952.3 (1617.1 to 2340.0)
All controls combined, n = 186	1688.1 (1423.3 to 2023.9)
COPD, n = 59	1905.8 (1519.8 to 2224.2)
Healthy controls, n = 127	1662.4 (1361.4 to 1917.1)

* Group differences examined using the Mann-Whitney U test; $p < 0.0001$ for RA vs all controls combined; $p = 0.0008$ for COPD versus healthy controls; $p = 0.54$ for RA versus COPD. COPD: chronic obstructive pulmonary disease.

observed no evidence of interactions between any of the CD14 tagging SNP and race influencing sCD14 levels.

DISCUSSION

CD14 serves several important and potentially distinct biologic functions that include endotoxin binding[2,36], mediation of cellular apoptosis[37,38,39], and regulation of lymphocyte activation and function[40,41], as well as acting as an acute-phase protein[5]. In support of its latter role, Bas and colleagues[5] observed significant correlations of serum sCD14 concentration with both CRP and IL-6 in patients with different forms of inflammatory arthritis. That report showed that IL-6, a proinflammatory cytokine that regulates the hepatic synthesis of acute-phase proteins, stimulated *in vitro* production of CD14 in liver tissues from non-RA donors[5]. Although we observed significant correlations of sCD14 concentrations with both ESR and hsCRP, other proinflammatory measures including IL-6 and TNF-α were not available from these patients and would be informative in future analyses. Consistent with findings from our study, Bas, *et al* also found that levels of circulating sCD14 were higher in patients with RA compared to controls[5]. Our study extends these findings by showing that differences in expression between RA cases and controls are evident even among RA patients with low levels of disease activity, suggesting that sCD14 may be a particularly sensitive acute-phase measure in RA. Similarities in serum concentrations observed in RA and COPD suggest that inflammatory pathways involving sCD14 may be implicated in both of these chronic conditions. Further, our results show that in the context of RA, sCD14 concentrations appear to be most strongly influenced by specific patient factors including older age, race, and higher levels of RA disease activity.

In contrast to its strong associations with measures of disease activity, our results do not provide evidence that genetic variation in *CD14* substantially influences sCD14 expression in RA. The absence of an association between *CD14* genotypes and sCD14 concentrations may not be surprising, given the equivocal results reported to date. While some studies have shown significant associations of the same tagging *CD14* SNP examined in our study with sCD14 concentrations[12,13,14,15], others failed to show any associations. In a recent study of patients with periodontal disease, *CD14*/–260 genotype showed no association with level of sCD14[8]. Koenig, *et al* reported significant associations of the same *CD14* genotype with sCD14 levels in patients with cardiovascular disease, an association that was absent in healthy controls[14]. It is possible that any genetic influences on this acute-phase protein response are simply overshadowed by other inflammatory stimuli in conditions such as RA and periodontal disease, recognizing that most studies reporting genetic associations with sCD14 level were completed in relatively healthy cohorts in the absence of systemic inflammation[12,13,15].

Consistent with other reports[8], in our study both older age and being white (vs African American) were associated with higher sCD14 concentrations in patients with RA, independent of other covariates. Sequential adjustments for *CD14* tagging SNP did not attenuate the associations observed for race, nor was there evidence of significant gene-race interactions affecting sCD14 concentrations. These data show that racial differences in *CD14* inheritance are unlikely to explain the observed differences in sCD14 expression. It is also notable that adjustments for different measures of disease activity did not attenuate the association of race with levels of sCD14, perhaps not surprisingly, because disease phenotypes in this population (including measures of disease activity) do not appear to vary substantially based on self-reported race[16]. The reasons for the observed racial differences in sCD14 expression are unclear, but could be related to variation in genes coding for other molecules that either directly or indirectly influence sCD14 synthesis or expression. The association of older age with higher sCD14 concentrations in this study is highly consistent with similar age-related changes reported in the expression of other acute-phase reactants. Age-related increases have been shown for ESR in addition to a number of specific acute-phase proteins including fibrinogen, alpha-1-antitrypsin, haptoglobin, and others[42,43,44].

Whether differences in the sCD14 acute-phase response have important clinical implications remains unknown, a possibility that warrants further study. Nicu and colleagues[8] recently speculated that sCD14 could serve as a potential "link" to explain the increased cardiovascular disease burden observed in patients with periodontal disease[45]. Soluble CD14 has been shown to transport cell-bound endotoxin to circulating lipoproteins[46], a complex that could ultimately promote foam-cell formation and the progression of atherosclerosis[47]. Whether sCD14 could serve as a serum biomarker predictive of future cardiac events or whether its increased expression could account for a portion of the excess cardiovascular morbidity seen in RA[48] remains unknown.

In summary, we have shown that levels of sCD14 are increased in RA relative to controls and are positively and

Table 3. Univariate associations of RA patient factors with sCD14 concentrations.

Variable	OR (95% CI)	p
Demographic characteristics		
Age at enrollment, yrs	1.02 (1.01 to 1.03)	< 0.001
Male sex	1.01 (0.72 to 1.42)	0.966
Race		
White	1.00	Referent
African American	0.47 (0.36 to 0.61)	< 0.001
Other non-white	0.83 (0.56 to 1.24)	0.360
≥ High school education	0.80 (0.61 to 1.05)	0.106
Smoking status		
Never	1.00	Referent
Former	1.09 (0.84 to 1.43)	0.506
Current	0.97 (0.73 to 1.30)	0.842
Body mass index	0.98 (0.96 to 1.00)	0.014
Comorbidity count	1.13 (1.06 to 1.21)	< 0.001
RA-related measures at enrollment		
Disease duration at enrollment	1.01 (1.00 to 1.01)	0.209
HLA-DRB1 SE-positive	1.28 (1.03 to 1.59)	0.028
ACPA-positive	1.17 (0.91 to 1.49)	0.220
Rheumatoid factor-positive	1.27 (0.95 to 1.69)	0.107
Subcutaneous nodules	1.33 (1.09 to 1.63)	0.006
hsCRP ≥ 3 mg/l	2.25 (1.79 to 2.84)	< 0.001
ESR	1.02 (1.01 to 1.02)	< 0.001
Tender joint count	1.02 (1.00 to 1.04)	0.023
Swollen joint count	1.06 (1.04 to 1.08)	< 0.001
Pain	1.02 (0.99 to 1.06)	0.221
Physician global	1.01 (1.00 to 1.01)	0.017
Patient global	1.00 (1.00 to 1.01)	0.376
MD-HAQ	1.24 (1.04 to 1.48)	0.019
DAS28 category		
< 2.6 (remission)	1.00	Referent
≥ 2.6 and < 3.2 (low activity)	1.34 (0.96 to 1.88)	0.090
≥ 3.2 and < 5.1 (moderate activity)	1.51 (1.17 to 1.96)	0.002
≥ 5.1 (high activity)	2.15 (1.60 to 2.89)	< 0.001
RAPID-3 score	1.08 (0.99 to 1.16)	0.068
Prednisone use	0.95 (0.77 to 1.17)	0.616
Methotrexate use	1.01 (0.82 to 1.24)	0.943
Anti-TNF-α use	0.93 (0.74 to 1.17)	0.551
CD14 genotypes		
rs2569190 (*CD14*/–260)		
GG	1.00	Referent
AG or AA	1.16 (0.94 to 1.44)	0.176
rs5744455 (*CD14*/–651)		
CC	1.00	Referent
CT or TT	0.91 (0.74 to 1.11)	0.351
rs2915863 (*CD14*/–1720)		
TT	1.00	Referent
CT or CC	1.24 (1.01 to 1.52)	0.039
rs2569193 (*CD14*/–2838)		
GG	1.00	Referent
AG or AA	1.27 (1.04 to 1.56)	0.019

OR, 95% CI, and p values generated using ordinal regression analysis. RA: rheumatoid arthritis; SE: shared epitope; ACPA: anticitrullinated protein antibody; hsCRP: high sensitivity C-reactive protein; ESR: erythrocyte sedimentation rate; MD-HAQ: Multidimensional Health Assessment Questionnaire; DAS: Disease Activity Score; RAPID: Routine Assessment of Patient Index Data; TNF: tumor necrosis factor.

significantly correlated in RA with other acute-phase responses including ESR and hsCRP. In patients with RA, sCD14 levels do not appear to be significantly influenced by genetic variation in CD14 but rather are more strongly associated with other patient factors that include older age, race, and inflammatory disease burden. Further studies are needed to elucidate the implications and potential effects of higher circulating levels of sCD14 in patients with RA.

Table 4. Multivariable associations of rheumatoid arthritis (RA) patient characteristics with higher quartiles of soluble CD14 (sCD14).

Variable	Clinical Model	Clinical Model plus rs2915863 (*CD14*/–1720)	Clinical Model plus rs2569193 (*CD14*/–2838)	Clinical Model plus rs2569190 (*CD14*/–260)	Clinical Model plus rs2915863 (*CD14*/–1720)
Age at enrollment, yrs	1.02 (1.00 to 1.03)†	1.02 (1.00 to 1.03)†	1.02 (1.01 to 1.03)†	1.02 (1.00 to 1.03)†	1.02 (1.00 to 1.03)†
Race					
White	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
African American	0.52 (0.38 to 0.71)††	0.49 (0.35 to 0.67)††	0.51 (0.37 to 0.70)††	0.53 (0.38 to 0.72)††	0.54 (0.39 to 0.75)††
Other non-white	0.95 (0.60 to 1.52)	0.95 (0.59 to 1.52)	1.10 (0.69 to 1.75)	0.96 (0.60 to 1.53)	0.98 (0.60 to 1.58)
Body mass index	0.98 (0.96 to 1.00)*	0.98 (0.96 to 1.00)	0.98 (0.96 to 1.00)*	0.98 (0.96 to 1.00)	0.98 (0.96 to 1.00)
hsCRP ≥ 3 mg/ml	2.09 (1.59 to 2.74)††	2.02 (1.54 to 2.66)††	2.12 (1.61 to 2.80)††	2.06 (1.57 to 2.71)††	2.07 (1.58 to 2.72)††
DAS28 category					
<2.6 (remission)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
2.6 to 3.2 (low activity)	1.39 (0.92 to 2.11)	1.38 (0.91 to 2.11)	1.35 (0.88 to 2.06)	1.40 (0.92 to 2.13)	1.40 (0.92 to 2.12)
3.3 to 5.1 (moderate activity)	1.50 (1.08 to 2.10)*	1.55 (1.10 to 2.17)*	1.46 (1.04 to 2.05)*	1.54 (1.10 to 2.16)*	1.55 (1.11 to 2.17)*
> 5.1 (high activity)	1.78 (1.22 to 2.59)†	1.84 (1.25 to 2.70)†	1.77 (1.21 to 2.59)†	1.84 (1.26 to 2.69)†	1.87 (1.28 to 2.75)†
rs5744455 (*CD14*/–651)					
TT	—	1.00 (Referent)	—	—	—
CT or CC		0.77 (0.60 to 0.99)*			
rs2569193 (*CD14*/–2838)					
GG	—	—	1.00 (Referent)	—	—
AG or AA			1.27 (1.00 to 1.61)		
rs2569190 (*CD14*/–260)					
GG	—	—	—	1.00 (Referent)	—
AG or AA				1.04 (0.80 to 1.35)	
rs2915863 (*CD14*/–1720)					
TT	—	—	—	—	1.00 (Referent)
CT or CC					1.11 (0.86 to 1.43)

*p < 0.05, †p < 0.01, ††p < 0.001; results from multivariable ordinal regression; all other values nonsignificant. hsCRP: high sensitivity C-reactive protein; DAS: Disease Activity Score.

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